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SELECTION AND APPLICATION OF SOME MICROALGAL STRAINS IN PENAEUS SHRIMP LARVICULTURE IN VIETNAM

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PhD THESIS ABSTRACT

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INTRODUCTION

The larviculture of Penaeus shrimp in Vietnam predominantly depends on the diatom *Thalassiosira weissflogii*. However, the practical application of *T. weissflogii* in shrimp hatcheries still faces several challenges.

In response to these issues, the present PhD research, titled "Selection and Application of Microalgal Strains in Penaeus Shrimp Larviculture in Vietnam," was conducted with the following objectives:

To investigate the selection and application of specific microalgal strains as both direct and indirect feed sources in the larviculture of whiteleg shrimp (*Penaeus vannamei*) and black tiger shrimp (*Penaeus monodon*) in Vietnam, as detailed following:

- ❖ To identify 2–3 novel microalgal strains/species with high potential as direct feed sources in Penaeus shrimp larviculture.
- ❖ To identify 2–3 novel microalgal strains/species with high potential as indirect feed sources in Penaeus shrimp larviculture.
- ❖ To optimize key culture conditions that are commonly used and easily implemented in commercial shrimp hatcheries in Vietnam.
- ❖ To evaluate the role of microalgae as an indirect feed source in Penaeus shrimp larval development.
- ❖ To determine the optimal microalgal feed ratio for larval rearing, thereby contributing to the development of standardized protocols for microalgal culture and application in Penaeus shrimp larviculture in Vietnam.

Scientific and Practical Significance of the Thesis:

This thesis provides a valuable scientific foundation on the growth characteristics and biochemical composition of selected microalgal strains/species applied in aquaculture in general, and Penaeus shrimp hatchery production in particular. The research presents empirical evidence on how specific culture conditions influence microalgal population growth and nutritional quality. Furthermore, it offers insights into the effects of microalgal feed rations on the larviculture performance of Penaeus shrimp in Vietnam. These findings are expected to support academic instruction, scientific research, and the sustainable development of the shrimp farming industry in Vietnam.

Conducted within the framework of a collaborative research and development project, the study was oriented toward practical application. The successful outcomes of the thesis have contributed to the establishment of a microalgal seed bank comprising diverse strains for hatchery use. The optimization of culture conditions provides a scientific basis for developing standardized microalgal production protocols. In addition, an effective Penaeus larviculture process was formulated, integrating results from microalgal feeding trials and experiments involving rotifer application.

CHAPTER 1. LITTERATURE REVIEW

1.1. Overview of microalgae

Microalgae are single-celled organisms found in a wide range of environments, including soil, rocks, and aquatic habitats such as freshwater and marine ecosystems. Some species may also live as parasites on other organisms. In aquatic ecosystems, which cover over 71% of the Earth's surface, microalgae exhibit rapid growth. Morphologically, microalgal cells can exist either as individual cells or in colonies. Their shape and size vary significantly depending on species and culture conditions. Unlike multicellular organisms, the growth of microalgae—and microorganisms in general—is typically characterized at the population level.

Microalgae possess a wide array of biochemical components that contribute to their nutritional value. Among these, lipid content and fatty acid profiles are of particular interest in both research and applied contexts. Protein content in microalgae can range widely, from 6% to 52% of dry cell weight depending on the species, with some exceptional cases reaching up to 71% [4, 8]. In addition to species-specific traits, several environmental and culture-related factors significantly influence intracellular protein content, including temperature, salinity, light conditions, pH, culture medium, and carbon dioxide availability.

Culture conditions also have a profound impact on both population growth and biochemical composition. The influence of light can be assessed through three key parameters: intensity, photoperiod (light-dark cycle), and spectral quality. Alongside light, temperature is a critical environmental factor that regulates most physiological and biochemical processes in microalgae, as in other organisms. Salinity affects both the growth and biochemical profiles of microalgae through direct and indirect mechanisms. Additionally, the choice of culture medium plays a crucial role in shaping microalgal population dynamics and biochemical characteristics, particularly in applied research such as aquaculture systems.

1.2. Overview of Penaeus shrimp

The shrimp family Penaeidae comprises four genera—Penaeus, Metapenaeus, Parapenaeopsis, and Metapenaeopsis—and includes numerous species within the taxonomic system. Embryonic development in penaeid shrimp encompasses multiple sub-stages, beginning with fertilization and continuing until hatching. The duration of embryonic development ranges from approximately 12 to over 20 hours, depending on the species and specific environmental conditions, particularly temperature. Upon completion of the embryonic phase, larvae progress through distinct developmental stages. Based on morphological features, swimming behavior, and feeding patterns,

larval development is typically categorized into four stages: nauplius, zoea, mysis, and postlarva [2].

The dietary protein requirement of shrimp larvae ranges from 30% to 56%, depending on factors such as the nature of the feed source, developmental stage, physiological condition, and various physicochemical characteristics of the environment [3]. While shrimp larvae and adults do not have a defined quantitative requirement for total lipids, lipid inclusion levels are generally recommended to be around 6–7.5%, with an upper limit of approximately 10%, as suggested by several studies [7]. More critically, the specific composition of lipid classes and fatty acid profiles plays a key role in larval nutrition [3]. In particular, three lipid-related components are consistently highlighted in the literature as essential for optimal larval development: long-chain (≥C20) polyunsaturated fatty acids (LC-PUFAs), phospholipids, and sterols [7]. Shrimp larvae of the genus Penaeus do not exhibit a direct requirement for carbohydrates. However, in aquaculture—particularly in shrimp hatchery diets—polysaccharides such as starch and dextrins are commonly incorporated as energy sources to partially substitute for high-cost protein ingredients. The carbohydrate requirement of shrimp larvae is estimated to range from approximately 7.5% to 33%, depending on species and culture conditions [5, 6].

1.3. Research on the application of microalgae in shrimp larviculture

1.3.1. Application of microalgae as direct feed in shrimp larval production

Microalgae serve as a vital live feed in shrimp larval production, owing to several key characteristics: (1) appropriate size and pigmentation, which stimulate feeding responses; (2) buoyancy, allowing them to remain suspended in the water column; (3) their role in maintaining water quality; and (4) their high nutritional value. Like other heterotrophic organisms, shrimp larvae require an adequate and balanced intake of proteins, lipids, carbohydrates, minerals, and vitamins to support growth and development. Carbohydrates function primarily as an immediate energy source, while proteins and essential amino acids are crucial for tissue formation and various metabolic processes. Lipids not only act as energy reserves but also play integral roles in cell membrane formation and the synthesis of steroid hormones.

1.3.2. Application of microalgae as indirect feed in shrimp larval production

In shrimp larviculture systems in Vietnam and many other countries, alive feeds commonly used during the larval rearing phase include microalgae and *Artemia* nauplii (instar I/II). As a result, research on the indirect application of microalgae as a feed component for shrimp larvae remains limited. However, the concept of utilizing microalgae to cultivate rotifers, which are then provided as feed for shrimp larvae, represents a promising and innovative approach. This strategy has the potential to enhance larval nutrition while improving feed management and sustainability in hatchery operations [1].

CHAPTER 2. MATERIALS AND METHODOLOGY

2.1. Subjects, Time, and Research Locations

– The subjects of the study are 8 microalgae strains from the Australian National Algae Culture Collection (ANACC), managed by the CSIRO, Hobart, Australia.

– The study was conducted from January 2021 to August 2023

– The study took place in the Microalgae Laboratory, Institute of Aquaculture, Nha Trang University; Minh Phu Aquaculture Seed Production Co., Ltd., An Hai – Ninh Phuoc – Ninh Thuan; and the Biochemical Analysis Laboratory, CSIRO, Hobart – Tasmania – Australia.

2.2. Research components

- Population growth and biochemical composition of potential microalgal strains.
- Effects of various culture conditions on the population growth and biochemical composition of selected algae strains.
- Investigation of using some microalgal strains for rotifer culture applying in shrimp larviculture.
- Effects of different algae feed portions on the growth, development, and health of Penaeus shrimp larvae.

2.3. Experimental design

- Experiment 1: Survey of population growth and biochemical composition of potential microalgal strains under Vietnamese conditions.
- Experiment 2: Influence of different nutrient media (f, f/2, and AGP-C) on population growth and biochemical composition of microalgal strains.
- Experiment 3: Interaction between light intensity and photoperiod on population growth and biochemical composition of microalgal strains
- Experiment 4: Influence of different temperatures on bichemical composition of microalgal strains.
- Experiment 5: Influence of different salinities on population growth and biochemical composition of microalgal strains.
- Experiment 6: Investigation of microalgal strains for rotifer culture applying in larviculture
- Experiment 7: Influence of different microalgal diets on the growth, development, and health of Penaeus shrimp larvae.

