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

Internship Report at Oceano Fresco

Tiago Miguel Paiva Baptista

2025

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Internship Report at Oceano Fresco

Tiago Miguel Paiva Baptista

Internship report for a Master's degree in Aquaculture

Master's report realized under the orientation of Specialist Professor Teresa Baptista
and the supervision of Scott Mactier

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Title: Internship Report at Oceano Fresco

Escola Superior de Turismo e Tecnologia do Mar – Peniche

Instituto Politécnico de Leiria

2025

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Acknowledgements

I began this Master's journey as a way of overcoming a difficult period in my life and turning it into an opportunity for growth. I wish to express my sincere gratitude to Oceano Fresco for the internship opportunity that made this work possible. I am deeply thankful to my academic advisor for their guidance, encouragement, and support throughout this process. My thanks also go to the dedicated hatchery team, whose patience, companionship, and knowledge greatly enriched my learning experience.

I would like to thank my family for their unwavering support and strength during this path, and my closest friends, Cláudio and TP, for their constant trust and belief in me. Finally, a very special acknowledgement goes to my grandfather Valdemar, my pride, whom I always carried in my heart and to whose memory I dedicate this achievement in part.

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Resumo

Este relatório de estágio, desenvolvido ao longo de um ano, entre 19 de junho de 2023 e 19 de junho de 2024, documenta o funcionamento operacional e as oportunidades de melhoria identificadas numa maternidade comercial de bivalves, com ênfase em *Ruditapes decussatus* e *Venerupis corrugata*. O estágio abrangeu todas as fases de produção, desde o condicionamento dos reprodutores até à produção larvar, pós-larvar e de semente, proporcionando uma compreensão abrangente das rotinas da maternidade e das práticas de biossegurança. As operações diárias, semanais e mensais foram acompanhadas em detalhe em cada unidade de produção, consolidando competências técnicas em maneio, alimentação, monitorização e controlo microbiológico.

Um componente central do estágio foi um projeto piloto que visou colmatar um dos maiores constrangimentos enfrentados pela maternidade neste período: a escassez de microalgas para alimentação. Tanques de cultivo de semente de 3000 L foram reaproveitados para a produção em batch de diatomáceas em grande volume. Ao longo de sucessivos ciclos de produção, testaram-se diferentes configurações, ajustando volume de trabalho, dosagens de nutrientes, sistemas de arejamento, suplementação com CO_2 e iluminação. Os resultados demonstraram que a eficiência do arejamento, o equilíbrio nutricional e a regulação do carbono são críticos para sustentar altas densidades celulares e a estabilidade da cultura. A introdução de CO_2 melhorou significativamente o desempenho e a inoculação de batch para batch revelou-se viável, aumentando a eficiência operacional e reduzindo a dependência da sala dos sacos. No final do projeto, foram alcançadas colheitas diárias de vários milhares de litros de cultura limpa, aliviando de forma significativa a escassez alimentar e apoiando a produção da maternidade.

O estágio também evidenciou áreas com potencial de otimização adicional, incluindo a implementação de quarentena e monitorização gonadal nos reprodutores, monitorização contínua da qualidade da água na sala das larvas, otimização das configurações dos sistemas de fixação na sala de pós-larvas, aperfeiçoamento dos protocolos de alimentação e expedição da semente, e reforço das práticas de higiene em operações transversais. Uma melhoria transversal identificada foi a digitalização dos registos operacionais, que permitiria aumentar a rastreabilidade e a capacidade preditiva.

Em conclusão, o estágio alcançou o seu duplo objetivo: consolidar competências técnicas e científicas através da integração total nas operações da maternidade e responder com sucesso ao desafio alimentar por meio de um ensaio industrial que evoluiu para uma estratégia de produção viável. A experiência proporcionou não só conhecimento prático, mas também perspetivas sobre o potencial de melhoria contínua e inovação na aquacultura de bivalves.

Palavras-chave: Aquacultura; Maternidade de bivalves; Produção de microalgas.

Abstract

This internship report, developed over a one-year period from June 19, 2023, to June 19, 2024, documents the operational workflow and improvement opportunities identified in a commercial bivalve hatchery, with emphasis on *Ruditapes decussatus* and *Venerupis corrugata*. The internship covered all production stages, from broodstock conditioning to larval, post-larval, and seed rearing, providing a comprehensive understanding of hatchery routines and biosecurity practices. Daily, weekly, and monthly operations were followed in detail across each production unit, consolidating technical skills in husbandry, feeding, monitoring, and microbiological control.

A central component of the internship was a pilot project addressing one of the most pressing bottlenecks the hatchery was facing at this period: the shortage of microalgae for feeding. Spare 3000 L seed tanks were repurposed for large-volume batch production of diatoms. Over successive production cycles, different configurations were tested, adjusting working volume, nutrient dosages, aeration systems, CO_2 supplementation, and lighting. Results demonstrated that aeration efficiency, nutrient balance, and carbon regulation are critical for sustaining high cell densities and culture stability. The introduction of CO_2 markedly improved performance, and batch-to-batch inoculation proved feasible, improving efficiency of operations and reducing dependence on the Upright Bag room. By the end of the project, daily harvests of several thousand liters of clean culture were achieved, significantly alleviating the feed shortage and supporting hatchery production.

The internship also highlighted areas for further optimization, including broodstock quarantine and gonadal monitoring, continuous larval water quality monitoring, optimization of setting system configurations in the post-larval room, refinement of seed feeding and shipment protocols, and reinforcement of hygiene practices across transversal operations. A cross-cutting improvement identified was the digitalization of operational records, which would enhance traceability and predictive capacity.

In conclusion, the internship achieved its dual aim: consolidating technical and scientific skills through full integration into hatchery operations, and successfully achieving the project through an industrial-scale trial that evolved into a viable production strategy. The experience provided not only practical expertise but also insights into the potential for continuous improvement and innovation in bivalve aquaculture.

Key-words: Aquaculture; Bivalve hatchery; Microalgae production.

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List of abbreviations

DGRM: Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos

EU: European Union

EUMOFA: European Market Observatory for Fisheries and Aquaculture

FAO: *Food and Agriculture Organization*

INE: Instituto Nacional de Estatística

MA: Marine Agar

OF: Oceano Fresco

TCBS: Thiosulfate Citrate Bile Salts Sucrose

UV: Ultraviolet Radiation

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I.Introduction

World aquaculture has played an increasingly important role in global food security, with steady growth in recent years. The sector has expanded not only in terms of production volume, but also in the diversity of farmed species and the adoption of more sustainable practices, with initiatives aimed at minimizing environmental impacts and ensuring long-term resilience. These advances include the development of aquaculture systems, such as water recirculation and integrated multi-trophic systems, as well as the use of more efficient and sustainable feeds, which reduce dependence on marine origin ingredients. This commitment to sustainability is essential if we are to satisfy the world's growing demand without jeopardizing aquatic ecosystems (FAO, 2024b).

Global aquaculture production reached an all-time high in 2022, totalling 130.9 million tonnes, including 94.4 million tonnes of aquatic animals and 36.5 million tonnes of algae. For the first time, aquaculture production of aquatic animals (51%) surpassed wild capture (49%), underscoring the sector's increasing importance meeting global demand (Figure 1). Of the total 185.4 million tonnes of aquatic animal production, approximately 89% (164.6 million tonnes) was destined for direct human consumption, with *per capita* consumption estimated at 20.7 kg of which aquaculture contributed 57% (Figure 2). This continued growth and the rising share of aquaculture in human consumption highlight its significance not only for food security but also for the sustainable development of global food value chains, providing affordable and nutritious sources of protein (FAO, 2024b).

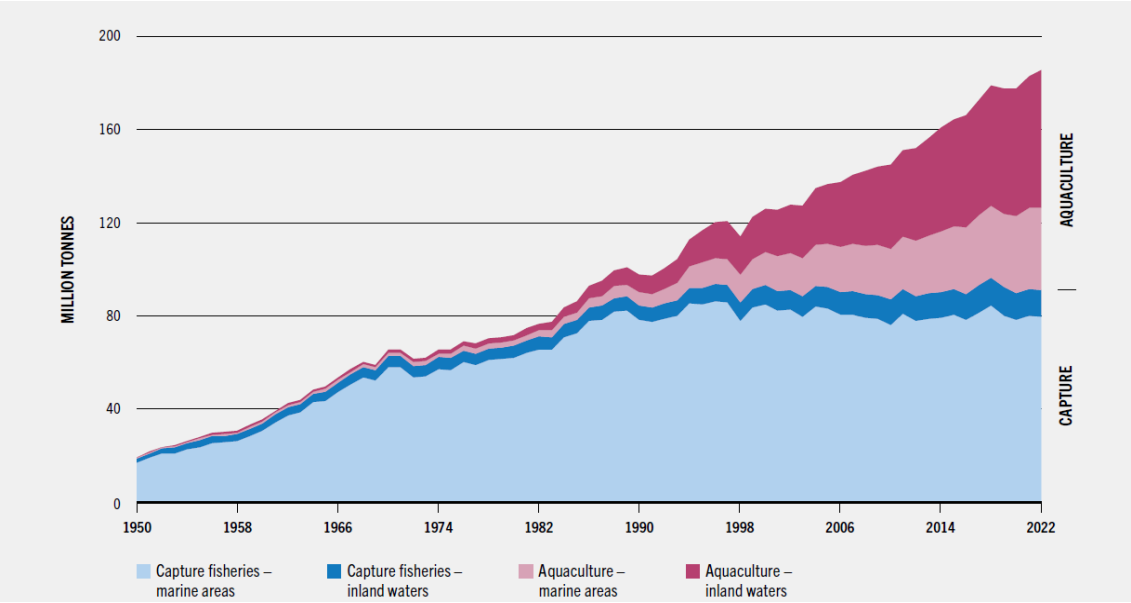

Figure 1. World fisheries and aquaculture production of aquatic animals (Source: FAO, 2024b - The State of World Fisheries and Aquaculture).

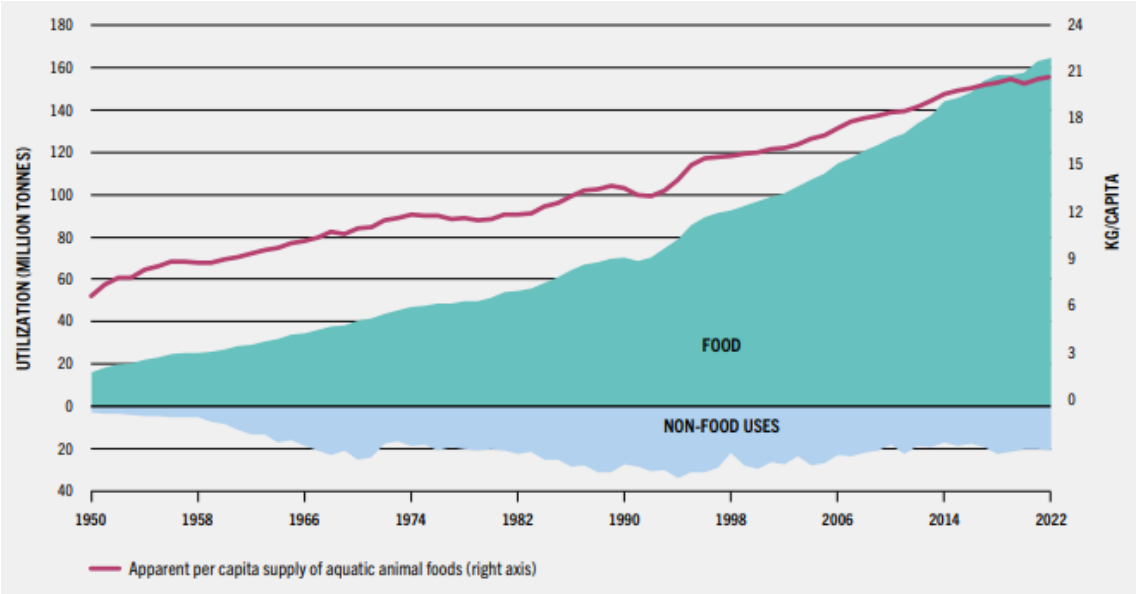

Figure 2. Utilization of fisheries and aquaculture production of aquatic animals (Source: FAO, 2024b - The State of World Fisheries and Aquaculture).

Asia was the region that contributed the most to global aquaculture production in 2022, accounting for 91.4% of the total, followed by Latin America and the Caribbean (3.3%), Europe (2.7%) and other regions. Among the ten largest producers of aquatic animals, China stands out, responsible for 36% of global production and consolidating its position as the world leader (FAO, 2024b).

Other notable producers include India, Indonesia and Peru, which have shown significant growth focusing primarily on freshwater fish and crustaceans. These countries play a key role in diversifying production, aligning with both regional and global needs (FAO, 2024b).

Fish represent the most farmed group of aquatic animals worldwide, accounting for 65.2% of global aquaculture production in 2022, followed by molluscs (20%) and crustaceans (13.5%). Within the fish group, carp dominate, representing half of total production due to their prominence in Asian markets and ease of cultivation. In molluscs, oysters rank first (37.4%) followed by clams (23.9%). Among crustaceans, shrimp lead with 62.2% of production. This species diversity demonstrates the versatility of aquaculture and its ability to meet the varied demands of the global market (FAO, 2024b).

Algae production, which reached 36.5 million tonnes in 2022, further reflects aquaculture's importance beyond food supply, contributing to sectors such as biofuels, cosmetics and food additives. This diversification enhances the broader economic and environmental benefits of aquaculture (FAO, 2024b).

This global panorama is mirrored in Europe, which accounts for 2.7% of world aquaculture production. The region is recognized for its species diversification and commitment to sustainable practices. Europe is a global benchmark in the adoption of technologies such as recirculating water systems (RAS) and integrated multi-trophic aquaculture (IMTA), which minimise environmental impacts and promote efficient resource use. These advances are reinforced by strict regulations that safeguard marine ecosystems and ensure the production of high-quality aquaculture products. As a result, Europe positions itself at the forefront of responsible aquaculture practices, responding to the growing global demand for sustainable products (FAO, 2024b).

This sustainable approach has enabled the sector to face historical challenges of decline and adapt to changes in the global aquaculture landscape. Although Europe contributes a smaller share of global production, its aquaculture has shown resilience and adaptability over the decades. Following a gradual decline in fisheries and aquaculture production since the 1980s, there was a significant recovery in the 2000s, albeit with some fluctuations in the subsequent years. In 2022, European aquaculture production of aquatic animals reached 3.5 million tonnes, representing a 7.1% increase compared with 2020 (FAO, 2024b).

Production is dominated by fish, which accounted for 66.9% (~2.4 million tonnes) of the total, followed by molluscs at 30.6% (~600.000 tonnes) and crustaceans at 0.2%. Among fish, salmon, trout, sea bass and sea bream are the most widely farmed species, while mollusc production is concentrated in mussels, oysters and clams. (FAO, 2024b).

Production is concentrated in countries such as Norway, the leading producer of salmon, as well as Spain and France, which are major producers of molluscs, and Greece and Italy, which dominate sea bream, sea bass and trout production. These countries play a key role in diversifying aquaculture production, supplying both regional and international markets. Through diversification and a strong focus on quality, Europe has consolidated its position as a benchmark in the supply of sustainable, high-quality aquaculture products to the global market (FAO, 2024b).

Within Europe, Portugal presents unique characteristics that reflect both the potential and the challenges of its aquaculture sector. The country has the highest consumption of fisheries and aquaculture products in the EU, although apparent per capita consumption has declined since its 2018 peak of nearly 61 kg live weight equivalent. In 2022, Portuguese households spent an average of 413€ on fisheries and aquaculture products, a figure almost three times higher than the EU average and 143€ above Spain, which ranked second (Figure 3). Furthermore, household expenditure in Portugal was more evenly distributed between fishery (44%) and aquaculture products (56%), compared with the EU average, where the ratio is usually 1:4 (EUMOFA, 2023).

In 2022, Portugal produced 18,688 tonnes of aquaculture products, valued at 168.5 million euros, representing increases of 6% and 7%, compared with 2021. These figures reflect significant growth, especially when compared with production levels a decade earlier, which were nearly two to three times lower (Eurostat, 2024).

By the end of the same year, Portugal had 1,290 licensed aquaculture establishments, 38 more than in the previous year. However, the number of breeding units remained stable, suggesting untapped potential for diversification and modernization within the sector (INE & DGRM, 2024).

Although Portugal contributes only a modest share to global and European production, the sector has shown consistent growth and exerts a strong cultural and economic impact, underscoring its potential to consolidate as a strategic pillar at the national level.

One of the licensed establishments is Oceano Fresco (OF), a sustainable seafood company that uses innovative aquaculture techniques to cultivate clam species native to Europe. These include *Venerupis corrugata* (pullet carpet shell) and *Ruditapes decussatus* (grooved carpet shell), which are currently in decline and threatened by exotic and invasive species, yet remain highly valued. Guided by a vision of regenerative

aquaculture, the company promotes its clams as among the most sustainable, healthy, and palatable foods in the world.

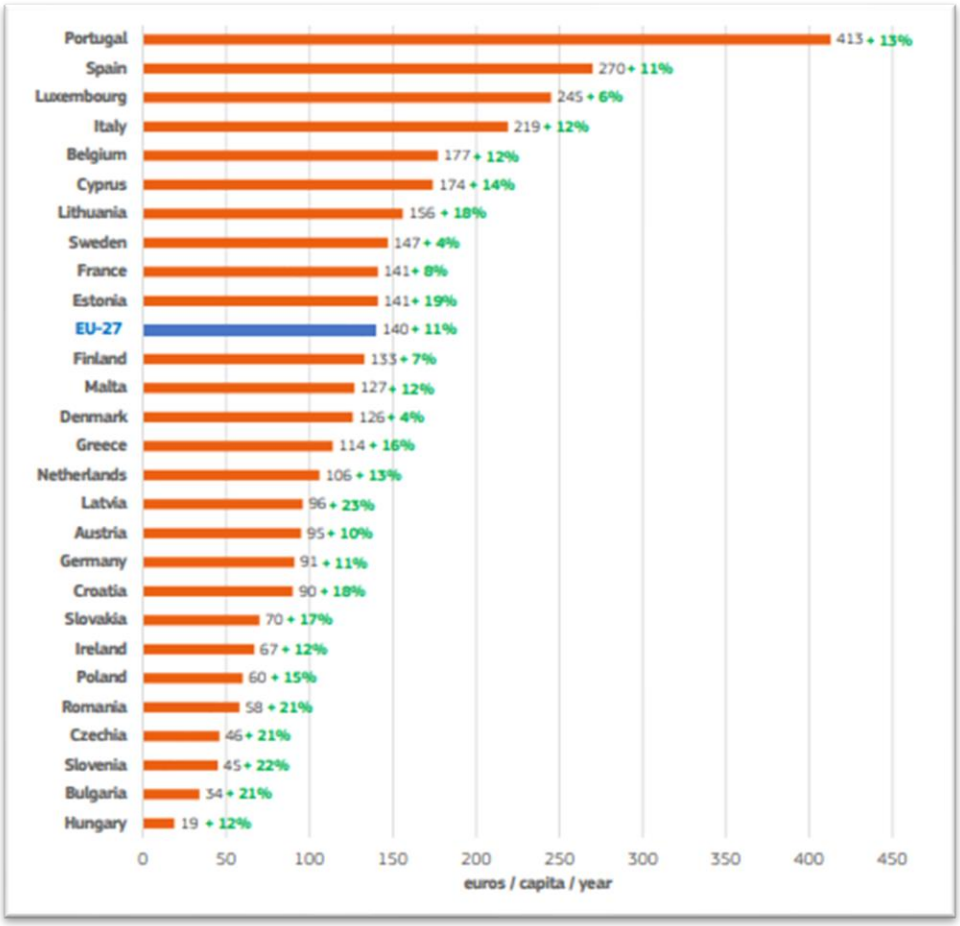

Figure 3. Per capita household nominal expenditure on fishery and aquaculture products in 2022 and % variation 2022/2021 (Source: EUMOFA, 2023).

