Latin American Journal of Aquatic Research, 52(4): 521-535, 2024
DOI: 10.3856/vol52-issue4-fulltext-3213

Research Article

Effect of diet on larval settlement, growth, and spat survival of the oyster *Crassostrea gigas* (Thunberg, 1793)

Hugo Moreira Gomes[1], Simone Sühnel[1], Carlos Henrique Araujo de Miranda Gomes[1] Eliziane Silva[1], Francisco Carlos da Silva[1] & Claudio Manoel Rodrigues de Melo[1]
[1]Laboratory of Marine Mollusks, Department of Aquaculture, Federal University of Santa Catarina Florianópolis, Brazil
Corresponding author: Claudio Manoel Rodrigues de Melo (claudio.melo@ufsc.br)

ABSTRACT. This study aimed to assess the impact of microalgal concentrations in diets on the settlement and metamorphosis stages of pediveliger larvae and spat of *Crassostrea gigas* oysters. Diets containing microalgal concentrations of 8, 12, and 16×10^4 cells mL^{-1} were administered for seven days during the larval settlement phase of pediveliger larvae. In the metamorphosis and postlarval cultivation phases, diets with microalgal concentrations of 8, 16, 24, and 32×10^4 cells mL^{-1} and a control group without food were tested for 14 and 21 days, respectively. Growth, yield, and survival were assessed every seven days. The diet comprised 30% *Isochrysis galbana* and 70% *Chaetoceros muelleri* microalgae. In the metamorphosis phase, the results revealed no significant differences in larval metamorphosis rate and survival across the tested diets. The concentrations exhibited similar survival in the spat with an initial average shell height of 0.657 ± 0.05 mm, with a significant difference only in growth. In the spat with an initial average shell height of 0.830 ± 0.12 mm, no differences in survival were observed among the tested concentrations. We concluded that diets of 12 and 16×10^4 cells mL^{-1} provide high rates of larval metamorphosis and spat yield during the larval settlement and metamorphosis phases. During the spat cultivation phases, diets of 32 and 24×10^4 cells mL^{-1} could optimize the cultivation time and yield of *C. gigas* spat in the laboratory. However, when considering survival alone, no advantage was found in providing a diet with a microalgal concentration above 8×10^4 cells mL^{-1} across all *C. gigas* oyster cultivation phases.

Keywords: *Crassostrea gigas*; Pacific oyster; food; bivalves; metamorphosis; microalgae; diet concentration

INTRODUCTION

The Pacific oyster *Crassostrea gigas* (Thunberg, 1793) has been introduced to numerous countries for aquaculture development. However, currently, the species *Crassostrea gigas* has been reclassified as *Magallana gigas* (Salvi et al. 2014, Salvi & Mariottini 2017); therefore, the World Register of Marine Species web (WoRMS, www.marinespecies.org), registers all Pacific cupped oysters in a new genus, *Magallana*. However, Bayne et al. (2017) claim that the segregation of *Crassostrea* in three genera is unwarranted. In addition, further molecular studies, with special highlights from Sigwart et al. (2021), attest to the preferable maintenance of *Crassostrea* as the sole genus in Crassostreinae, referring to the other genera *Magallana and Talanostrea* as subgenera. Therefore, following these later studies, we will keep the species name *C. gigas* for this study. This species is known for its adaptability to various coastal environments and exceptional zootechnical performance (Miossec et al. 2009). It is among the oyster species of significant

Associate Editor: Cesar Lodeiros

importance in the worldwide production of bivalve mollusks (Botta et al. 2020, Garlock et al. 2020, FAO 2022). In 2020, it accounted for 3.4% of the global mollusk production, yielding 610,300 t (FAO 2022).

Santa Catarina, a state in Brazil, is the country's leading oyster producer. In 2020, the state's estimated oyster production was approximately 2,165.1 t, with 98.3% being *C. gigas* oysters (EPAGRI 2022), derived from laboratory-produced spat. The settlement and metamorphosis stages of bivalve mollusks, produced under laboratory conditions, are of profound importance owing to the high larval mortality rate during these stages (Helm & Bourne 2004).

Feeding is crucial during the settlement and metamorphosis of bivalve mollusks (Rico-Villa et al. 2006). Rico-Villa et al. (2009) identified three distinct phases in the food intake capacity of these animals throughout the larval to metamorphosis process. These phases include a mixotrophic phase characterized by low and constant ingestion (from D-veliger larvae to 110 µm), an exotrophic phase marked by a significant increase in ingestion (from umbonate larvae to 300 µm), and a metamorphosis phase where ingestion is reduced (in larvae above 300 µm).

Microalgae serves as the primary nutritional source for bivalve mollusks (Cheng et al. 2020), providing a significant amount of polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic (EPA - 20:5 n-3) and docosahexaenoic (DHA - 22:6 n-3). These PUFAs contribute significantly to the development and health of these organisms (Holden & Patterson 1991, Sühnel et al. 2012, Gao et al. 2016). Diets incorporating more than two microalgae species are more beneficial for bivalve growth due to their varying levels of EPA and DHA (Helm & Bourne 2004, Cheng et al. 2020). In laboratory settings, mollusk production necessitates careful consideration of the microalgal diets provided, and factors such as quality, quantity, concentration, cell size, biochemical composition, amino acid composition, and vitamin composition are of utmost importance. These factors directly influence spat growth and yield through their impact on ingestion and digestion (Martínez-Fernández et al. 2006, Rico-Villa et al. 2006). The microalgae species *Isochrysis galbana* and *Chaetoceros muelleri* are frequently used in bivalve diets (Enright et al. 1986, Laing & Millican 1986, O'Connor et al. 1992) because of their high nutritional values (Napolitano et al. 1990). These species have been found to enhance the zootechnical performance of bivalve mollusks effectively (Galley et al. 2010, Noble et al. 2015).

The nutritional significance of microalgae in bivalve mollusk diets has prompted numerous studies investigating their impact on the survival and larval development of *C. gigas* in laboratory settings (Thompson & Harrison 1992, Brown & Robert 2002, Ponis et al. 2008). This research is crucial for ensuring the health of these organisms and achieving high yields. However, studies in the literature examining the concentration of microalgae during the settlement, metamorphosis, and postlarval phases of *C. gigas* are scarce (Rico-Villa et al. 2006, 2009).

The growth of bivalves is directly influenced by the concentration of microalgae in their diets, among other factors (Velasco & Barros 2008). Therefore, employing an optimal concentration ensures successful production under laboratory conditions. Conversely, without the proper knowledge to administer the correct concentration, these animals' survival and growth rates could be adversely affected (Doroudi & Southgate 2000).

A comprehensive understanding of diet's impact on the rate of metamorphosis, growth, survival, and yield on the settlement, larval metamorphosis, and postlarval phases of the oyster *C. gigas* is essential. Hence, this study aimed to assess the influence of diets with varying microalgal concentrations on the rate of metamorphosis, growth, yield, and survival during the pediveliger larval metamorphosis phases, as well as the postlarval phase of the *C. gigas* oyster in a laboratory setting (Fig. 1).

MATERIALS AND METHODS

In this study, we assessed the impact of diets with concentrations of 8×10^4 cells mL^{-1} (C8), 12×10^4 cells mL^{-1} (C12), and 16×10^4 cells mL^{-1} (C16) on the rate of metamorphosis, growth, yield, and survival during the last part of the larval stage (pediveliger larvae and metamorphosis), produced according to internal protocol, over seven days. The larvae were subjected to three epinephrine baths (0.33 g L^{-1} solution in distilled water, diluted 1:9 in filtered sea water; Silveira et al. (2011)) every 48 h to induce metamorphosis. We also evaluated the impact of diets with microalgal concentrations of 8×10^4 cells mL^{-1} (C8), 16×10^4 cells mL^{-1} (C16), 24×10^4 cells mL^{-1} (C24), and 32×10^4 cells mL^{-1} (C32) concerning a control group without feeding (NF) on the growth, yield, and survival of postlarval stage (spat) using spat with an initial average shell height of 0.657 ± 0.05 mm (retained in the 510 µm diagonal mesh sieve) over 14 days. The same parameters were assessed in the postlarval stage using spat with an initial average shell height of 0.830 ± 0.12 mm standard deviation (SD) (retained on the 700 µm

