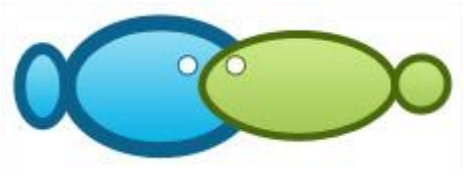

Effects of initial density, nutrient medium, salinity and light intensity on the growth of microalgae *Nannochloropsis oculata*

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Abstract. A study was conducted to determine the effect of the initial density, nutrient medium, salinity and light intensity on the growth of the microalgae *Nannochloropsis oculata*. Microalgae were grown in 20 L plastic cans at 4 initial density levels, namely, 6.5×10^4 cells mL^{-1} (T1), 7.5×10^4 cells mL^{-1} (T2); 8.5×10^4 cells mL^{-1} (T3) and 9.5×10^4 cells mL^{-1} (T4). Results showed that the cultured *N. oculata* algae reached the highest maximum density on the 10^{th} day of culture at 8.5×10^4 cells mL^{-1} (T3) with an equilibrium phase more stable than in the 3 other treatments. Three nutrient media were tested, namely: Walne, F/2, and TT3. *N. oculata* algae cultured in F/2 medium were the fastest to reach the highest maximum density, on the 10^{th} day of culture, and exhibited a stable equilibrium phase. Four salinity levels were also tested: 20, 25, 30 and 35‰. Results showed that at a salinity of 30‰, *N. oculata* reached the highest density on the 9^{th} day of culture and exhibited a stable equilibrium phase compared with other experimental salinity levels. Four levels of light intensity were tested, namely: 2,000 lux (T1), 3,000 lux (T2), 4,000 lux (T3) and 5,000 lux (T4). Results showed that the group treated at a light intensity of 3000 lux was the fastest to reach the highest density on the 10^{th} day of culture, with a fairly stable, balanced phase. In conclusion, the conditions that enabled the highest maximum density for *N. oculate* were an initial density of 8.5×10^4 cells mL^{-1}, the use of F/2 medium at 30‰ salinity and 3,000 lux of light intensity.
Key Words: day of culture, maximum density, optimum salinity, optimum light intensity.

Introduction. Microalgae are the first link in the aquatic food chain and are indispensable source of live food in the hatchery technology as well as in the commercial farming of aquatic species. They serve especially as feed for all growth stages of mollusc bivalves, larval stage of some crustaceans and fishes. *Nannochloropsis oculata* is a species of microalgae of a small size (2-4 μm), with relatively high nutritional content (Wikfors 2001; Binh & Thuy 2018). According to Zittelli et al (2003), *N. oculata* has a high content of eicosapentaenoic acid (EPA; 3.2%), ascorbic acid (0.8%) and are rich in vitamin B_{12}, being able to meet the development needs of aquatic animals at early stages of development. It is considered an important food source for rotifers, some fish larvae and other crustaceans (Trung et al 2009). Factors that affect the growth and development of microalgae include the initial density, dissolved nutrient, salinity and light intensity (Phuong et al 2018; Tram et al 2018). In this study, we determined the effects of these factors to identify the most suitable condition for microalgae growth.

Material and Method

Algal source. *N. oculata* was imported from the Institute of Aquaculture Research North Central (Nghe An) and stored at Department of Plant Cell Technology, Institute of Biotechnology, Hue University, Vietnam. Algae were inoculated in 500 mL bottles of clean treated water and cultured in F/2 medium with 24 h aeration at 33‰ salinity and pH=8. The algae were cultured until the density increased and were again inoculated to create primary seed pots.

Effects of initial density on growth of N. oculata. Four different initial densities were tested, namely: 6.5×10^4 cells mL^{-1}, 7.5×10^4 cells mL^{-1}, 8.5×10^4 cells mL^{-1} and 9.5×10^4 cells mL^{-1}. The treatments were completely randomized, with 3 replicates for each treatment. Plastic cans of 20 L were used as containers. The environmental parameters were consistent among treatments: temperature 25-270°C, pH=8, light intensity 2,000 lux, light cycle 24/24 and F/2 algal nutrient medium.