Production begins with clam reproduction in the BioMarine Center (Nazaré, Portugal), a breeding facility officially inaugurated in 2021 (Figure 4). Within the center, successive stages take place, including larval and seed rearing. The young clams are then transferred to the world's first open sea clam farm, located off the coast of Lagos (Portugal), where they feed exclusively on naturally available microalgae until reaching adulthood.

Founded in 2015, the company has been supported by funding from its founders, public subsidies and venture capital investors who share its transformative vision of a 'Blue Economy' for Europe and beyond.

It was within the BioMarine Center's breeding unit that this internship took place. This unit is divided into several production, laboratories and outdoor areas, including sections for seed, larvae, broodstock and microalgae, as well as an inoculum chamber, research and production laboratories, technical area, and other smaller specialized spaces.

The internship involved acquiring hands-on experience with all production-related procedures across the different sections of the unit. This was the primary objective. A second objective was to contribute to solving a practical challenge faced by the company.

At the time, the hatchery required additional algae production capacity to sustain the scaling up of seed output and enable the development of new projects and ideas. To address this need, I started the batch production of microalgae, thereby fulfilling the internship's second objective.

Figure 4. Oceano Fresco' Biomarine Center in Nazaré, Portugal (Source: screen capture from Imagens de Marca, 2025).

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II. Bivalves' hatchery operations – Oceano Fresco's production methods and daily operation for a technician

This section provides a summary of the different methodologies used in a bivalve aquaculture company and describes the operational methods adopted by Oceano Fresco.

1. Supplying the hatchery with high quality water (Technical area)

The objective of this area is straightforward: to supply the hatchery with high-quality, clean seawater at the desired temperature while also providing air and CO_2 (Figure 5).

Figure 5. Oceano Fresco' technical area south (left) and north side (right) (Source: Author, 2025).

a. Methodologies

Bivalve aquaculture operations frequently rely on sub-sand intake wells (seawater wells) to capture seawater, taking advantage of the natural filtration provided by sand. This method ensures a stable, nutrient-rich water supply with minimal fluctuations in temperature and salinity, conditions that are ideal for sensitive larval stages (Helm et al., 2004). Alternatively, seawater may be drawn through offshore intake pipelines sited at depth (often below the seasonal thermocline) to buffer short-term fluctuations in temperature and salinity, reduce the entrainment of plankton blooms and fouling organisms, and provide a more stable inflow for intensive hatchery operations. In

temperate areas, intakes may need to be placed at depths up to ~20 m; locating them near the coastline shortens pipe runs while still reaching sufficient stable water (Helm et al., 2004). Some hatcheries also use treated industrial effluents, applying preliminary filtration and water conditioning (e.g., pH adjustment and ammonia removal) before use in production (Helm et al., 2004).

In bivalve hatcheries, coarse mechanical filtration is commonly applied using rapid sand filters or multi-layer beds to remove larger particles (>20–40 µm) and protect early larval stages (Helm et al., 2004; Shingare et al., 2019). The water then undergoes fine filtration through cartridge or bag filters, or via self-cleaning rotating drum filters, which provide continuous removal of suspended solids and particles without the need for manual intervention (Helm et al., 2004). In more advanced systems – particularly in hatcheries – membrane ultrafiltration (~0.02 µm) is employed to remove gametes, viruses, and pathogens. This process is often combined with air-backwash cleaning to maintain system efficiency (Cordier et al., 2019).

Following physical filtration, water used in bivalve hatcheries typically undergoes complementary disinfection to remove pathogenic microorganisms, thereby protecting larvae and juveniles (Helm et al., 2004). The most widely used method is ultraviolet (UV) radiation, which damages DNA and effectively inactivates bacteria, viruses, and protozoa without chemical additives, making it an industry standard (Helm et al., 2004). Alternatively, some systems apply electrolytic seawater treatment, which generates hypochlorite *in situ* and has demonstrated high efficacy in reducing *Vibrio* spp. and other pathogens, while minimizing residual chemical accumulation (Jorquera et al., 2002).

Treated wastewater from bivalve hatcheries is generally discharged back into the marine environment after internal treatment systems remove suspended solids and reduce nutrient loads. Research shows that bivalve filter feeders can effectively remove fine suspended particles and organic matter from aquaculture wastewater effluents (Zhou et al., 2014). Moreover, integrated RAS systems combining bivalves with filtration mechanisms can further contribute to effluent management (Myers, 2006). Although waste products such as pseudo-faeces may accumulate locally, areas with strong hydrodynamic circulation exhibit low deposition rates and minimal environmental impacts (Gallardi, 2014). In integrated systems like IMTA, bivalves contribute to natural remediation by filtering particulate nutrients during production and facilitating nutrient removal through harvest, thereby reducing overall pollution discharge (Kjerulf Petersen et al., 2019). These practices reflect the sector's commitment to environmental sustainability by ensuring that effluents are managed without overburdening coastal ecosystems.

b. How Oceano Fresco operates

The area is equipped with two reservoirs, each with a capacity of 40,000 liters, which receive seawater via two collection pumps installed 700 meters from the hatchery at a depth of 20 meters (Figure 6). This seawater undergoes a three-stage filtration process to ensure optimal quality before entering the hatchery, thereby minimizing the risk of diseases, contamination, and potential mortality outbreaks (Figure 7).

The filtration process consists of:

- Sand filters, which remove larger suspended solids,

- Cartridge filters, arranged in a series of decreasing filtration precision: two filters of 1 µm, one of 5 µm, and one of 10 µm, allowing progressive removal of finer particles,
- UV filter, which disinfects the water by inactivating pathogenic microorganisms.

Figure 6. Hatchery reservoirs of 40,000 liters (Source: Author, 2025).

Figure 7. The three-stage filtration, sand filters (left), cartridge filters (middle) and UV filters (right) (Source: Author, 2025).

Seawater from the reservoirs is pumped through several water pumps and passes subsequently through the sand filters, the cartridge filter system, and finally the UV filter. Two parallel lines of cartridge and UV filters operate alternately every week, allowing for regular cleaning and maintenance.

Following filtration and disinfection, the water is ready for use within the hatchery. Prior to distribution, it can be optionally heated or cooled depending on operational needs (Figure 8).

The system includes three distinct water lines:

- Ambient line, delivering water at its natural temperature,
- Warm line, supplying heated water,
- Temperate line, providing water at an intermediate temperature achieved by mixing ambient and heated water to meet specific setpoints.

Figure 8. Technical area' water pumps (left) and heaters (right) (Source: Author, 2025).

These three lines allow precise temperature control, supplying different areas within the hatchery and ensuring optimal conditions for each production stage.

c. Technical area operations

The maintenance team carries out the following operations in the technical area, sometimes with the support of a production technician. Two main routine tasks are usually performed every Thursday: reservoir cleaning and cartridge filter replacement. Additionally, periodic cleaning and disinfection of water lines are performed according to the monthly maintenance schedule.

- Reservoir cleaning

Reservoir cleaning is performed one reservoir at a time to ensure that the hatchery´s water supply is never interrupted. While one reservoir is being cleaned, the other continues to supply water.

Once emptied, the reservoir interior is visually inspected through the access hatch. If sand or other deposits are observed on the bottom, suction cleaning is conducted. Every three weeks, in addition to cleaning the bottom, the reservoir walls are cleaned and disinfected using sodium hypochlorite, a long-handle broom, and a pressure washer (Figure 9). After cleaning, the reservoir is refilled, and any residual sodium hypochlorite is diluted as the reservoir reaches operational capacity. Once the first reservoir reaches approximately 90% capacity, the second reservoir undergoes the same cleaning procedure.

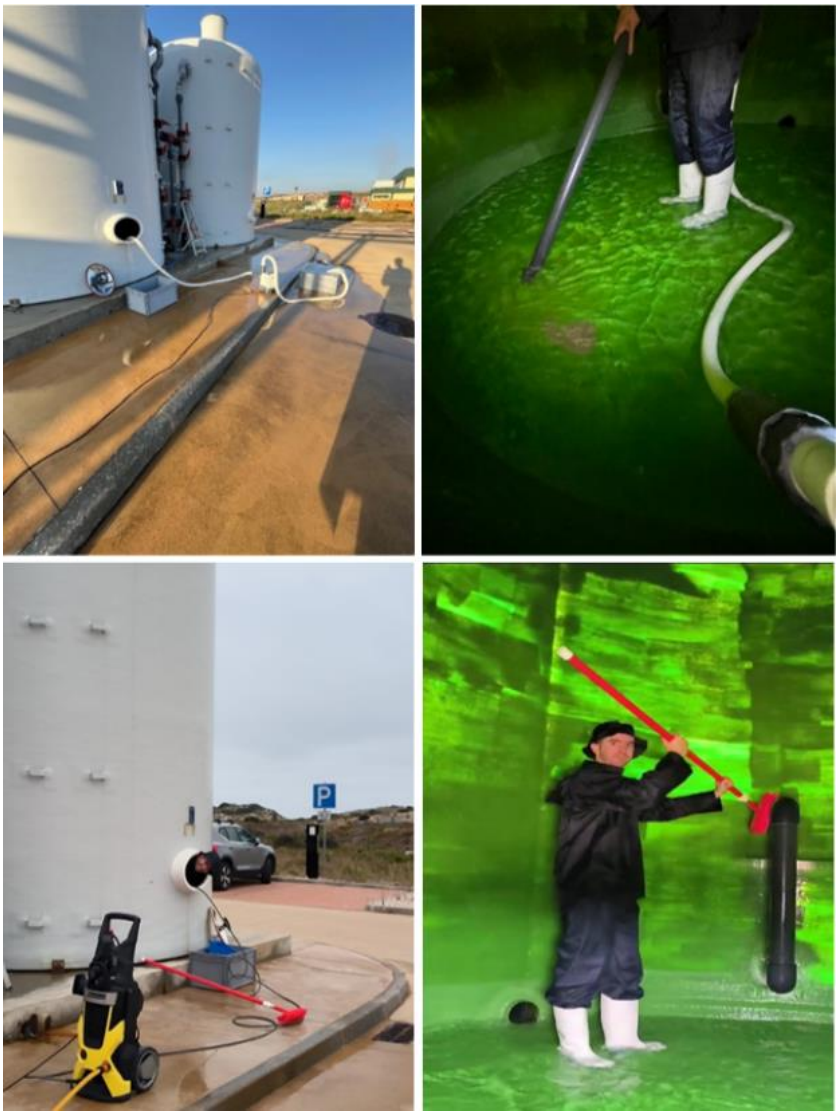

Figure 9. Reservoir cleaning operation – access via opened hatch (top left), deposits suction (top right), cleaning equipment (bottom left) and wall cleaning (bottom right) (Source: Author, 2025).

- Cartridge filter replacement

Cartridge filter replacement is typically performed concurrently with reservoir cleaning. The process involves removing used cartridges from the active filtration line and installing new filters in the alternate line. The used cartridges are then cleaned with fresh water and sodium hypochlorite and are stored without filters for the next cycle. The newly installed filters remain in service until the next scheduled replacement the following week (Figure 10).

Figure 10. Cartridge filter replacement operation – removing used cartridges (top left), cleaning them with sodium hypochlorite (top right), putting new filter (bottom left) and installing the cartridge in the new line (bottom right) (Source: Author, 2025).

- Cleaning and disinfection of water lines

As part of the monthly maintenance routine, water lines are periodically cleaned and disinfected to prevent biofilm buildup and the proliferation of undesirable bacteria, which could compromise operational efficiency over time.

The process involves circulating cleaning agents through the selected water line, including oxalic acid, sodium hypochlorite and sodium thiosulfate for neutralization.

The cleaning solution is prepared in a 1,000 L tank, with a working volume of 600–700 L and introduced into the system immediately downstream of the UV filter. It circulates through the entire length of the water line, including passages inside the hatchery, until it reaches the exit point. If the line targeted for cleaning is currently supplying the hatchery, water flow is first redirected to an alternative line to maintain a continuous supply. After the cleaning cycle, the treated water line is usually left to dry before being returned to service.

2. Storage, maintenance and production of high-density microalgae inocula (Inoculation chamber)

This room serves as the company's microalgae bank, where microalgae strains are stored and maintained (Figure 11). The primary objective is to maintain this bank and to produce high-quality, high-density microalgae inocula under near-axenic conditions. These inocula are used both for larval rearing and for scaling up microalgae production.

Figure 11. Inoculation chamber (left) and the microalgae strains bank (right) (Source: Author, 2025).

Microalgae cultures progress through characteristic microbial growth phases that directly influence their suitability as inocula. Following inoculation, cells undergo a lag phase, during which they adapt to the new environment with little or no cell division. This is followed by the exponential phase, characterized by rapid cell division and maximum growth efficiency, if light, nutrients, and dissolved CO_2 are supplied in sufficient amounts. For hatchery purposes, inocula are ideally harvested and transferred during the late exponential or early stationary phase, when both cell density and biochemical quality are optimal. Prolonged culture leads to the stationary phase, where growth plateaus due to nutrient limitation, CO_2 depletion, or light restriction, and ultimately to the decline phase, marked by increased cell mortality and reduced feed quality. Careful timing of inoculum transfer is therefore essential to ensure robust, high-quality starter cultures for subsequent scaling (Andersen, 2005; Borowitzka & Moheimani, 2013; Richmond & Hu, 2013).

a. Methodologies

The inoculation room is a critical space in a bivalve hatchery, where axenic microalgae cultures are established and maintained to provide high-quality, reliable nutrition for early larval stages (Helm et al., 2004). The process begins with the isolation and purification of strains: environmental or existing culture samples are processed using techniques such as serial dilution, micropipette isolation, or plating on selective media to obtain unialgal cultures free from contaminants, which are verified through microscopic inspection and bacterial contamination tests (Fernandez-Valenzuela et al., 2021; Helm et al., 2004). To maintain axenic conditions, all equipment, culture media, and seawater are sterilized using filtration (0.2 µm), autoclaving, or UV irradiation (Helm et al., 2004).

Once axenic conditions are ensured, inoculum (typically 5–10% v/v) is transferred into batch or semi-continuous culture systems using flasks or carboys ranging from 2 to 25 L. Batch systems are favoured for more sensitive species, while semi-continuous systems are preferred for more robust flagellates because they extend culture duration and maintain exponential growth (Helm et al., 2004). The inocula are then progressively scaled up to larger volumes (50–200 L or more), with strict control of light, temperature, pH (regulated with CO_2), and aeration to ensure dense, stable, and contaminant-free cultures suitable for larval feeding or further mass production (Helm et al., 2004).

Recent methodological advances, particularly fluorescence-activated cell sorting (FACS), have improved the efficiency of axenic microalgal culture isolation, enabling faster and more precise separation of target strains for aquaculture applications (Sensen et al., 1993; Cho et al., 2013; Doppler et al., 2021)

b. How Oceano Fresco operates

The company maintains a diverse collection of selected microalgae strains, ranging from flagellates to diatoms. These include flagellates: *Tetraselmis suecica* (*Ts*), *Tisochrysis lutea* (*T-iso*), *Isochrysis galbana* (*Ig*), *Diacronema lutheri* (*Dl*); and diatoms: —*Chaetoceros gracilis* (*Chg*), *Chaetoceros calcitrans* (*Chc*), *Chaetoceros muelleri*, *Skeletonema costatum,* and *Thalassiosira weissflogii.* Among these, the first five are actively in production, while the remaining strains are currently under study.

All strains are initially maintained in T-flasks of two sizes: 40 mL and 160 mL. These are positioned horizontally, limiting their working volumes to approximately 10 mL and 50 mL, respectively. The smaller flasks serve as first-generation stock, while the larger flasks are used to inoculate Erlenmeyer flasks. T-flasks are refreshed every two months by replacing half of their volume with fresh culture medium (Figure 12).

Each strain is also maintained in an Erlenmeyer flask, which is used to inoculate two additional Erlenmeyer flasks for a subsequent scale-up model (Figure 13). The standard inoculation protocol for Erlenmeyer flasks involves transferring 60 mL of culture from one of the two Erlenmeyer flasks into two new Erlenmeyer flasks after 7 days of growth, supplemented with 240 mL of fresh culture medium. This medium is prepared using a defined ratio of Varicon® medium and filtered seawater adjusted to 30ppt. The remaining culture is then used to inoculate a 5 L balloon containing 4L of autoclaved seawater and enriched with 10 mL/L of Varicon® medium.

After a 7-day growth period, the culture in this balloon is used to inoculate new balloons (up to five), each containing 4 L of autoclaved seawater, 500 mL of culture, and the same 10 mL/L concentration of Varicon® medium. After another 7 days, these balloons are ready for use either to inoculate cultures in the Upright Bag Room or, in the case of *Chc*, to feed the larvae directly.

For diatom strains, such as *Chaetoceros spp.*, silicate supplementation is essential. A concentration of 1 mL/L of silicate is added at each inoculation step — 240 µL for Erlenmeyer flasks and 4 mL for each balloon.