2.4. Data Collection Methodology

- The microalgal population growth
- Lipid and fatty acid composition analysis methods
- Method for determining rotifer population growth
- Method for determining the growth and development of shrimp larvae

2.5. Data analysis

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Population growth and biochemical composition of potential microalgae strains (Exp 1)

3.1.1. Population growth of some potential microalgae strains

Among the tested microalgal strains, *Nannochloropsis oceanica* exhibited the highest growth performance in terms of maximum cell density (MCD), reaching $78.92 \pm 5.63 \times 10^6$ cells/mL on day 8 of the cultivation cycle. This value was approximately 5.5 times greater than that of the second-ranking strain, *Phaeodactylum tricornutum*, which achieved a maximum biomass of $14.37 \pm 2.04 \times 10^6$ cells/mL (Table 3.1). Ranking third was the marine diatom *Chaetoceros muelleri*, with an MCD of $8.26 \pm 0.35 \times 10^6$ cells/mL. Differences in MCD among these strains were statistically significant at the $\alpha = 0.05$ level. In contrast, *Thalassiosira weissflogii* exhibited the lowest maximum cell density, reaching only $0.60 \pm 0.04 \times 10^6$ cells/mL. This value was significantly lower than those of *N. oceanica*, *P. tricornutum*, and *C. muelleri*, but not significantly different from the remaining microalgal strains.

Table 3.1. Summary of population growth of some microalgal strains (Min-Max Mean±SD, n=3).

Strains	MCDs ($\times 10^6$ cell/mL)	EGRs (/day)	Max SGRs (/day)
Chaetoceros calcitrans CS-178	4.11-4.6 4.37±0.25^{a}	0.31-0.34 0.33±0.1^{a}	0.53-0.62 0.57±0.04^{a}
Chaetoceros muelleri CS-176	7.88-8.55 8.26±0.35^{b}	0.68-0.75 0.71±0.03^{c}	0.75-1.01 0.89±0.13^{b}
Isochrysis galbana CS-186	3.40-3.81 3.60±0.20^{a}	0.54-0.61 0.58±0.04^{b}	0.57-0.70 0.63±0.06^{a}
Nannochloropsis oceanica CS-179	72.50-83.00 78.92±5.63^{d}	0.92-1.06 0.98±0.07^{e}	1.09-1.36 1.24±0.14^{c}
Phaeodactylum tricornutum CS-29	12.50-16.55 14.37±2.04^{c}	0.79-0.92 0.88±0.07^{de}	1.05-1.17 1.11±0.06^{bc}
Tisochrysis lutea CS-177	3.70-4.20 3.88±0.28^{a}	0.41-0.69 0.57±0.16^{b}	0.45-0.76 0.64±0.17^{a}
Thalassiosira pseudonana CS-173	3.49-4.61 4.11±0.57^{a}	0.70-0.87 0.81±0.09^{cd}	0.94-1.18 1.06±0.12^{bc}
Thalassiosira weissflogii CS-871	0.56-0.64 0.60±0.04^{a}	0.70-0.77 0.72±0.04^{c}	0.73-1.09 0.95±0.19^{b}

3.1.2. Biochemical composition of some potential microalgal strains

Significant variation was observed in the biochemical composition of the studied microalgal strains (Table 3.2). Total lipid content ranged from 16 to 90 mg/g dry weight among the strains. The haptophyte algae *Tisochrysis lutea* and *Isochrysis galbana* exhibited the highest lipid levels, with values of 90.3 mg/g and 61.1 mg/g, respectively. These were followed by the eustigmatophyte *Nannochloropsis oceanica*, which had a total lipid content of 55 mg/g.

In contrast, the lowest lipid concentrations were found in the marine diatoms, particularly *Thalassiosira weissflogii* and *T. pseudonana*, both of which recorded total lipid contents of 16 mg/g. Notably, in most treatments, polar lipids accounted for a predominant proportion of the total lipid fraction, ranging from 87.2% to 97.3%.

Table 3.2. Fatty acid profile (% TFA, n=2) and lipid class composition (% total lipid) in some microalgal strains

Species	***C. calcitrans***	***C. muelleri***	***I. galbana***	***N. oceanica***
Strain	CS-178	CS-176	CS-186	CS-179
Fatty acid (% TFA)				
20:4ω6 ARA	2.3 ±2.9	10.6 ±10.7	Tr	6.5 ±1.5
20:5ω3 EPA	21.2 ±11.3	0.7 ±0.3	0.5 ±0.1	30.0 ±7.4
22:6ω3 DHA	0.8 ±0.0	0.5 ±0.2	10.1 ±0.8	Tr
FA Group				
∑SFA	20.7	27.2	31.3	27.5
∑MUFA	27.6	25.9	19.2	26.6
∑PUFA	38.9	35.1	41.1	40.4
∑LC-PUFA	22.0	1.1	10.6	30.0
Lipid class				
Wax ester*	Tr	0.2	2.2	Tr
Triacylglycerol	Tr	2.0	2.5	Tr
Free fatty acid	2.5	5.7	0.0	2.5
Sterol	1.1	1.3	0.9	1.1
Polar lipid	96.3	90.8	94.4	96.3
Total lipid (*mg/g*)	**55.0**	**43.7**	**61.1**	**55.0**

3.2. Effects of various cultivation conditions on the growth and biochemical composition of microalgal strains.

3.2.1. Influence of different nutrient media (f, f/2, and AGP-C) on population growth and biochemical composition of potential strains (Exp 2)

❖ *Influence of different nutrient environments on the growth and biochemical composition of Chaetoceros muelleri*

The diatom *Chaetoceros muelleri* exhibited optimal growth in the supplemented **f** nutrient medium, followed by the commercial AGP-C medium, and lastly the standard **f/2** medium, as reflected across all measured parameters—maximum cell density (MCD), exponential growth rate (EGR), and maximum specific growth rate (Max SGR). One-way ANOVA analysis from the second trial revealed statistically significant differences among the three treatments for all growth parameters (Table 3.3).

Table 3.3. Summary of growth results of *Chaetoceros muelleri* in three different culture mediums (Min-Max, Mean±SD, n=4).

	Medium		
	f	**f/2**	**AGP-C**
1st			
MCDs *(×10⁶ cell/mL)*	11,45-12,51	8,26-8,91	9,29-10,11
	12,01±0,48^{c}	8,54±0,30^{a}	9,81±0,37^{b}
EGRs *(/day)*	0,76-0,92	0,66-0,76	0,70-0,79
	0,84±0,07^{b}	0,72±0,05^{a}	0,74±0,04^{a}
Max SGRs *(/day)*	1,12-1,32	0,73-1,08	1,02-1,13
	1,21±0,10^{b}	0,88±0,15^{a}	1,08±0,05^{b}
2nd			
MCDs *(×10⁶ cell/mL)*	9,61-10,13	7,46-8,21	9,81-10,03
	9,95±0,23^{c}	7,79±0,31^{a}	9,92±0,11^{b}
EGRs *(/day)*	0,74-0,87	0,64-0,77	0,68-0,77
	0,79±0,06^{c}	0,70±0,06^{a}	0,73±0,04^{b}
Max SGRs *(/day)*	0,92-1,24	0,71-0,97	0,78-0,91
	1,03±0,14^{c}	0,82±0,11^{a}	0,84±0,07^{b}

With regard to lipid composition, the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents were substantially lower in the f and f/2 treatments compared to the AGP-C treatment. In contrast, polyunsaturated fatty acids (PUFAs), particularly long-chain PUFAs (LC-PUFAs), were more abundant in the NT-2.1 and NT-2.2 treatments. A similar pattern was observed for the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Specifically, EPA content was highest in treatments NT-2.1 and NT-2.2, at 24.27% and 20.94% of total

fatty acids (TFA), respectively, while only 10.48% TFA was recorded in NT-2.3. Likewise, DHA content in NT-2.1 and NT-2.2 was 1.13% and 1.42% TFA, respectively—substantially higher than in NT-2.3, which showed only 0.80% TFA. A different trend was observed for arachidonic acid (ARA). ARA content was markedly higher in NT-2.2 and NT-2.3, at 5.3% and 4.2% TFA, respectively, compared to just 1.64% in NT-2.1.