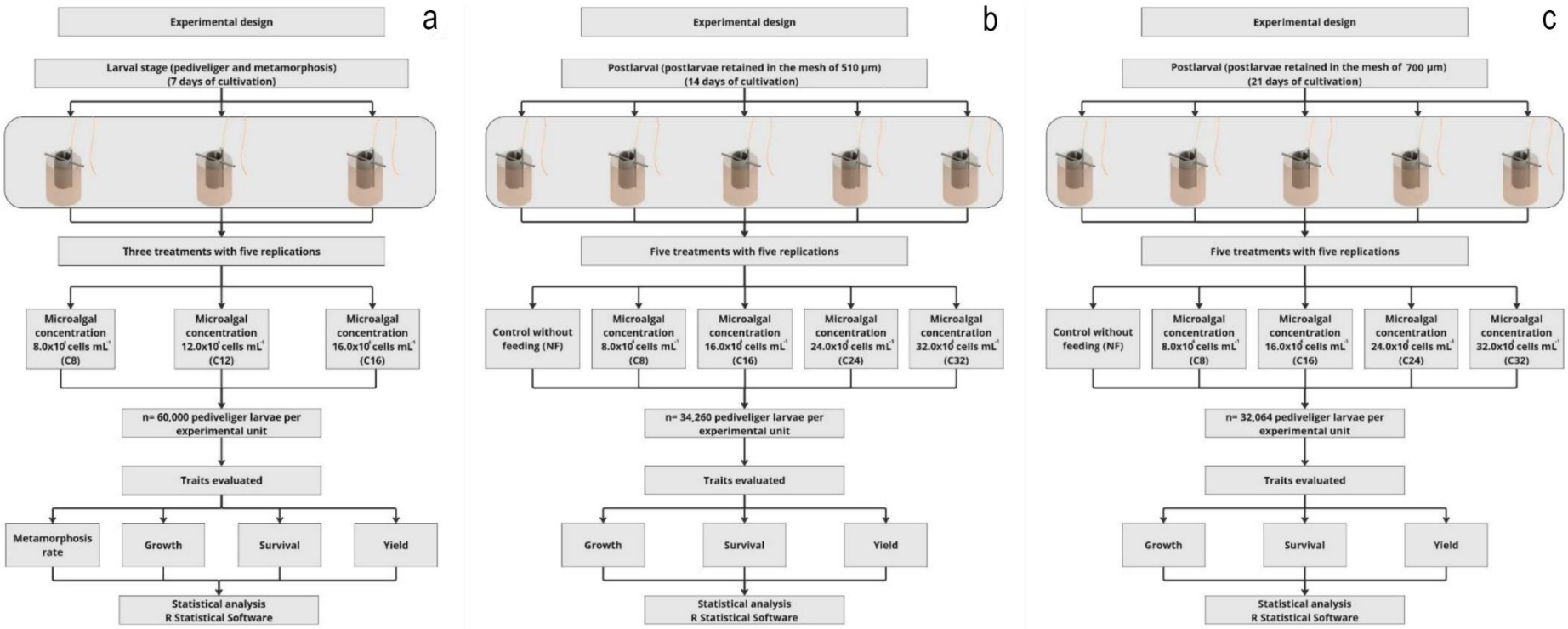

Figure 1. Schematic of the experimental design. Stages: a) last part of the larval stage, b) postlarval stage using larvae >510 µm, and c) postlarval stage using larvae >710 µm (c). See the yield definition in the penultimate paragraph of the material and methods section.

diagonal mesh sieve) over 21 days. Summering, these concentrations were tested using two spat sizes of the postlarval stage, and samples were taken every seven days. At each cultivation phase, we assessed individuals from different *C. gigas* spat production batches at the Laboratory of Marine Mollusks of the Federal University of Santa Catarina (LMM/UFSC, by its Portuguese acronym).

The diet comprised the microalgae *I. galbana* and *C. muelleri* in 30 and 70% proportions. These microalgae were cultivated in a semi-continuous culture system housed in 100 L bags, utilizing Conway culture medium at a concentration of 1 mL L^{-1}. A silicate solution was used for *C. muelleri*. The experiments were conducted following a completely randomized design. Three treatments (C8, C12, and C16) were used in the larval study, each with five replications. The spat studies involved five treatments (C8, C16, C24, C32, and NF), each with five replications.

The experimental units (EU; Fig. S1) were constructed from PVC pipes, each with a diameter of 150 mm, a height of 240 mm, and a bottom area of 0.15 m^2. These units were lined at the bottom with a mesh screen with an opening of 180 µm. The EUs were situated individually within a 20 L plastic bucket to create a downwelling system.

During the last part of the larval stage, each EU was populated with 66,000 (360.74 ± 19.82 µm; height mean ± SD) pediveliger larvae. In the postlarval stage, each EU was populated by 34,260 spat.

The daily maintenance of the EUs involved a complete water change, cleaning the buckets with a Tahiti lime (*Citrus aurantifolia*) solution (Carvalho et al. 2013), and removing sediments and potential encrustations from the EUs, larvae, and spat using low-pressure saltwater jets. Following routine maintenance, microalgae diets were provided daily in the morning. The water temperature (ºC), pH, and salinity were measured daily after the water change, utilizing a pHmeter (AT355, Alfakit) and a refractometer (RHS-10ATC).

Larvae and spat were screened weekly using sieves with diagonal mesh openings of 370, 510, 700, 970, 1,560, and 2,000 µm, selected based on the cultivation phase and postlarval size. The dimensions of the sieve meshes utilized in the study were ascertained using trigonometric ratios (diagonal, side 1, and side 2), facilitated by a microscope (Zeiss-Axiolab 5) and a magnifying glass (Dino Lite-AM-423x). The diagonal measurement was employed to determine the size of the mesh openings.

The impact of the diets (C8, C12, and C16) on the metamorphosis rate, growth, yield, and survival of pediveliger larvae and spat was assessed during the last part of the larval stage (pediveliger larvae and metamorphosis), following seven days of cultivation.

For the postlarval stage (using spat ≥510 µm), the impact of diets (C8, C12, C16, C32, and NF) was evaluated by calculating the average growth, yield, and survival after 7 and 14 days of cultivation. For spat ≥700 µm, these parameters were assessed after 7, 14, and 21 days of cultivation.

The metamorphosis rate was assessed based on the quantity of live spat retained on the 370 and 510 µm sieves relative to the number of larvae present in each EU. Biometric measurements were taken from 450 individuals (150 specimens per treatment) to evaluate growth during the last part of the larval stage (pediveliger larvae and metamorphosis).

During the cultivation of postlarval spat, biometric data from 1,786 and 2,280 spat were gathered proportionally from the sieves of varying mesh sizes used in the weekly screenings. The shell's height and average length were measured using ZEN Blue 3.2 and Dino Capture 2.0 image analysis software, as described by Galtsoff (1964).

During the last part of the larval stage (pediveliger larvae and metamorphosis), yield was determined by comparing the total number of spat retained in the 510 µm sieve to the number of larvae in each EU. The yield was calculated for spat ≥510 µm by comparing the total number of spat retained on the 970 µm diagonal mesh sieve to the number of populated spat in each EU. The yield was determined for spat ≥700 µm by comparing the total spat retained in the 2,000 µm diagonal mesh sieve to the number of spat in each EU.

Survival (number of live larvae or spat at the time of evaluation – number of larvae or spat introduced into the EU) during the cultivation stages was calculated. For this calculation, three samples of spat, each with a dry volume of 200 µL, were collected from each EU. The live spat in these samples was then quantified and averaged.

Throughout the study, approximately 305,027 individuals of *C. gigas* were quantified. The counting process was conducted in a Sedgewick Rafter chamber, utilizing a Zeiss-Axiolab 5 optical microscope.

Statistical analysis

The data on metamorphosis, growth, yield, and survival were subjected to an analysis of variance (ANOVA). The Shapiro-Wilk and Bartlett tests analyzed the normality and homoscedasticity of variance errors.

When the prerequisites for ANOVA were satisfied and significant disparities were identified among the examined diets, Tukey's parametric test was employed to distinguish the means. If the assumptions of normality and homoscedasticity of error variance were not met, the data were analyzed using the non-parametric Kruskal-Wallis test, succeeded by the Wilcoxon test (see Table S1 for details).

In every instance, a significance level (α) of 5% was employed. All statistical analyses were conducted using the RStudio® software, version 3.6.1.

RESULTS

Rate of metamorphosis and growth

The metamorphosis rate of larvae remained consistent across all treatments, with average rates (±SD) of 88.0 ± 8.6, 99.8 ± 0.5, and 97.4 ± 3.1% observed in C8, C12, and C16, respectively (Fig. 2). Notably, significant differences among treatments ($P < 0.05$) were evident in shell height and length after seven days. The highest averages were found in treatments with elevated concentrations of microalgae (Fig. 2).