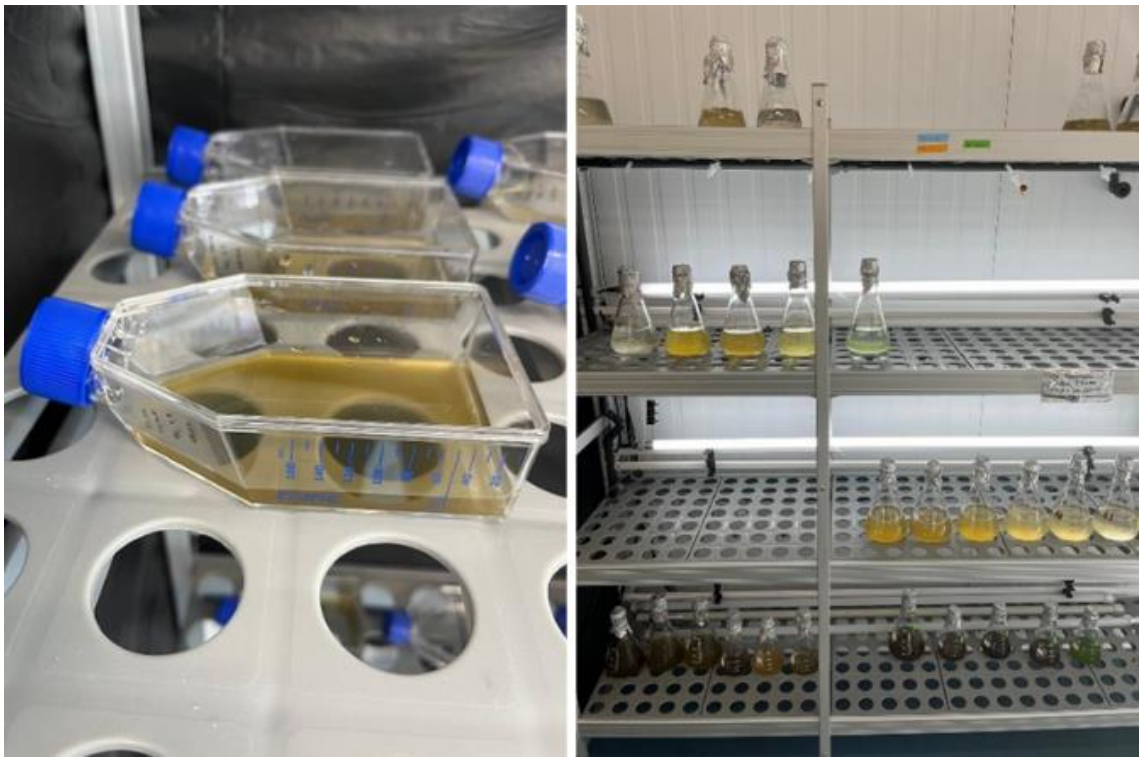

Figure 12. T-flask placed horizontally (left) and strains stored in Erlenmeyer's shelf (right) (Source: Author, 2025).

In terms of handling and hygiene (Figure 14):

- All Erlenmeyer flask transfers are performed inside a laminar flow chamber to maintain sterility.
- Balloon inoculations are carried out near an open flame to minimize the risk of contamination.

All inoculated balloons are then stored on shelves and racks equipped with continuous aeration, CO_2 supply, and controlled lighting to support optimal algal growth (Figure 15).

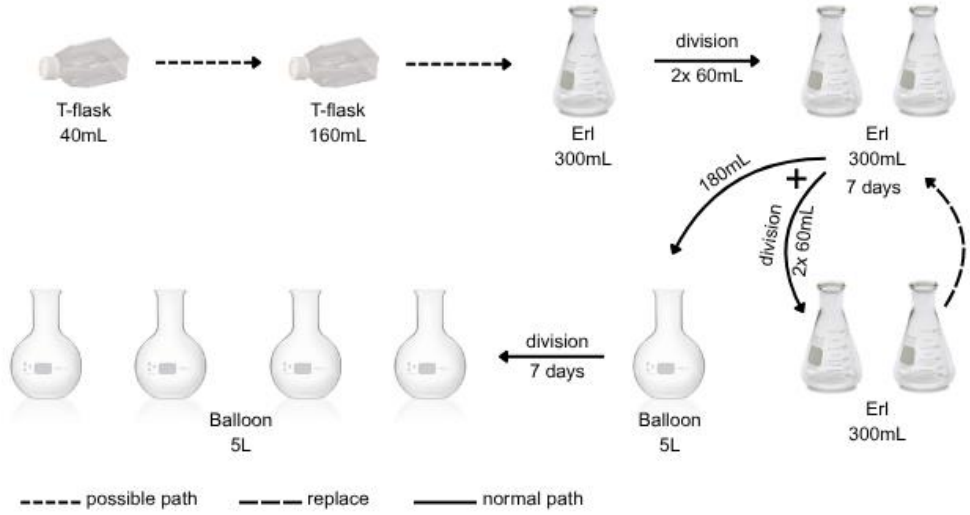

Figure 13. Scale-up model for Erlenmeyer flask and balloon divisions used in the company (Source: Author, 2025).

Figure 14. Laminar flow chamber (left) and a balloon ready for inoculation near a laboratory blowtorch (right) (Source: Author, 2025).

Figure 15. Balloon shelves supplied with aeration, CO_2 and light (Source: Author, 2025).

c. Inoculation chamber operations

Currently, the only daily operations carried out in the inoculation chamber are microalgae counts, which are necessary to calculate the diet for the larvae and the needed food for the broodstock, and the division of Erlenmeyer flasks and balloons (Figure 16).

The microalgae counting task begins with the collection of samples from selected upright bags of *Chg*, *T-iso* and *DI*, located in the Upright Bag room whenever there are larvae to feed. If no larvae are present, only the harvested microalgae mix from the bag room (collected overnight) is counted. Samples are first observed under a microscope to assess cell quality and check for possible contamination. If contamination is detected in a bag, an alternative bag of the same species is selected and inspected to ensure culture quality before being used to feed larvae. Next, the samples are diluted (with dilution factors ranging from 1:5 to 1:10 for bags and up to 1:27 for balloons) and placed in Neubauer chambers for counting. The counting method follows a straightforward formula:

$$\left(\frac{\boldsymbol{average\ count}}{\mathbf{4}}\right)/\mathbf{0.1} * \boldsymbol{dillution\ factor} = \boldsymbol{n^{o}\ cells}/\mu\boldsymbol{L}$$

The division of Erlenmeyer flasks and balloons follows the previously described scale-up model. The division of *Chc* is performed daily, whereas the remaining strains are divided on two dedicated days per week — typically Tuesdays and Thursdays — to

optimize the workload. On Tuesdays, *Chg*, *Dl*, and *Ig* are divided, followed by *T-iso* and *Ts* on Thursdays.

Figure 16. Setup for microalgae counts (left) and the divided balloons (right) (Source: Author, 2025).

Throughout the week, additional tasks include replenishing fresh culture medium and silicates as needed, as well as cleaning and preparing all glassware used in the process (Figure 17).

Figure 17. Glassware to be autoclaved (left) and glassware ready to use (right) (Source: Author, 2025).

3. Large-scale microalgae production for hatchery feeding (Upright Bag room)

The Upright Bag room is vital to the hatchery's functionality, ensuring a continuous supply of high-quality microalgae to nourish clams at all developmental stages, including larval, seed, and broodstock phases (Figure 18).

Figure 18. Upright Bag room, showcasing the six existing lines of bags and their respective harvest tank (Source: Author, 2025).

a. Methodologies

In large-scale microalgae production for aquaculture, different systems are employed depending on operational scale, budget, and desired algae quality. The two main categories are open systems and closed systems, with some hybrid approaches in between. Open systems, such as raceway ponds and algae bags, are widely used due to their low capital and operational costs, ease of scalability, and simple construction (Novoveská et al., 2023). Algae bags—although physically enclosed—are usually classified as semi-open systems because they are exposed to environmental variables and lack the strict control of fully closed photobioreactors (PBRs) (Zhang et al., 2024). Raceways typically consist of shallow, oval channels with paddle wheels to maintain circulation. They are suited for large volumes but carry a higher risk of contamination and evaporation, achieving biomass concentrations of around 0.5 g/L (Novoveská et al., 2023) (Figure 19).

On the other hand, closed systems, such as PBRs—including tubular, flat-panel, and vertical column configurations—enable superior control over temperature, light

exposure, gas exchange, and sterility. These systems often achieve higher biomass concentrations (e.g., 2–6 g L^{-1}) and are ideal for producing high-quality cultures for hatcheries, despite higher installation and maintenance costs (Razzak et al., 2024). Hybrid systems, such as combined raceway-PBR setups, are being developed to leverage the benefits of both approaches by enhancing productivity while reducing contamination risk (Kubar et al., 2025). Additionally, alternative setups, including suspended bags or vertical greenwalls are being explored for their balance between cost-efficiency and ease of handling at hatchery scale (Novoveská et al., 2023) (Figure 20).

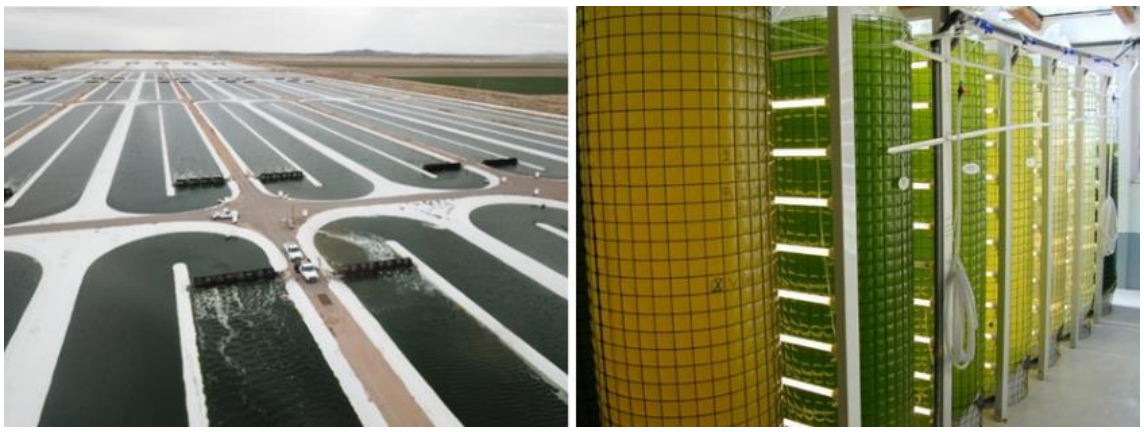

Figure 19. Examples of open microalgae production systems used in aquaculture: Raceway pond (left) (Photo by Qualitas, via U.S. Department of Energy); Algae bag system (right) (Photo by TerraVia, via Fish Farming Expert).

Figure 20. Examples of closed microalgae production systems used in aquaculture: Green wall (left) and tubular photobioreactor (right) at Necton, Portugal (Photos by Benjamin Schmid, via ProFuture).

Beyond the type of culture system, operational mode is another key factor in hatchery-oriented production. Batch culture remains the most common approach: cultures are inoculated, grown through the lag, exponential, and stationary phases, and harvested before the decline phase. Vessels are then cleaned and reinoculated, making this a discontinuous yet flexible system (Andersen, 2005; Richmond & Hu, 2013). Semi-continuous culture involves harvesting a fraction of the culture at intervals and replenishing it with fresh medium, thereby maintaining growth near the exponential phase for extended periods. Continuous culture, in contrast, achieves steady-state production by constantly supplying fresh medium and withdrawing culture at a fixed

dilution rate, maximizing productivity and maintaining stable cell physiology. Although semi-continuous and continuous regimes can yield higher and more consistent biomass, they require stricter biosecurity and technical control, which explains why batch culture remains dominant in commercial bivalve hatcheries (Borowitzka & Moheimani, 2013; Richmond & Hu, 2013).

Ultimately, the choice of system depends on the species being cultivated, the production scale, and the quality for downstream applications, such as larval diets or broodstock feeding in bivalve aquaculture.

b. How Oceano Fresco operates

The Upright Bag room is dedicated to the continuous large-scale production of five microalgae species: *Ts*, *T-iso*, *Ig*, *Dl* and *Chg*. As shown in Figure 18, a total of 84 vertical plastic bags—made of low-density polyethylene (900 × 1900 × 0.2 mm)—are arranged in a wire support structure divided into six lines, with 14 bags per line. Each line is assigned to a specific species, except *Chg*, which occupies two lines due to its higher demand.

The system operates in continuous harvest mode. Each line is connected to a dedicated 100 L harvest tank equipped with a submerged pump, which transfers the harvested microalgae to three external 1000 L storage tanks (Figure 21).

Bag inoculation is done using the balloons from the Inoculation chamber. Before inoculation, the bags are inflated and filled overnight with approximately 50 L of filtered seawater mixed with nutrients. The inoculation itself is performed through the top of the bag using a sharp tube to puncture it and pour in the culture from the balloon.

Figure 21. Harvest (left) and external storage (right) tanks (Source: Author, 2025).

After inoculation, each bag receives around 50 L of seawater per day, reaching the target harvest volume of approximately 280 L after about five days. At this point, a harvest connection is made using a thin plastic hose (the harvest hose) attached to the harvest pipeline, initiating continuous harvest. Bags are typically maintained for 1 to 1.5 months, after which they are discarded due to aging, contamination, or structural collapse (Figure 22).

Water for the bag room is sourced from the Technical Area, following UV filtration. It passes sequentially through two cartridge filters (0.2 µm and 1 µm) before entering an internal pasteurizer. From there, it is distributed through a system of glass pipelines, supplying each bag via individual capillary tubes.

A dosing pump injects nutrients into the water immediately after the pasteurizer at a rate of approximately 10 L per day. CO_2 is injected into the aeration line at 0.6 L/min and distributed to each bag to ensure proper gas exchange and optimal growth conditions (Figure 23).

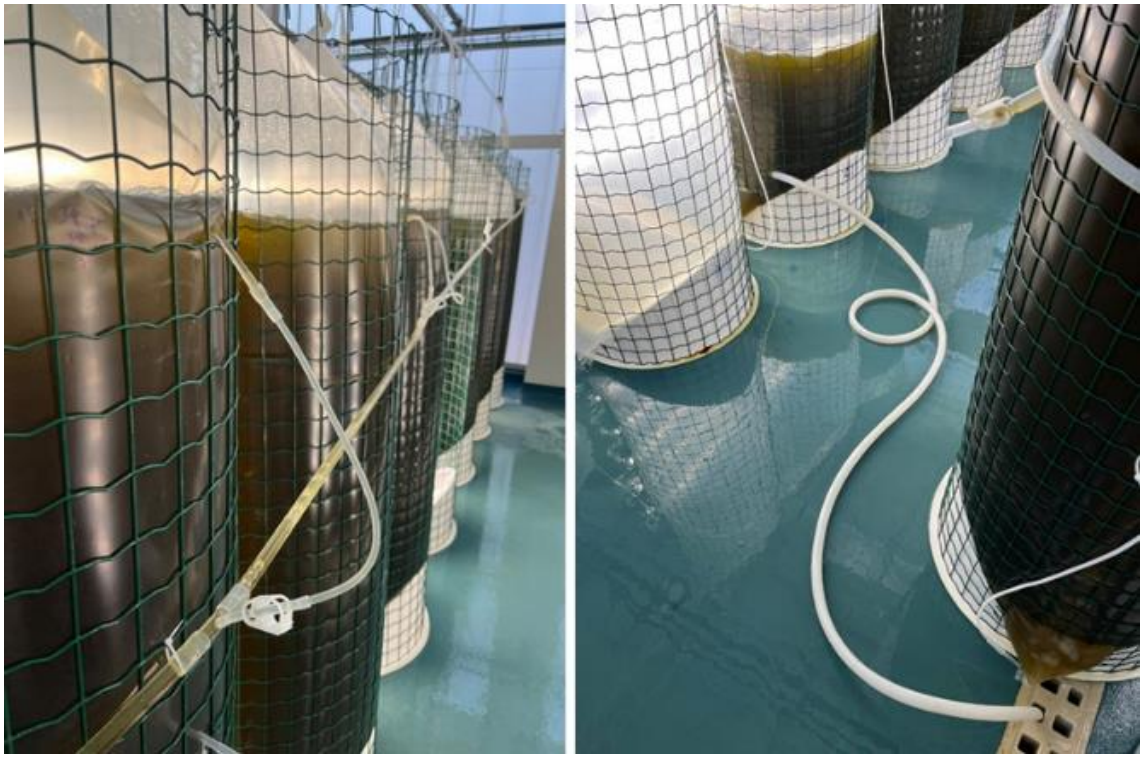

Figure 22. Harvest hose connect to the harvest pipeline (right) and a bag discard (left) (Source: Author, 2025).

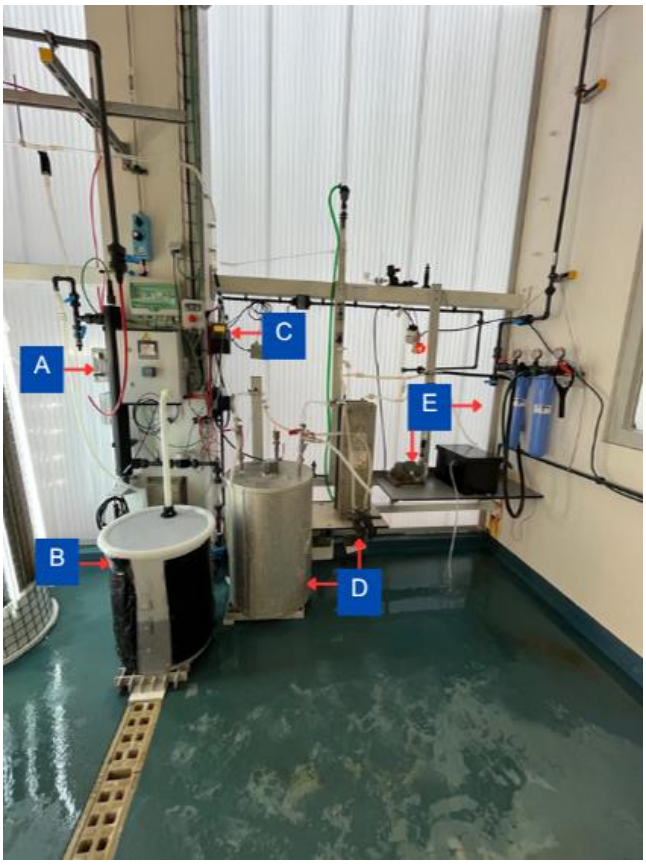

Figure 23. Supply system: CO_2 flowmeter (A), nutrients tank (B), nutrients dose pump (C), pasteurizer and heat exchanger (D), water pump and cartridge filters (E) (Source: Author, 2025).

c. Upright Bag Room operations

This room requires strict operational routines to ensure continuous and safe microalgae production. These routines are divided into daily, weekly, and biweekly tasks.

- Daily operations

Each day begins with a visual inspection of the room to ensure that all systems are operating correctly. Following this, the harvest pumps of each line are recirculated inside boxes, and the harvest tanks are cleaned. Recirculation is performed using sodium hypochlorite for approximately 30 minutes and then neutralized with sodium thiosulfate. During this process, the microalgae transfer pipes of each of the six lines - connecting the bag room to the external 1000 L storage tanks - are flushed with freshwater (Figure 24).

After recirculation, the pumps are returned to the harvest tanks and reconnected to the transfer pipes to transfer any remaining culture. Once emptied, the tanks are cleaned with Virkon™ and freshwater. During cleaning, all bag harvest hoses are temporarily closed and only reopened once the harvest tanks have been fully cleaned and the pumps reinstalled. Additionally, the sieves in each harvest tank—which remove residues such as clumps of dead microalgae from the incoming culture—are placed in small boxes with sodium hypochlorite solution kept in the room for disinfecting materials.

These sieves are reinstalled in the harvest tanks before reopening the bag harvest hoses (Figure 24).

Other minor tasks include:

- Checking which bags are near harvest volume and require a harvest connection.
- Counting and recording the number of bags currently harvesting.
- Updating operational data logs.