❖ *Influence of different culture mediums on the growth and biochemical composition of Phaeodactylum tricornutum*

In the first experimental trial, the highest maximum cell density (MCD) and exponential growth rate (EGR) during the logarithmic phase were observed in the f-supplemented nutrient medium (NT-2.4), reaching $21.54 \pm 2.62 \times 10^6$ cells/mL and 0.95 ± 0.05 day^{-1}, respectively. This was followed by the AGP-C medium (NT-2.6), which recorded MCD and EGR values of $18.22 \pm 0.89 \times 10^6$ cells/mL and 0.86 ± 0.04 day^{-1}, respectively. The lowest values were obtained in the f/2-supplemented medium (NT-2.5), with an MCD of $14.81 \pm 1.82 \times 10^6$ cells/mL and an EGR of 0.78 ± 0.02 day^{-1}. These differences were statistically significant at the $\alpha = 0.05$ level. In contrast, the differences in maximum specific growth rates (Max SGR) were less pronounced. Both NT-2.4 and NT-2.6 exhibited comparable and significantly higher Max SGRs of 1.07 and 0.98 day^{-1}, respectively, compared to NT-2.5, which recorded a Max SGR of 0.90 day^{-1} (Table 3.3).

The biochemical composition of *Phaeodactylum tricornutum* varied notably across the different culture media used in the experiment. In contrast to *Chaetoceros muelleri* in the previous trial, *P. tricornutum* exhibited the highest total lipid content when cultured in the AGP-C nutrient medium (NT-2.6), reaching 103.3 mg/g (dry weight). This was followed by the f/2-supplemented medium (NT-2.5), which produced a lipid content of 92.7 mg/g. The lowest lipid accumulation was observed in the f-supplemented medium (NT-2.4), with a total lipid content of 65.1 mg/g.

The distribution of lipid classes—including polar lipids, sterols, and triacylglycerols—was generally consistent across treatments, with polar lipids overwhelmingly dominating the profile, comprising 94–96% of total lipids. Notably, the f-supplemented nutrient medium (NT-2.4) yielded the highest proportions of sterols and triacylglycerols, reaching 1.6% and 1.8% of total lipids (%TL), respectively. In contrast, the lowest concentrations of sterols and free fatty acids were observed in the AGP-C commercial nutrient medium (NT-2.6), indicating a more simplified lipid profile under these culture conditions.

Table 3.4. Summary of growth results of *P. tricornutum* in three different supplemented nutrient media (Min-Max, Mean±SD, n=4).

	Medium		
	f	**f/2**	**AGP-C**
1st			
MCDs *(×10⁶ cell/mL)*	18.46-24.61	12.86-16.50	17.36-19.25
	21.54±2.62[c]	14.81±1.82[a]	18.22±0.89[b]
EGRs *(/day)*	0.90-1.01	0.76-0.80	0.82-0.90
	0.95±0.05[c]	0.78±0.02[a]	0.86±0.04[b]
Max SGRs *(/day)*	0.97-1.13	0.85-0.97	0.98-1.05
	1.07±0.07[b]	0.90±0.05[a]	0.98±0.15[b]
2nd			
MCDs *(×10⁶ cell/mL)*	13.68-16.06	8.60-13.51	14.40-16.26
	14.96±1.07[b]	10.25±2.21[a]	15.19±0.85[b]
EGRs *(/day)*	0.87-0.90	0.64-0.76	0.77-0.83
	0.87±0.90[c]	0.69±0.06[a]	0.80±0.03[b]
Max SGRs *(/day)*	0.94-1.02	0.75-0.84	0.87-0.90
	0.97±0.04[c]	0.79±0.04[a]	0.87±0.90[b]

❖ *Impact of different nutrient mediums on growth and biochemical composition of Thalassiosira weissflogii*

In the first experiment, the maximum cell densities (MCDs) of *Thalassiosira weissflogii* varied significantly across the three nutrient-enriched media. The f-supplemented medium (NT-2.7) yielded the highest MCD at $0.67 \pm 0.04 \times 10^6$ cells/mL, followed by the AGP-C commercial medium (NT-2.9) with $0.52 \pm 0.04 \times 10^6$ cells/mL, and lastly, the f/2-supplemented medium (NT-2.8) with $0.40 \pm 0.04 \times 10^6$ cells/mL. These differences were statistically significant at the $\alpha = 0.05$ level. This trend was fully replicated in the second experiment, reinforcing that among the tested media, the f-supplemented formulation was the most conducive to *T. weissflogii* growth, while the f/2-supplemented medium consistently supported the lowest cell density.

In terms of exponential growth rate (EGR) and maximum specific growth rate (Max SGR), both experiments confirmed the superior performance of the f-supplemented medium. However, in the first experiment, the EGR and Max SGR values between the f/2-supplemented (NT-2.8) and AGP-C (NT-2.9) treatments did not differ significantly. Specifically, NT-2.8 exhibited an EGR of 0.70 ± 0.05 /day and a Max SGR

of 0.71 ± 0.05 /day, while NT-2.9 recorded slightly higher values at 0.89 ± 0.11 /day and 0.93 ± 0.10 /day, respectively (Table 3.5).

The total lipid content was highest in the f/2-supplemented medium (NT-2.8), reaching 34.72 mg/g. In contrast, the lowest lipid content was observed in the AGP-C medium (NT-2.9), at 31.43 mg/g. Notably, compared to the results from Experiment 1, the lipid content in this experiment was approximately double. Among the lipid classes, triacylglycerols and sterols were most abundant in NT-2.9, whereas the lowest levels of free fatty acids and triacylglycerols were recorded in NT-2.7. The polar lipid fraction remained relatively high and stable in NT-2.7 and NT-2.8, accounting for approximately 92–93% of total lipids (TL), while this proportion decreased to 88.6% in NT-2.9.

Overall, the results of Experiment 2 demonstrated that the culture medium had a substantial impact on the population growth and biochemical composition of the three diatom species: *Chaetoceros muelleri*, *Phaeodactylum tricornutum*, and *Thalassiosira weissflogii*. Among the tested media, the f-supplemented formulation consistently supported the best growth performance across all three diatom species. Furthermore, the different culture media appeared to influence lipid biosynthesis and fatty acid accumulation in species-specific ways, with each diatom exhibiting distinct responses in both total lipid content and lipid class composition.

Table 3.5. Summary of growth results of *T. weissflogii* in three different supplemented nutrient mediums (Min-Max, Mean±SD, n=4)

	Medium		
	f	**f/2**	**AGP-C**
1st			
MCDs *(×10^6 cell/mL)*	0.64-0.74	0.34-0.44	0.49-0.56
	0.67±0.04^c	0.40±0.04^a	0.52±0.04^b
EGRs *(/day)*	0.89-0.97	0.65-0.75	0.68-0.79
	0.93±0.04^b	0.70±0.05^a	0.71±0.05^a
Max SGRs *(/day)*	1.16-1.30	0.75-1.01	0.80-1.06
	1.26±0.07^b	0.89±0.11^a	0.93±0.10^a
2nd			
MCDs *(×10^6 cell/mL)*	0.96-1.10	0.60-0.68	0.79-0.92
	1.03±0.06^c	0.63-0.04^a	0.84±0.06^b
EGRs *(/day)*	0.90-1.04	0.73-0.86	0.82-0.89
	0.97±0.05^c	0.76±0.06^a	0.86±0.04^b
Max SGRs *(/day)*	1.12-1.23	0.84-0.98	0.92-1.08
	1.16±0.05^c	0.90±0.06^a	1.02±0.07^b

3.2.2. Interaction between light intensity and photoperiod on population growth and biochemical composition of potential microalgal strains (Exp 3)

❖ *Interaction between light intensity and photoperiod on population growth and biochemical composition of C. muelleri*

The interaction between light intensity (LI) and photoperiod (PP) on the growth of *Chaetoceros muelleri* was clearly evident when analyzing the population growth rate during the logarithmic phase (EGRs). Multivariate linear regression analysis, based on the model [EGRs = 0.82×LI + 1.91×PP – 1.25×(LI×PP)], yielded statistically significant results ($R^2 = 0.901$, $p < 0.001$). Among the predictor variables, photoperiod exerted the greatest influence on EGRs, as indicated by a high standardized coefficient ($\beta = 1.91$, $t = 5.8$, $p < 0.001$). This suggests that increasing the photoperiod significantly enhanced the growth rate of *C. muelleri* populations during the exponential phase. Light intensity also had a positive and statistically significant effect ($\beta = 0.82$, $t = 3.3$, $p = 0.003$), further contributing to improved algal growth. Importantly, the interaction term between light intensity and photoperiod was also statistically significant ($\beta = –1.25$, $t = –3.07$, $p = 0.005$), indicating a diminishing return effect. Specifically, the negative coefficient implies that the stimulatory impact of increasing light intensity on growth rate becomes less pronounced when the photoperiod is extended. This suggests a nonlinear interaction in which the combined effect of high light intensity and prolonged photoperiod may lead to suboptimal or plateaued growth responses over time.

