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Preliminary evaluation of a raceway photobioreactor for mass culture of microalgae for Peruvian scallop, *Argopecten purpuratus* postlarvae

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Feeding microalgae during the larval and post-larval stages of bivalve mollusks is necessary to overcome the high mortality rates that occur during metamorphosis. Proper selection of the microalgae species and strain to be used by considering its nutritional value, cell density, culture time, and type of culture system to improve bivalve productivity is of high importance. In this study a microalgae recirculating culture system was evaluated. The system consisted of an open horizontal photobioreactor inside a hatchery raceway tank (12 m long, 2 m wide, and 0.5 m high) with a central division of 10 m and a driving paddle that generated a continuous flow of water at a speed of 1.25 m/s. The microalgae species cultivated in the photobioreactor were strains of *Nannochloris* spp. and *Phaeodactylum* spp., with the former reaching a significantly higher cell density of 19.37 $\pm$ 1.31 (10^6 cell mL^{-1}) than the latter, which obtained a cell density of 1.41$\pm$ 1.31 (10^6 cell mL^{-1}). The reproductive cycle for *Nannochloris* spp. was 59 d, whereas that for *Phaeodactylum* spp. was 19 d. This study demonstrates that, although both strains of species of microalgae can be cultivated utilizing a horizontal photobioreactor system in hatcheries, *Nannochloris* spp. strains offer productive advantages including culture duration.

KEYWORDS

aquaculture nutrition, live feed, marine aquaculture, microalgal growth, raceway photobioreactor

1 Introduction

Aquaculture is an economic activity of great importance in many countries as it provides a source of high-quality animal protein and contributes to food security and economic development (FAO, 2022). The culture of the Peruvian scallop, *Argopecten purpuratus*, a bivalve mollusk, contributes to the economic, social, and environmental development of countries that engage in this activity, such as Spain, China, Japan, the United States, Panama, Peru, and Chile (Kluger et al., 2019). However, the success of culturing this species, as well as other bivalves, depends on several factors such as cultivation technology, diet, and diseases that can influence the yield and profitability of the crop (FAO, 2016). One of the significant challenges facing aquaculture production of *A. purpuratus* is the availability and continuous supply of feed during the larval and post-larval stages of this species (FAO, 2016). The culture of *A. purpuratus* is a complex process that varies between 14 and 18 months, consisting of the production stages: larval (20–35 days), post-larval (40–55 days), pre-fattening (2–3 months), fattening (8–12 months), and harvesting (1–2 months) (Ministerio de la Producción, 2022). The larval stage, when metamorphosis to postlarvae occurs, from 20 to 35 days, is critical because of the physiological and anatomical changes that deplete energy reserves (Crisóstomo et al., 2023). Mortality at this stage can increase between 23% and 77% on the second day after larval attachment and between 20% and 50% on the fifth day after attachment (Farías, 2008; Yupanqui-Ccallata, 2018; Rojas et al., 2023). To control the mass mortality of *A. purpuratus* larvae by pathogenic Vibrio outbreaks, a diet rich in high-density unsaturated fatty acids (HUFA) is necessary to improve survival from 75-80%.

A. purpuratus individuals filter microalgae to provide the nutrients necessary for normal development (Carreño et al., 2012; Castro-Bustamante, 2018). The type of microalgae chosen for mollusk feeding must comply with a series of characteristics such as high cell density, culture time, adaptability to variable environmental conditions, and nutritional composition (Vivanco et al., 2014). Among the different microalgae strains evaluated, *Nannochloris* spp. strains present advantages of adaptation to varying salinities (Witt et al., 1981), low sensitivity to light and nutrient changes, and for example, strains KMMCC-119 and 395 can be cultured at temperatures up to 30°C (Bae and Hur, 2011). *Nannochloris* spp. has been used to feed mollusks such as Pacific oysters, *Crassostrea gigas*, being suitable for mass culture (Rodríguez-Pesantes, 2020). However, among the disadvantages of its cultivation is contamination with other microalgae species due to temperature variations, such as *Phaeodactylum* spp. at 25°C and *Skeletonema* spp. at 18°C (Witt et al., 1981), ciliates (Hue et al., 2020; Zhao et al., 2021), and rotifers (Deruyck et al., 2019; Hong et al., 2024). *Nannochloris* spp. have been reported to reach an average protein content of 42%, carbohydrate content of 11%, and lipid content of 15% under batch culture conditions in controlled laboratory environments, typically maintained at 20–25°C and at moderate to high light intensities (Witt et al., 1981; Ben-Ammar et al., 2024). Biochemical analysis of strain KMMCC-119 revealed the presence of nervonic acid (C24:1, omega-9 monounsaturated fatty acid), which is essential in medicinal plants and in the biosynthesis of myelin cells for the maintenance and development of the nervous system (Saadaoui et al., 2016), thus favoring the development and health of *C. gigas* postlarvae. On the other hand, *Phaeodactylum tricornutum* is a marine diatom whose relevance lies in the production of omega-3 fatty acids, with great emphasis on the production of EPA, whose composition ranges from 14 to 32% (García et al., 2021). Therefore, both *Nannochloris* spp. and *Phaeodactylum* spp. cultures have potential as food for bivalve postlarvae; however, there are differences in the growth rate and nutritional composition of microalgae that could affect their value as food, which is a critical factor for their respective use (Zafra-Trelles, 2017). A balanced diet for *A. purpuratus* includes the supply of different species of microalgae, such as *Isochrysis galbana, Diacronema lutheri, Chaetoceros calcitrans, Chaetoceros gracilis Nannochloris maculata, Nannochloris* sp, *Nannochloropsis oculata*, *Dunaliella tertiolecta*, and *Tetraselmis suecica* (Ministerio de la Producción, 2022). However, in batch culture conditions, *Isochrysis galvana, Chaetoceros gracilis, Thlassiosira pseudonana*, and *Tetraselmis maculata* do not exceed an average density of 4.9 x 10^6 cel mL^{-1}, which does not meet the high cell density requirement needed for large-scale live feed production (Azaldi and Montoya, 2001) *Nannochloris* spp. present a growth rate significantly higher than 129 L^{-1} d^{-1} (Dogaris et al., 2015). Thus, when deciding which microalgae strain to cultivate, factors such as the high density of the culture and the time at which it will reach the maximum cell density should be taken into account, since the microalgal culture time should coincide with the larval and post-larval culture time of *A. purpuratus* in the hatchery for up to 90 days (Avendaño et al., 2001; Ministerio de la Producción, 2022), elements that are determinant for continuous and axenic production.