For postlarval spat ≥510 µm, after seven days of experimentation, the height and length of animals under treatments C32 and C24 did not differ ($P > 0.05$). However, these animals were significantly larger ($P < 0.05$) than those under treatments C8, C16, and NF. Additionally, the height and length of animals in these treatments significantly differed ($P < 0.05$) from each other (Fig. 3). After 14 days, the height and length of the animals in treatments C32, C24, C16, and C8 did not significantly differ ($P > 0.05$). However, these parameters were significantly greater ($P < 0.05$) than those of the animals in the control treatment without food (NF) (Fig. 3).

For postlarval spat ≥700 µm, significant growth differences ($P < 0.05$) were observed only in the height of the individuals after seven days of cultivation (Fig. 4). Following 14 days of cultivation, significant differences ($P < 0.05$) in both height and length were noted among the treatments. The average height and length of animals subjected to diets C32 and C24 did not significantly differ ($P > 0.05$), with the highest averages recorded. The height and length of animals in treatments C8 and C16 exhibited statistical differences ($P < 0.05$) only in length across the two treatment groups (Fig. 4). During these two analysis periods (after 7 and 14 days), no individuals were retained on the 1,560 and 2,000 µm sieves in the treatment where the animals were not fed (NF).

Following 21 days of cultivation, the height and shell length of organisms exposed to treatments C32 and C24 differed significantly ($P < 0.05$) compared to those observed in organisms subjected to treatments C8, C16, or NF. Significant statistical differences ($P < 0.05$) were also identified in organisms' average height and length across treatments C8, C16, and NF. Organisms fed with treatments C32 and C24 demonstrated the greatest average height and length (Figs. 3-4).

Yield

During the last part of the larval stage, significant statistical differences ($P < 0.05$) were observed between the yield of animals subjected to treatments C12 and C8. The highest yields were recorded for treatments C16 and C12, with respective values of 97.4 ± 2.9 and 99.8 ± 0.5%. Conversely, treatment C8 presents the lowest result, yielding 88 ± 8.7% (Fig. 5).

For the postlarval cultivation phase, stated with spat retained in the 510 µm diagonal mesh sieve, the yield of spat did not vary significantly among treatments with microalgae after 7 or 14 days of cultivation. The yields for C32, C24, C16, and C8 were 72.0 ± 3.4, 68.0 ± 3.6, 69.0 ± 3.6, and 66.0 ± 3.5%, respectively, after seven days and 73.0 ± 3.2, 70.0 ± 3.5, 71.0 ± 6.5, and 72.0 ± 3.2%, after 14 days. However, these yields were significantly different ($P < 0.05$) when compared to the yields of spat from the control treatment without feeding, which were 23.0 ± 3.6 and 28.0 ± 5.1%, respectively, at 7 and 14 days of cultivation (Fig. 6).

For the postlarval cultivation phase, started with spat retained in the 700 µm diagonal mesh sieve, no significant differences were observed for spat yield between the C32 (18.0 ± 2.5%) and C24 (16.0 ± 5.7%) treatments after seven days of cultivation. Both treatments significantly ($P < 0.05$) outperformed the C16 (8.0 ± 2.6%) and C8 (3.0 ± 5.9%) treatments in terms of yield. Furthermore, the C16 and C8 treatments also significantly ($P < 0.05$) differed from each other (Fig. 7).

Following 14 days of cultivation, the highest yield was noted in C32 (73.0 ± 2.5%), which demonstrated significant differences ($P < 0.05$) compared to other dietary treatments. The yields from treatments C24, C16, and C8 also significantly differed ($P < 0.05$) from each other, with respective yields of 60.0 ± 5.7, 32.0 ± 2.6, and 13.0 ± 5.9% (Fig. 7).

Upon completion of the study, which spanned 21 days of cultivation, no significant differences were observed in spat yield between treatments C32 (83.0 ± 2.4%) and C24 (79.0 ± 4.7%). However, both these treatments significantly differed ($P < 0.05$) from treat-

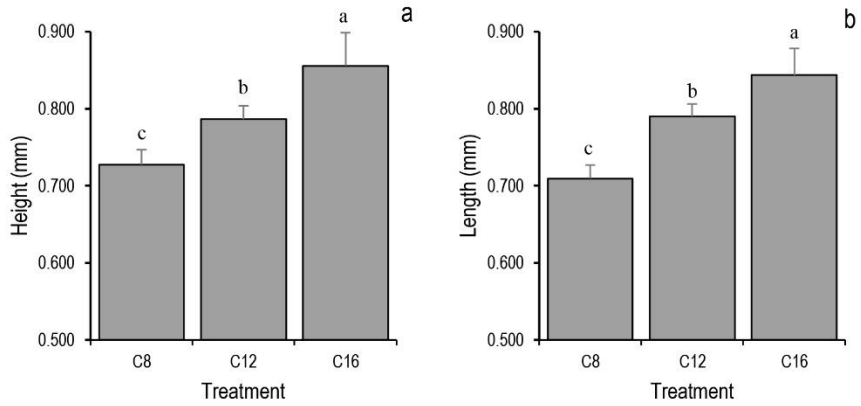

Figure 2. a) Shell height (mm), and b) shell length (mm) during the larval stage (pediveliger and metamorphosis) of the Pacific oyster *Crassostrea gigas* fed with different microalgal concentrations. Different letters indicate statistical differences for each parameter using Tukey's test ($P < 0.05$). Diets: C8, 8×10^4 cells mL^{-1}; C12, 12×10^4 cells mL^{-1}; C16, 16×10^4 cells mL^{-1}. Diets were composed of 30% *Isochrysis galbana* and 70% *Chaetoceros muelleri*.

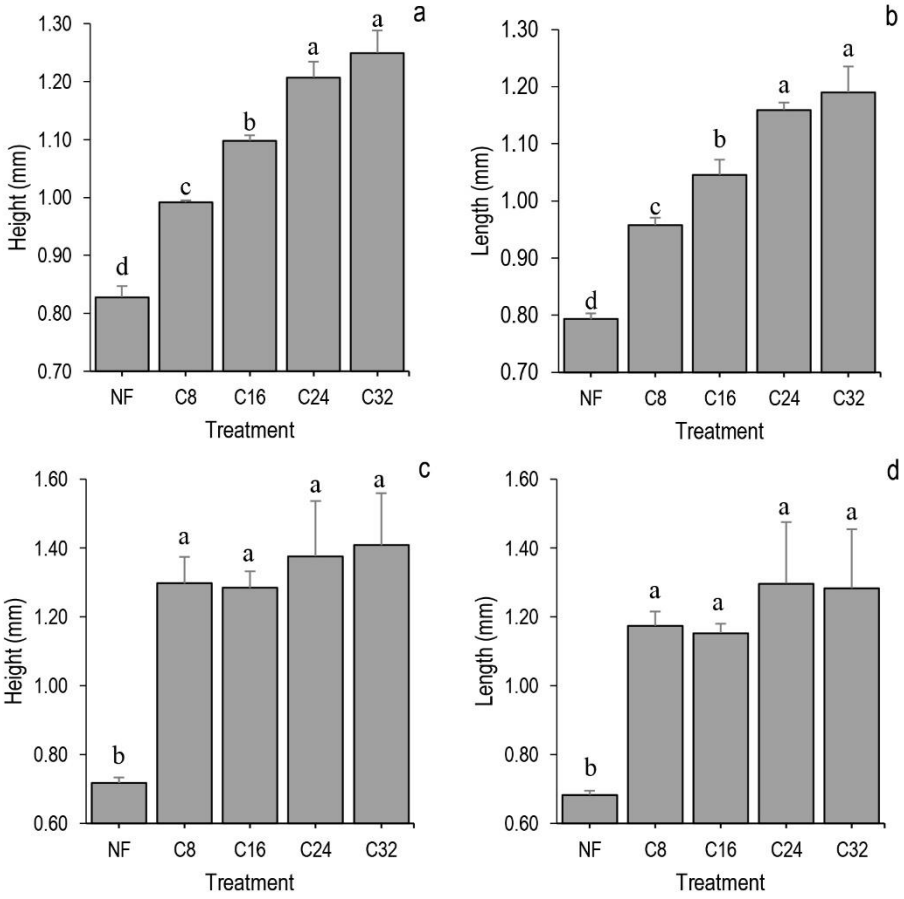

Figure 3. Shell height (mm) and shell length (mm) at a-b) seven days, and c-d)14 days of the postlarval stage of the Pacific oyster *Crassostrea gigas* fed with different microalgal concentrations of microalgae in the early spat phases. Postlarval phase: initial average (± standard deviation) shell height of 0.657 ± 0.05 mm. For height after seven days (a), different letters indicate statistical differences using Tukey's test ($P < 0.05$). For length and height after 7 (b) and 14 (c-d) days, different letters represent statistical differences for the Wilcoxon test ($P < 0.05$). Diets: NF, no food; C8, 8×10^4 cells mL^{-1}; C16, 16×10^4 cells mL^{-1}; C24, 24×10^4 cells mL^{-1}; C32, 32×10^4 cells mL^{-1}. The diets comprised 30% of *Isochrysis galbana* and 70% of *Chaetoceros muelleri*.