Table 3.6. Population growth of *C. muelleri* in 9 treatments

Treatments	**MCDs *(×10^6 cell/mL)***	**EGRs *(/day)***	**Max SGRs *(/day)***
NT-3.1	7.85-9.15	0.87-0.90	0.99-1.16
(75µE12hL:12hD)	8.38±0.68	0.88±0.02	1.10±0.10
NT-3.2	8.93-10.25	1.05-1.11	1.20-1.35
(75µE18hL:6hD)	9.63±0.67	1.08±0.03	1.29±0.07
NT-3.3	9.50-10.13	1.21-1.33	1.16-1.43
(75µE24hL:0hD)	9.76±0.33	1.27±0.06	1.27±0.14
NT-3.4	8.88-9.15	0.91-0.96	1.19-1.35
(100µE12hL:12hD)	9.02±0.14	0.93±0.03	1.29±0.09
NT-3.5	9.45-10.43	1.08-1.17	1.32-1.48
(100µE18hL:6hD)	9.92±0.49	1.14±0.05	1.42±0.09
NT-3.6	9.55-11.05	1.19-1.24	1.19-1.22
(100µE24hL:0hD)	10.15±0.7	1.21±0.03	1.20±0.02
NT-3.7	9.68-10.65	0.93-1.04	1.34-1.62
(125µE12hL:12hD)	10.13±0.49	0.98±0.05	1.50±0.15
NT-3.8	8.65-11.03	1.07-1.18	1.33-1.54
(125µE18hL:6hD)	9.77±1.19	1.11±0.06	1.46±0.11
NT-3.9	9.45-10.13	1.17-1.26	1.37-1.56
(125µE24hL:0hD)	9.75±0.34	1.22±0.05	1.44±0.10

In general, lipid content exhibited a direct, positive correlation with both increasing light intensity and photoperiod. Under initial conditions of low light intensity and short photoperiod (75 µE/m²/s and 12h light:12h dark), a moderate increase in both parameters to 100 µE/m²/s and an 18-hour photoperiod resulted in a substantial rise in total lipid content—approximately 20%. A further elevation to 125 µE/m²/s and continuous illumination (24h light) led to an additional, albeit smaller, increase in lipid accumulation. A similar trend was observed in triacylglycerol levels, which also increased progressively under enhanced light and photoperiod conditions. In contrast, sterol content demonstrated an inverse relationship, showing a gradual decline as light intensity and photoperiod increased.

❖ *Interaction between light intensity and photoperiod on population growth and biochemical composition of P. tricornutum*

The results of linear regression analysis revealed notable effects of light intensity, photoperiod, and their interaction on the growth dynamics of *P. tricornutum* populations. The regression model predicting the exponential growth rate (EGR) during the logarithmic phase—$EGR = 0.78 \times LI + 1.39 \times PP - 0.66 \times (LI \times PP)$—was statistically significant ($p < 0.001$), explaining approximately 86% of the variance in EGR ($R^2 = 0.860$).

The influence of light conditions on the biochemical composition of *P. tricornutum* varied across different lipid components. Notably, total lipid content exhibited a significant decline with increasing light intensity. When light intensity increased from 75 to 100 µE/m²/s, a marked reduction in total lipid content was observed, particularly under extended photoperiods (18h light:6h dark and continuous light). A similar downward trend was recorded for polar lipids, which also decreased substantially under higher light intensities.

In terms of population growth, photoperiod demonstrated a strong stimulatory effect. As photoperiod increased, the EGR of *P. tricornutum* also increased significantly ($\beta = 1.39$, $t = 3.6$, $p = 0.002$). While elevated light intensity also promoted growth, its effect was comparatively weaker ($\beta = 0.72$, $t = 2.42$, $p = 0.024$). Although the interaction term between light intensity and photoperiod exhibited a negative coefficient, indicating a potential diminishing return at high levels of both factors, this interaction was not statistically significant in the case of *P. tricornutum* ($p = 1.82$), in contrast to the pattern observed in *C. muelleri*.

Table 3.7. Results of population growth of *P. tricornutum* in 9 treatments (Min-Max, Mean±SD, n=3).

	MCDs *(×10^6 cell/mL)*	**EGRs *(/day)***	**Max SGRs *(/day)***
NT-3.10	15.43-22.25	0.79-0.82	1.07-1.22
(75µE12hL:12hD)	18.64±3.43	0.81±0.01	1.14±0.08
NT-3.11	15.75-21.25	0.94-0.96	1.04-1.08
(75µE18hL:6hD)	18.50±2.75	0.95±0.01	1.06±0.02
NT-3.12	15.25-21.75	0.96-1.02	1.11-1.21
(75µE24hL:0hD)	18.67±3.26	1.00±0.03	1.16±0.05
NT-3.13	16.75-17.50	0.85-0.89	0.90-1.04
(100µE12hL:12hD)	17.25±0.43	0.86±0.02	0.95±0.08
NT-3.14	19.50-20.50	0.91-0.95	1.05-1.17
(100µE18hL:6hD)	19.92±0.52	0.94±0.02	1.11±0.06
NT-3.15	21.00-22.50	1.06-1.10	1.32-1.40
(100µE24hL:0hD)	21.75±0.75	1.09±0.03	1.36±0.04
NT-3.16	16.75-17.50	0.93-0.94	1.14-1.19
(125µE12hL:12hD)	17.17±0.38	0.93±0.00	1.16±0.03
NT-3.17	19.75-21.25	0.93-0.98	1.00-1.13
(125µE18hL:6hD)	20.50±0.75	0.96±0.03	1.08±0.08
NT-3.18	20.43-21.50	1.02-1.10	1.11-1.21
(125µE24hL:0hD)	20.80±0.61	1.07±0.05	1.16±0.05

❖ *Interaction between light intensity and photoperiod on population growth and biochemical composition of T. weissflogii*

The interaction between light intensity and photoperiod on the population growth of *T. weissflogii* was clearly evident and statistically robust. This effect was consistently observed across all measured growth parameters, including exponential growth rate (EGR), maximum cell density (MCD), and specific growth rate (SGR). The multiple linear regression model describing the relationship among light intensity, photoperiod, and EGR was defined as: **EGR = 1.22×LI + 2.12×PP – 1.65×(LI×PP)**. The model demonstrated high statistical significance ($p < 0.001$) and explained approximately 79.2% of the variation in EGR ($R^2 = 0.792$). Both photoperiod and light intensity exhibited significant and positive effects on the EGR of *T. weissflogii* populations. Similar to the trend observed in *C. muelleri*, the photoperiod exerted a stronger influence on population growth than light intensity. Furthermore, the interaction term between light intensity and photoperiod was statistically significant ($\beta = –1.65$, $t = –2.8$, $p = 0.01$), suggesting that the stimulatory effect of increasing photoperiod on EGRs was modulated by the duration of light exposure. This indicates a diminishing return in growth rate under conditions of simultaneous high light intensity and extended photoperiod.

Table 3.8. Results of population growth of *T. weissflogii* in 9 treatments (Min-Max, Mean±SD, n=3).