One aspect of microalgae cultivation technology involves the proper selection of the type of photobioreactor for microalgae cultivation, depending on its ease of operation, versatility in automation, low contamination, evaporation rate, fluctuating temperatures, nutrient limitation, culture medium, homogenization efficiency, and mass harvesting properties that can influence microalgal productivity (Brennan and Owende, 2010; Amaro et al., 2011; Handler et al., 2012). That is, superior photobioreactors allow optimal control of the culture conditions (Wang et al., 2012; Tham et al., 2023). Although several studies have demonstrated the feasibility of continuous microalgal production in raceway systems under greenhouse and hatchery conditions — particularly in the southeastern United States (Rusch and Malone, 1998; Theegala et al., 1999; Rusch and Christensen, 2003)— challenges remain in achieving consistently high-density production over extended periods under semi-controlled environments suitable for mollusk hatcheries. These studies have provided valuable insights into the management of environmental variables, but maintaining stable, high biomass levels over larval rearing cycles still requires further optimization. For instance, Bounnit et al. (2020) cultivated *Nannochloris* spp. QUCCCM31 in parallel 1^{-L} photobioreactors under controlled laboratory conditions, achieving a biomass productivity of 226 mg L^{-1} d^{-1}, demonstrating low-scale laboratory cultivation. However, to

support large scale hatchery production of bivalves, technological advances in mass cultivation of strains under controlled conditions must be developed. These studies suggest that recirculating, open-air, horizontal photobioreactors can be more efficient than open raceways for the cultivation of different species of microalgae. However, low productivity and high costs require improved design and modeling of the technology for mass cultivation of microalgae in recirculating conditions. Therefore, scalability of microalgae cultivation in open raceway tanks within a greenhouse or hatchery is suggested.

The present study aimed to compare the culture of two species of marine microalgae, *Phaeodactylum* spp. and *Nannochloris* spp., in a horizontal photobioreactor, located inside a recirculating bivalve culture hatchery, for the feeding of *A. purpuratus* larvae and postlarvae. The cell density and periodicity of the cultured microalgae were evaluated to determine their viability of mass culture.

2 Materials and methods

2.1 Design and dimensions of the microalgae culture raceway

The raceway photobioreactor was developed at the Centro de Investigaciones Costeras of the University de Atacama (CIC-UDA) in Chile. It consisted of a 12 m long, 2 m wide and 0.75 m high "hippodrome" type raceway (capacity 15 m^3), with a central division of 10 m and a microalgal culture storage capacity of 10 m^3 operating with an allowable culture height of 0.5 m (Table 1).

TABLE 1 Operational parameters of the horizontal photobioreactor for marine microalgae culture.

Parameter	Value	Notes
Raceway useful volume	10 m^3	
Raceway dimensions	10 m (length) × 2 m (width) × 0.5 m (depth)	
Paddle wheel blade angle	45°	Optimized for horizontal flow
Paddle wheel speed	30 rpm	Adjusted to achieve target velocity
Surface water velocity	~1.25 m/s	Verified by CFD modeling
Culture temperature	14.31 ± 1.03°C	Semi-controlled environment
Light intensity	1067.47 ± 443.82 lux	Natural light filtered through hatchery
Salinity	34.53 ± 0.38 PSU	Measured weekly
Aeration system	0.5 HP blower	To enhance mixing and oxygenation

The raceway culture tank was constructed using 100% reinforced fiberglass, internally coated with a nutritional grade isophthalmic gelcoat barrier. This material was specifically selected to guarantee the safety of microalgae cultures, avoiding the release of potentially contaminating or toxic compounds.

Water movement inside the raceway was generated using a paddle system. The water movement within the raceway was generated using a rotary vane system with a three-phase geared motor. The paddle wheel consisted of 4 flat blades with a 45° inclination, mounted on a horizontal shaft driven by a three-phase geared motor operating at 30 revolutions per minute (rpm). The blade design and rotation speed were determined through Computational Fluid Dynamics (CFD) simulations using *Salome v7*, an open-source platform for pre- and post-processing in numerical simulations, particularly useful for geometry creation and mesh preparation. Mesh generation was carried out with *snappyHexMesh*, a meshing utility within the OpenFOAM suite that generates predominantly hexahedral (hex) meshes by iteratively refining and snapping the mesh to complex CAD geometries. This approach ensures high-quality mesh resolution near surfaces and in regions of interest. The resulting mesh enabled accurate modeling of fluid flow and pressure distribution around the blades. These simulations informed the optimization of blade geometry and rotation speed, enhancing hydrodynamic efficiency and achieving a surface circulation velocity of approximately 1.25 m/s. This velocity was higher than that typically reported for open raceways (0.20–0.30 m/s), to ensure sufficient suspension of microalgae cells in a controlled indoor environment while avoiding excessive shear stress. Literature indicates that microalgae such as *Nannochloris* spp. and *Phaeodactylum* spp. can tolerate shear rates up to 100–300 s^{-1} without significant damage (Brennan and Owende, 2010). The system was modeled to maintain shear rates within safe thresholds across most of the raceway volume. Figure 1 illustrates the design and dimensions of the culture system. Theoretical modeling and evaluation of the culture system were conducted to determine the dynamics of the particles in the raceway and the general behavior of the fluid in the system to estimate the correct arrangement of velocities and flows within the raceway.

Both were elaborated with the *Salome v7.1* software and the *snappyHexMesh* tool, generating the dynamic geometry of the microalgae culture raceway tank. To validate the computational fluid dynamics (CFD) simulation results, experimental measurements of surface water velocity were conducted using a portable vane anemometer (Model DEF-123). Measurements were taken at five strategic locations along the longitudinal axis of the photobioreactor, near the water surface (0–5 cm depth).