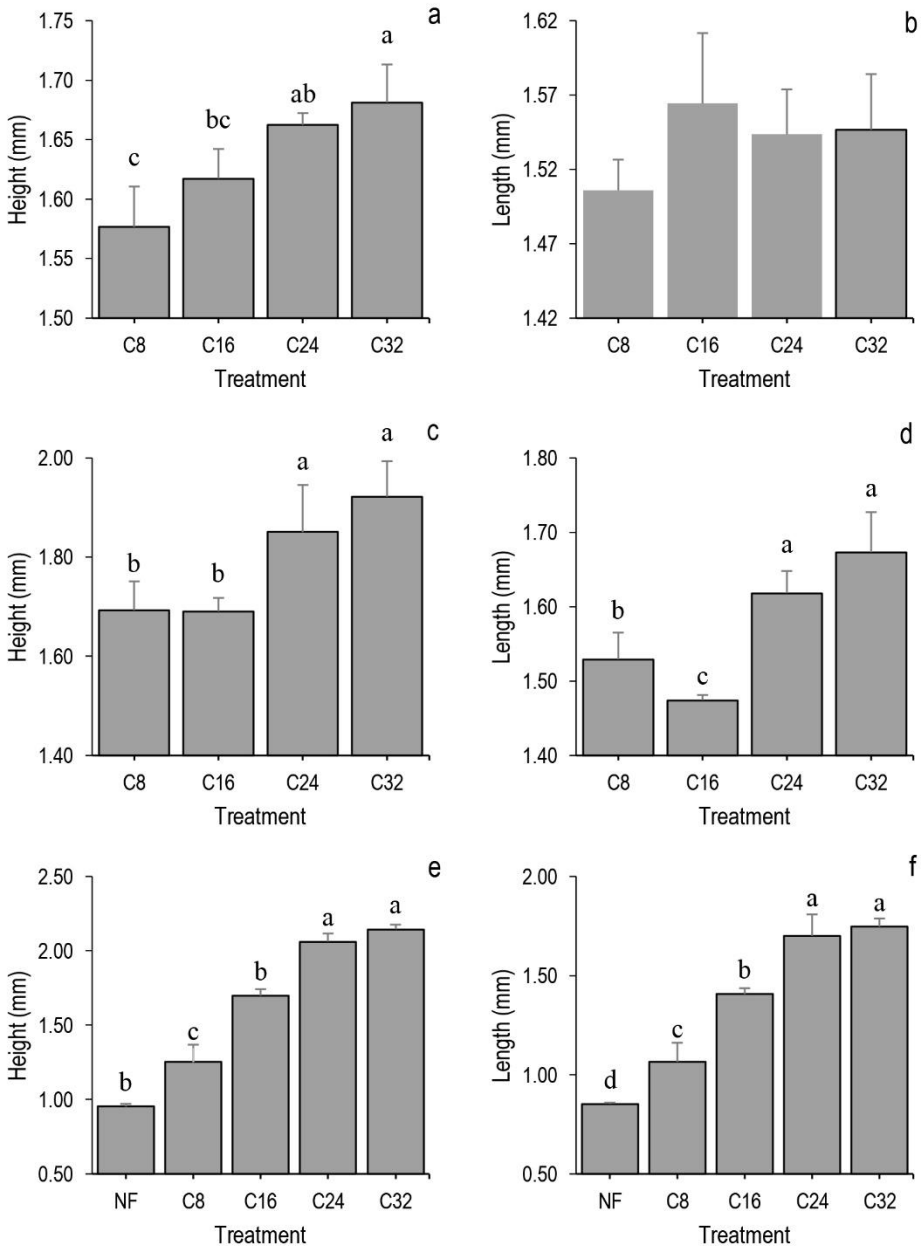

Figure 4. Shell height (mm) and shell length (mm) at a-b) seven days, c-d) 14 days, and e-f) 21 days of the postlarval stage of the Pacific oyster *Crassostrea gigas* fed with different microalgal concentrations. Postlarval phase: initial average (± standard deviation) shell height of 0.820 ± 0.12 mm. In the graphs for height after seven days (a) and height after 14 days (b), different letters indicate statistical differences using Tukey's test ($P < 0.05$). The absence of letters represents that there were no significant differences between treatments. In the graphs of height (c, e) and length (d, f) after 14 and 21 days, different letters represent statistical differences using the Wilcoxon test ($P < 0.05$). Diets: NF, no food; C8, 8×10^4 cells mL^{-1}; C16, 16×10^4 cells mL^{-1}; C24, 24×10^4 cells mL^{-1}; C32, 32×10^4 cells mL^{-1}. The diets comprised 30% of *Isochrysis galbana* and 70% of *Chaetoceros muelleri*.

ments C16 (65.0 ± 2.7%) and C8 (27.0 ± 8.6%), which also exhibited significant differences ($P < 0.05$) between each other (Fig. 7). At this cultivation stage, the animals in the treatment group that did not receive food (NF) failed to produce spat that could be retained on the 2,000 µm sieve, thereby indicating a lack of spat yield. So, they were not graphed.

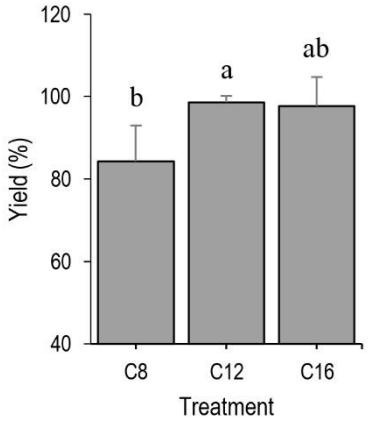

Figure 5. Yield rate of the Pacific oyster *Crassostrea gigas* fed with different concentrations of microalgae in the settlement and larval metamorphosis stages. Different letters indicate statistical differences using the Wilcoxon test ($P < 0.05$). Diets: C8, 8×10^4 cells mL^{-1}; C12, 12×10^4 cells mL^{-1}; C16, 16×10^4 cells mL^{-1}. Diets comprised 30% of *Isochrysis galbana* and 70% of *Chaetoceros muelleri*.

Survival

During the last part of the larval stage, survival rates did not significantly differ in response to diets with varying concentrations. Specifically, survival rates of 92.0 ± 4.4, 94.0 ± 1.1, and 95.0 ± 1.6% were observed in C8, C12, and C16, respectively.

Following a 14-day cultivation period started using spat ≥510 µm, the survival rates of animals fed at C32 (73.0 ± 3.6%), C24 (70.0 ± 3.9%), C16 (71.0 ± 7.2%), or C8 (72.0 ± 3.3%) did not significantly differ from each other. However, these rates were significantly higher ($P < 0.05$) than the survival rate of animals in the NF control treatment, which was 28.0 ± 5.2% (Fig. 8a).

In the postlarval cultivation phase, stated with spat ≥710 µm, no significant differences were observed in spat survival across treatments with food (C32, C24, C16, C8) after 21 days of cultivation. The only significant differences ($P < 0.05$) were noted between the animals subjected to the C32 treatment (86.0 ± 2.8%) and C8 (84.0 ± 2.2%) when compared to the spat of the NF treatment (76.0 ± 4.5%). The spat from the C24 and C16 treatments exhibited survival rates of 81.0 ± 4.0 and 83.0 ± 4.7%, respectively, and these rates did not significantly differ from that of the NF treatment (Fig. 8b).

The cultivation conditions, including temperature, pH, and salinity, were optimal for producing *C. gigas* oyster larvae and spat during all three phases (Table 1).