	MCDs *(×10^6 cell/mL)*	EGRs *(/day)*	Max SGRs *(/day)*
1st			
NT-3.19	0.19-0.19	1.15-1.18	1.31-1.50
(75µE12hL:12hD)	0.19±0.00	1.17±0.01	1.43±0.10
NT-3.20	0.20-0.21	1.51-1.75	1.44-1.61
(75µE18hL:6hD)	0.20±0.00	1.66±0.13	1.55±0.10
NT-3.21	0.23-0.23	1.81-1.83	1.41-1.70
(75µE24hL:0hD)	0.23±0.00	1.82±0.01	1.61±0.17
NT-3.22	0.20-0.20	1.21-1.31	1.95-2.05
(100µE12hL:12hD)	0.20±0.00	1.28±0.06	2.00±0.05
NT-3.23	0.22-0.23	1.77-1.78	1.84-1.95
(100µE18hL:6hD)	0.22±0.00	1.78±0.01	1.88±0.06
NT-3.24	0.22-0.23	1.71-1.89	1.67-2.08
(100µE24hL:0hD)	0.22±0.01	1.83±0.10	1.92±0.22
NT-3.25	0.20-0.21	1.40-1.62	1.95-2.16
(125µE12hL:12hD)	0.22±0.00	1.53±0.12	2.04±0.11
NT-3.26	0.22-0.23	1.61-1.84	1.95-1.98
(125µE18hL:6hD)	0.23±0.00	1.76±0.13	1.97±0.02
NT-3.27	0.18-0.19	1.72-1.93	1.73-1.88
(125µE24hL:0hD)	0.19±0.00	1.80±0.12	1.82±0.07

Similarly, the linear regression analyses for the remaining growth parameters yielded statistically significant models. The regression equation for predicting maximum cell density (MCD) was: **MCD = 2.18×LI + 3.37×PP – 3.77×(LI×PP)**, and for maximum specific growth rate (SGR): **SGR = 1.22×LI + 2.12×PP – 1.65×(LI×PP)**. In both cases, the models demonstrated strong statistical significance ($p < 0.001$), with consistent patterns across all examined factors. Specifically, light intensity and photoperiod each had significant and positive effects on the growth parameters, while their interaction exhibited a significant negative influence.

The effects of light intensity and photoperiod on the biochemical composition of *Thalassiosira weissflogii* were evident across several key components. An increase in light intensity was associated with enhanced synthesis and accumulation of total lipids. For example, under a photoperiod of 12h light : 12h dark, total lipid content rose from 44.08 mg/g at 75 µE/m²/s to 50.51 mg/g at 100 µE/m²/s, and further increased to 56.60 mg/g at 125 µE/m²/s. A similar trend was observed for sterol content, indicating a positive correlation with increasing light intensity. In addition, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and triacylglycerols also showed marked increases under elevated light conditions, further supporting the stimulatory effect of light intensity on lipid biosynthesis in *T. weissflogii*.

3.2.3. Influence of different temperatures on biochemical composition of selected algae strains (Exp 4)

❖ *Effect of temperature of temperature on biochemical composition of C. muelleri*

The total lipid (TL) content in this diatoms exhibited a clear upward trend with increasing temperature, rising from 43.73 mg/g at 20°C (NT-4.1) to 50.33 mg/g at 25°C (NT-4.2), and reaching a peak of 61.3 mg/g at 30°C (NT-4.3). A similar pattern was observed for sterol content, which increased modestly from 1.3% TL at 20°C to 1.7% TL at 30°C.

With regard to fatty acid composition, arachidonic acid (AA) content in *Chaetoceros muelleri* consistently increased with temperature elevation. In contrast, eicosapentaenoic acid (EPA) levels displayed a non-linear pattern. The highest EPA content was recorded at 25°C (NT-4.2) with 34.27% of total fatty acids (TFA), followed by 26.53% at 20°C (NT-4.1), and significantly declined to 18.83% at 30°C (NT-4.3). Meanwhile, docosahexaenoic acid (DHA) content remained relatively stable across the three temperature regimes.

In terms of broader fatty acid classes, saturated fatty acids (SFAs) were more abundant at higher temperatures (30°C), whereas lower temperatures (20°C) favored an increase in monounsaturated fatty acids (MUFAs). Notably, elevated proportions of polyunsaturated fatty acids (PUFAs), including long-chain PUFAs (Lc-PUFAs), were observed at the intermediate temperature of 25°C, indicating optimal conditions for PUFA biosynthesis at this temperature.

❖ *Effect of temperature of temperature on biochemical composition of P. tricornutum*

Similar to *Chaetoceros muelleri*, the total lipid content in the silicified microalga *Phaeodactylum tricornutum* increased markedly with rising environmental temperatures. Specifically, total lipid content nearly doubled, rising from 45.54 mg/g at 20°C to 89.7 mg/g at 30°C. A similar upward trend was observed in wax ester content, which increased modestly from 0.37 mg/g to 0.7 mg/g over the same temperature range. Notably, triacylglycerol and free fatty acid levels peaked at 25°C, while sterol and polar lipid fractions were relatively reduced at this intermediate temperature.

In contrast to the pattern observed for total lipid accumulation, the content of eicosapentaenoic acid (EPA), polyunsaturated fatty acids (PUFAs), and long-chain polyunsaturated fatty acids (Lc-PUFAs) was inversely related to temperature. These components were most abundant at lower temperatures and declined progressively with

increasing thermal conditions. Interestingly, the highest concentration of monounsaturated fatty acids (MUFAs) was detected at the intermediate temperature of 25°C, suggesting a temperature-dependent shift in fatty acid biosynthesis pathways.

❖ *Effect of temperature of temperature on biochemical composition of T. weissflogii*

Consistent with the trends observed in the two previously mentioned diatom species, the total lipid content in *Thalassiosira weissflogii* also increased with rising environmental temperatures. Specifically, total lipid content rose from 16.07 mg/g at 20°C (NT-4.7) to 28.6 mg/g at 30°C (NT-4.9). Due to technical limitations, some data on lipid class composition could not be collected for the NT-4.9 treatment; thus, a comprehensive assessment of temperature-induced changes in lipid class distribution was not possible. However, when comparing the two available temperature conditions—20°C and 25°C—triacylglycerol and polar lipid levels were found to be higher at 25°C, whereas wax esters, free fatty acids, and sterols were more abundant at 20°C.

The influence of temperature on the fatty acid composition of *T. weissflogii* exhibited distinct patterns compared to *C. muelleri* and *P. tricornutum*. Notably, the levels of arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) did not show a consistent trend across treatments. However, temperature-induced variation in fatty acid groups was more evident. Elevated levels of polyunsaturated fatty acids (PUFAs) and long-chain polyunsaturated fatty acids (Lc-PUFAs) were observed at 25°C, while saturated fatty acid (SFA) content increased at 30°C. The 20–25°C temperature range favored the accumulation of monounsaturated fatty acids (MUFAs), indicating a potential temperature-dependent adjustment in membrane lipid composition.

3.2.4. Influence of different salinities on population growth and biochemical composition of selected algal strains (Exp 5)

❖ *Effects of different salinities on the growth and biochemical composition of C. muelleri*

The findings of this study demonstrate that salinity exerts a significant influence on the growth of *Chaetoceros muelleri*, particularly with respect to maximum cell density (MCD). As shown in Table 3.18, the highest MCD values were recorded in the 25 ppt salinity treatment (NT-5.2) across both experimental trials, reaching 17.70 ± 0.33 and $19.52 \pm 0.52 \times 10^6$ cells/mL, respectively. These differences were statistically significant at the 0.05 level. In contrast, the lowest MCD in the first trial was observed in the 30 ppt treatment (NT-5.3), with a value of $12.82 \pm 0.50 \times 10^6$ cells/mL. In the

second trial, the lowest MCD was recorded at 20 ppt (NT-5.1), also with statistical significance at the 0.05 level.

However, when considering other growth indicators such as exponential growth rates (EGRs) and maximum specific growth rates (Max SGRs), salinity did not appear to significantly affect the growth performance of *C. muelleri*. In the first and second trials, EGRs ranged from 1.64 to 1.71 and 1.79 to 1.88 day^{-1}, respectively. Likewise, Max SGRs varied between 1.86 and 1.91 day^{-1} in the first trial, and between 1.90 and 2.06 day^{-1} in the second. One-way ANOVA revealed no statistically significant differences in these growth parameters among treatments.

In terms of biochemical composition, *C. muelleri* exhibited a clear trend of enhanced lipid synthesis and accumulation in response to increasing salinity. The total lipid content rose progressively from 145.33 mg/g at 20 ppt (NT-5.1) to 156.30 mg/g at 25 ppt (NT-5.2), and further to 182.17 mg/g at 30 ppt (NT-5.3). A similar pattern was evident in triacylglycerol levels, which increased substantially from 4.75% at 20 ppt to 10.24% and 29.15% at 25 ppt and 30 ppt, respectively.

Conversely, polar lipid content exhibited an inverse relationship with salinity. The proportion of polar lipids relative to total lipids decreased from 81.52% at 20 ppt to 81.15% at 25 ppt, and markedly declined to 58.05% at 30 ppt. These findings suggest that elevated salinity promotes lipid accumulation—particularly in storage lipids such as triacylglycerols—while concurrently reducing the relative abundance of structural lipids.

Table 3.9. Population growth of *C. muelleri* in three treatments of salinity (Min-Max, Mean±SD, n=4).