2.2 Microalgae cultivation

The cultured microalgal strains utilized in this study (*Phaeodactylum* spp. and *Nannochloris* spp.) were supplied by CIC- UDA. For all culture stages, seawater treated by three filters of 5µm, 2 µm, and 1 µm, irradiated with UV light, disinfected with

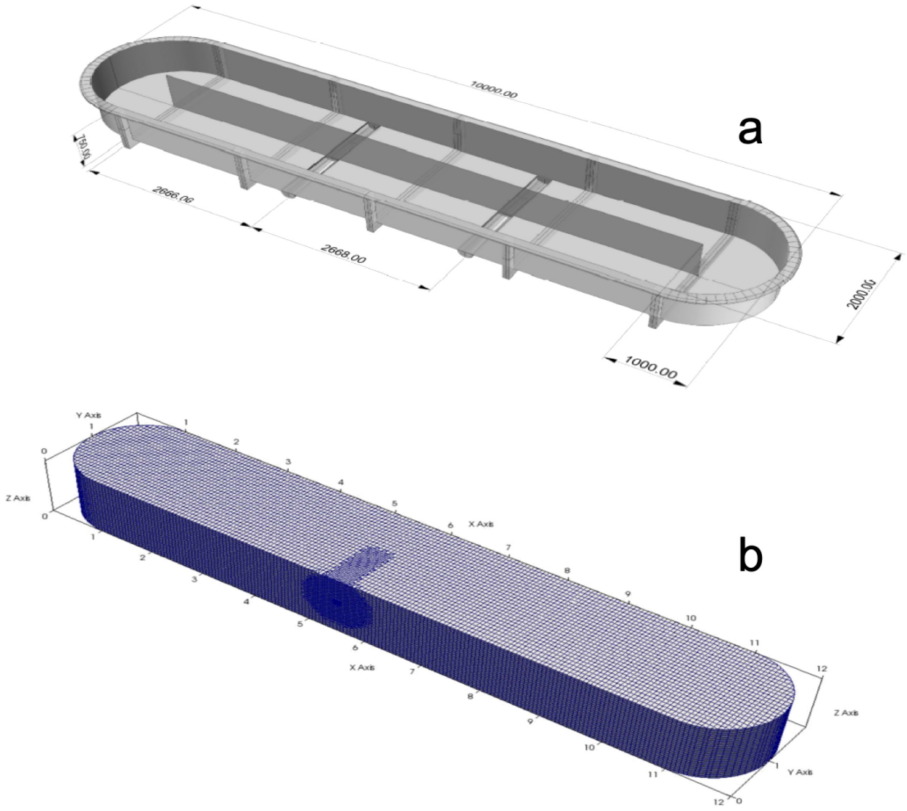

FIGURE 1
Three-dimensional design and meshing of the horizontal photobioreactor. **(a)** Top view of the 3D model showing paddle wheel (marked) and central divider. **(b)** Meshing of the culture raceway generated with 265,468 nodes.

0.25 mL L^{-1} of 2.5% sodium hypochlorite, and neutralized with 0.1 mL L^{-1} of 24.81% sodium thiosulfate was used. In addition, seawater was enriched at all stages of the culture scale-up with F/2 Guillard medium (Guillard, 1975) at a cell density of 1mL L^{-1} (Méndez-Ancca et al., 2020). Cultures were inoculated at initial densities of 2.16×10^5, 3.33×10^5, 1.83×10^5, and 7.17×10^5 cells mL^{-1} for Batches 1, 2, 3, and 4 (grown under static conditions without continuous addition of nutrients), respectively, determined by Neubauer chamber counting. The system operated in batch mode, without recirculation or scheduled daily harvesting.

Besides the initial addition of 1 mL L^{-1} F/2 medium (water-soluble mineral medium for the cultivation of marine microalgae), weekly supplementation with 0.5 mL L^{-1} of concentrated F/2 medium was applied to sustain nutrient levels during the experiment. To prevent and monitor potential contamination during long-term cultivation, several strategies were employed. Seawater used for culture preparation underwent triple-stage filtration (5 µm, 2 µm, 1 µm), ultraviolet (UV) light disinfection, chlorination with 0.25 mL L^{-1} of 2.5% sodium hypochlorite, and subsequent neutralization with 0.1 mL L^{-1} of 24.81% sodium thiosulfate. During the cultivation period, weekly microscopic inspections were performed on samples collected from different points of the photobioreactor to detect potential contamination by other microalgae species, bacteria, or zooplankton such as rotifers. No significant contamination events were detected during the 59-day cultivation of *Nannochloris* spp., ensuring culture viability and stability throughout the experimental period. (Table 2).

Microalgae cultures were grown under semi-controlled conditions, maintaining a constant temperature, natural illumination, and dissolved oxygen levels, and cultures were aerated with a system powered by one regenerative air blower of 0.5 HP (GEBIAO, Model: GB-370, China). The raceway tank was aerated using a perforated polyethylene tube positioned along the interior side wall of the tank. The tube was perforated with holes spaced every 10 cm and extended along the entire length of the raceway. The aeration configuration ensured uniform air distribution throughout the raceway tank, promoting effective suspension of microalgal cells and maintaining adequate dissolved oxygen levels during algal cultivation. A transparent polycarbonate roof allowed natural solar radiation to penetrate the raceway tank. No artificial lighting or temperature control systems were employed during the experimental period.

Light intensity and temperature were monitored twice daily (at 08:00 h and 16:00 h) using a digital lux meter (Extech Instruments,

TABLE 2 Summary of contamination control and monitoring methods applied during microalgal cultivation.

Monitoring Method	Description	Frequency
Triple-stage filtration	10 µm → 5 µm → 1 µm filtration of seawater	Once (before use)
UV disinfection	UV irradiation after filtration	Once (before use)
Chemical disinfection	0.25 mL L^{-1} sodium hypochlorite, neutralized with 0.1 mL L^{-1} sodium thiosulfate	Once (before use)
Microscopic inspection	Detection of contaminant microalgae, bacteria, or rotifers	Weekly

Model: LT300), and a calibrated digital thermometer (Gain Express, Model: THE-27), respectively.