DISCUSSION

Rate of metamorphosis and growth

The diets provided to *C. gigas* larvae during the last part of the larval stage (concentrations 8, 12, and 16×10^4 cells mL^{-1} for treatments C8, C12, and C16, respectively) resulted in high metamorphosis rates. In contrast, diets with concentrations of 12, 20, and 40 cells μL^{-1} negatively impacted the larval metamorphosis of this species (Rico-Villa et al. 2009). This outcome is likely due to the availability of adequate energy reserves to fulfill the needs of these individuals during metamorphosis (Haws et al. 1993). Literature reports suggest that the rate of larval metamorphosis in bivalve mollusks is contingent on the concentration of microalgae in their diet, evidenced by the results for *Ostrea edulis*, which was fed microalgae at concentrations ranging from 7.0×10^{-11} to 3.5×10^{-9} cells mL^{-1} (Robert et al. 2017). The findings of this study, in conjunction with the studies mentioned above, underscore the decisive role of microalgal concentration in the diets of bivalve mollusks for successful settlement and larval metamorphosis. Other research (Rico-Villa et al. 2006, 2009, Pettersen et al. 2010, Rodríguez-Pesantes et al. 2022) also indicates that the combination of microalgae and the cell concentration at which they are offered to bivalve mollusks directly influence the settlement phase, larval metamorphosis, and survival of these animals.

In the present study, we observed a gradual increase in larvae's height and shell length, which was in correlation with the rise in microalgal concentration in their diets. These findings align with those of Tabelskaya & Kalinina (2021), who found that *C. gigas* larvae exposed to elevated microalgal concentrations exhibited the most significant growth. Rico-Villa et al. (2009) noted that the highest larval growth of *C. gigas* was associated with the highest microalgal concentrations (20 and 40 cells μL^{-1}). However, the average shell length growth of the larvae in this study was greater than reported by Rico-Villa et al. (2009).

Varying dietary concentrations of microalgae are directly correlated with the growth of bivalve mollusks (Velasco & Barros 2008, Linard et al. 2011). This finding is consistent with those from the present study. Despite resulting in reduced height and length growth in larvae, spat of *C. gigas* compared to those under diets C24 and C36, diets with microalgal concentrations of C8 facilitated continuous growth. This growth was not observed in the food-deprived animals (NF). Animals subjected to this control treatment exhibited negligible

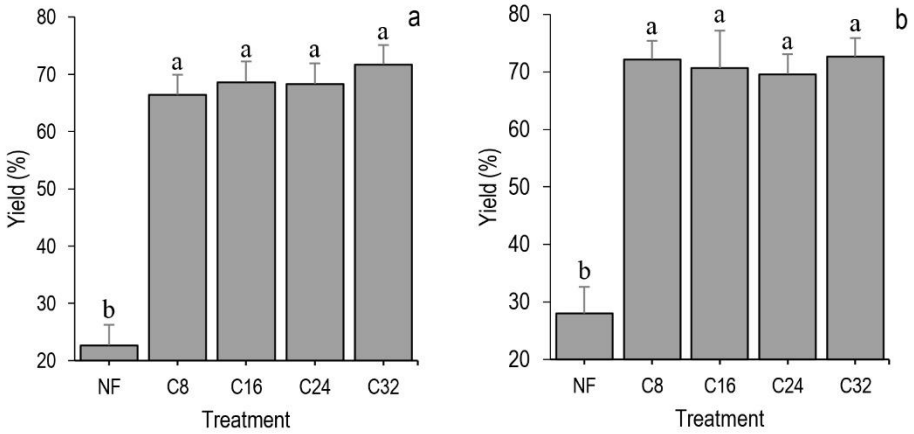

Figure 6. Yield rate of postlarval of the Pacific oyster *Crassostrea gigas* at a) seven days and b) 14 days of cultivation fed with different concentrations of microalgae. Postlarval phase: initial average (± standard deviation) shell height of 0.657 ± 0.05 mm. Different letters indicate statistical differences using Tukey's test ($P < 0.05$). Diets: NF, no food; C8, 8×10^4 cells L^{-1}; C16, 16×10^4 cells mL^{-1}; C24, 24×10^4 cells mL^{-1}; C32, 32×10^4 cells mL^{-1}. The diets comprised 30% of *Isochrysis galbana* and 70% of *Chaetoceros muelleri*.

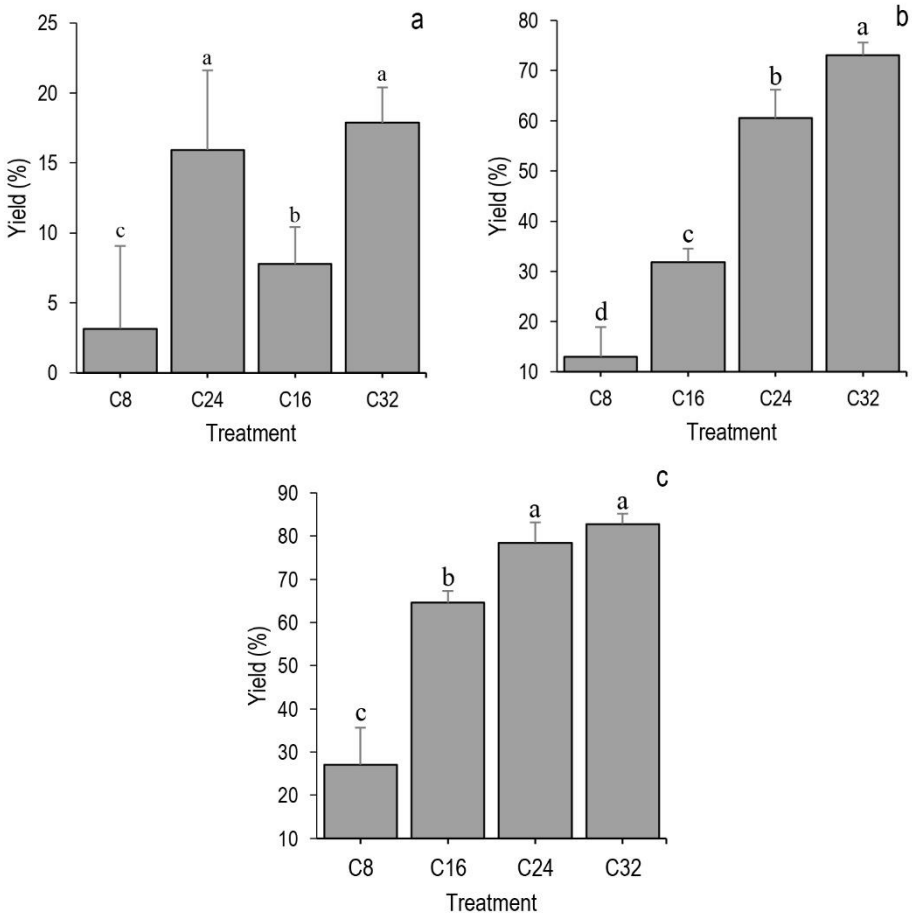

Figure 7. Yield rate of postlarval of the Pacific oyster *Crassostrea gigas* at a) seven days, b) 14 days, and c) 21 days of cultivation fed with different concentrations of microalgae. Postlarval phase: initial average (±standard deviation) shell height of 0.820 ± 0.12 mm. Different letters indicate statistical differences using Tukey's test ($P < 0.05$). Diets: C8, 8×10^4 cells mL^{-1}; C16, 16×10^4 cells mL^{-1}; C24, 24×10^4 cells mL^{-1}; C32, 32×10^4 cells mL^{-1}. Diets were composed of 30% *Isochrysis galbana* and 70% *Chaetoceros muelleri*.

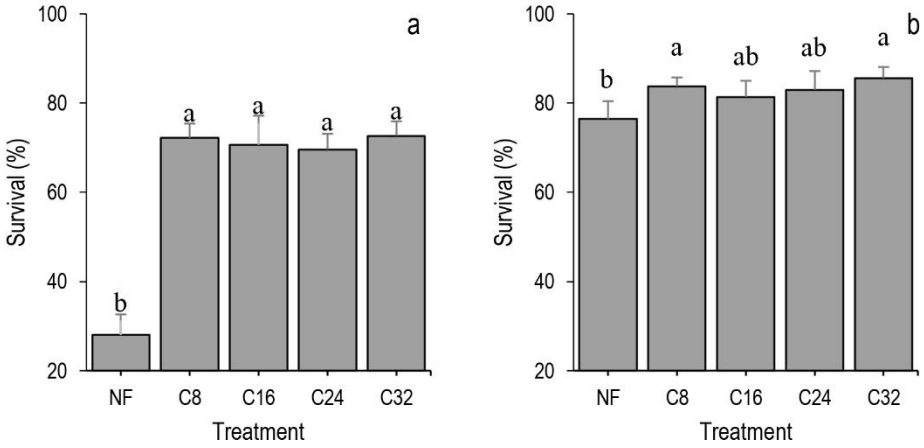

Figure 8. Survival rates for the Pacific oyster *Crassostrea gigas* at a) seven days and b) 14 days of the postlarval stage fed with different concentrations of microalgae. Different letters indicate statistical differences using Tukey's test ($P < 0.05$). Diets: NF, no food; C8, 8×10^4 cells mL^{-1}; C16, 16×10^4 cells mL^{-1}; C24, 24×10^4 cells mL^{-1}; C32, 32×10^4 cells mL^{-1}. Postlarval phase: initial average (±standard deviation) shell height of 0.657 ± 0.05 mm; late spat phase: initial average (±standard deviation) shell height of 0.820 ± 0.12 mm. The diets comprised 30% of *Isochrysis galbana* and 70% of *Chaetoceros muelleri*.

growth in height and length during the postlarval cultivation phase. These findings align with Moran & Manahan (2004), who reported minimal growth in animals deprived of food for 21 days.