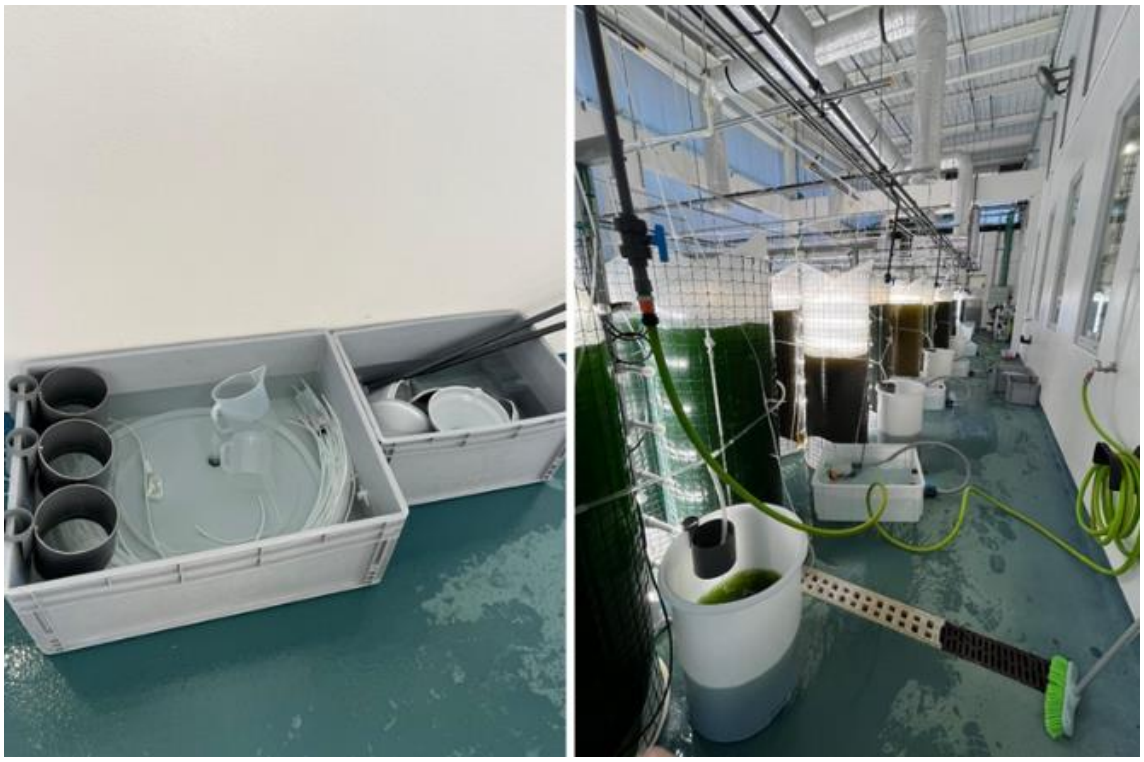

Figure 24. Disinfection tank (left) and daily operations – pump recirculation and flush of the transfer pipes (right) (Source: Author, 2025).

- Weekly operations

Several critical tasks are performed throughout the week: preparation of silicate and nutrient solutions, silicate supplementation, bag harvesting and inoculations, deep cleaning on Tuesdays, and transfer line maintenance and general cleaning on Thursdays.

Nutrient solutions are prepared by mixing 1 kg Varicon® bags with distilled water at a ratio of 1 kg per 10 L, producing 80 L of solution, which is sufficient for approximately one week of operation. Silicate solutions are prepared by diluting a concentrated stock solution in distilled water to a final concentration of 30 g/L of metasilicates. These are stored in jerricans for use throughout the week.

On Mondays, Wednesdays, and Fridays, each *Chg* bag receives 100 mL of silicate solution (equivalent to 50 mL per day), which is injected using a syringe. Before injection, harvesting from the bag is temporarily closed to allow proper mixing of the silicates with the culture.

Bag replacement is carried out on two days of the week, usually involving two bags per species. *Chg*, *DL*, and *Ig* bags are dropped on Mondays while *T-iso* and *Ts* bags are processed on Wednesdays. Bag dropping involves puncturing each bag at mid-height (to avoid collecting sediment at the bottom) and transferring the contents into the harvest tank via a hose, while discarding residual material into the drain. After the drop, the empty bags are removed, and their bases are cleaned and disinfected. Fresh bags are then placed in the same positions, inflated with air, and filled with pasteurized seawater overnight in preparation for inoculation the following day (Figure 25).

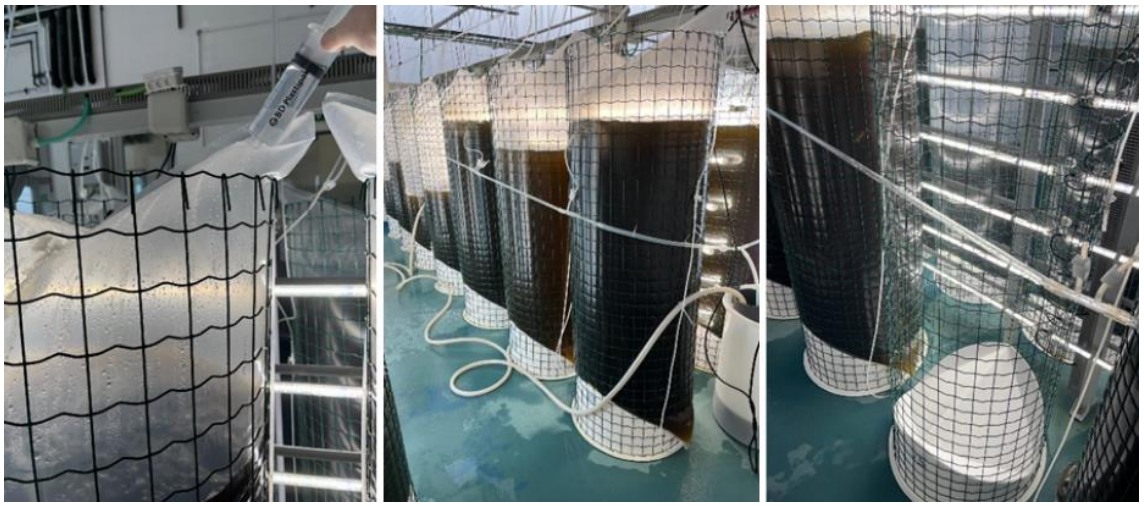

Figure 25. Silicates injection (left), bag drop (middle) and, a cleaned and disinfected spot to receive the new bag (right) (Source: Author, 2025).

Inoculations take place on Tuesdays and Thursdays using balloons from the Inoculation chamber, following the procedure described in section (3.b).

Every Tuesday, the room undergoes one of two deep-cleaning protocols, designated as type 1 and type 2, alternating on a 1-1-2 weekly cycle.

- **Type 1 cleaning - Steam cleaning**

Prior to cleaning, the system is drained, the pump and water supply are shut off, the pasteurizer is isolated, the harvest connections from the bags are closed, and the steam machine is connected. Steam is then applied through the water pathway of the system in isolated segments, which are sealed after each step. The process begins at the pasteurizer's heat exchanger, where each segment receives approximately 10 minutes of steam. Next, each of the six lines is cleaned sequentially: steam is applied to the water pipelines and capillaries for 20 minutes, followed by 20 minutes to the harvest pipelines. Finally, the harvest hoses are steamed in batches of 2 to 3, maintaining pressure and preventing culture backflow. Once steaming is complete, the pump and water supply are reactivated, allowing water to pass through the pasteurizer and discharge onto the floor. The system may only be reactivated once the pasteurizer stabilizes at 85–90°C under continuous water flow (Figure 26).

- **Type 2 cleaning – Acid and sodium hypochlorite cleaning**

This procedure follows the same initial steps as Type 1 but terminates after the pasteurizer's heat exchanger has been treated. During steaming, the harvest hoses and

capillaries are removed from the bags. A 200 L oxalic acid solution is then pumped through the glass pipelines, harvest hoses, and bag capillaries. With each line loaded the only exit point is through the capillaries. Once the acid solution is fully dispensed, the system is flushed with fresh water and sanitized with a 200 L sodium hypochlorite solution, applied in the same manner as the acid. Finally, a 100 L sodium thiosulfate solution is circulated—not to load the system, but to pass through and neutralize residual sodium hypochlorite. Hypochlorite test kits are used at the capillaries to confirm the absence of chlorine before the system is returned to operation, following the same final steps as in Type 1 cleaning (Figure 27).

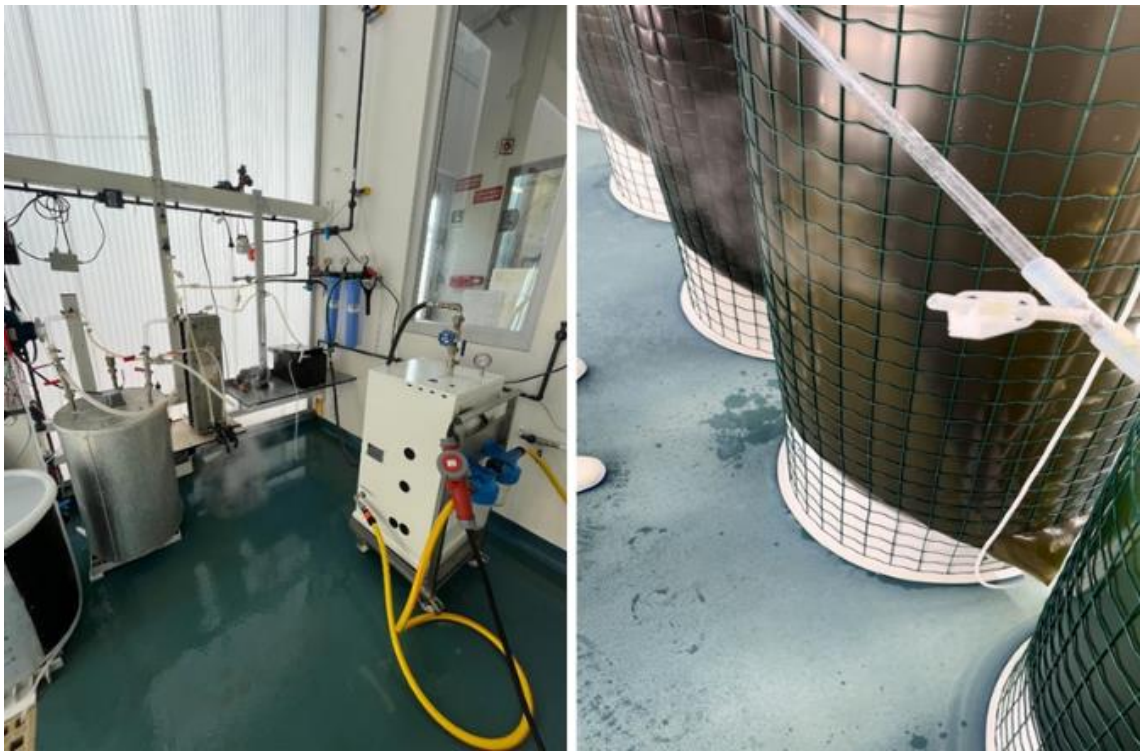

Figure 26. Steam cleaning: heat exchanger side (left) and harvest hoses (right) (Source: Author, 2025).

Figure 27. Acid and sodium hypochlorite cleaning: solution tank and cleaning pump (left), loading the lines (middle) and, solution dripping from the capillaries (right) (Source: Author, 2025).

The use of protective equipment—specifically a face visor and thick gloves—is mandatory during both cleaning protocols due to handling of sodium hypochlorite, acid, and steam at high temperature and pressure. In addition, cartridge filters are replaced every two weeks, on the same day as cleaning, and before reopening the seawater supply.

On Thursdays, a dedicated cleaning is performed on the microalgae transfer line, which transports cultures from the Upright Bag room to the external 1000 L storage tanks. The procedure begins with flushing the line with a sodium hypochlorite solution, followed by sodium thiosulfate. Both solutions are introduced from the harvest tanks and discharged into the drain next to the storage tanks.

Every two weeks, the cleaning includes the passage of a sponge through the transfer line to thoroughly remove deposits from the internal walls of the PVC pipes. This process starts at the harvest tank connection of each line, which is cleaned one at a time. For each line, the check valve at the top is removed—since the sponge cannot pass through it—and temporarily replaced with a standard valve. A small amount of sodium hypochlorite solution is then flushed through the line, after which the sponge is retrieved. The removed check valve is manually cleaned with a brush soaked in Virkon™, then reassembled and reinstalled. Once sponge has been passed through all lines and the check valves are back in place, the transfer line is flushed again with sodium hypochlorite solution as in non-sponge weeks, followed by sodium thiosulfate to neutralize any residual chlorine.

Additionally, on Thursdays, the two sodium hypochlorite boxes located in the room are replaced, and some material is stored. These boxes are used for disinfecting equipment such as harvest hoses, air hoses, bag drop hoses, and inoculation tools.

4. Receiving, maintaining, and conditioning of clam broodstock to produce high-quality eggs (Broodstock room)

This room is dedicated to the maintenance and conditioning of wild broodstock collected from different regions of Portugal—such as Aveiro, Óbidos, and the Algarve—as well as from international sources. Its primary purpose is to ensure the optimal physiological condition of the animals, thereby maximizing the success of scheduled spawning events and achieving high egg yields and hatching rates from fully matured broodstock (Figure 28).

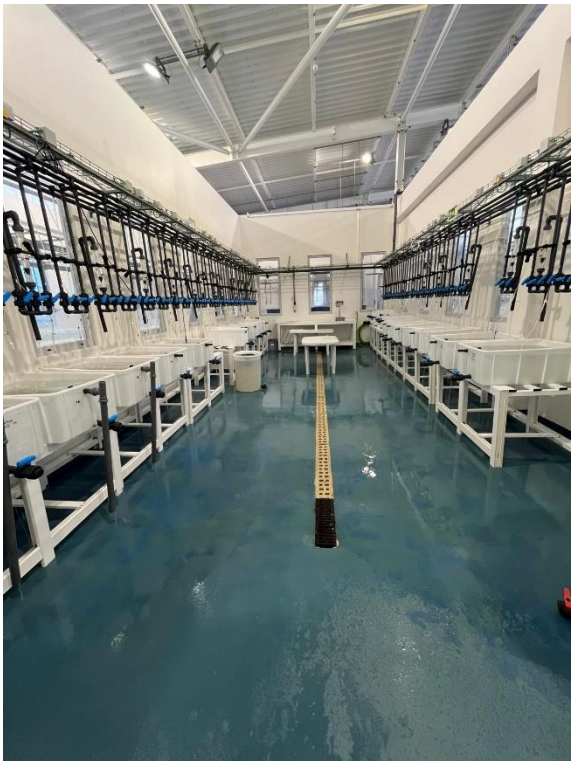

Figure 28. Oceano Fresco' broodstock room (Source: Author, 2025).

a. Ecology of cultivated species

- *Venerupis corrugata* (Gmelin, 1791)

The pullet carpet shell (*Venerupis corrugata*) (Figure 29) is a benthic infaunal bivalve inhabiting sandy or muddy-sand substrates, generally in sheltered coastal areas, where it burrows 2–5 cm into the sediment (Macho et al., 2016). Its geographic range extends from the Atlantic coasts of Norway and the British Isles to the Mediterranean and northwest Africa, including estuarine and lagoon environments with good water exchange (Rivero et al., 2020). This species is an active suspension feeder, consuming phytoplankton and organic particles, thereby contributing to nutrient cycling and acting as a natural biofilter in coastal systems (Shumway et al., 2003; FAO, 2024a). This ecological function makes it relevant for coastal ecosystem functioning and integrated multi-trophic aquaculture (IMTA) systems, where it can act as a biofilter (Shumway et al., 2003).

The species generally tolerates water temperatures between 10 and 25 °C and salinities of 25–35 ppt. However, exposure to sudden or extreme deviations—especially low salinity (≤15 ppt) combined with high temperatures (32–35 °C)—can result in significant mortality and impaired feeding activity (Rato et al., 2022). In aquaculture, *V. corrugata* is valued for its relatively fast growth and adaptability to extensive and semi-intensive farming systems. Larval rearing trials in recirculating aquaculture systems (RAS) have shown higher survival, growth, and metamorphosis rates at densities up to 40 larvae/mL, with cultivation times reduced by approximately two days compared with static systems (Joaquim et al., 2016).

- *Ruditapes decussatus* (Linnaeus, 1758)

The grooved carpet shell (*Ruditapes decussatus*) (Figure 29) is a benthic infaunal bivalve inhabiting sandy to muddy-sand substrates in intertidal and shallow subtidal zones, typically at depths of 10–20 m (FAO, 2024a; Macho et al., 2016). It is native to the eastern Atlantic, ranging from the British Isles to Senegal, and occurs throughout the Mediterranean and the Macaronesian archipelagos (FAO, 2024a). As a suspension feeder, it filters phytoplankton and organic particles from the water column, thereby contributing to nutrient cycling, sediment stabilization, and the ecological balance of coastal habitats (Shumway et al., 2003; Rato et al., 2022). Owing to its ecological role and high commercial value *R. decussatus* is considered a priority species in European bivalve aquaculture, particularly in southern Europe (FAO, 2024a).

R. decussatus tolerates water temperatures of approximately 5-28 °C and salinities of 20-40 ppt, showing relative resilience to moderate environmental fluctuations (FAO, 2024a; Rato et al., 2022). However, experimental studies have shown that temperatures above 30 °C, especially when combined with salinity levels below 15 ppt, result in pronounced reductions in feeding activity and increased mortality (Rato et al., 2022). Juveniles are susceptible to low salinity under high temperature, whereas higher salinity conditions confer greater tolerance to thermal extremes (Rato et al., 2022). At the physiological level, *R. decussatus* exhibits metabolic adjustments during thermal stress, including upregulation of glycolysis and shifts toward anaerobic metabolism to meet energy demands. These responses indicate both acute stress responses and compensatory adaptation. Nevertheless, prolonged exposure to unfavourable conditions can overwhelm these mechanisms, ultimately reducing survival (Papadopoulos et al., 2025).

Figure 29. *V. corrugata* (left) and *R. decussatus* (right) (Source: adapted from Aquanostra, retrieved from https://www.aquanostra.pt/loja-online).

b. Methodologies

The reception of new broodstock represents a critical step prior to conditioning for *V. corrugata* and *R. decussatus*. Upon arrival at the hatchery, broodstock should be transported under stable conditions, ideally at temperatures close to those of the

collection site, using insulated containers and, if required, sealed ice packs to maintain thermal stability (Helm et al., 2004). Sudden changes in temperature or salinity during transport and reception should be avoided to minimize physiological stress (Helm et al., 2004). All incoming batches must be recorded, including date of arrival, origin, number of individuals, and biometric data such as shell length and total weight, along with visual inspection for shell damage, parasites, or excessive biofouling (Helm et al., 2004).