2.3 Statistical analysis

Due to the logistical constraints associated with operating a single large volume photobioreactor (10,000 L), each batch constituted an independent cultivation event without replication. As such, traditional inferential statistical analyses (e.g., ANOVA or mixed-effects models) were not applied. Growth data were analyzed descriptively, with means and standard deviations calculated for each batch to identify overall trends and variability.

3 Results

3.1 Modeling and construction of the microalgae cultivation raceway tank

The fluid velocities inside the raceway at different levels measured from the bottom of the raceway are presented in Figure 1. The direction of rotation of the paddle is indicated by arrows. It was observed that the greatest water movement occurred in the upper strata (0.50 m depth – velocities up to 1.25 m/s), while water movement decreased approaching a depth of 0.1 m with velocities decreasing to 0.0 m/s. Higher fluid velocity generated greater turbulence in the sector where the rotated vanes rotate. The analysis of water movement in the photobioreactor showed that there were places where eddy-like closed rotating movements were formed, and these occurred mostly at the ends of the raceway. Caution should be taken in monitoring extreme zones, especially zones closest to the bottom of the raceway, because near bottom zones represent an area where particles settle. Therefore, it is advisable to install aeration in these areas to avoid accumulation of particles and dead zones in the raceway (Figure 2).

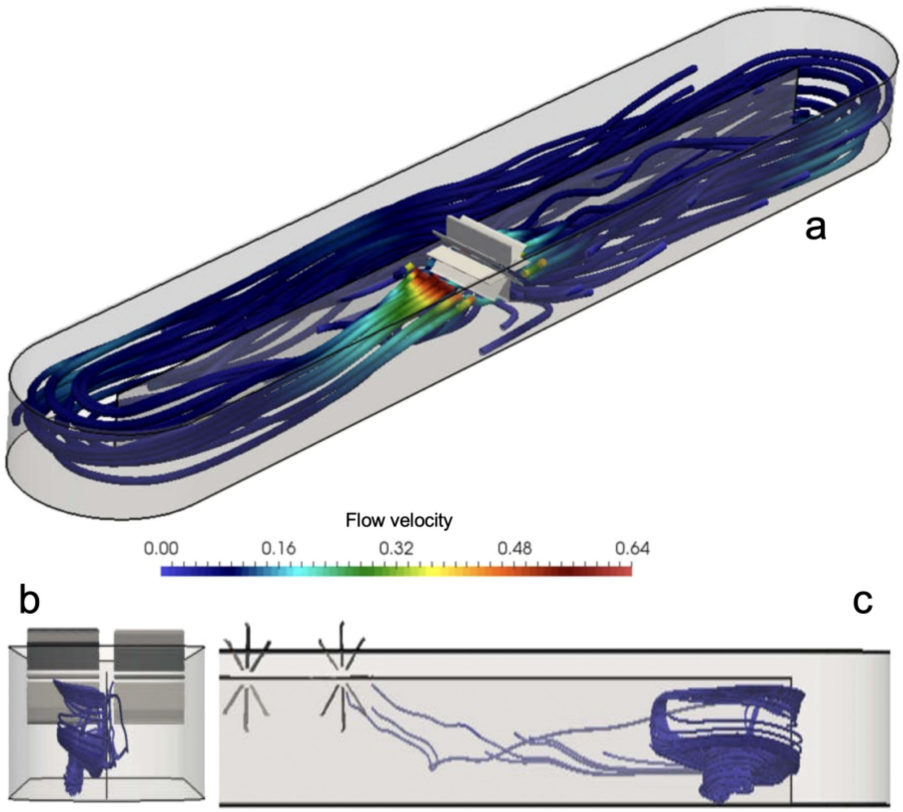

FIGURE 2
Particle trajectory inside the raceway photobioreactor **(a)** isometric view showing water circulation **(b)** frontal view highlighting paddle wheel position **(c)** longitudinal view showing the drainage point locations.

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After modeling and prediction of the maximum water depth that the experimental raceway could maintain the water mass of the microalgae culture raceway in a moving state, the microalgae culture raceway was built and installed in the premises of CIC-UDA; the horizontal fiberglass culture raceway tank, 10 m long, 2 m wide and 0.5 m useful, contained 10 m^3 of microalgae culture. There were also two zones of solid accumulation and flushing drainage (Figures 3, 4). Experimental validation of the fluid dynamic model using surface velocity measurements yielded an average water flow of 1.21 ± 0.08 m/s, confirming the accuracy of the simulated flow pattern and supporting the reliability of the CFD predictions (Figure 3).

3.2 Microalgae cultivation

The averages of the physical parameters of the culture were: water temperature 14.31 ± 1.03°C, light intensity 1067.47 ± 443.82 lux, salinity 34.53 ± 0.38 PSU, and dissolved oxygen 8.79 ± 0.88 mg L^{-1}. Throughout the experimental period (June–October 2017), temperature and light intensity were monitored daily inside the hatchery where the photobioreactor was located. As shown in Figure 5, temperature fluctuated naturally between approximately 13°C and 16°C, while light intensity varied from around 700 to 1500 lux depending on the month. Lower temperatures and reduced light levels were recorded during July, which corresponded to the austral winter period, whereas higher values were observed in September. Despite these natural fluctuations, both parameters remained within acceptable ranges for the cultivation of *Nannochloris* spp. And *Phaeodactylum* spp., supporting stable microalgal growth. When interpreting differences in growth curves among batches, particularly between microalgae species, it is important to consider the potential impact of ambient temperature and light intensity variations alongside the intrinsic growth characteristics of the microalgae. Under these conditions, *Phaeodactylum* spp. survived in the three batches of cultivation of this species in the months of June, July, and August, and *Nannochloris* spp. survived in a single batch over 59 days.