	20 ppt	**25 ppt**	**30 ppt**
1st Experiment			
MCDs *(×10⁶ cell/mL)*	14.83-15.95	17.23-17.98	12.33-13.33
	15.38±0.48[b]	17.70±0.33[c]	12.82±0.50[a]
EGRs *(/day)*	1.57-1.68	1.66-1.75	1.61-1.67
	1.64±0.05	1.71±0.04	1.64±0.03
Max SGRs *(/day)*	1.75-1.94	1.84-1.92	1.89-1.94
	1.86±0.09	1.88±0.04	1.91±0.02
2nd Experiment			
MCDs *(×10⁶ cell/mL)*	15.65-16.45	18.75-19.88	16.48-17.45
	15.97±0.38[a]	19.52±0.52[c]	17.05±0.47[b]
EGRs *(/day)*	1.79-1.92	1.88-2.00	1.88-1.96
	1.86±0.06	1.96±0.05	1.93±0.03
Max SGRs *(/day)*	1.80 -2.00	1.90-2.15	1.89-2.07
	1.90±0.08	2.06±0.11	1.99±0.08

❖ *Effects of different salinities on the growth and biochemical composition of P. tricornutum*

A salinity level of 25 ppt (NT-5.5) significantly enhanced the population growth of *Phaeodactylum tricornutum* compared to the other treatments—NT-5.4 (20 ppt) and NT-5.6 (30 ppt). In the first experiment, the maximum cell density (MCD) observed in the NT-5.5 treatment was $49.81 \pm 1.95 \times 10^6$ cells/mL, which was significantly higher than the $39.00 \pm 3.42 \times 10^6$ cells/mL recorded in NT-5.4 ($p < 0.05$). Similarly, in the second experiment, MCD in the 25 ppt treatment was markedly greater than those recorded in both the 20 ppt and 30 ppt treatments. Moreover, when considering additional growth indicators—exponential growth rates (EGRs) and maximum specific growth rates (Max SGRs)—salinity levels of 25 ppt and 30 ppt were more favorable for population growth compared to 20 ppt.

In contrast to the experimental outcomes observed for *C. muelleri*, the biochemical composition of *P. tricornutum* displayed notable variation across different salinity treatments. Specifically, total lipid content ranged from 129.43 to 139.02 mg/g among the three experimental conditions. As presented in Table 3.21, total lipid content in *P. tricornutum* showed a decreasing trend with increasing salinity. This pattern was consistent across multiple lipid classes, including wax esters, triacylglycerols, and free fatty acids. Conversely, polar lipid content tended to increase with higher salinity levels, suggesting a potential structural adaptation to osmotic stress. The sterol content remained relatively consistent across treatments, with only minor differences detected.

Table 3.10. Population growth of *P. tricornuitum* in three treatments of salinity (Min-Max, Mean±SD, n=4)

	20 ºC	**25 ºC**	**30 ºC**
1st Experiment			
MCDs *(×10⁶ cell/mL)*	34.50-42.50	48.25-52.50	46.75-48.75
	39.00±3.42[a]	49.81±1.95[b]	47.75±0.91[b]
EGRs *(/day)*	1.02-1.05	1.12-1.18	1.10-1.13
	1.03±0.01[a]	1.16±0.03[c]	1.11±0.02[b]
Max SGRs *(/day)*	1.18-1.39	1.40-1.57	1.34-1.55
	1.25±0.10[a]	1.50±0.09[b]	1.44±0.09[b]
2nd Experiment			
MCDs *(×10⁶ cell/mL)*	37.25-40.25	41.25-45.50	38.75-41.25
	38.63±1.25[a]	43.44±1.75[b]	40.25±1.22[a]
EGRs *(/day)*	1.23-1.31	1.37-1.44	1.35-1.42
	1.26±0.03[a]	1.41±0.03[b]	1.39±0.03[b]
Max SGRs *(/day)*	1.48-1.58	1.63-1.69	1.61-1.67
	1.53±0.05[a]	1.66±0.03[b]	1.64±0.03[b]

❖ *Effects of different salinities on the growth and biochemical composition of T. weissflogii*

Regarding maximum cell densities (MCDs), statistically significant differences were observed among the three salinity treatments in both experimental trials. The highest MCDs were recorded at a salinity of 25 ppt, reaching $1.45 \pm 0.06 \times 10^6$ cells/mL in the first trial and $1.35 \pm 0.11 \times 10^6$ cells/mL in the second trial. At 20 ppt and 30 ppt salinities, MCDs in the first trial were 1.25 ± 0.07 and $1.23 \pm 0.03 \times 10^6$ cells/mL, respectively, while corresponding values in the second trial were 1.06 ± 0.09 and $1.11 \pm 0.12 \times 10^6$ cells/mL, respectively.

Overall, the biochemical composition of *Thalassiosira weissflogii* exhibited minimal variation across the tested salinity levels. Total lipid content ranged from 46.7 to 59.8 mg/g across all three treatments. Polyunsaturated fatty acids (PUFAs), particularly non-methylene interrupted PUFAs, predominated in the fatty acid profile, accounting for 37.17% to 38.78% of total fatty acids (TFA). Consistent with previous studies, *T. weissflogii* demonstrated high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), highlighting its nutritional value.

Table 3.11. Population growth of *T. weissflogii* in three salinity treatments (Min-Max, Mean±SD, n=4).

	20 ppt	**25 ppt**	**30 ppt**
1st Experiment			
MCDs *(×10⁶ cell/mL)*	1.18-1.34	1.39-1.54	1.18-1.25
	1.25±0.07[a]	1.45±0.06[b]	1.23±0.03[a]
EGRs *(/day)*	1.07-1.33	1.42-1.46	0.97-1.07
	1.21±0.13[b]	1.43±0.02[c]	1.01±0.05[a]
Max SGRs *(/day)*	1.45-1.61	1.50-1.70	1.35-1.61
	1.52±0.07	1.60±0.08	1.46±0.12
2nd Experiment			
MCDs *(×10⁶ cell/mL)*	0.96-1.16	1.24-1.50	0.98-1.24
	1.06±0.09[a]	1.35±0.11[b]	1.11±0.12[a]
EGRs *(/day)*	1.00-1.29	1.26-1.39	1.19-1.35
	1.15±0.12[a]	1.31±0.06[a]	1.27±0.07
Max SGRs *(/day)*	1.27-1.39	1.39-1.79	1.29-1.50
	1.32±0.05	1.56±0.18	1.41±0.11

3.3. Investigation of microalgal strains for rotifer culture applying in larviculture

3.3.1. Growth of Brachionus plicatitis population in different microalal feeding regimes.

Overall, *Nannochloropsis oceanica* emerged as the most suitable microalgal diet for promoting rotifer population growth in this study. The highest maximum cell density (MCD) was observed in the *N. oceanica* treatment (NT-6.1), reaching approximately 738 individuals/mL. This was followed by the mixed-algae treatment (NT-6.3), with 685 individuals/mL, and the *Tisochrysis lutea* treatment (NT-6.2), which recorded the lowest value at 614 individuals/mL. These differences were statistically significant ($p < 0.05$). Similarly, the maximum specific growth rate was highest in the *N. oceanica* treatment (0.53/day), significantly exceeding the rates observed in the *T. lutea* (0.38/day) and mixed-algae (0.37/day) treatments. The proportion of egg-carrying rotifers remained low during the initial two days of culture, ranging from 0.28 to 0.38 (data not shown). However, this proportion increased markedly from day 3 to day 5 of the cultivation cycle, reaching between 0.42 and 0.53.

Table 3.12. Growth of rotifers in various treatments (Min-Max, Mean±SD, n=4)

Variables	Treatments		
	NT-6.1	**NT-6.2**	**NT-6.3**
MCDs *(inds/mL)*	700-760	580-630	670-700
	738 ± 18^{c}	614 ± 23^{a}	685 ± 13^{b}
Max SGRs *(/day)*	0.47-0.59	0.38-0.57	0.37-0.40
	0.53 ± 0.05^{b}	0.44 ± 0.08^{a}	0.38 ± 0.01^{ab}

3.3.2. Study of using rotifer as a livefeed stock in white leg shrimp larval culture

❖ <u>*Survival rate by stages*</u>

In general, the survival rate of *Penaeus vannamei* larvae declined progressively throughout the rearing cycle. During the early developmental stages (Z_1 and Z_2), survival rates were relatively uniform across treatments. Specifically, survival during the Z_1 stage ranged from 91% to 98% across all experimental groups, with minimal variation. By the Z_2 stage, however, differences began to emerge. Treatments supplemented with rotifers at densities of 75 (NT-6.5) and 100 individuals/larva/day (NT-6.6) exhibited higher survival rates of $75.2 \pm 1.32\%$ and $81.6 \pm 5.9\%$, respectively. In contrast, the treatment with 50 individuals/larva/day (NT-6.4) recorded a survival rate of $67.32 \pm 6.01\%$, while the control group (without rotifer supplementation) achieved approximately

64.2 ± 12.27%. Despite these differences, they were not statistically significant (*p > 0.05*).