Cleaning procedures generally involve gentle mechanical removal of sediments and fouling organisms, followed, when appropriate, by short-term immersion in approved disinfectant solutions, such as diluted sodium hypochlorite at safe concentrations, prior to thorough rinsing and reuse. These procedures reduce the risk of pathogen introduction and maintain hygiene standards (Helm et al., 2004).

A quarantine period in separate tanks is recommended, during which mortality and water quality parameters are closely monitored (Helm et al., 2004). Gradual acclimation to hatchery conditions, particularly temperature and salinity, ensures smooth physiological adjustment. Feeding with high-quality microalgae from the time of arrival supports recovery of energy reserves and promotes gonadal redevelopment (Abbas et al., 2018).

Once acclimated, broodstock are conditioned in flow-through tank systems with trays placed over gravel substrates, allowing natural burrowing behaviour and efficient feeding (Helm et al., 2004). Water can be supplied either filtered or unfiltered—unfiltered seawater provides a natural and diverse seston diet, whereas filtered systems ensure greater stability and biosecurity (Helm et al., 2004). Routine maintenance during conditioning includes removal of faeces and pseudofaeces, siphoning of settled waste, and regular monitoring of water quality parameters such as temperature, salinity, dissolved oxygen, and pH (Helm et al., 2004).

Feeding regimes are critical for gametogenesis. In *R. decussatus*, monoalgal diets of *Chc* or *Ts*, and especially their mixture, promote faster gonadal maturation and improved spawning success compared with *Nannochloropsis oculata* or unfed controls (Abbas et al., 2018). Mixed diets combining diatoms and flagellates provide balanced lipid and protein profiles, supporting high-quality gamete production (Abbas et al., 2018). In *V. corrugata*, broodstock condition index and oocyte lipid content are strongly correlated with larval survival during the first 24 h (*D*-larval stage), emphasizing the importance of sustained nutritional input during conditioning (Joaquim et al., 2016). Daily feed rations during broodstock conditioning are typically calculated as a percentage of the dry meat weight of adults, with values of 2–4% most applied (Helm et al., 2004). This percentage should be adjusted according to biomass, algal cell concentration, species-specific clearance rates, and reproductive stage, since rations exceeding 6% can hinder successful gonadal conditioning, whereas underfeeding may delay maturation (Helm et al., 2004).

Temperature manipulation is the primary tool for stimulating maturation and synchronizing spawning. In *R. decussatus*, gradual increases in temperature to approximately 20 °C accelerate gametogenesis, reduce the number of degree-days required for spawning, and increase larval survival rates compared with constant-temperature regimes (Ojea et al., 2008). Conditioning initiated in autumn, with a progressive thermal ramp, can induce spawning up to four months earlier than under natural conditions (Ojea et al., 2008).

Spawning preparation involves transferring broodstock to spawning tanks under optimal temperature and salinity conditions, with elevated microalgal concentrations provided for several days before induction (Helm et al., 2004). After spawning, broodstock are returned to recovery tanks supplied with optimal feeding and stable environmental conditions to promote further gamete development if repeated spawning events are desired (Helm et al., 2004).

c. How Oceano Fresco operates

The room is equipped with shelves holding 22 plastic boxes, which are used to maintain clams in a flow-through system. The two species are kept separate, with one species placed on the left side of the room and the other on the right, as shown on Figure 28. Each box can accommodate a maximum biomass of 3 kg and is supplied with seawater at a flow rate of 60 L h^{-1}, aeration via bubbling, and a semi-continuous supply of microalgal feed (Figure 30). To ensure full traceability of Oceano Fresco's production, each broodstock batch - distinguished by origin and date of entry - is kept separate, thereby linking marketable clams and seed production back to their parental stock.

The system enables seawater supply at two different temperatures to support broodstock conditioning and handling. Cold water (18 °C) is used to prevent spontaneous spawning, whereas warm water (20–21 °C) is applied to promote gamete maturation. Feed consists of a mixed algal diet supplied from the Upright Bag room via a dedicated feed line. Algae are collected daily, diluted with seawater, and stored in a 1000 L tank located inside the broodstock room. From this tank, feed is pumped to the designated boxes.

Feeding is controlled by a timer that switches the pump on and off in 15-minute intervals, providing feed input for 15 minutes followed by 15 minutes pause. The daily feed requirement for the room is calculated based on the total broodstock biomass and the mean cell density of the algae mixture, to supply a ration equivalent to 4% of the animals´ dry biomass weight. The flow rate of feed delivered to each box (mL 10 s^{-1}) is also measured to ensure proper distribution. When specific boxes are assigned to fasting, this is accounted for in the feed requirement calculations, as it affects the total demand and distribution for the entire system.

Figure 30. Supply of the broodstock boxes (Source: Author, 2025).

d. Broodstock room operations

The broodstock room operates under a structured routine designed to maintain optimal environmental conditions for *V. corrugata* and *R. decussatus*, ensure biosecurity, and support animal welfare. These operations follow established protocols in bivalve aquaculture for hygiene, health management, and the physiological preparation of broodstock for spawning. Tasks are organized according to their frequency, encompassing daily, weekly, and monthly procedures.

- Daily operations

At the start of each day, the technician assigned to the broodstock room performs a visual inspection of the system, checking air supply, water flow, feed delivery, and overall system functionality. Each box is then emptied, and the clams are sprayed with freshwater while remaining in their boxes. The boxes are subsequently refilled with seawater, and any dead clams are removed, stored in a freezer, and later collected by a licensed company for organic waste disposal. Mortality data are updated, and fresh feed is transferred to the room. A final inspection is conducted at the end of the daily routine to ensure all systems are operating correctly and that no tasks have been overlooked.

- Weekly operations

Twice a week, the boxes undergo cleaning and disinfection with Virkon™. During this procedure, clams are removed from the boxes and washed with freshwater while inside their trays on a designated worktable (Figure 31). Additionally, the feed line is cleaned and disinfected once per week by sequentially pumping oxalic acid solution, sodium hypochlorite, and sodium thiosulfate through the system. Each solution is prepared in approximately 175 L of freshwater within a 200 L tank and pumped separately for around 30 minutes. During this process, all feed hoses are removed from the boxes and reinserted upon completion of cleaning.

Figure 31. Weekly operations: Cleaning and disinfection with Virkon™ (left) and freshwater spraying of clams (right) (Source: Author, 2025).

- Monthly operations

Monthly tasks may include the reception of new broodstock, preparation for shipping, screening, sampling, and pre-spawning preparation. Upon reception, broodstock typically arrive packed in mesh bags or, in some cases, hermetically sealed boxes. The animals are washed, counted, weighed, and evenly distributed into boxes without feed for two days fasting period, during which species identity is confirmed through screening. For shipping, broodstock are packed in mesh bags, weighed, and returned to their boxes without feed until dispatched the following day. Transport is carried out in hermetically sealed boxes with ice packs and damp cloth to maintain suitable environmental conditions (Figure 32). Broodstock are shipped to the company's

offshore farm in Lagos, where they are kept until required for spawning, at which point they are returned to the hatchery.

Approximately one week before a planned spawning event, selected broodstock are sampled, and gonadal status is assessed (Figure 32). From this point until spawning, handling and temperature fluctuations are minimized to prevent premature gamete release. One to two days before spawning, broodstock are placed on a fasting regime, and on the day before spawning they are transferred to hermetically sealed boxes with ice packs and damp cloth to maintain low temperatures. This practice reduces the risk of premature gamete release and enhances the thermal shock effect when broodstock are subsequently introduced into the spawning tanks. Whenever broodstock are received, shipped, prepared for spawning, or returned from spawning, feed distribution to each box is adjusted to reflect changes in biomass or fasting requirements.

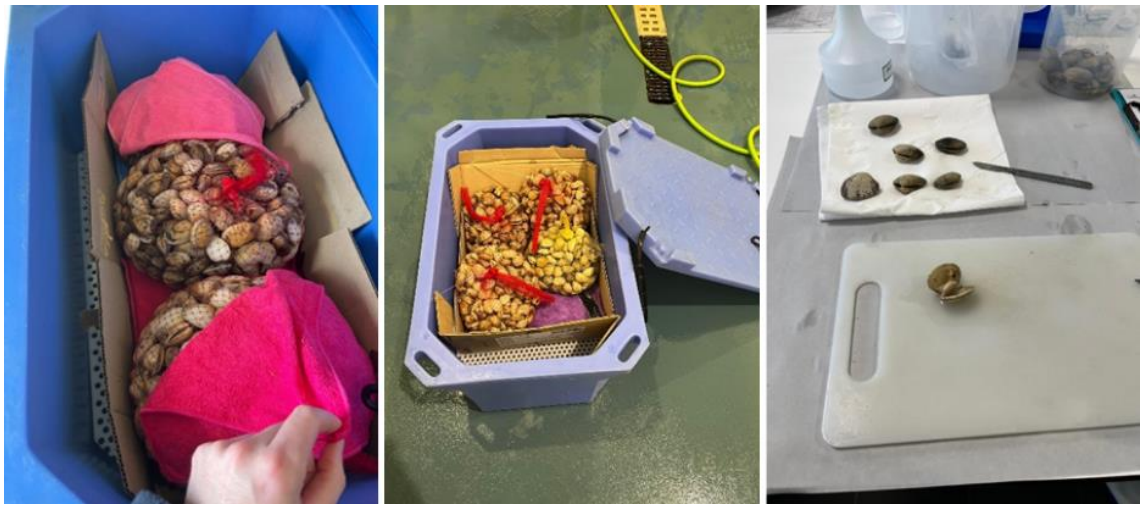

Figure 32. Broodstock monthly operation: reception/shipping (left), pre-spawning preparation (middle) and assessment of gonadal status (right) of broodstock (Source: Author, 2025).

5. Spawning, egg harvesting and hatching, and the first steps on larvae rearing (Larvae room); Rearing of post-larvae in closed systems (Post-larvae room)

The objective for the Larvae room is to optimize the larval cycle, achieving high performance in both the quantity and quality of larvae. This involves maximizing outcomes from spawns that yield a high number of eggs per clam and a high hatching rate (Figure 33). As the Larvae room hosts the first and most delicate stages of development, its successful operation is critical to overall hatchery production, since failure at this stage can compromise the entire production cycle.

The Post-larvae room aims to provide optimal conditions for larval settlement, metamorphosis, and early post-larvae rearing, serving as a transitional phase before transfer to the seed room (Figure 33).

Figure 33. Larvae (left) and post-larvae (right) room (Source: Author, 2025).

a. Larvae development cycle

The development cycle of *V. corrugata* and *R. decussatus* under hatchery conditions follows the typical pattern of heterodont bivalves, progressing from gamete release through fertilization and embryogenesis followed by larval stages, and culminating in post-larval settlement. Fertilization occurs externally in the water column, after which embryos undergo rapid cleavage and progress through the blastula stage, reaching the trochophore stage within 12–18 hours post-fertilization (Figure 33) (Helm et al., 2004).

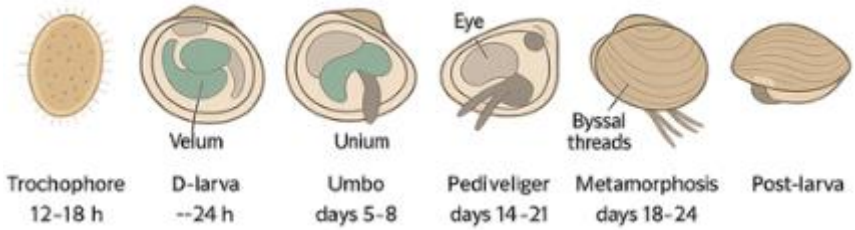

Figure 34. Larval development cycle of V. corrugata and *R. decussatus* under hatchery conditions, showing the main morphological stages: trochophore, D-larva (prodissoconch I), umbo stage (prodissoconch II), pediveliger, metamorphosis, and post-larva. Times indicated correspond to optimal rearing conditions at 18–22 °C and 30–35 ppt salinity. Adapted from Helm et al. (2004), Joaquim et al. (2016).

The trochophore is a free-swimming, ciliated stage that develops into the D-larva (straight-hinge stage) within approximately 24 hours, characterized by the secretion of

the initial bivalve shell, the prodissoconch I (PI) (Helm et al., 2004). At this point, larvae begin exogenous feeding, relying on microalgae such as *Ig* and *Chc* (Abbas et al., 2018).

Over the next 5–8 days, larvae progress through the early veliger and umbo stages, forming the prodissoconch II (PII) shell and developing more complex organs, including a functional velum for swimming and feeding, a developing foot, and a rudimentary digestive system (Helm et al., 2004). In *R. decussatus*, the umbo stage typically occurs around days 6–8 at 20 °C, while in *V. corrugata* it may occur slightly earlier under optimal conditions (Joaquim et al., 2016).

The pediveliger stage represents the final larval phase before metamorphosis. It is characterized by a well-developed foot, the appearance of an eyespot, and progressive reduction of the velum as larvae prepare for settlement (Helm et al., 2004). Under optimal hatchery conditions (18–22 °C, stable salinity of 30–35 ppt, and abundant algal availability), pediveligers usually appear between days 14 and 21 post-fertilization, with complete metamorphosis into post-larvae occurring within 18–24 days (Helm et al., 2004; Joaquim et al., 2016).

Metamorphosis involves profound morphological and physiological changes, including loss of the velum, further foot development for crawling, and the secretion of byssal threads for temporary attachment to substrates (Helm et al., 2004). Successful settlement produces post-larvae, which transition into juvenile clams capable of benthic life and filter feeding at the sediment-water interface (Helm et al., 2004). Under hatchery conditions, the complete larval cycle of *V. corrugata* and *R. decussatus* is generally completed in 18–24 days, although suboptimal temperature, salinity, or diet can extend this period (Joaquim et al., 2016).

b. Methodologies

Once broodstock have been conditioned and prepared, selected individuals are transferred to dedicated spawning tanks within the larvae rearing facility. Spawning induction is initiated by applying thermal stimulation — typically a sudden or gradual increase of 3–5 °C above the holding temperature — to replicate natural environmental cues that trigger gamete release (Helm et al., 2004). In some cases, the addition of diluted gamete suspensions from actively spawning individuals can be used to synchronize spawning events through chemical signalling (Helm et al., 2004). Throughout the induction process, clean, aerated seawater at optimal salinity (30–35 ppt) is maintained, and gametes are continuously monitored to ensure efficient fertilization. Fertilized eggs are collected via gentle sieving through a 30–40 µm mesh, rinsed to remove excess sperm and debris, and transferred to incubation tanks for early embryonic development before stocking in larval rearing systems (Helm et al., 2004).

Larval culture of bivalves such as *V. corrugata* and *R. decussatus* is carried out under controlled hatchery conditions, with the goal of maximizing survival, growth, and successful metamorphosis into post-larvae. The most widely used approach is the static batch system, in which larvae are reared in tanks of 100–500 L filled with filtered and UV-treated seawater. Partial or complete water exchanges are performed every 24–48 h, depending on stocking density and water quality (Helm et al., 2004). While this method allows precise control of environmental and feeding parameters and is suitable for small-

scale or experimental rearing, it is labour-intensive and water quality can deteriorate rapidly if cleaning schedules are not strictly maintained (Helm et al., 2004).

The flow-through system maintains a continuous inflow of clean seawater into larval tanks, usually at rates equivalent to 50–200 % of tank volume per day. This stabilizes temperature, salinity, dissolved oxygen, and pH, while diluting metabolic waste products (Rico-Villa et al., 2006). The method reduces handling and allows higher larval densities, but requires precise adjustment of algal dosing to compensate for feed dilution caused by continuous water exchange (Rico-Villa et al., 2006).

The semi-static or 'drip-feed' system combines aspects of both batch and flow-through methods. Larval tanks receive a continuous low-volume inflow of seawater pre-mixed with microalgae, while waste is periodically siphoned and partial water changes are performed, typically once per day (Helm et al., 2004; Rico-Villa et al., 2006). This approach helps maintain stable water quality, reduces feed losses, and minimizes labour compared to static batch systems, making it suitable for medium-scale larval production (Helm et al., 2004; Rico-Villa et al., 2006).

Water quality parameters are critical for successful larval rearing. For both *V. corrugata* and *R. decussatus*, optimal conditions include temperatures of 18–22 °C, salinities of 30–35 ppt and pH maintained around 8.0–8.2 (Helm et al., 2004). Dissolved oxygen should remain above 6 mg L^{-1} to prevent metabolic stress, and total ammonia nitrogen (TAN) should be kept below 0.1 mg L^{-1} to avoid toxicity (Shingare et al., 2019). Deviations from these ranges can lead to reduced feeding activity, delayed development, and increased mortality (Helm et al., 2004; Rico-Villa et al., 2009).

Larval stocking densities are a critical parameter for optimizing growth and survival. For both species, initial densities at the D-larva stage typically 10–15 larvae mL^{-1}, progressively reduced to 2–5 larvae mL^{-1} by the pediveliger stage to limit competition for food and space (Helm et al., 2004; Rico-Villa et al., 2006). Overstocking can reduce growth rates, increase deformities, and elevate susceptibility to bacterial infections, whereas understocking may result in inefficient use of tank capacity (Rico-Villa et al., 2006).

Grading and sieving are performed to separate larvae by size, remove dead individuals and debris, and limit competition between size classes. Sieving is typically performed every 2–3 days during early stages and daily during later veliger stages. Mesh sizes are selected according to developmental stage: ~45–55 µm for early D-larvae, ~80–100 µm for umbo-stage larvae, and 150–180 µm for late veligers approaching metamorphosis (Helm et al., 2004). Gentle handling during sieving is crucial to avoid shell damage and stress, which can significantly impact survival (Helm et al., 2004; Rico-Villa et al., 2009).

Feeding regimes are based on live microalgae, with mixed diets providing balanced profiles of essential fatty acids, amino acids, and sterols required for larval development (Brown et al., 1997). Common species for *V. corrugata* and *R. decussatus* include the diatoms *Chc* and *Skeletonema costatum*, and the flagellates *Ig* and *Ts* (Abbas et al., 2018). Algal cell concentrations are adjusted to larval stage and biomass: D-larvae are typically fed 30,000–50,000 cells mL^{-1}, increasing to 80,000–100,000 cells mL^{-1} at late veliger stages to meet the elevated energetic demands of metamorphosis (Helm et al., 2004).

The duration of the larval cycle is influenced by temperature, food availability, and species-specific growth rates. Under optimal conditions, *R. decussatus* and *V. corrugata* typically reach the pediveliger stage within 14–21 days post-fertilization, with complete metamorphosis into post-larvae occurring within 18–24 days (Helm et al., 2004; Joaquim et al., 2016). Suboptimal environmental conditions may extend this period whereas optimal conditions promote faster and more uniform settlement (Helm et al., 2004; Joaquim et al., 2016).