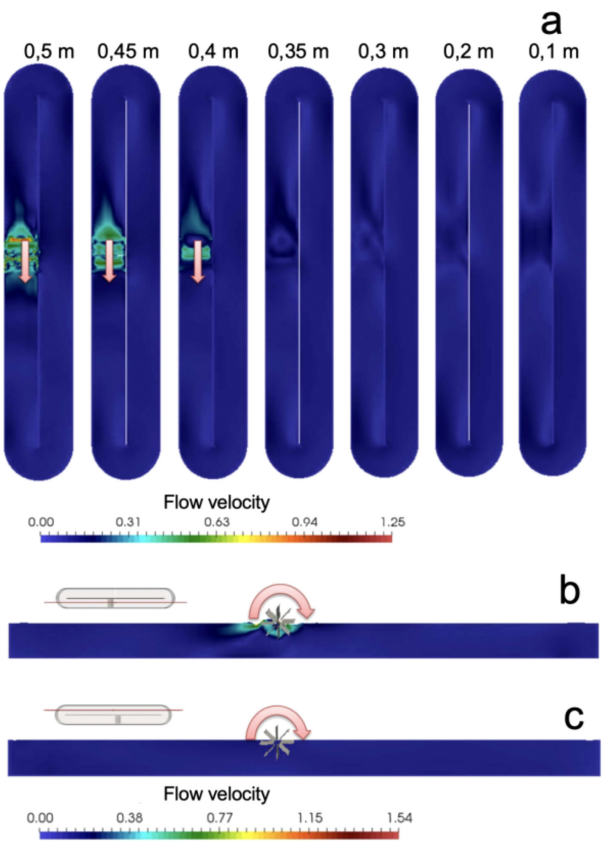

FIGURE 3
Fluid velocity (m/s) at different depths inside the photobioreactor. **(a)** Different levels from the bottom of the raceway tank, **(b)** Side with rotating paddle, **(c)** Side without rotating paddle.

Throughout the experimental period, natural fluctuations in environmental parameters were recorded (Figure 5), with lower temperatures and light intensities observed during July (Batch 2). These environmental variations coincided with reduced microalgal growth performance during this batch, suggesting that seasonal factors influenced cell density. Therefore, when interpreting differences in growth curves among batches, particularly between species, it is important to consider the potential impact of ambient temperature and light intensity variations alongside the intrinsic growth characteristics of microalgae. It was also observed that the lowest performance in terms of duration and cell density of microalgae were observed in Batch 2 (July), this performance could be mainly explained by the low temperatures of this month and the low light intensity at this time of the year.

Table 3 shows the parameters of the microalgae *Phaeodactylum* spp. massively cultivated in the periods of June, July, and August, appreciating in each period that the maximum growth was 2.60, 1.80, and 5.51 (10^6 cell mL^{-1}) correlating to 14 days, 6 days, and 14 days of culture for *Phaeodactylum* spp.; and 36 days of culture for *Nannochloris* spp. strains, proportional to the periods mentioned above. For the microalgae *Phaeodactylum* spp., an increase in cell density was observed in August; however, the average cultivation period was short (14 days), a discontinuous static cultivation of short duration, which is not optimal for the long cultivation period (90 days) of *A. purpuratus* in hatcheries. From Figure 6, it can be seen why a fourth assay (Batch 4) was carried out with *Nannochloris* spp., which lasted for two months in September and October (59 days), showing that maximum growth was 36.56 (10^6 cell mL^{-1}), and the cell density reached at 36 days was very similar to the maximums (4–6 x 10^7 cell mL^{-1}) reported by Mohammady et al. (2022) for the raceway culture of *Nannochloris* spp.

As shown in Figure 3, regarding growth of *Phaeodactylum* spp., the maximum cell density obtained (Batch 3) was 5 (10^6 cell mL^{-1}), which is in contrast with the results obtained for *Nannochloris* spp. (Batch 4), in which the highest cell density was 35 (10^6 cell mL^{-1}). According to statistical analysis, the mean cell concentration (cell mL^{-1}) of the *Nannochloris* spp. strain was higher (M= 19.37 x10^6, SE= 1.31 x10^6), than that of the *Phaeodactylum* spp. strain (M=1.41 x10^6, SE= 1.31x10^6), specifically 13.5 times higher according to Levene's test.