Survival differences among treatments became more pronounced in the later developmental stages (M_2, PL_1, and PL_{12}). At the M_2 stage, the highest survival rates were again observed in the NT-6.5 and NT-6.6 treatments, both of which were significantly higher than the control group that did not receive rotifer supplementation (*p < 0.05*).

❖ *Body length and CV*

As the larvae transitioned to the M_2 stage, differences in larval length began to emerge. Specifically, treatments with rotifer diets at 75 individuals/larval/day (NT-6.5) and 100 individuals/larval/day (NT-6.6) showed better growth in length compared to the other two treatments. However, this difference was not statistically significant at the 0.05 level. This trend became more pronounced when the larvae progressed to the PL_1 stage. The NT-6.5 and NT-6.6 treatments resulted in significantly greater larval lengths compared to the other treatments (*p<0.05*).

Table 3.13. Body length (mm) and CV PL_{12} (%) of larval in treatments

Larval	Treatment			
	NT-6.4	**NT-6.5**	**NT-6.6**	**NT-6ĐC**
Z_2	1.74 ± 0.19	1.79 ± 0.11	1.81 ± 0.08	1.76 ± 0.08
M_2	3.39 ± 0.20	3.53 ± 0.25	3.60 ± 0.13	3.30 ± 0.24
PL_1	4.89 ± 0.53^a	5.15 ± 0.61^b	5.19 ± 0.67^b	4.82 ± 0.82^a
PL_{12}	9.58 ± 1.09	8.97 ± 0.87	9.49 ± 0.69	9.16 ± 1.15
CV PL_{12}	10.69 ± 4.38	9.67 ± 3.12	6.62 ± 2.87	11.35 ± 2.88

❖ *Development time of larvae (hours)*

The results concerning the effects of rotifer-enriched diets on the developmental progression of *Penaeus vannamei* larvae are detailed in Table 3.26 (full thesis version). Overall, larval development time was relatively consistent across treatments and replicates. However, the duration of development was notably longer than what is typically observed under commercial hatchery conditions. This extended period is likely attributable to suboptimal environmental factors, particularly the relatively low ambient temperature. Furthermore, the small volume of the rearing tanks may have contributed to increased temperature fluctuations, thereby further delaying larval development.

Among the treatments, the control group and NT-6.4 exhibited prolonged developmental timelines. In contrast, larvae reared under NT-6.5 and NT-6.6 conditions—supplemented with rotifers at 75 and 100 individuals/larva/day, respectively—demonstrated improved development rates. Observational data during the trial also indicated that larvae in

these two treatments transitioned through developmental stages more rapidly, although quantitative metrics for these observations were not showed.

Table 3.14. Development time of white leg shrimp larvae

	Treatment			
	NT-6.4	**NT-6.5**	**NT-6.6**	**NT-6ĐC**
Z_2	37.33±0.29	37.55±0.09	37.33±0.29	37.16±0.29
M_2	144.5±0.29	144.0±0.00	144.67±0.00	146.28±0.19
PL_1	218.50±0.33	217.50±0.17	216.83±0.17	222.75±0.30

❖ *Residual of environmental shock*

The health status of *Penaeus vannamei* larvae at the PL12 stage, assessed through two key indicators—salinity shock resistance and formalin tolerance—is presented in Table 3.27. Overall, no statistically significant differences were observed in shock resistance among the experimental treatments. Larvae demonstrated high survival rates following 30 minutes of exposure to both formalin (100 ppm) and hypoosmotic salinity stress (0 ppt). These findings indicate that all treatments met the quality standards for postlarval shrimp seed stock as defined by both the hatchery facility and the relevant regulatory frameworks in Vietnam. The uniform rearing conditions maintained across all treatments from PL1 to PL12 are likely a major contributing factor to the consistent health performance observed.

Table 3.15. Residual of environmental shock of larvae (%)

	Treatment			
	NT-6.4	**NT-6.5**	**NT-6.6**	**NT-6ĐC**
Salinity	93,00±2,00	94,33±1,53	95,00±3,61	91,67±1,53
Formalin	100±0,00	100±0,00	100±0,00	100±0,00

❖ *Lipid drops and gut: muscle ratio*

Two commonly employed indicators for evaluating the health status of PL12 post-larvae are the muscle-to-gut ratio and the lipid content in the hepatopancreas, assessed through the presence of oil droplets. A higher muscle-to-gut ratio is generally associated with superior larval condition, reflecting robust muscular development relative to digestive tract size. Similarly, hepatopancreas lipid reserves are classified on a scale from "A" to "E", with the "A" grade denoting abundant lipid accumulation—indicative of optimal nutritional status—while the "E" grade reflects depleted reserves and poor physiological condition. In the present study, treatments receiving higher levels of rotifer supplementation exhibited improved muscle-to-gut ratios and more favorable hepatopancreas lipid classifications, suggesting enhanced larval health under these feeding regimes.

3.4. Effects of different algae food portions on the growth, development, and health of Penaeus shrimp larvae (Exp 7)

❖ *Survival rates by stages*

In the study on *Penaeus monodon*, various microalgal diet combinations (NT-7.1 to NT-7.6) did not exhibit a statistically significant influence on larval survival during the Z_2 stage. At this early developmental phase, survival rates remained consistently high across all six treatments, ranging from 89.9% to 99.7%. However, as the larvae progressed to the M_2 stage, marked differences in survival became evident, with treatments incorporating dual-species microalgal diets (NT-7.4, NT-7.5, and NT-7.6) yielding significantly higher survival rates compared to those provided with a single microalgal species (NT-7.1, NT-7.2, and NT-7.3) *($p < 0.05$*).

These differences became more pronounced at the PL*1* stage. The NT-7.4 (Tw + Cm) and NT-7.5 (Tw + Pt) treatments achieved the highest survival rates at 91.02% and 88.76%, respectively. NT-7.6 (Cm + Pt) followed with a survival rate of approximately 79.52%. In contrast, the treatment using only *Phaeodactylum tricornutum* recorded the lowest survival rate at 67.86%. These differences were statistically significant (*$p < 0.05$*). This trend persisted until the end of the rearing period, with survival rates in NT-7.4 and NT-7.5 remaining significantly higher than those in the other treatments, except for the single-species treatment using *Chaetoceros muelleri*.

In contrast to *P. monodon*, survival rate disparities in *Penaeus vannamei* larvae emerged as early as the Z*2* stage. Notably, NT-7.10 (Tw + Cm) and NT-7.12 (Cm + Pt) exhibited the highest survival rates, at 98.34% and 99.77%, respectively. Conversely, the lowest survival rates were observed in the single-species treatments using *C. muelleri* (92.13%) and *P. tricornutum* (89.94%). From the M*2* stage onward, survival trends mirrored those observed in *P. monodon*, with the multi-species microalgal diets (NT-7.10 and NT-7.11) consistently supporting superior larval survival. In contrast, single-species diets—particularly those based solely on *P. tricornutum*—were associated with reduced survival outcomes in *P. vannamei*.

❖ *Body length and CV*

At the Z2 stage of *Penaeus monodon* larvae, treatments incorporating multi-species microalgal diets as well as the single-species *Thalassiosira weissflogii* resulted in significantly greater larval lengths compared to those receiving single-species diets of *Chaetoceros muelleri* or *Phaeodactylum tricornutum* ($p < 0.05$). A similar trend was

observed in *Penaeus vannamei* larvae, wherein treatments using multi-species combinations—particularly *T. weissflogii* + *C. muelleri* (Tw+Cm) and *T. weissflogii* + *P. tricornutum* (Tw+Pt)—consistently promoted superior larval growth relative to other treatments.

As with survival outcomes, the differences in larval length became increasingly evident in the later developmental stages, especially at PL1. Notably, the NT-7.4 treatment, which utilized a combination of *T. weissflogii* and *C. muelleri*, produced significantly longer larvae than most other dietary treatments ($p < 0.05$).