In recent years, recirculating aquaculture systems (RAS) have been adapted for bivalve larval rearing. These systems integrate mechanical and biological filtration, with UV sterilization, allowing seawater reuse, improving biosecurity, and reducing water demand (Joaquim et al., 2016). Experimental trials with *V. corrugata* larvae have demonstrated that RAS can maintain growth and survival rates comparable to flow-through methods, while allowing more consistent environmental control and minimizing pathogen introduction (Joaquim et al., 2016).

c. How Oceano Fresco operates and room operations

- Larvae room

The larvae room is equipped with eight 2000L and twelve 450L cylindroconical rearing tanks operated under a static batch system. Each larval rearing cycle begins with spawning. As described in section 4.d, conditioned broodstock, subjected to a two-day fasting regime and kept for 24 hours in dry, cold conditions inside hermetically sealed boxes with ice packs and a damp cloth, are transferred on the spawning day to 450 L spawning tanks, where they are distributed in trays (Figure 34). The thermal shock caused by immersion in water at approximately 22 °C is usually sufficient to trigger spawning. If this is not initiated, additional stimulation can be applied by opening a few individuals and releasing gonadal contents into the tank water, along with a small quantity of microalgae (Figure 35). Once spawning starts in one tank, broodstock can be moved between tanks to stimulate those that have not yet released gametes. Males typically release gametes before females. Within a few hours, the water becomes clouded with gametes, and eggs are present in high concentrations. At this stage, broodstock are removed and returned to the broodstock room for recovery.

Following broodstock removal, spawning tanks are drained and eggs are collected by sequential sieving through 40 µm mesh (to remove faecal material and debris) and through 25 µm mesh (to retain the eggs). A sample from each tank is used to estimate egg numbers, which are subsequently distributed according to density protocols into pre-filled 2000 L or 450 L rearing tanks, depending on the production objective. Research and development (R&D) cycles are generally carried out in 450 L tanks, whereas production-oriented cycles use 2000 L tanks (Figure 35). Spawning tanks are subsequently cleaned and disinfected with Virkon™, followed by oxalic acid, with freshwater rinses in between steps. This procedure completes “day zero” of a new cycle.

On day one, tanks containing eggs receive an initial algal ration, while new tanks are simultaneously filled with seawater in preparation for the following day. Day two marks the first “drop” of the cycle, when tanks are drained to harvest newly hatched larvae and evaluate hatching success. The first task of the day is to record water

temperature and pH from both the room supply and each tank — those containing larvae and those filled the previous day. The pH of the tanks prepared the previous day is adjusted to 8.2 with sodium carbonate, after which algal rations are added according to protocol. Larval diets consist of mixed microalgal blends supplied from selected bags in the Upright Bag room and supplemented with balloons from the Inoculation chamber. A hatching rate of 25–30% is generally considered satisfactory. Subsequent drops are performed every two days until settlement. Before each drop, a 500 mL sample is collected from each tank for microscopic observation, assessing larval development, swimming activity, mortality, gut fullness and coloration, lipid reserves, and possible contamination, as well as to measure at least ten individuals for growth monitoring.

Figure 35. Spawn: Broodstock receiving thermal shock (left), stimulation with gonadal content (middle) and the 2000L rearing tanks (right) (Source: Author, 2025).

During each drop, larvae are collected by sieving, starting with 50 µm mesh on day two and progressing to a 175 µm mesh around day fourteen. Once a tank is emptied, the sieves are transferred to the workbench for grading. Graded larvae are placed in beakers, gently homogenized, and three subsamples are taken with a micropipette and preserved with a drop of formaldehyde for counting. These counts are used to estimate the total number of larvae per size class, after which larvae are redistributed into pre-filled tanks according to the density protocol (Figure 36). Operations are organized to optimize efficiency through overlapping tasks: once grading begins for one tank, the drop of the next tank can be initiated, and while this second tank is dropping, its sample can be examined under the microscope. This workflow minimizes downtime and ensures continuity throughout drop days. If the largest size class of larvae reaches the target density, smaller larvae are discarded; if not, a portion of the smaller size class may be retained. After all, drops and redistributions are completed, emptied tanks and associated equipment are cleaned and disinfected. Data on larval abundance and size for each tank are recorded on the room's tracking board. In the afternoon, larval tanks receive their scheduled feeding (Figure 37).

In summary, on non-drop days, routine tasks include filling new tanks for the next day, recording water parameters, conducting morning and afternoon feedings,

performing larval observations in all tanks, and replacing the cartridge filter supplying water to the room weekly (Figure 37). On drop days, these tasks are maintained with one exception: instead of filling new tanks, technicians adjust the pH of newly prepared tanks, carry out larval drops, perform grading and counting, and complete cleaning and disinfection of emptied tanks and equipment (Figure 37).

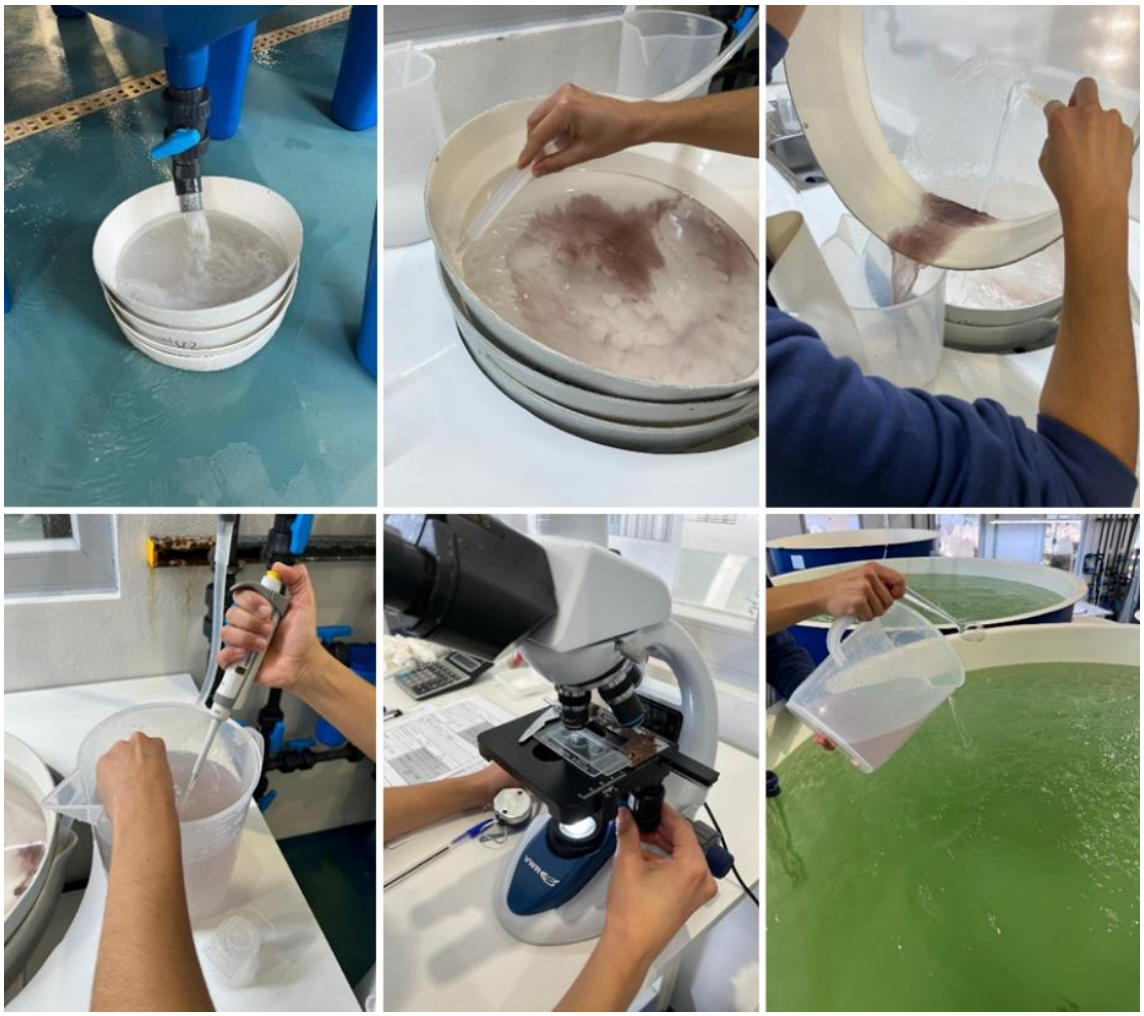

Figure 36. Drop procedure from start to finish (represented the 1st drop of a cycle) (Source: Author, 2025).

At the end of the larval cycle, competent larvae ready for settlement are transferred to the Post-larvae rearing room to continue their development. Following the transfer, the larvae room undergoes a complete cleaning and disinfection process. In addition to the routine cleaning of all tanks with Virkon™ and oxalic acid and the disinfection of all equipment, the water supply line is also cleaned and disinfected using oxalic acid, sodium hypochlorite, and sodium thiosulfate solutions. After the cleaning is completed, the room is sealed and remains closed until the start of the next production cycle.

Figure 37. Data board (represented day 12) (left); cleaned and disinfected material (middle); and the weekly cartridge filter change (right) (Source: Author, 2025).

- Post-larvae room

This room contains eight 450 L cylindroconical tanks (identical to those in the larval room) and two 3000 L square tanks (identical to those in the seed room), all operating under a static batch system. It receives larvae from the larval room when they are competent to settle, typically at 175 µm in size. Larvae are placed into a setting system (mesh-bottom cylinders with aeration), which are then positioned inside the rearing tanks. Each 450 L tank accommodates one setting system, while each 3000 L tank holds six (Figure 38). Metamorphosis occurs in these systems, and development continues until the post-larvae reach approximately 300 µm in size, which generally requires 1–2 weeks depending on growth rates. Before being transferred to the seed room, post-larvae are graded using the same sieving procedures applied in the Larvae room, but with larger mesh sizes, to ensure uniformity and optimise growth in the next rearing phase. Once graded, they are moved to the seed room to undergo the final hatchery stage before transferring out of the facility.

Daily operations in this room follow the same water parameter monitoring, feeding, and observation routines as in the Larvae room. The workflow alternates between two main operational sequences. On one day, the setting systems are removed from the tanks, and the settled larvae are gently sprayed with seawater before being returned to their tanks; new tanks are also filled in preparation for the next day. On the following day, the newly prepared tanks are adjusted to pH 8.2 with sodium carbonate, supplied with algae according to protocol, and stocked with post-larvae transferred from the origin tanks into new setting systems (Figure 39). The origin tanks are then emptied, cleaned, and disinfected following the same procedures applied in the Larvae room. The cartridge filter supplying water to the room is replaced weekly.

At the end of each post-larval cycle, the room, tanks, equipment, and water line are cleaned and disinfected following the same protocol used in the Larvae room.

Figure 38. Setting systems inside a 450L (left) and a 3000L (right) rearing tank (Source: Author, 2025).

Figure 39. Post-larvae operations: filling new tanks (left), adjusting pH with sodium carbonate (middle), and gathering microalgae to feed the post-larvae (right) (Source: Author, 2025).

6. Fast rearing of clam seeds in open water flow through systems (Seed room)

The objective of the seed room is to provide optimal conditions for consistent seed growth while minimizing mortality, from the time individuals are transferred from the Post-larvae room until they reach the size required for shipment (Figure 40).

Figure 40. OF' Seed room (Source: adapted from GoParity, *Bivalves Hatchery III* page, retrieved [2025], from https://goparity.com/project/bivalves-hatchery-iii-490).

a. Methodologies

The hatchery-based seed rearing phase begins immediately after post-larvae complete metamorphosis, at sizes of approximately 250–300 µm, and continues until they reach a size suitable for transfer to nursery systems. During this phase, the main objective is to promote rapid growth and high survival while producing uniform cohorts suitable for subsequent on-growing. Depending on species, system design, and environmental conditions, seed rearing in hatcheries typically lasts between four and eight weeks (Helm et al., 2004).

Several rearing systems are employed, including static tanks with regular water changes, flow-through raceways, downweller and upweller systems. The choice depends on operational scale, infrastructure, and growth objectives (Helm et al., 2004; FAO, 2024a). Static tanks are simple and low cost but require frequent cleaning and water exchange to maintain water quality (Helm et al., 2004). Flow-through raceways provide more stable conditions and support higher stocking densities but demand greater infrastructure and water supply (Helm et al., 2004). Downwellers deliver water from above through a screened base, allowing food-rich water to pass downward across the seed; this design is particularly effective immediately after metamorphosis, as it enhances settlement and early post-settlement survival at high densities (Utting & Spencer, 1991). Upwellers, in contrast, deliver water from beneath the seed, maintaining gentle movement and high oxygenation, enhancing waste removal and supporting faster growth in subsequent nursery stages (Helm et al., 2004; Utting & Spencer, 1991).

Optimal environmental parameters are essential to achieve the target size within the desired timeframe. Water temperature should be maintained within the species' preferred range — generally 18–22 °C for *R. decussatus* and *V. corrugata* — with salinity between 30–35 ppt, dissolved oxygen above 6 mg L^{-1}, and pH between 8.0 and 8.2 (Helm et al., 2004; FAO, 2024a).

Continuous or semi-continuous feeding with a balanced microalgal diet is required, often using mixed species such as *Ig*, *Chaetoceros* spp., and *Ts*, which together provide essential fatty acids and support robust growth (Brown et al., 1997; Laing et al., 1987). Feeding regimes are tailored to the developmental stage, with rations typically calculated either as a percentage of seed fresh weight or based on cell concentrations in the rearing water, often ranging between 4 % and 8 % of seed wet weight per day under hatchery conditions (Helm et al., 2004). These regimes are particularly critical during early seed phase, when metabolic demands are high but selective feeding capacity is limited. Consistent and appropriate rations are therefore essential to ensure survival and growth (Brown et al., 1997; Laing et al., 1987).

Routine husbandry practices are equally critical to maintain seed quality. Regular grading, usually once or twice per week, is performed using mesh sieves to separate seed into size classes, thereby reducing competition and preventing size dominance within tanks (Helm et al., 2004; Utting & Spencer, 1991). Cleaning of tank surfaces and removal of faeces and biofilm are necessary to prevent biofouling and maintain water flow. Mortality is recorded after each grading or cleaning event to monitor seed health and performance trends. The frequency of water renewal, or turnover rates in flow-through systems, is adjusted according to biomass and feed input, with higher exchange rates used as stocking densities increase (Helm et al., 2004).

The target size for seed at the time of hatchery transfer typically ranges between 2–4 mm, depending on production strategy and environmental predictability in the receiving nursery (Helm et al., 2004; Utting & Spencer, 1991). In some operations, seed is transferred earlier (around 1 mm) to reduce pressure on hatchery systems, particularly space and recirculation capacity; however, this strategy requires nurseries with stable conditions and abundant natural microalgal food. Other operations retain seed until 3–4 mm, especially when external conditions are less predictable or when mortality risk during the nursery phase is high (Helm et al., 2004; Utting & Spencer, 1991).

Once seed reach the selected transfer size, a final grading is performed to ensure uniformity, minimize handling stress during transport, and optimize survival and growth in the nursery phase (Helm et al., 2004; FAO, 2024a). This final stage in the hatchery marks the transition from the controlled hatchery environment to land-based or offshore nurseries, where environmental and operational conditions differ significantly (Helm et al., 2004; FAO, 2024a).

b. How Oceano Fresco operates

The seed room contains twenty 3000 L flow-through rearing tanks, each fitted with six setting systems. Tanks receive seawater at a flow rate of approximately 60 L min^{-1}, supplied directly from the technical area at a temperature of around 18 °C. Seed enter this stage at approximately 300 µm in size, with the target size for shipment set at 2.4 mm.

Feeding is provided continuously from 1000 L storage tanks, which are supplied with microalgae from the Upright Bag room. Rations are calculated based on cell concentration, with a target of 60 × 10^3 cells mL^{-1} per rearing tank. Each tank receives feed via a dedicated thin hose connected to the main feed line of the room.

Grading of seed batches is conducted every one to two weeks, depending on operational schedules. A series of mesh sieves, ranging from 300 µm to 2.4 mm, is used to separate the seed into size categories. Grading is performed manually by trained technicians. Following grading, seeds are redistributed into clean setting systems according to their size class, and these are placed into cleaned tanks to continue the rearing cycle.

c. Seed room operations

Daily operations in the seed room include the removal of setting systems from the tanks to allow cleaning of both seed and mesh. Cleaning is performed by gently spraying seawater to remove faeces and biofouling. The 1000 L storage tanks supplying microalgae to the room are also cleaned daily to maintain feed quality and prevent contamination. On Mondays, Wednesdays, and Fridays, tanks are additionally emptied, rinsed with freshwater, cleaned with Virkon™, and rinsed again before being refilled.

On Tuesdays, the room's microalgae supply line is cleaned using oxalic acid, sodium hypochlorite, and sodium thiosulfate solutions, following the same protocol applied in other rooms. During this procedure, all feed hoses are disconnected from the tanks and reconnected once cleaning is completed.

Grading operations are performed as needed, depending on management schedules. For each selected batch, setting systems are removed individually, and seeds are transferred to stacked grading sieves on the workbench. After grading, seeds are placed in smaller bottom-mesh cylinders, separated by size, to allow excess water to drain before being weighed. Five samples from each size class are taken to assess survival and mortality and estimate total numbers (Figure 41). Based on density charts, seeds are then redistributed into clean setting systems and returned to freshly cleaned tanks. All previously used setting systems, sieves, and equipment are cleaned with a sponge and Virkon™, rinsed with freshwater, and stored.

When the cumulative quantity of seed reaches the target size of 2.4 mm and the offshore farm in Lagos is prepared to receive them, all seed of this size — from all batches — is removed from their tanks and setting systems, placed in mesh bags, drained of excess water, and weighed. Shipment timing also depends on favourable sea conditions, as the receiving team must deploy the seed immediately upon arrival. The bags are then placed in hermetically sealed boxes or Styrofoam containers for transport to Lagos, where the seed will continue growing to market size (Figure 42).