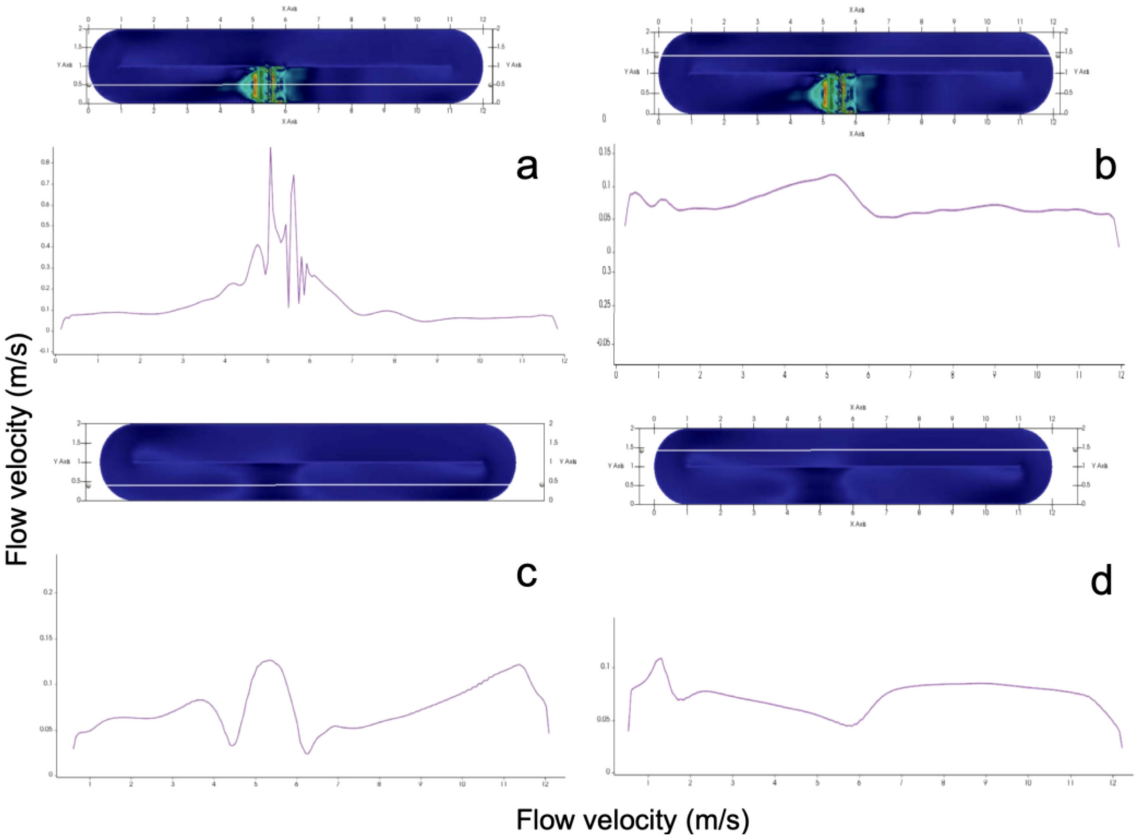

FIGURE 4
Fluid velocity field (m/s) at different depths inside the photobioreactor **(a)** Surface layer (0.5 m depth), **(b)** Intermediate layer (0.4 m depth), **(c)** Lower layer (0.3 m depth), **(d)** Bottom layer (0.1 m depth). Color scale indicates velocity magnitude (m/s).

4 Discussion

The present investigation demonstrated that the fluid dynamics in the culture raceway, specifically at a water velocity of 1.25 m/s, had a significant impact on the growth and productivity of the microalgae *Phaeodactylum* spp. *and Nannochloris* spp. The increased water movement in the upper strata of the raceway enhanced the dispersion of microalgae and their exposure to light and nutrients, which favors growth and productivity (Prieto et al., 2005; Pedruzi et al., 2020). However, specific areas of horizontal raceways, such as the ends and bottom, where eddies form and particles settle, can affect the distribution and density of microalgae (Marcé et al., 2007). Documenting that the pattern of water movement favors microalgae growth in a large-scale horizontal photobioreactor provides valuable insight for optimizing the design and operation of these systems.

Unlike conventional open raceway systems, which are exposed to fluctuating environmental conditions and higher contamination risks, the horizontal photobioreactor designed in this study operates within a controlled hatchery environment. This setting allows for greater stability of critical parameters such as temperature, salinity, illumination, and water quality. In addition, prior to the physical construction of the system, hydrodynamic modeling using *Salome v7.1* and the *snappyHexMesh* tool was conducted to optimize internal water flow, minimize dead zones, and improve particle suspension throughout the culture volume. This approach differs substantially from previous raceway designs, which often rely on empirical layouts without detailed fluid dynamics optimization. As a result, the system enabled the continuous culture of *Nannochloris* sp. for 59 days, covering approximately two-thirds of the hatchery cycle of *A. purpuratus* larvae and postlarvae. These innovations support the scalability and reliability of microalgae production systems intended for intensive aquaculture hatcheries.

Large-scale outdoor continuous microalgae culture studies are costly and difficult to perform (Novoveská et al., 2023). Therefore, the design of raceways used for mass cultivation in hatcheries can be improved (Costa and de Morais, 2014). Raceways are the most inexpensive method for mass microalgae production (Chisti, 2008), require little energy to move paddles, and are easy to clean (Ugwu et al., 2008). Horizontal photobioreactors inside greenhouses or hatcheries with transparent covers in which sunlight can be used as a stimulant for the growth of microalgae stimulate growth under axenic conditions (Duque-Granda et al., 2019), and accessorily coupled with air conditioning or an automatic heater to avoid

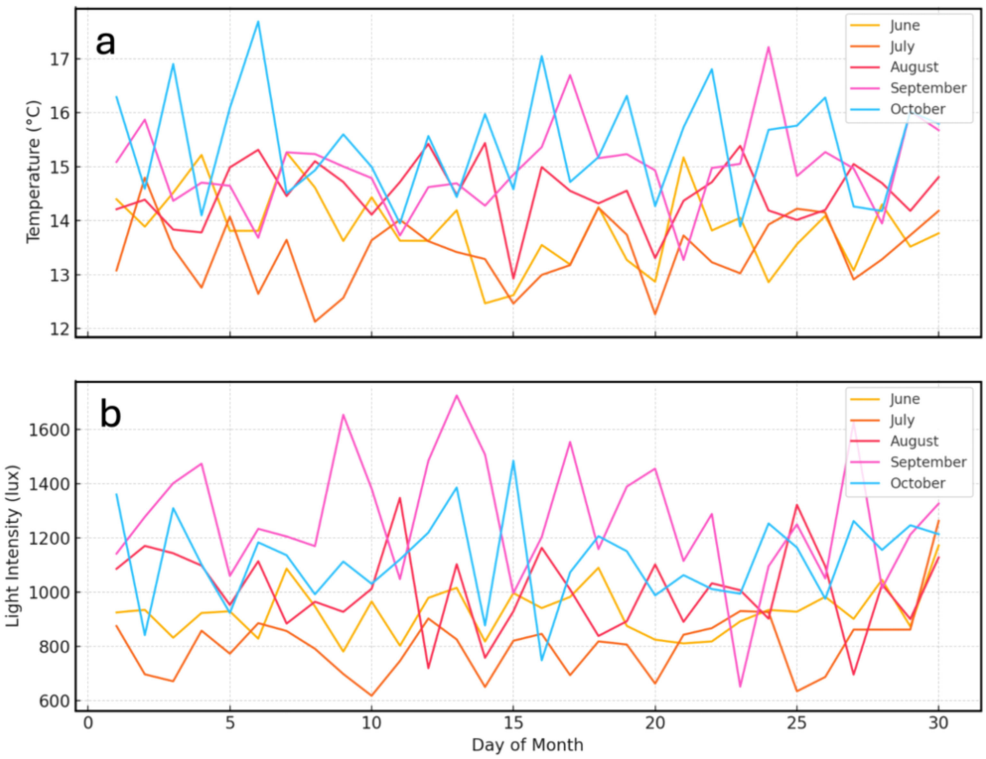

FIGURE 5
Daily variation of **(a)** temperature and **(b)** light intensity inside the hatchery from June to October 2017, recorded under natural light conditions with an approximate photoperiod of 11 hours light and 13 hours dark (11L:13D).

temperature fluctuations during the night, allow greater control of cultivation conditions and higher productivity compared to other cultivation systems.