Studies have demonstrated that the concentration of microalgae in diets can impact the growth of various species. This has been observed in pearl oysters *Pinctada margaritifera* (Yukihira et al. 1998, Linard et al. 2011), *Pinctada maxima* (Yukihira et al. 1998), and mussels *Mytilus galloprovincialis* (Pettersen et al. 2010).

Yield

The current study reveals that the highest concentrations yield the most metamorphosed larvae and spat in the shortest cultivation time, making these concentrations suitable for cultivating *C. gigas* spat in a laboratory setting. Understanding the requisite food concentration to satisfy the needs of the species being cultivated under laboratory conditions (Doroudi & Southgate 2000) and determining the suitable diet at each stage of animal development (Fernández-Pardo et al. 2016) is paramount because the concentration administered to each species is unique. For instance, a food concentration ranging between 10 and 20×10^3 cells mL^{-1} is ideal for the development of *Pinctada margaritifera* (Yukihira et al. 1998), for the growth of the sand clam larvae *Venerupis corrugata* (Gmelin 1791), diets containing microalgae at concentrations of 0.025, 0.030, and 0.075 cells mL^{-1} have been shown to maximize animal development (Fernández-Pardo et al. 2016).

In the postlarval phase, diets with microalgae at concentrations of 8 and 16×10^4 cells mL^{-1} yielded significantly lower results with developed spat. However, these diets were sufficient to meet the animals' needs and provide high yields. A longer cultivation period would be required to achieve a larger shell size (height and length), consequently increasing production costs. These findings are consistent with those reported in previous studies (Yukihira et al. 1998, Linard et al. 2011), where animals subjected to diets with lower food concentrations (800 cells mL^{-1}) exhibited continuous, albeit slower, growth.

Survival

Although no statistical difference was observed in the last part of the larval stages, diets with the highest microalgal concentrations (12 and 16×10^4 cells mL^{-1}) facilitated high survival rates of *C. gigas* larvae in this study. These findings align with those documented in the literature for the same species (Tabelskaya & Kalinina 2021) and for the larvae of the benthic mollusk *Clinocardium nuttalli* (Liu et al. 2010). The data suggest that diets with sufficient microalgal concentrations, which can be fully ingested and digested, positively influence the survival and growth of bivalve mollusk larvae, supporting previous literature findings (Yang et al. 2021). Conversely, diets with microalgal concentrations exceeding the larvae's

Table 1. Mean ± standard deviation (SD) of temperature (ºC), pH, and water salinity during cultivation. Diets: NF, no food; C8, 8×10^4 cells mL^{-1}; C12, 12×10^4 cells mL^{-1}; C16, 16×10^4 cells mL^{-1}; C24, 24×10^4 cells mL^{-1}; C32, 32×10^4 cells mL^{-1}). Postlarval phase: initial average (±SD) shell height of 0.657 ± 0.05 mm; spat ≥510 µm; postlarval phase: initial average (±SD) shell height of 0.820 ± 0.12 mm; spat ≥710 µm. The diets comprised 30% of *Isochrysis galbana* and 70% of *Chaetoceros muelleri*.

Last part of the larval stage			
Treatment	Temperature (°C)	pH	Salinity
C8	23.7 ± 0.35	8.1 ± 0.04	35.3 ± 0.70
C12	23.7 ± 0.35	8.0 ± 0.05	35.6 ± 1.02
C16	23.8 ± 0.40	8.0 ± 0.04	35.5 ± 0.60
Postlarval phase with spat ≥510 µm			
Treatment	Temperature (°C)	pH	Salinity
NF	21.7 ± 0.37	8.1 ± 0.03	35.8 ± 0.74
C8	21.8 ± 0.51	8.1 ± 0.05	35.6 ± 0.85
C16	21.7 ± 0.41	8.1 ± 0.05	35.7 ± 0.73
C24	21.7 ± 0.43	8.1 ± 0.04	35.8 ± 0.75
C32	21.7 ± 0.37	8.1 ± 0.05	35.8 ± 0.75
Postlarval phase with spat ≥710 µm			
Treatment	Temperature (°C)	pH	Salinity
NF	17.8 ± 1.63	8.0 ± 0.06	35.0 ± 0.58
C8	17.8 ± 1.60	8.0 ± 0.20	35.0 ± 0.54
C16	17.7 ± 1.61	8.0 ± 0.08	35.1 ± 0.56
C24	17.7 ± 1.63	8.0 ± 0.09	35.1 ± 0.57
C32	17.8 ± 1.61	8.0 ± 0.10	35.0 ± 0.63

requirements can result in decreased survival rates, as noted in the larvae of the oyster *P. margaritifera* (Doroudi & Southgate 2000) and *Mulinia lateralis* (Say, 1822) (Yang et al. 2021).

The current study revealed a direct correlation between the quantity and concentration of microalgae in the diets provided at all stages of larval and mollusk spat production and the survival and growth of these organisms. Similar findings have been reported for the survival and growth of *Argopecten nucleus* and *Nodipecten nodosus* larvae (Velasco & Barros 2008), *Panopea generous* juveniles (Liu et al. 2016) and *Crassostrea corteziensis* (Rivero-Rodríguez et al. 2007).

This study examined the survival rates of unfed spat across various phases. Spat ≥710 µm demonstrated higher survival rates than their ≥510 µm spat counterparts. This suggests that an individual's pre-fasting endogenous reserves, size, and age influence their ability to survive for extended periods without food. Previous studies (Sastry 1962, Hickman & Gruffydd 1971, Elston 1980, Rodriguez et al. 1990) have reported that bivalve mollusk larvae may survive for several days during the larval metamorphosis period without feeding, relying solely on their energy reserves. Moran & Manahan (2004) found that *C. gigas* D-stage veliger larvae could survive for over 30 days without food. However, His & Seaman (1992) reported total larval mortality after six days of starvation, while Robert et al. (2017) reported a mean survival rate of 58.13% at six days of culture and total mortality at 13 days of culture for *Ostrea edulis* larvae. For *Perna perna* (Linnaeus, 1758), *Mesodesma mactroides*, and *Venerupis philippinarum* spat, Vieira et al. (2021) reported mean survival rates of 91, 98, and 84%, respectively, when individuals were not fed for a period between 21 and 35 days. These studies indicate that, in the absence of food, larval or juvenile survival rates vary depending on the species and cultivation conditions. However, it is generally understood that animals will reduce their metabolism without food, subsisting on their endogenous energy reserves until feeding is resumed or mortality occurs (Yan et al. 2009).

This study's findings indicate that microalgal diets at concentrations of 12 and 16×10^4 cells mL^{-1} are appropriate during the last part of the larval stage. These concentrations offer high metamorphosis and survival rates and promote larval growth, resulting in high yields. Conversely, microalgal diets at 24 and 32×10^4 cells mL^{-1} concentrations are more effective during the post-larval cultivation phase. These concentrations ensure high survival rates and decrease the animals' growth time, reducing production costs.

CONCLUSION

High rates of larval metamorphosis and yield of *C. gigas* spat can be achieved by providing diets with microalgae at concentrations of 12 and 16×10^4 cells mL^{-1}. Given the lower food requirement, a concentration of 12×10^4 cells mL^{-1} is suggested for this cultivation phase. During the postlarval cultivation phase, it is advisable to use a diet with a concentration of 24×10^4 cells mL^{-1}. This concentration has been found to optimize cultivation time, promoting rapid growth and high yields. These outcomes were also observed at the highest tested concentration of 32×10^4 cells mL^{-1}. The survival rate of the oyster *C. gigas* remained unaffected across all cultivation phases, regardless of the concentrations tested, except for treatments not fed in the last larval phase. Consequently, when considering survival alone, there is no necessity to provide a diet with a microalgal concentration exceeding 8×10^4 cells mL^{-1}.