A one-way ANOVA analysis confirmed that the diet composed of *T. weissflogii* and *C. muelleri* was the most effective in promoting larval growth, as evidenced by a statistically significant increase in larval length when compared to the remaining experimental diets.

Table 3.16 Body length (mm) and CV (%) of Penaeus larvae

	Black tiger shrimp					
	NT-7.1	**NT-7.2**	**NT-7.3**	**NT-7.4**	**NT-7.5**	**NT-7.6**
Z_2	2.16 ± 0.03^{b}	2.06 ± 0.04^{a}	2.00 ± 0.07^{a}	2.26 ± 0.03^{b}	2.23 ± 0.05^{b}	2.17 ± 0.08^{b}
M_2	4.16±0.06	4.08±0.28	3.91±0.16	4.29±0.06	4.21±0.28	4.05±0.17
PL_1	6.04 ± 0.10^{b}	5.80 ± 0.09^{a}	6.10 ± 0.11^{bc}	6.29 ± 0.10^{d}	5.94 ± 0.09^{ab}	6.24 ± 0.11^{cd}
PL_{10}	10.81±0.05	10.24±0.43	10.62±0.55	10.36±0.27	10.49±0.20	10.61±0.24
KLK PL_{10}	0.76±0.03	0.59±0.11	0.76±0.07	0.65±0.10	0.66±0.01	0.75±0.09
	White leg shrimp					
	NT-7.7	**NT-7.8**	**NT-7.9**	**NT-7.10**	**NT-7.11**	**NT-7.12**
Z_2	1.85 ± 0.03^{ab}	1.83 ± 0.04^{a}	1.77 ± 0.07^{a}	2.02 ± 0.03^{c}	2.00 ± 0.04^{c}	1.94 ± 0.07^{bc}
M_2	3.77±0.05	3.70±0.24	3.56±0.14	3.88±0.05	3.81±0.24	3.67±0.14
PL_1	5.37 ± 0.09^{cd}	5.07 ± 0.08^{a}	5.33 ± 0.09^{bc}	5.49 ± 0.09^{d}	5.19 ± 0.08^{ab}	5.45 ± 0.09^{cd}
PL_{12}	10.14 ± 0.1^{c}	9.72 ± 0.3^{b}	9.19 ± 0.1^{a}	11.28 ± 0.1^{e}	10.86 ± 0.3^{d}	10.32 ± 0.1^{c}
CV PL_{12}	10.2±2.12	11.04 ±3.38	10.7±4.38	7.62±1.87	8.62±2.87	9.35±2.88

❖ *Development time of larvae*

Observing the development time of larvae across different stages revealed some distinctions compared to the two parameters of survival rate and larval length. The research findings presented in Table 3.30 indicated that treatments incorporating *Thalassiosira weissflogii* (NT-7.1, NT-7.4, NT-7.5 for *Penaeus monodon* and NT-7.7,

NT-7.10, NT-7.11 for *Penaeus vannamei*) resulted in shorter development times for the larvae compared to the other treatments.

By the time of PL_1, treatments with *T. weissflogii* in the diet reduced the development time by more than 2 days for *P. monodon* and approximately 1 day for *P. vannamei*. However, this result does not completely contradict the observations related to survival rate and larval length. In other words, the treatments combining *T. weissflogii* and *Chaetoceros muelleri* still produced favorable results in terms of larval development time for both species of shrimp in the study.

Table 3.17 Development time (hours) of Penaeus larvae

	Black tiger shrimp					
	NT-7.1	**NT-7.2**	**NT-7.3**	**NT-7.4**	**NT-7.5**	**NT-7.6**
Z_2	29.11±0.10	30.28±0.25	29.83±0.58	29.19±0.27	29.00±0.00	30.22±0.10
M_2	142.06±0.1	213.39±0.4	180.2±15.0	145.22±0.1	145.11±0.1	189.94±8.1
PL_1	204.56±0.1	280.89±8.5	276.9±0.1	204.50±0.0	204.50±0.0	268.7±13.6
	White leg shrimp					
	NT-7.7	**NT-7.8**	**NT-7.9**	**NT-7.10**	**NT-7.11**	**NT-7.12**
Z_2	35.00±0.00	35.33±0.14	35.72±0.69	32.67±0.29	33.17±0.29	34.11±0.09
M_2	136.17±0.3	137.22±0.4	143.17±0.3	134.17±0.3	134.33±0.6	135.33±0.6
PL_1	182.67±0.3	183.72±0.4	189.67±0.3	160.80±0.0	164.00±1.4	180.80±1.4

Overall, the findings from Experiment 7 demonstrated that dietary composition exerted a substantial influence on the performance of *Penaeus* shrimp larviculture. Among the tested treatments, the combination of two diatom species, *Thalassiosira weissflogii* and *Chaetoceros muelleri*, as a live feed yielded the most favorable outcomes. This specific microalgal combination consistently supported superior larval survival, growth (as indicated by body length), and accelerated development across both *P. monodon* and *P. vannamei*. These results underscore the potential advantages of multi-species microalgal diets in enhancing larval quality and improving hatchery performance.

CHAPTER 4. CONCLUSION AND RECOMMEDATONS

CONCLUSION

4.1 - The study examined the growth and biochemical composition of eight microalgal strains introduced from CSIRO, revealing that all strains adapted and thrived well under Vietnamese conditions. Their biochemical profiles were rich and suitable for aquaculture purposes. Among these, three strains (*Thalassiosira weissflogii*, *Phaeodactylum tricornutum*, and *Chaetoceros muelleri*) were identified as optimal direct food sources for shrimp larvae. Additionally, *Nannochloropsis oceanica* and *Tisochrysis lutea* were selected as indirect feed for culturing rotifers used in shrimp hatcheries.

4.2 - The environmental culture conditions have various affected on the microalgal growth and biochemical composition:

The three mediums f, f/2, and AGP-C proved highly suitable for culturing the three diatom species. Nutritional media significantly influenced population growth and the biochemical composition of microalgal cells. Among these, f medium consistently yielded the highest results for most species in this study. Different microalgal species demonstrated distinct trends in biosynthesis and biochemical accumulation.

The interaction between light intensity and photoperiod significantly influenced microalgal population growth, though the effects varied among species. *T. weissflogii* exhibited the most pronounced response, followed by *C. muelleri* and *P. tricornutum*. While the growth of microalgal populations generally increased with higher light intensity and longer photoperiods, the interaction of these two factors was inversely related to growth. The impact of light conditions on biochemical composition also varied depending on the specific species.

Temperature notably affected both microalgal population growth and cell biochemical composition. The optimal range for cultivation was 25–30°C, which maximized growth across all studied species. The influence of temperature on biochemical composition differed by species and specific temperature thresholds.

Salinity had a significant impact on the growth and biochemical composition of diatom cells. A salinity range of 25–30 ppt was optimal for cultivating the studied microalgae. Variations in salinity also caused species-specific changes in biochemical composition.

4.3 - *N. oceanica* and *T. lutea* were highly suitable for rotifer cultivation, with *N. oceanica* yielding superior results in population growth. Incorporating rotifers into the larval rearing process for *Penaeus vannamei* significantly improved performance, with optimal rotifer feeding rates of 75–100 individuals per shrimp larva per day.

4.4 - *T. weissflogii*, *P. tricornutum*, and *C. muelleri* were identified as excellent feed sources for shrimp larvae. Combining *T. weissflogii* and *C. muelleri* resulted in the highest larval rearing performance, improving growth, development, and overall larval quality across all measured parameters.

RECOMMENDATIONS

❖ This study is part of an applied research program. The ability to apply research results to practical production is considered a top criterion. This approach also reveals certain limitations, particularly in terms of academic rigor. Therefore, the first recommendation is to conduct more in-depth studies in this field, such as molecular biology research, algae cell biology, and intracellular physiological reactions, or study of isolating and applying the local microalgal strains in Penaeus larviculture in Vietnam.

❖ In terms of applied algae research, further examination of parameters such as selective feeding of larvae, digestive mechanisms, and absorption with each type of food would be beneficial.

❖ From a technical perspective, further research on cultivation technology should be conducted to further improve production efficiency, such as multi-stage algae cultivation technologies or membrane-based cultivation technologies.

❖ Finally, under favorable conditions, further in-depth biochemical analyses should be conducted. For example, in algae, this includes amino acid composition, plant growth hormones and other parameters. For shrimp larvae, analyses could focus on immune response, gut tissue sectioning, and digestive enzyme systems, and other biochemical parameters including FA and lipid class composition and content.

LIST OF PUBLICATIONs

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