In this study, the maximum cell density result for *Nannochloris* spp. was 19.37 ± 10.20 (10^6 cells mL^{-1}), reached on day 36 of culture at a temperature of 14.31 ± 1.03°C and salinity of 34.53 ± 0.38 PSU; maintaining similarity with the density recorded by Nava (2012) who cultivated *Nannochloris* spp. obtaining the maximum cell density of 19.6 x10^6 cells mL^{-1} on day 08 of culture at a temperature of 25 ± 1°C and 25 PSU. Both experiments differed in temperature and salinity at 9.33°C and 10 PSU, respectively. Likewise, Bae and Hur (2011) cultured *Nannochloris* spp. (KMMCC-395) in 250 mL flasks at 30°C, 30 PSU and 100 µm m^{-2} s^{-1} showed a high cell density of 10.733 x 10^4 cell mL^{-1} after seven days of culture. In similar studies on a smaller scale (Cho et al., 2007) inoculated microalga *Nannochloris oculata* placed in 250 mL flasks, maintaining a temperature of 25°C and salinity of 30 PSU, reached a maximum cell density of 308.0 ± 11.51 x 10^6 cells mL^{-1}, at 21 ± 0.6 days of culture. Similarly, other authors cultivating pilot photobioreactors obtained a biomass of 3.685 g L^{-1} from the strain *Nannochloris* spp (Saadaoui et al., 2016). From these studies, it is deduced that the increase in temperature and decrease in salinity favor the rapid growth of *Nannochloris* spp. However, to achieve a continuous supply of food in the larval and post-larval stages of *A. purpuratus*, it is necessary to prolong the life of the strain employed.

Regarding the microalga *Phaeodactylum* spp., in the present experiment a cell density of 1.41 x10^6 ± 1.31 x10^6 was obtained at a temperature of 14.31 ± 1.03°C, light intensity 1067.47 ± 443.82 lux and salinity of 34.53 ± 0.38 PSU. Similarly, Jerí (2018) cultured *P. tricornutum* in a volume of 475 mL, with high CO2 injections (25%)

TABLE 3 Physical, chemical, and growth parameters of *Phaeodactylum* spp. and *Nannochloris* spp. culture.

Batch	Specie	Temperature (°C)	Dissolved oxygen (mg/mL)	Light intensity (lux)	Days of culture	Day of max. growth	Cell density (10^6 cell/mL)	SGR (d^{-1})
1	*Phaeodactylum* spp.	15.11 ± 0.96	8.89 ± 1.39	1282 ± 399.09	17	14	2.60	17.77
2	*Phaeodactylum* spp.	13.61 ± 0.92	8.48 ± 0.65	670.75 ± 450.91	10	4	1.80	42.16
3	*Phaeodactylum* spp.	15.24 ± 0.77	9.36 ± 0.86	711.76 ± 39.33	14	12	5.52	28.37
4	*Nannochloris* spp.	14.89 ± 0.96	8.35 ± 0.31	1159.41 ± 195.55	59	34	36.56	11.56

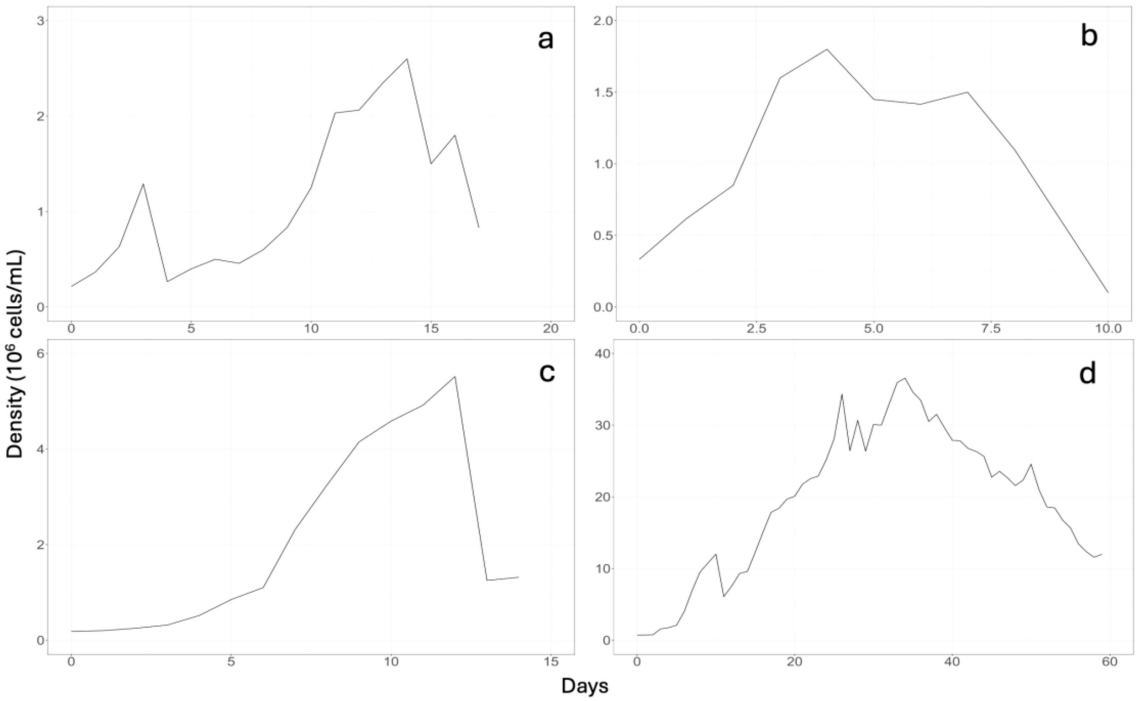

FIGURE 6
Growth curves for *Phaeodactylum* spp. and *Nannochloris* spp. during cultivation in the horizontal photobioreactor. **(a)** Batch 1, **(b)** Batch 2, **(c)** Batch 3 for *Phaeodactylum* spp. and **(d)** Batch 4 for *Nannochloris* spp.