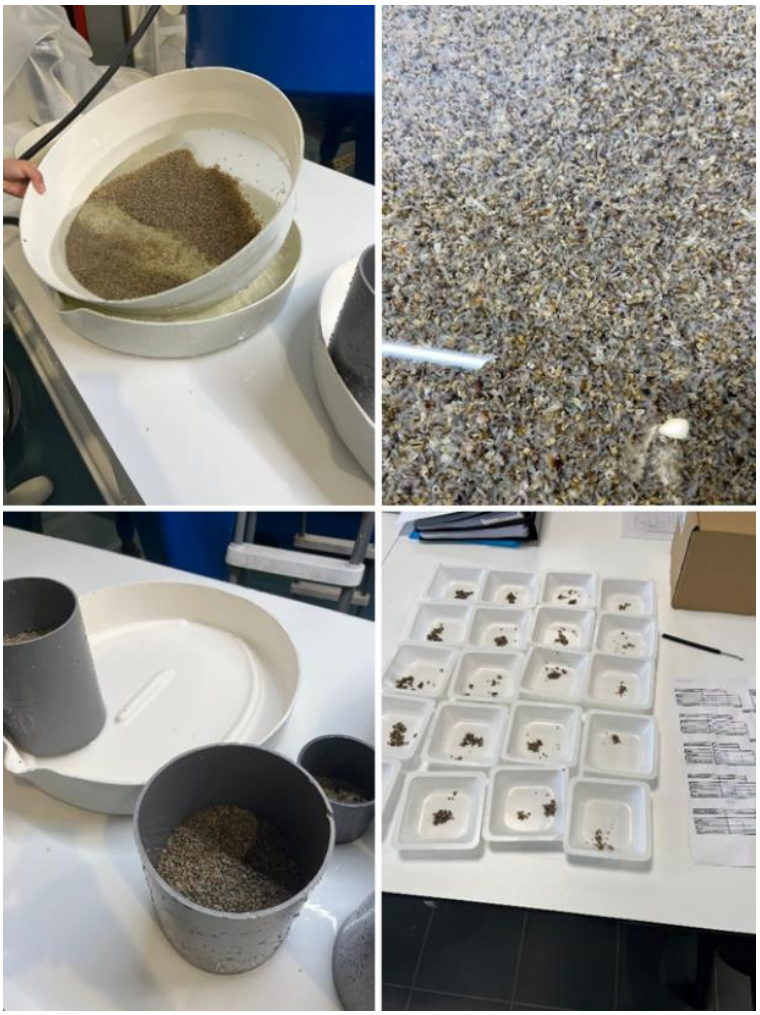

Figure 41. Seed grading operation: graded seeds (top), collected in bottom-mesh cylinders to be weighed (bottom left) and samples (bottom right) (Source: Author, 2025).

Figure 42. Seed shipment operation: gathered seed (left), seed in mesh bags (middle), shipment Styrofoam box (right) (Source: Author, 2025).

7. Other operations

In addition to the routine daily and weekly operations conducted in each room, several supplementary tasks take place across the hatchery. These include cleaning of miscellaneous equipment and floors, repair or construction of tools such as sieves when time allows, data entry into the Cloud system, preparation of various solutions and culture media including Thiosulfate Citrate Bile Salts Sucrose (TCBS) and Marine Agar (MA), and, most importantly, microbiological sampling and analysis.

Microbiological monitoring is performed every Monday, when TCBS and MA samples are collected from several points across the hatchery. These points include running seawater outlets in each room, the technical area, and the harvest lines in the Upright Bag room (Figure 43). TCBS medium is used to detect the presence of *Vibrio* spp., while MA is employed to assess total bacterial load. Sample processing takes place in the laminar flow cabinet located in the Inoculation chamber, and plates are incubated in a controlled-temperature incubator for 72 hours before readings (Figure 44).

In addition, on Mondays, Wednesdays, and Fridays, samples are taken from the harvest tanks in the Upright Bag room for TCBS monitoring. Likewise, samples are collected from larval tanks on drop days to ensure early detection of potential bacterial issues. In both cases, sterile micropipettes are used to directly inoculate TCBS plates, eliminating the need for laminar flow cabinet processing.

Figure 43. Microbiology sampling: post-UV running seawater of Technical area (left) and post-cartridge filter in Larvae room (middle); TCBS plates for the harvest tanks sampling of the Upright Bag room (right) (Source: Author, 2025).

Figure 44. Sample processing in the laminar flow cabinet (left), storing in the controlled-temperature oven (middle), and reading (right) (Source: Author, 2025).

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III. Batch production of microalgae at large volumes

This section addresses the second objective of the internship, which focuses on overcoming a specific challenge and providing a practical solution for the company. The objective in this context was to increase microalgae production, both in volume and consistency, to meet the growing demands of the hatchery and ensure a reliable feed supply.

1. Why? Problem & solutions

The company faced an urgent need to increase microalgae production to sustain seed production at an expanded scale. Microalgae represent the fundamental feed source for all developmental stages in bivalve hatcheries, with both quantity and quality directly affecting survival, growth, and reproductive performance (Helm et al., 2004; FAO, 2024b). At the time, production in the Upright Bag room was insufficient to meet daily requirements, creating a bottleneck in hatchery operations. As a result, seed growth rates and broodstock performance were negatively impacted, limiting the hatchery´s ability to scale up production or conduct trials with new methodologies.

Few immediate solutions were available to overcome this constraint. The hatchery had previously experimented with large outdoor pools for algal production; however, exposure to open-air conditions resulted in poor culture stability and contamination risks, rendering them unsuitable for reliable production. Moreover, such systems were inadequate for the cultivation of diatoms — the primary target for this intervention — given that two-thirds of the Upright Bag room's output consisted of flagellates.

An alternative option was identified within the facility: the use of the 3000 L seed tanks, some of which were available at that time. Unlike the outdoor pools, these tanks were located indoors, ensuring greater control over environmental conditions and lowering contamination risks. This approach enabled the testing of large-volume batch production of microalgae under controlled hatchery conditions, intending to achieve cell concentrations of at least 3,000–4,000 cells mL^{-1} — or higher if possible — to adequately meet the demands of broodstock and seed. This approach formed the basis of the present project.

2. Batch production methodology

Batch culture is one of the most widely applied methodologies for microalgal production in bivalve hatcheries, particularly when large volumes are required to meet the feeding demands of broodstock, larvae, and seed. In this method, microalgae are inoculated into a sterilized culture vessel containing nutrient-enriched seawater and allowed to grow through the characteristic microbial growth phases: lag, exponential, stationary, and decline (Borowitzka & Moheimani, 2013; Andersen, 2005). During the lag phase, cells adapt to the new environment before entering exponential growth, in which cell division and biomass accumulation occur at maximum rates under optimal

conditions. As nutrients become limiting or metabolic wastes accumulate, cultures transition into the stationary phase, during which growth slows and cell quality may decrease, eventually leading to the decline phase characterized by increased mortality and heightened contamination risk (Andersen, 2005).

For hatchery purposes, cultures are typically harvested during the late exponential or early stationary phase, when both cellular density and biochemical quality are optimal, yet before nutrient depletion leads to loss of lipid and protein reserves (Borowitzka & Moheimani, 2013). Harvesting can be performed either by direct transfer of culture to feed tanks or by concentration methods when higher densities are required. After harvest, culture vessels are thoroughly cleaned and disinfected before reinoculation, making batch culture a discontinuous but flexible system well suited for short-term high-volume production (Guillard, 1975; Borowitzka & Moheimani, 2013).

The main advantages of batch culture are its relative simplicity, low infrastructure requirements, and the ability to exert strict control over culture conditions at the start of each cycle, thereby reducing contamination risk compared to continuous systems. However, productivity is inherently limited by the discontinuous nature of the method, and regular labour input is required for cleaning, preparation, and reinoculation (Richmond & Hu, 2013). Despite these constraints, batch production remains a cornerstone of hatchery microalgae supply, particularly for diatoms such as *Chg*, which are highly valued for their digestibility, lipid content, and suitability as feed for bivalve seed and broodstock (Helm et al., 2004; Brown et al., 1997).

3. Adapted methodology and Experimental design

As mentioned above, spare 3000 L seed tanks were repurposed for the batch production of a diatom species of microalgae. No formal experimental design was established beforehand, as this initiative was primarily an industrial-scale trial aimed at quickly addressing the urgent feed shortage. Consequently, operational parameters were adjusted progressively, cycle by cycle, in response to observed culture performance. In addition, exploratory assessments were carried out in some cycles to complement the evaluation of culture stability. These included microbiological analyses to monitor bacterial load dynamics using MA and TCBS plates (performed in two cycles) and the use of nitrate test strips on a few occasions to verify nutrient availability during growth.

A few key decisions were made prior to initiating the trial, including the number of tanks to use, the species to culture, the cycle duration, working volume per tank, inoculum source and volume, and the setup for aeration, light, and CO_2 supply. The project began with the inoculation of a single 3000 L tank using culture from a *Chg* upright bag. Aeration was initially provided through custom-made PVC pipe bars built in-house and connected to the hatchery's air supply. Illumination was supplied by two LED lamps per tank (Figure 45). At this initial stage, no CO_2 supplementation was provided, as the Seed room had not yet been connected to the hatchery's CO_2 line; this was implemented later in the project.

Over successive cycles, several prototypes (Pro) were tested. Adjustments included modifications to working volume and inoculum volume, as well as the addition

of silicates and Varicon®. Alternative aeration systems were trialled, including replacement of the PVC aeration bars with a 2 m nano bubbling diffuser for general aeration and a 20 cm nano bubbling diffuser dedicated to CO_2 injection. Lighting intensity and distribution were also tested under different configurations (Table I). The duration of each batch cycle was generally about seven days, although slight variations occurred depending on culture performance and operational adjustments.

Figure 45. Batch tank configuration with PVC aeration pipe bars and LED lamps (Source: Author, 2024).

Table I - Batch prototypes configuration. Summary of conditions tested during the batch culture trials (ON = system in use; OFF = system not in use).

Prototype (Pro)	Starting date	Water volume (L)	Inoculum volume (L)	Light & Aeration	CO_2	Varicon® (L)	Silicates (L)	Test
1.1	28-Dec-2023	1000	150	ON	OFF	5	1	-
1.2	5-Jan-2024	1800	300	ON	OFF	15	3	Two-week duration
1.3	18-Jan-2024	1500	150	ON	OFF	11.25	2.25	-
1.4	29-Jan-2024	1500	150	ON	OFF	7.5	2.25	-
1.5	6-Feb-2024	1500	150	ON	OFF	7.5	2.25	-
1.6	20-Feb-2024	1500	150	ON	OFF	1.5	2.25	-
1.7	29-Feb-2024	1500	150	ON	OFF	1.5	2.25	-
1.8	2-Apr-2024	1500	200-250	ON	OFF	1.5	1.5	Less silicates
1.9	9-Apr-2024	1500	200-250	ON	OFF	1.5	1.5	Less silicates
2.1	7-Mar-2024	1500	200-250	ON	OFF	1.5	2.25	New air diffuser
3.1	21-Mar-2024	1500	200-250	ON	ON	1.5	2.25	New CO_2 diffuser
3.2	28-Mar-2024	1500	300	ON	ON	1.8	2.25	Inoculation from 3.1
4.1	20-Mar-2024	1500	200	ON	OFF	1.5	2.25	4 lights

4. Results & Discussion

The key parameter for evaluating batch performance was the final cell density (cells mL^{-1}). At the end of each cycle, density values were recorded and used to guide adjustments in subsequent trials. Additional qualitative factors, such as cell morphology and spine visibility, presence of contaminants, and general cleanliness of the tank and culture, were also considered. Complete density records are in Appendix.

Most *Chg* upright bags used as inoculum were already contaminated with *Ts*, justifying the presence of this species in some cultures. CO_2 injection was first introduced in Pro 3.1, following the connection of the Seed room to the hatchery's CO_2 supply line.

- Initial trials without CO_2

Pro 1.1, conducted in December 2023, reached a cell density of 2,325 Kcell mL^{-1}. Although not fully satisfactory, this was considered acceptable for a first attempt. Microscopic examination confirmed clean cultures with well-defined cell spines. However, pH increased above 9 from day 2 onwards — a common outcome in the absence of CO_2 supplementation, since photosynthetic activity rapidly depletes dissolved inorganic carbon and causes alkaline drift (Richmond & Hu, 2013; Borowitzka & Moheimani, 2013). Such alkaline conditions reduce photosynthetic efficiency and cell division rates, thereby limiting culture productivity and compromising nutritional quality (Helm et al., 2004). Feeding stressed algae to, for example, oyster larvae has been shown to increase deformities, reduce feeding and swimming ability, and delay development (Vignier et al., 2021)."

Pro 1.2 tested a two-week cycle, with adjustments to volume and nutrient supplementation (Varicon® and silicates). Although density fluctuated after the first week, the culture ultimately reached 2,685 Kcell mL^{-1} by day 14, compared to 1,935 Kcell mL^{-1} at day 7. This suggested that longer cycles could achieve higher densities at a larger volume; however, two-week durations were operationally impractical due to increased tank usage and higher contamination risk.

Pro 1.3 reduced both working volume and cycle duration compared to Pro 1.2, while returning to the inoculum conditions of Pro 1.1. This trial achieved the highest density so far, 3,608 Kcell mL^{-1} at day 10. However, contamination by ciliates and *Ts* were detected, likely resulting from contaminated inoculum.

Pro 1.4 tested an 8-day cycle with reduced Varicon® addition. Maximum density remained below 2,500 Kcell mL^{-1}, with the stationary phase reached by day 5. Microbiological assessment, initiated in this trial, revealed *Vibrio* contamination, not present in the inoculum, with bacterial load increasing progressively before stabilising mid cycle. Similar trends were obtained in subsequent monitored trials (Table II).

Pro 1.5 repeated the Pro 1.4 configuration, showing comparable microbiological results, but achieved a higher peak density of 3,260 Kcell mL^{-1} by day 6 before declining. Nitrate test strips indicated no nutrient limitation (250 mg L^{-1}).

Pro 1.6 lowered nutrient levels to 1 mL L^{-1} and shortened the cycle to 6 days. The poor outcome was primarily due to aeration failure, resulting in insufficient mixing. In addition, the culture was heavily contaminated with ciliates and *Ts*, carried over from the

inoculum, further compromising performance. This trial clearly demonstrated the importance of reliable aeration for maintaining culture performance.

Pro 1.7 repeated the configuration of Pro 1.6 with corrected aeration. This trial produced 3,000 Kcell mL^{-1} in only 6 days, representing the most efficient outcome to date, given the shorter duration and lower nutrient input compared to Pro 1.3. Nitrate test strips indicated almost complete nutrient consumption (10-25 mg L^{-1}).

Table II – Microbiology assessment: MA with different dilution factors (nº of colonies represented) and TCBS (* = Negative in the bag used to inoculate).

Day	Pro 1.4			Pro 1.5			
	MA (-2)	MA (-3)	TCBS	MA (-1)	MA (-2)	MA (-3)	TCBS
0	1	0	+ *	62	1		+ *
1	17	8					+
2	93	31				28	
3	146	115			108	31	
4		89	+				
5							
6					113	49	+
7	176	27	+				

- Aeration and lighting tests

Pro 2.1 replaced the PVC aeration bars with a 2 m nano bubbling diffuser. Despite using a high inoculum volume, the results were unsatisfactory. The diffuser created weak water movement and excessive surface foam, which likely reduced light penetration and limited culture growth. Due to these limitations, this configuration was discarded (Figure 46).

Pro 4.1 tested increased illumination by using four lamps per tank instead of two. Although cell densities were comparable to earlier Pro 1 trials, the stronger lighting led to higher contamination, a 1 °C temperature increase, and visibly dirtier tanks by the end of the cycle. This configuration was therefore also rejected (Figure 46).

Figure 46. Batch Pro 2.1 (left) and Pro 4.1 (right) testing aeration and light respectively (Source: Author, 2024).

- Introduction of CO_2

Progress initially remained slow, with only one cycle completed per week. By switching to two tanks and running two cycles per week, the project accelerated. Pro 3.1 marked the first trial incorporating CO_2 injection, using a 20 cm nano bubbling diffuser. The impact was substantial: final cell density reached 4,515 Kcell mL^{-1}, the highest obtained so far. The culture was exceptionally clean, pH remained stable at 7.6, and nitrate levels were nearly depleted by harvest, confirming the essential role of CO_2 in optimizing algal growth.

Pro 3.2 tested inoculating a new batch with culture from Pro 3.1 rather than upright bags, while maintaining CO_2 supplementation and an adjusted Varicon® dosage of 1.2 mL L^{-1}. Final density was slightly lower at 3,640 Kcell mL^{-1} but still among the best results. Although CO_2 stability was more challenging to maintain, this trial demonstrated that batch-to-batch inoculation is feasible, simplifying logistics (Figure 47). This marked the beginning of routine scaling-up of operations, which is described in detail below.

Figure 47. Batch inoculation (Source: Author, 2024).

- Testing other species

Curiosity-driven trials were also conducted to assess the system´s versatility. Using the Pro 1.7 configuration without silicates, the flagellates *Ig* and *T-iso* achieved final densities of 2,543 and 3,080 Kcell mL^{-1}, respectively. These results demonstrated the feasibility of producing flagellates with this system if needed.

Further tests with reduced silicate dosages (1 mL L^{-1}) in *Chg* resulted in poorer performance compared to the Pro 1.7 standard, leading to abandonment of this configuration.

The other diatom routinely produced in the hatchery, *Chc*, was tested under Pro 3 conditions with balloon inoculum and doubled Varicon® dosage. The trial achieved excellent final density (4,095 Kcell mL^{-1}), indicating strong potential for batch culture of this species. Initially, only one tank was equipped with CO_2 and therefore dedicated to *Chc*, while *Chg* remained under the Pro 1.7 setup. After the CO_2 system was installed in all tanks in May 2024, both species were tested in parallel.

- Scaling up

Following the advances achieved with Pro 3.2, the project entered the scaling-up phase. From this point, batches were harvested daily on a 7-day cycle using the Pro 1.7 configuration. By early May 2024, two tanks were being inoculated and harvested each day, representing an additional 3000–3600 L of microalgae per day for the hatchery. After installation of a permanent CO_2 supply with air stones in all tanks, culture performance improved significantly (Figure 48). Optimized configurations stabilized at 1.5 mL L^{-1}

silicates and 1.5 mL L^{-1} Varicon®. Although further tests suggested slightly better results for *Chg* with reduced silicates, this modification was not pursued due to limited replicates (Table III).

Table III – Average cell density (Kcell mL^{-1}) in batch culture under different conditions. Comparison between no CO_2, CO_2 supplementation, and CO_2 + reduced silicates (ND = not defined).

Specie	CO_2		
	OFF	ON	ON + Less silicates
Chc	ND	4709	4817
Chg	3117	4871	5188
	Cell Density (Kcell mL^{-1})		

Figure 48. Batch culture in production mode (Source: Author, 2024).

From this stage onward, the project could already be considered a success, although opportunities for further optimization remained. Fine-tuning key parameters such as nutrient and silicate dosages, CO_2 supply, and light configuration — while balancing penetration depth with culture volume and mixing dynamics — will be essential to maximise cell densities and efficiency, ensuring optimal use of resources.

IV.Final considerations

This internship provided a comprehensive overview of the operational workflow of a bivalve hatchery, covering all production stages from broodstock conditioning to seed rearing. It offered the opportunity not only to acquire practical skills and in-depth knowledge of the methodologies employed in each room, but also to participate directly in addressing one of the hatchery's most pressing challenges: the shortage of microalgae for broodstock and seed.

The project on large-volume batch production of microalgae in 3000 L seed tanks demonstrated that significant increases in algal supply can be achieved within a controlled hatchery environment. Initially conceived as an urgent industrial-scale trial, the approach rapidly evolved into a functional production strategy, with significant improvements observed following the introduction of CO_2 supplementation. The results confirmed the critical roles of effective aeration, balanced nutrient supply, and carbon regulation in sustaining high cell densities and maintaining culture stability. Furthermore, the project showed the feasibility of batch-to-batch inoculation, reducing dependence on the Upright Bag room and simplifying logistics. By the end of the project, daily harvests of several thousand liters of clean culture were achieved, effectively alleviating the bottleneck in feed availability and supporting broodstock performance, larval growth, and seed rearing. This method also enabled a scale-up of algal production, though with some constraints, including the large footprint required and the relatively high workload demand for managing multiple large-volume tanks. Overall, the initiative resulted in a 100% increase in algal production capacity at the hatchery and sustained a 30% increase in seed output during 2024, clearly demonstrating both industrial and biological relevance.