Credit author contribution

Gomes, H.M.: methodology, formal analysis, investigation, and writing - original draft - preparation. Sühnel, S.: methodology, investigation, writing - review & editing - preparation. Gomes, C.H.A.M., Silva, F.C. & Silva, E.: writing - review & editing - preparation. De Melo, C.M.R.: conceptualization, funding acquisition, super-vision, methodology, project administration, and writing - review & editing - preparation. All the authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

ACKNOWLEDGMENTS

This study was financed by the "Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001" and by the Federal University of Santa Catarina (249/2016). The authors also thank the "National Council for Scientific and Technological Development - Brazil (CNPq), which provided a scholarship to Claudio De Melo.

REFERENCES

Bayne, B.L., Ahrens, M., Allen, S.K., Anglès-D'auriac, M., Backeljau, T., Beninger, P., et al. 2017. The proposed dropping of the genus *Crassostrea* for all Pacific cupped oysters and its replacement by a new genus *Magallana*: A dissenting view. Journal of Shellfish Research, 36: 545-547. doi: 10.2983/035.036.0301

Botta, R., Asche, F., Borsum, J.S. & Camp, E.V. 2020. A review of global oyster aquaculture production and consumption. Marine Policy, 117: 103952. doi: 10.1016/j.marpol.2020.103952

Brown, M. & Robert, R. 2002. Preparation and assessment of microalgal concentrates as feeds for larval and juvenile Pacific oyster (*Crassostrea gigas*). Aquaculture, 207: 289-309. doi: 10.1016/S0044-8486(01)00742-6

Carvalho, Y.B.M., Ferreira, J.F., da Silva, F.C. & Bercht, M. 2013. Factors influencing larval settlement of the Atlanticlion's paw scallop, *Nodipecten nodosus*. Journal of Shellfish Research, 32: 719-723. doi: 10.2983/035.032.0313

Cheng, P., Zhou, C., Chu, R., Chang, T., Xu, J., Ruan, R., et al. 2020. Effect of microalgae diet and culture system on the rearing of bivalve mollusks: Nutritional properties and potential cost improvements. Algal Research, 51: 102076. doi: 10.1016/j.algal.2020.102076

Doroudi, M.S. & Southgate, P.C. 2000. The influence of algal ration and larval density on growth and survival of blacklip pearl oyster *Pinctada margaritifera* (L.) larvae. Aquaculture Research, 31: 621-626. doi: 10.1046/j.1365-2109.2000.00483.x

Elston, R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica*. Proceedings of the National Shellfisheries Association, 70: 65-93.

Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI). 2022. Síntese anual da agricultura de Santa Catarina 2020-2021. EPAGRI, Santa Catarina.

Enright, C.T., Newkirk, G.F., Craigie, J.S. & Castell, J.D. 1986. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. Journal of Experimental Marine Biology and Ecology, 96: 1-13. doi: 10.1016/0022-0981(86)90009-2

Fernández-Pardo, A., da Costa, F., Rial, D., Nóvoa, S., Martínez-Patiño, D. & Vázquez, J.A. 2016. Use of response surface methodology to determine optimum diets for *Venerupis corrugata* larvae: Effects of ration and microalgal assemblages. Aquaculture, 452: 283-290. doi: 10.1016/j.aquaculture.2015.11.005

Food and Agriculture Organization (FAO). 2022. The state of world fisheries and aquaculture 2022. Towards blue transformation. FAO, Rome.

Galley, T.H., Batista, F.M., Braithwaite, R., King, J. & Beaumont, A.R. 2010. Optimization of larval culture of the mussel *Mytilus edulis* (L.). Aquaculture International, 18: 315-325. doi: 10.1007/s10499-009-9245-7

Galtsoff, P.S. 1964. The American oyster *Crassostrea virginica* Gmelin. Fishery Bulletin of the Fish and Wildlife Service, 64: 219-238.

Gao, X., Li, X., Zhang, M., Chi, L., Song, C. & Liu, Y. 2016. Effects of LED light quality on the growth, survival, and metamorphosis of *Haliotis discus* hannai Ino larvae. Aquaculture Research, 47: 3705-3717. doi: 10.1111/are.12980

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., et al. 2020. A global blue revolution: Aquaculture growth across regions, species, and countries. Reviews in Fisheries Science & Aquaculture, 28: 107-116. doi: 10.1080/23308249.2019.1678111

Haws, M.C., DiMichele, L. & Hand, S.C. 1993. Biochemical changes and mortality during metamorphosis of the Eastern oyster, *Crassostrea virginica*, and the Pacific oyster, *Crassostrea gigas*. Molecular Marine Biology and Biotechnology, 2: 207.

Helm, M.M. & Bourne, N. 2004. Hatchery culture of bivalves: A practical manual. FAO, Rome.

Hickman, R.W. & Gruffydd, L.D. 1971. The histology of the larvae of *Ostrea edulis* during metamorphosis. Fourth European Marine Biology Symposium, Bangor.

His, E. & Seaman, M.N.L. 1992. Effects of temporary starvation on the survival, and on subsequent feeding and growth, of oyster (*Crassostrea gigas*) larvae. Marine Biology, 114: 277-279. doi. 10.1007/BF00 349530

Holden, M.J. & Patterson, G.W. 1991. Absence of sterol biosynthesis in oyster tissue culture. Lipids, 26: 81-82. doi: 10.1007/BF02544030

Laing, I. & Millican, P.F. 1986. Relative growth and growth efficiency of *Ostrea edulis* L. spat fed various algal diets. Aquaculture, 54: 245-262. doi: 10.1016/ 0044-8486(86)90270-X

Linard, C., Gueguen, Y., Moriceau, J., Soyez, C., Hui, B., Raoux, A., et al. 2011. Calcein staining of calcified structures in pearl oyster *Pinctada margaritifera* and the effect of food resource level on shell growth. Aquaculture, 313: 149-155. doi: 10.1016/j.aquaculture.2011.01.008

Liu, W., Gurney-Smith, H., Beerens, A. & Pearce, C.M. 2010. Effects of stocking density, algal density, and temperature on growth and survival of larvae of the basket cockle, *Clinocardium nuttallii*. Aquaculture, 299: 99-105. doi: 10.1016/j.aquaculture.2009.11.023

Liu, W., Pearce, C.M., McKinley, R.S. & Forster, I.P. 2016. Nutritional value of selected species of microalgae for larvae and early post-set juveniles of the Pacific geoduck clam, *Panopea generosa*. Aquaculture, 452: 326-341. doi: 10.1016/j.aquaculture.2015.10.019

Martínez-Fernández, E., Acosta-Salmón, H. & Southgate, P.C. 2006. The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae. Aquaculture, 257: 491-503. doi: 10.1016/j.aquaculture.2006.03.022

Miossec, L., Deuff, R.L. & Goulletquer, P. 2009. Alien species alert *Crassostrea gigas* (Pacific oyster). ICES Cooperative Research Report, 299: 42.

Moran, A.L. & Manahan, D.T. 2004. Physiological recovery from prolonged "starvation" in larvae of the Pacific oyster *Crassostrea gigas*. Journal of Experimental Marine Biology and Ecology, 306: 17-36. doi: 10.1016/j.jembe.2003.12.021

Napolitano, G.E., Ackman, R.G. & Ratnayake, W.M.N. 1990. Fatty acid composition of three cultured algal species (*Isochrysis galbana*, *Chaetoceros gracilis*, and *Chaetoceros calcitrans*) used as food for bivalve larvae. Journal of the World Aquaculture Society, 21: 122-130. doi: 10.1111/j.1749-7345.1990.tb00532.x

Noble, W.J., Benkendorff, K. & Harris, J.O. 2015. Growth, settlement, and survival of *Dicathais orbita* (Neogastropoda, Mollusca) larvae in response to temperature, diet, and settlement cues. Aquaculture Research, 46: 1455-1468. doi: 10.1111/are.12298

O'Connor, W.A., Nell, J.A. & Diemar, J.A. 1992. The evaluation of twelve algal species as food for juvenile Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley). Aquaculture, 108: 277-283. doi: 10.1016/0044-8486(92)90112-X

Pettersen, A.K., Turchini, G.M., Jahangard, S., Ingram, B.A. & Sherman, C.D.H. 2010. Effects of different dietary microalgae on survival, growth, settlement and fatty acid composition of blue mussel (*Mytilus galloprovincialis*) larvae. Aquaculture, 309: 115-124. doi: 10.1016/j.aquaculture.2010.09.024