under laboratory conditions at a temperature of 24 ± 1°C and a light intensity of 1500 lx, obtaining a cell density of 1. 67 x10^6 cell mL^{-1}. In another study Arenas (2023) performed a semi-continuous culture (15 L) of *P. tricornutum* for 19 days obtaining a higher density than the previous studies of 3.473 x10^3 ± 375.33 cell mL^{-1}, probably due to the semi-continuous operating conditions. Meiser et al. (2004) managed to harvest from a *Phaeodactylum* spp strain 1.37 g L^{-1} biomass (including CO2). Hall et al. (2003), collected up to 1.4 g L^{-1} d^{-1} of biomass, growing the same strain in a helical tubular photobioreactor. However, both cell density and biomass of *Phaeodactylum* spp. were lower than those *of Nannochloris* spp.

TABLE 4 Energy consumption and biomass production efficiency of the hatchery-based horizontal photobioreactor.

Parameter	Value	Notes
Paddle wheel motor power	0.37 kW	0.5 HP motor
Aeration blower power	0.37 kW	0.5 HP blower
Total installed power	0.74 kW	
Operation time	24 h/day for 59 days	Continuous operation
Total energy consumption	1,047.84 kWh	0.74 kW × 24 h × 59 days
Photobioreactor volume	10,000 L (10 m^3)	
Estimated dry biomass yield	5 kg	0.5 g/L as reference value
Specific energy consumption	210 kWh/kg dry biomass	

One limitation of the present study is that *Phaeodactylum* spp. and *Nannochloris* spp cultures were conducted during different seasonal periods and not under identical environmental conditions. Consequently, observed differences in cell density and culture duration between the two species may partially reflect variations in ambient temperature and light intensity rather than exclusively species-specific responses or photobioreactor performance. Future studies should consider cultivating both species simultaneously under controlled environmental conditions and apply statistical corrections for seasonal effects to better isolate species-specific growth characteristics. The superior performance of *Nannochloris* spp. observed in this study can be attributed to its well-documented physiological resilience to environmental stress. Previous studies have reported that *Nannochloris* spp. strains exhibit high tolerance to salinity fluctuations (Witt et al., 1981) and maintain stable growth rates under varying temperature regimes, up to 30°C (Bae and Hur, 2011). Moreover, strains of *Nannochloris* spp. are characterized by a relatively low sensitivity to light intensity variations, which enables sustained productivity even under suboptimal irradiance conditions (Saadaoui et al., 2016). These physiological traits likely conferred a survival advantage to *Nannochloris* spp. strains over *Phaeodactylum* spp. strains in a semi-controlled hatchery environment, where natural seasonal changes in temperature and light were unavoidable. Consequently, the longer culture duration and higher cell density achieved with the *Nannochloris* spp. strain in this study could be

attributed not only to the photobioreactor design but also to the intrinsic robustness of the species. In terms of productivity of the microalgae strains *Phaeodactylum* spp., the results showed that the maximum growth in the periods of June, July, and August was relatively short (12 days) on average, not optimal for the long culture period (90 days) of *A. purpuratus* in hatcheries. In contrast, the culture of the *Nannochloris* spp. stain in this study had a culture period of two months (59 days).

An approximate energy consumption analysis was conducted to assess the preliminary productive viability of the system (Table 4). The combined power consumption of the paddle wheel motor (0.37 kW) and aeration blower (0.37 kW) correspond to the nominal rated power specified by the equipment manufacturers, rather than directly measured consumption. Assuming continuous operation over 59 days, the total estimated energy consumption was 1,047.84 kWh. The estimated dry biomass yield of approximately 5 kg was calculated based on an average biomass concentration of 0.5 g/L– a typical production value for *Nannochloris* spp. under optimal culture conditions—multiplied by the effective working volume of the photobioreactor (10,000 L). Accordingly, the specific energy consumption was calculated at around 210 kWh per kilogram of biomass produced. Although this value may appear high compared to large-scale open raceway systems, it reflects the improved control of environmental parameters, and the axenic conditions achieved in the hatchery-based photobioreactor. Future optimizations, such as implementing variable-speed drives, intermittent aeration, and real-time energy monitoring, could further reduce energy demands, enhancing the scalability and economic feasibility of the system for industrial aquaculture applications.

It should be noted that in this study, the biochemical composition of the cultivated microalgae was not analyzed, as the primary focus was on validating the photobioreactor's operational performance under hatchery conditions. Future research should include nutritional analyses of the biomass of microalgae produced and evaluate the feasibility of incorporating algal pastes into the raceway system to optimize larval feeding strategies and enhance operational flexibility. Despite the limitations associated with single-batch large-volume cultivations, the results of this study provide valuable insights for optimizing future operations. Future work will include replicated small-scale photobioreactor trials to statistically validate observed growth dynamics before scaling to full production systems.

5 Conclusions

The duration, density, and productivity of the culture of microalgae strains determined that the ideal species for cultivation in a horizontal photobioreactor are *Nannochloris* spp. strains, fulfilling the productive requirements of *A. purpuratus* culture, allowing 67.68% of its productive cycle of 90 days under hatchery conditions., The culture of *Phaeodactylum* spp. strains in Kallwall and bottles (traditional method) may be included in hatchery operations, however this microlagae species has a short useful life and does not coincide with the productive process of *A. purpuratus*.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RC: Formal Analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. RP: Data curation, Formal Analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. JH: Data curation, Methodology, Writing – review & editing. SM: Formal Analysis, Resources, Writing – review & editing. OA: Methodology, Supervision, Writing – review & editing. EC: Methodology, Writing – review & editing.

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Conflict of interest

Author JH was employed by Finfish Aquaculture Sociedad Anónima Cerrada.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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