The results also highlighted areas for further optimization. Future improvements should focus on fine-tuning Varicon® and silicate dosages to better match the metabolic demand of the cultures while monitoring nitrate dynamics closely; ensuring stable CO_2 supply across all tanks to prevent pH drift and maintain consistent growth; and evaluating light penetration in relation to water column depth and aeration patterns to optimize photosynthetic efficiency without compromising culture cleanliness. Strengthening contamination control through stricter hygiene routines in tanks and equipment, along with systematic microbiological monitoring of inocula and cultures, will also be key. While low levels of opportunistic species are expected in open systems, rigorous cleaning protocols and careful selection of inocula can minimize their impact on culture performance.

Beyond the microalgae project, the internship reinforced the importance of integrating detailed routine operations with flexibility for troubleshooting and innovation. The strict operational discipline maintained across all hatchery rooms — from broodstock to seed — ensures reliable production cycles, while targeted problem-solving initiatives such as this project demonstrate the potential for continuous improvement.

Several aspects of the hatchery workflow revealed opportunities for improvement that could further enhance production efficiency and biosecurity. In the broodstock room, establishing a dedicated quarantine area for newly received individuals would reduce the risk of introducing pathogens. In the longer term, relocating the broodstock facility to a

separate building could further enhance biosecurity by physically separating adult bivalves, which naturally carry higher microbial loads, from the more vulnerable larval and seed stages (Duthie, 2010). Additionally, implementing more standardized feeding protocols in the days preceding spawning could help ensure that broodstock consistently reach gamete release with adequate energy reserves and uniform physiological condition, reducing variability in spawning outcomes (Wilson et al., 1996). Strengthening gonadal monitoring would also improve the capacity to anticipate reproductive peaks and plan spawning events with greater accuracy.

In the larval room, continuous monitoring of temperature, pH, and dissolved oxygen, ideally linked to alarm systems, would allow rapid responses to deviations in these critical parameters, providing greater stability during the most delicate stage of production. In the post-larval room, optimizing the configuration of setting systems — including their position within tanks and associated aeration patterns — could promote more homogeneous settlement and uniform growth across batches (Helm, et al., 2004). For the seed room, improvements could include refining feeding protocols so that algal rations are adjusted to the biomass present in each tank, rather than applying identical quantities across tanks with varying stocking densities. In addition, testing alternative water flow regimes (e.g., variable flow rates) could also provide insights into their effects on seed growth and quality, supporting further refinement of rearing protocols (Helm et al., 2004).

At the level of microbiology and transversal operations, reinforcing hygiene and cleaning protocols remains critical to minimize contamination risks. Preventive maintenance of essential equipment should be systematically documented to ensure traceability and consistency. Greater emphasis should also be placed on the dry-out phase of production materials between cycles: after cleaning and disinfection, items such as tanks, sieves, and hoses should be thoroughly dried (ideally for at least five days in sun-lit conditions), following the internationally recognized *Clean–Drain–Dry* protocol to eliminate resilient biofilms and pathogens (U.S. Fish & Wildlife Service [USFWS], 2022).

In the longer term, pilot trials with semi-continuous culture systems could also be explored as a potential evolution of the batch approach, combining high productivity with greater operational stability. Finally, a cross-cutting improvement that could significantly enhance overall efficiency is the digitalization of operational records. Integrating currently paper-based data — including larval observations, drop results, seed grading records, broodstock monitoring, and equipment maintenance — into a unified digital platform would allow comparative analyses across cycles, facilitate traceability, and improve the predictive capacity across the hatchery.

In conclusion, the internship achieved its twofold objective: consolidating technical skills across the hatchery's production cycle and successfully addressing the feed bottleneck through large volume batch production of microalgae. At the same time, it highlighted concrete areas for future improvement, spanning broodstock biosecurity, larval monitoring, post-larval settlement, seed management, and transversal operations. By addressing these opportunities, the hatchery can strengthen its resilience, increase production efficiency, and create the conditions for future expansion and innovation in bivalve aquaculture.

Since the completion of the internship, further progress has been achieved in the hatchery's batch production system. Cultures are now inoculated directly from newly

acquired photobioreactors (PBRs), allowing greater control over inocula quality. The cultivation cycle has been shortened to five days while still achieving clean cultures at densities around 3000 K cell mL^{-1}. Optimized CO_2 regulation maintains pH values within 7.8–8.0 at harvest, ensuring nutritional stability of the feed. Furthermore, investment in new tanks—currently being implemented—will expand batch capacity by 50%, while freeing the former seed tanks for their original purpose. The harvesting regime has also shifted to continuous mode, reducing reliance on storage tanks and streamlining operations. These developments, alongside my current full-time integration within the hatchery team, demonstrate how the project has transitioned from an experimental trial into a consolidated production strategy with lasting impact.

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V.References

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VI.Appendix

Batch culture density records (ND = not defined).

Date Inoculation	Innoculation (origin)	Specie	CO2 (yes/no)	Date harvested	Culture duration (days)	Volume (L)	Static Harvest Concentration (Kcell/mL)
28-Dec-2023	Bag	*Chg*	no	05/jan/24	8	1150 L	2325 KCell/mL
5-jan-2024	Bag	*Chg*	no	18/jan/24	13	2100 L	2685 KCell/mL
18-jan-2024	Bag	*Chg*	no	29/jan/24	11	1650 L	3608 KCell/mL
29-jan-2024	Bag	*Chg*	no	6-Feb-2024	8	1650 L	2530 KCell/mL
6-Feb-2024	Bag	*Chg*	no	14-Feb-2024	8	1650 L	2640 KCell/mL
20-Feb-2024	Bag	*Chg*	no	26-Feb-2024	6	1650 L	1462 KCell/mL
29-Feb-2024	Bag	*Chg*	no	07/mar/24	7	1650 L	3000 KCell/mL
7-mar-2024	Bag	*Chg*	no	14/mar/24	7	1750 L	2375 KCell/mL
8-mar-2024	Bag	*Ig*	no	15/mar/24	7	1750 L	2543 KCell/mL
11-mar-2024	Bag	*T-iso*	no	18/mar/24	7	1750 L	3080 KCell/mL
14-mar-2024	ND	*Chg*	no	21/mar/24	7	ND	2380 KCell/mL
19-mar-2024	ND	*Chg*	no	26/mar/24	7	ND	2340 KCell/mL
20-mar-2024	Bag	*Chg*	no	27/mar/24	7	1700 L	2562 KCell/mL
21-mar-2024	Bag	*Chg*	yes	28/mar/24	7	1750 L	4515 KCell/mL
22-mar-2024	ND	*Chg*	no	29/mar/24	7	ND	2740 KCell/mL
23-mar-2024	Other tank	*Chg*	no	30/mar/24	7	1800 L	2270 KCell/mL
24-mar-2024	Other tank	*Chg*	no	31/mar/24	7	1800 L	2690 KCell/mL
25-mar-2024	ND	*Chg*	no	1-Apr-2024	7	ND	3690 KCell/mL
26-mar-2024	ND	*Chg*	no	2-Apr-2024	7	ND	2940 KCell/mL
27-mar-2024	ND	*Chg*	no	3-Apr-2024	7	ND	3240 KCell/mL
28-mar-2024	Other tank	*Chg*	yes	4-Apr-2024	7	1800 L	3640 KCell/mL
29-mar-2024	ND	*Chg*	no	5-Apr-2024	7	ND	2895 KCell/mL
30-mar-2024	Other tank	*Chg*	no	6-Apr-2024	7	1800 L	3135 KCell/mL
31-mar-2024	Other tank	*Chg*	no	7-Apr-2024	7	1800 L	2430 KCell/mL
1-Apr-2024	Bag	*Chg*	no	8-Apr-2024	7	1750 L	3360 KCell/mL
2-Apr-2024	Bag	*Chg*	no	9-Apr-2024	7	1750 L	3080 KCell/mL
3-Apr-2024	Bag	*Chg*	no	10-Apr-2024	7	1750 L	3400 KCell/mL
4-Apr-2024	Bag	*Chg*	no	11-Apr-2024	7	1750 L	2970 KCell/mL
5-Apr-2024	Baloon	*Chc*	yes	12-Apr-2024	7	1500 L	4095 KCell/mL
7-Apr-2024	Other tank	*Chg*	no	14-Apr-2024	7	1800 L	2187 KCell/mL
8-Apr-2024	Bag	*Chg*	no	15-Apr-2024	7	1750 L	2730 KCell/mL
9-Apr-2024	Bag	*Chg*	no	16-Apr-2024	7	1750 L	2889 KCell/mL
10-Apr-2024	Bag	*Chg*	no	17-Apr-2024	7	1750 L	4005 KCell/mL
11-Apr-2024	Bag	*Chg*	no	18-Apr-2024	7	1750 L	3150 KCell/mL
12-Apr-2024	Other tank	*Chc*	yes	19-Apr-2024	7	1800 L	4000 KCell/mL
13-Apr-2024	Other tank	*Chg*	no	20-Apr-2024	7	1800 L	3039 KCell/mL
14-Apr-2024	Other tank	*Chg*	no	21-Apr-2024	7	1800 L	3213 KCell/mL
15-Apr-2024	Bag	*Chg*	no	22-Apr-2024	7	1750 L	3287 KCell/mL
16-Apr-2024	Bag	*Chg*	no	23-Apr-2024	7	1750 L	4230 KCell/mL

17-Apr-2024	Bag	*Chg*	no	24-Apr-2024	7	1750 L	3285 KCell/mL
18-Apr-2024	Other tank	*Chg*	no	25-Apr-2024	7	1800 L	3125 KCell/mL
19-Apr-2024	Other tank	*Chc*	yes	26-Apr-2024	7	1800 L	0 KCell/mL
20-Apr-2024	Bag	*Chg*	no	27-Apr-2024	7	1750 L	3013 KCell/mL
21-Apr-2024	Other tank	*Chg*	no	28-Apr-2024	7	1800 L	3240 KCell/mL
22-Apr-2024	Bag	*Chg*	no	29-Apr-2024	7	1750 L	3510 KCell/mL
23-Apr-2024	Bag	*Chg*	no	30-Apr-2024	7	1750 L	1995 KCell/mL
24-Apr-2024	Bag	*Chg*	no	1-May-2024	7	1750 L	2887 KCell/mL
25-Apr-2024	Bag	*Chg*	no	2-May-2024	7	1750 L	3300 KCell/mL
26-Apr-2024	Bag	*Chg*	no	3-May-2024	7	1750 L	3615 KCell/mL
27-Apr-2024	Other tank	*Chg*	no	4-May-2024	7	1800 L	3090 KCell/mL
28-Apr-2024	Other tank	*Chg*	no	5-May-2024	7	1800 L	3663 KCell/mL
29-Apr-2024	Bag	*Chg*	no	6-May-2024	7	1750 L	3390 KCell/mL
30-Apr-2024	Bag	*Chg*	yes	7-May-2024	7	1750 L	3975 KCell/mL
1-May-2024	Other tank	*Chg*	no	8-May-2024	7	1800 L	3270 KCell/mL
2-May-2024	Bag	*Chg*	no	9-May-2024	7	1750 L	2900 KCell/mL
3-May-2024	Bag	*Chg*	no	10-May-2024	7	1750 L	3263 KCell/mL
4-May-2024	Other tank	*Chg*	no	11-May-2024	7	1800 L	2600 KCell/mL
5-May-2024	Other tank	*Chg*	no	12-May-2024	7	1800 L	2863 KCell/mL
6-May-2024	Bag	*Chg*	no	13-May-2024	7	1750 L	3270 KCell/mL
7-May-2024	Bag	*Chg*	yes	14-May-2024	7	1750 L	4125 KCell/mL
8-May-2024	Bag	*Chg*	no	15-May-2024	7	1750 L	3375 KCell/mL
9-May-2024	Bag	*Chg*	no	16-May-2024	7	1750 L	2940 KCell/mL
10-May-2024	Bag	*Chg*	no	17-May-2024	7	1750 L	3165 KCell/mL
11-May-2024	Other tank	*Chg*	no	18-May-2024	7	1800 L	2775 KCell/mL
12-May-2024	Other tank	*Chg*	no	19-May-2024	7	1800 L	2910 KCell/mL
13-May-2024	Bag	*Chg*	no	20-May-2024	7	1750 L	2640 KCell/mL
14-May-2024	Bag	*Chg*	yes	21-May-2024	7	1750 L	5580 KCell/mL
15-May-2024	Bag	*Chg*	yes	22-May-2024	7	1750 L	4563 KCell/mL
15-May-2024	Bag	*Chg*	yes	22-May-2024	7	1750 L	ND
16-May-2024	Bag	*Chg*	yes	23-May-2024	7	1750 L	4050 KCell/mL
16-May-2024	Bag	*Chc*	yes	23-May-2024	7	1750 L	6180 KCell/mL
17-May-2024	Bag	*Chg*	yes	24-May-2024	7	1750 L	5580 KCell/mL
17-May-2024	Bag	*Chg*	yes	24-May-2024	7	1750 L	5505 KCell/mL
18-May-2024	Other tank	*Chg*	yes	25-May-2024	7	1800 L	5520 KCell/mL
18-May-2024	Other tank	*Chg*	yes	25-May-2024	7	1800 L	5960 KCell/mL
19-May-2024	Other tank	*Chg*	yes	26-May-2024	7	1800 L	3860 KCell/mL
19-May-2024	Other tank	*Chg*	yes	26-May-2024	7	1800 L	2660 KCell/mL
20-May-2024	Bag	*Chg*	yes	27-May-2024	7	1750 L	5260 KCell/mL
20-May-2024	Baloon	*Chc*	yes	27-May-2024	7	1500 L	5300 KCell/mL
21-May-2024	Bag	*Chg*	yes	28-May-2024	7	1750 L	6420 KCell/mL
21-May-2024	Bag	*Chg*	yes	28-May-2024	7	1750 L	5480 KCell/mL
22-May-2024	Bag	*Chg*	yes	29-May-2024	7	1750 L	4500 KCell/mL
22-May-2024	Bag	*Chg*	yes	29-May-2024	7	1750 L	5160 KCell/mL
23-May-2024	Other tank	*Chc*	yes	30-May-2024	7	1750 L	4420 KCell/mL
23-May-2024	Bag	*Chg*	yes	30-May-2024	7	1750 L	5160 KCell/mL

24-May-2024	Bag	*Chg*	yes	31-May-2024	7	1750 L	4800 KCell/mL
24-May-2024	Bag	*Chg*	yes	31-May-2024	7	1750 L	4980 KCell/mL
25-May-2024	Other tank	*Chg*	yes	01/jun/24	7	1800 L	3600 KCell/mL
25-May-2024	Other tank	*Chg*	yes	01/jun/24	7	1800 L	4400 KCell/mL
26-May-2024	Other tank	*Chg*	yes	02/jun/24	7	1800 L	4360 KCell/mL
26-May-2024	Other tank	*Chg*	yes	02/jun/24	7	1800 L	4240 KCell/mL
27-May-2024	Baloon	*Chc*	yes	03/jun/24	7	1500 L	4380 KCell/mL
27-May-2024	Baloon	*Chc*	yes	03/jun/24	7	1500 L	5560 KCell/mL
28-May-2024	Other tank	*Chg*	yes	04/jun/24	7	1800 L	4840 KCell/mL
28-May-2024	Other tank	*Chg*	yes	04/jun/24	7	1800 L	4880 KCell/mL
29-May-2024	Bag	*Chg*	yes	05/jun/24	7	1750 L	4620 KCell/mL
29-May-2024	Bag	*Chg*	yes	05/jun/24	7	1750 L	5120 KCell/mL
30-May-2024	Other tank	*Chg*	yes	06/jun/24	7	1800 L	4260 KCell/mL
30-May-2024	Other tank	*Chg*	yes	06/jun/24	7	1800 L	4280 KCell/mL
31-May-2024	Baloon	*Chc*	yes	07/jun/24	7	1500 L	4603 KCell/mL
31-May-2024	Baloon	*Chc*	yes	07/jun/24	7	1500 L	4428 KCell/mL
1-jun-2024	Other tank	*Chg*	yes	08/jun/24	7	1650 L	5285 KCell/mL
1-jun-2024	Other tank	*Chg*	yes	08/jun/24	7	1650 L	4060 KCell/mL
2-jun-2024	Other tank	*Chg*	yes	09/jun/24	7	1650 L	5000 KCell/mL
2-jun-2024	Other tank	*Chg*	yes	09/jun/24	7	1650 L	3940 KCell/mL
3-jun-2024	Other tank	*Chc*	yes	10/jun/24	7	1650 L	5260 KCell/mL
3-jun-2024	Bag	*Chg*	yes	10/jun/24	7	1750 L	6000 KCell/mL
4-jun-2024	Bag	*Chg*	yes	11/jun/24	7	1750 L	4160 KCell/mL
4-jun-2024	Baloon	*Chc*	yes	11/jun/24	7	1500 L	2580 KCell/mL
5-jun-2024	Bag	*Chg*	yes	12/jun/24	7	1750 L	4700 KCell/mL
5-jun-2024	Bag	*Chc*	yes	12/jun/24	7	1750 L	4940 KCell/mL
6-jun-2024	Bag	*Chg*	yes	13/jun/24	7	1750 L	3900 KCell/mL
6-jun-2024	Baloon	*Chc*	yes	13/jun/24	7	1500 L	3720 KCell/mL
7-jun-2024	Other tank	*Chg*	yes	14/jun/24	7	1650 L	5260 KCell/mL
7-jun-2024	Other tank	*Chc*	yes	14/jun/24	7	1650 L	5420 KCell/mL
8-jun-2024	Other tank	*Chg*	yes	15/jun/24	7	1650 L	6360 KCell/mL
8-jun-2024	Other tank	*Chg*	yes	15/jun/24	7	1650 L	6420 KCell/mL
9-jun-2024	Other tank	*Chg*	yes	16/jun/24	7	1650 L	5760 KCell/mL
9-jun-2024	Other tank	*Chg*	yes	16/jun/24	7	1650 L	5720 KCell/mL
10-jun-2024	Other tank	*Chg*	yes	17/jun/24	7	1650 L	4280 KCell/mL
10-jun-2024	Baloon	*Chc*	yes	17/jun/24	7	1500 L	4380 KCell/mL
11-jun-2024	Bag	*Chg*	yes	18/jun/24	7	1750 L	5080 KCell/mL
11-jun-2024	Baloon	*Chc*	yes	18/jun/24	7	1500 L	4980 KCell/mL
12-jun-2024	Bag	*Chg*	yes	19/jun/24	7	1750 L	4560 KCell/mL
12-jun-2024	Bag	*Chg*	yes	19/jun/24	7	1750 L	4220 KCell/mL