Ponis, E., Parisi, G., Chini-Zittelli, G., Lavista, F., Robert, R. & Tredici, M.R. 2008. *Pavlova lutheri*: Production, preservation and use as food for *Crassostrea gigas* larvae. Aquaculture, 282: 97-103. doi: 10.1016/j. aquaculture.2008.06.010

Rico-Villa, B., Pouvreau, S. & Robert, R. 2009. Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. Aquaculture, 287: 395-401. doi: 10.1016/j. aquaculture.2008.10.054

Rico-Villa, B., Le Coz, J.R., Mingant, C. & Robert, R. 2006. Influence of phytoplankton diet mixtures on microalgae consumption, larval development and settlement of the Pacific oyster *Crassostrea gigas* (Thunberg). Aquaculture, 256: 377-388. doi: 10.1016/ j.aquaculture.2006.02.015

Rivero-Rodríguez, S., Beaumont, A.R. & Lora-Vilchis, M.C. 2007. The effect of microalgal diets on growth, biochemical composition, and fatty acid profile of *Crassostrea corteziensis* (Hertlein) juveniles. Aquaculture, 263: 199-210. doi: 10.1016/j.aquaculture. 2006.09.038

Robert, R., Vignier, J. & Petton, B. 2017. Influence of feeding regime and temperature on development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae. Aquaculture Research, 48: 4756-4773. doi: 10.1111/are.13297

Rodriguez, J.L., Sedano, F.J., García-Martín, L.O., Pérez-Camacho, A. & Sánchez, J.L. 1990. Energy metabolism of newly settled *Ostrea edulis* spat during metamorphosis. Marine Biology, 106: 109-111. doi: 10.1007/BF02114680

Rodríguez-Pesantes, D., Reyes, J., Márquez, A., Sonnenholzner, S., Hende, S. & Van Den, S. 2022. Early life cycle description and effects of microalgal diets on larval and post-larval development of the mangrove cockle *Anadara tuberculosa*, (Sowerby, 1833). Aquaculture Research, 53: 590-602. doi: 10.1111/are.15603

Salvi, D. & Mariottini, P. 2017. Molecular taxonomy in 2D: a novel ITS2 rRNA sequence structure approach guides the description of the oysters" subfamily Saccostreinae and the genus *Magallana* (Bivalvia: Ostreidae). Zoological Journal of Linnean Society, 179: 263-276. doi: 10.1111/zoj.12455

Salvi, D., Macali, A. & Mariottini, P. 2014. Molecular phylogenetics and systematics of the bivalve family Ostreidae based on rRNA sequence-structure models and multilocus species tree. Plos One, 9: e116014. doi: 10.1371/journal.pone.0108696

Sastry, A.N. 1962. The development and external morphology of pelagic larval and post-larval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. Bulletin of Marine Science, 15: 417-435.

Sigwart, J.D., Wong, N.L.W.S. & Esa, Y. 2021. Global controversy in oyster systematics and a newly described species from SE Asia (Bivalvia: Ostreidae: Crassostreinae). Marine Biodiversity, 51: 1-16. doi: 10.1007/s12526-021-01203-x

Silveira, R., Silva, F., Gomes, C., Ferreira, J. & Melo, C.M.R. 2011. Larval settlement and spat recovery rates of the oyster *Crassostrea brasiliana* (Lamarck, 1819) using different systems to induce metamorphosis. Brazilian Journal of Biology, 71: 557-562. doi: 10.1590/s1519-69842011000300029

Sühnel, S., Lagreze, F., Zanette, G., Magalhães, A.R.M. & Ferreira, J.F. 2012. Effect of the fatty acid EPA and DHA in the conditioning of the scallop *Nodipecten nodosus* (Linné, 1758). Aquaculture, 330-333: 167-171. doi. 10.1016/j.aquaculture.2011.12.013

Tabelskaya, A.S. & Kalinina, M.V. 2021. Growth and survival of the hatchery larvae of pacific oyster *Crassostrea gigas* under different concentrations of microalgae and salinity in conditions of southern Primorye. Izvestiya TINRO, 201: 723-734. doi: 10.26428/1606-9919-2021-201-723-734

Thompson, P.A. & Harrison, P.J. 1992. Effects of monospecific algal diets of varying biochemical composition on the growth and survival of Pacific oyster (*Crassostrea gigas*) larvae. Marine Biology, 113: 645-654. doi: 10.1007/BF00349708

Velasco, L.A. & Barros, J. 2008. Experimental larval culture of the Caribbean scallops *Argopecten nucleus* and *Nodipecten nodosus*. Aquaculture Research, 39: 603-618. doi: 10.1111/j.1365-2109.2008.01917.x

Vieira, G.C., Hawkyard, M., Langdon, C., Sühnel, S., Scaranto, B.M., Ferreira, J.P.R., et al. 2021. Replacement of living microalgae with a dried alfalfa chloroplast product in diets for the brown mussel (*Perna perna*), yellow clam (*Mesodesma mactroides*) and Manila clam (*Venerupis philippinarum*). Aquaculture Nutrition, 27: 2307-2319. doi: 10.1111/anu.13364

Yan, X., Zhang, Y., Huo, Z., Yang, F. & Zhang, G. 2009. Effects of starvation on larval growth, survival, and metamorphosis of Manila clam *Ruditapes philippinarum*. Acta Ecologica Sinica, 29: 327-334. doi: 10.1016/j.chnaes.2009.09.012

Yang, Z., Huang, X., Wang, H., Pan, H., Wang, X., Teng, M., et al. 2021. Effects of microalgae diets and stocking density on larval growth, survival and metamorphosis of dwarf surfclam, *Mulinia lateralis*. Aquaculture, 536: 736440. doi: 10.1016/j.aquaculture.2021.736440

Yukihira, H., Klumpp, D.W. & Lucas, J.S. 1998. Comparative effects of microalgal species and food concentration on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima* (Bivalvia: Pteriidae). Marine Ecology Progress Series, 171: 71-84. doi: 10.3354/meps171071

Received: January 30, 2024; Accepted: April 12, 2024

SUPPLEMENTARY MATERIAL

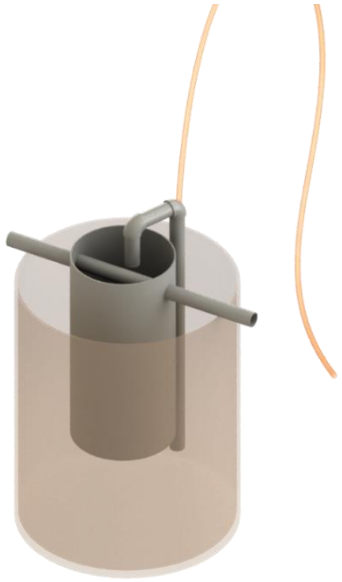

Figure S1. Overview of the downwelling system. The experimental units were constructed from PVC pipes, each with a diameter of 150 mm, a height of 240 mm, and a bottom area of 0.15 m^2. These units were lined at the bottom with a mesh screen with an opening of 180 µm. To create a downwelling system, the experimental units were situated individually within a 20 L plastic bucket.

Table S1. Parameters evaluated and statistical analyses applied in the study.

Last part of the larval stage	
Trait analysed	Test used
Metamorphosis Rate	Kruskal-Wallis; Wilcoxon
Growth (Fig. 2)	ANOVA. Tukey
Yield (Fig. 5)	Kruskal-Wallis; Wilcoxon
Survival	Kruskal-Wallis; Wilcoxon
Postlarval phase with spat ≥510 µm	
Trait analysed	Test used
Growth after 7 days of culture (Fig. 3)	ANOVA. Tukey (height, Fig. 2a) Kruskal-Wallis; Wilcoxon (length, Fig. 2b)
Growth after 14 days of culture (Fig. 3c-d)	Kruskal-Wallis; Wilcoxon (height and length)-
Yield after 7 and 14 days of culture (Fig. 6a-b)	ANOVA. Tukey
Survival (Fig. 8a)	ANOVA. Tukey
Postlarval phase with spat ≥ 710 µm	
Trait analysed	Test used
Growth after 7 and 14 days of culture (Fig. 4a-b)	ANOVA. Tukey (height)
Growth after 14 and 21 days of culture (Fig. 4c-f)	Kruskal-Wallis; Wilcoxon (height and length)
Yield after 7, 14 e 21 days of culture (Fig. 7a-c)	ANOVA. Tukey
Survival (Fig. 8b)	ANOVA. Tukey