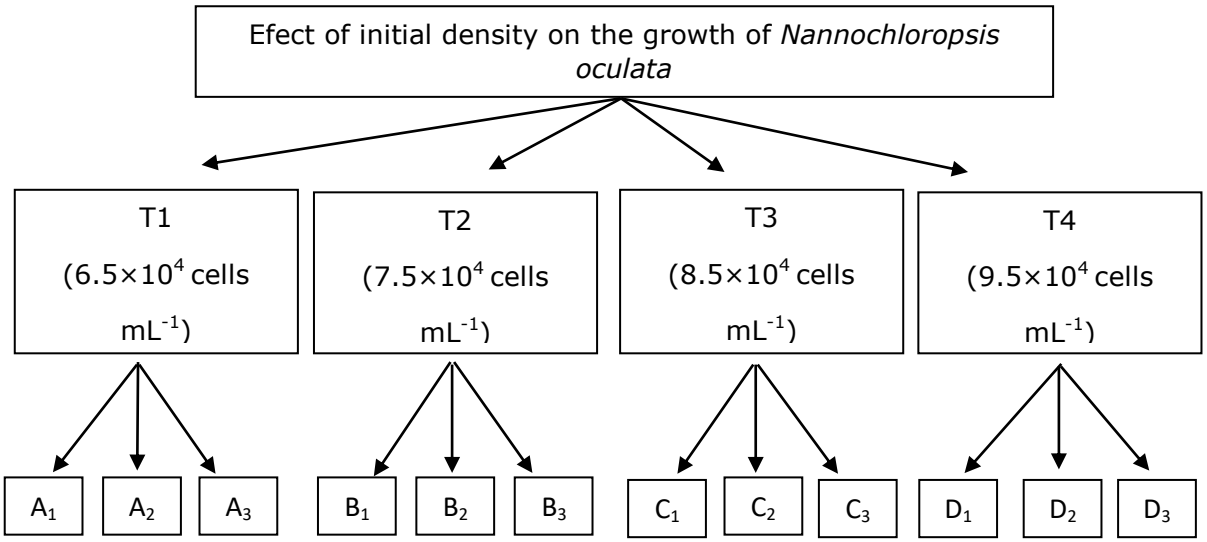

Figure 1. Schematic diagram of the experimental design: the effects of the initial density on the growth of *Nannochloropsis oculata*.

Effects of nutrient medium on growth of N. oculata. Three media were tested, namely: F/2 medium (treatment 1), Walne medium (treatment 2) and TT3 medium (treatment 3). The three culture media were completely randomized, being distributed in 9 plastic cans of 20 L volume, then each medium was replicated 3 times. Ambient conditions were as follows: temperature 25-270°C, pH=8, initial density $8.5x10^4$ cells mL^{-1}, light intensity 2,000 lux and light cycle 24/24 h.

Effects of salinity on the growth of N. oculata. Four salinity levels were tested, namely, treatment 1 at 20‰, treatment 2 at 25‰, treatment 3 at 30‰ and treatment 4 at 35‰. The treatments were completely randomized, being distributed in 12 plastic cans of 20 L volume, then each treatment was replicated 3 times. The environmental conditions were similarly arranged in the treatments: light intensity 2,000 lux, lighting cycle 24/24 h, temperature 25–27°C, pH=8 and initial density of $8.5x10^4$ cells mL^{-1}.

Effects of light intensity on growth of N. oculata. The experiment was conducted at 4 different light intensity levels, namely, treatment 1 at 2,000 lux, treatment 2 at 3,000 lux, treatment 3 at 4,000 lux and treatment 4 at 5,000 lux. The treatments were completely randomized, being distributed in 12 plastic cans of 20 L volume, then each treatment was replicated 3 times. Environmental conditions were similar in all treatments: nutrient medium F/2, light cycle 24/24h, temperature 25-27°C and pH=8.

The monitoring indicators included water environment parameters (temperature, pH, salinity, light intensity) and algal growth (density of algae through tests). The methods for determining the monitoring indicators are: the temperature and pH were measured with a Hanna HI98127 measuring pen; the light intensity was measured with an Extech light intensity meter; the cell density was determined by a Sedgewick Rafter counting chamber (capacity of 1 mL, with 1,000 count cells) and a microscope with a ×10 magnification. Algae samples were taken 1 time day^{-1}, at 8-8:30 am and each time 1 mL of medium was collected. Algae samples were placed in an Ependorf and stabilized with a Lugol's Neutral solution (Nga 2007; Cong et al 2014; Lien et al 2018).

The method of determining the density of algae was the counting chamber: the algae sample was shaken; the pasteur pipette was used to aspire the sprayed algae sample into the counting chamber, which has been covered and the sample left to settle for a while and then counted. Cells were counted inside the plot of the counting chamber under the microscope at x10 magnification. Each algae sample was counted 3 times, the formula for calculating the number of *N. occulata* cells was as described in literature (Mai 2009; Lien et al 2018; Phuong et al 2018):

$$\text{Cell density (cells mL}^{-1}) = \frac{Cx1000}{A\ x\ D\ x\ F}$$

Where:
C - countable cells;
A - area of each cell (1 mm^2);
D - height of each plot (1 mm);
F - number of plots to be counted.

Statistical analyses. Study data were collected and the mean and standard deviation were calculated using the Microsoft Excel 2015. Data were processed with the SPSS 20 software, using the one-way analysis of variance (One-Way ANOVA) and the Duncan test to find the differences between treatments (at a significance level $p<0.05$).

Results and Discussion

Effect of initial density on growth of N. oculata. The initial densities, closely related to the biomass type, and the durations of the proliferation at the maximum and optimum densities are characteristics of the algae species. There are species that require a large initial density and other require only a low initial density. In addition, depending on the production needs, one could increase the initial density at the appropriate threshold density to shorten the time to reach the maximum biomass (the more cells involved in the cleavage, the higher the density), shortening the time of the induction phase. In other cases, if it is necessary to maintain the culture of algae over a long period of time, a suitable culture density is necessary. The experiment results related to the effect of the initial density on the growth of *N. oculata* algae is shown in Table 1 and Figure 2.

Table 1

Density of *Nannochloropsis oculata* at different initial densities

Days	*Density (10^4 cells mL^{-1})*			
	6.5 x 10^4	*7.5 x 10^4*	*8.5 x 10^4*	*9.5 x 10^4*
1	6.50^{a}±0.02	7.50^{b}±0.02	8.50^{c}±0.01	9.50^{d}±0.02
2	8.37^{a}±0.04	12.07^{b}±0.06	13.32^{c}±0.05	14.34^{d}±0.28
3	13.08^{a}±0.12	18.98^{b}±0.26	22.78^{c}±0.49	25.12^{d}±0.23
4	25.12^{a}±0.68	40.12^{b}±0.58	43.96^{c}±0.14	60.72^{d}±0.01
5	45.11^{a}±0.30	68.51^{b}±0.28	95.27^{c}±0.58	107.24^{d}±1.38
6	79.04^{a}±0.47	93.67^{b}±0.48	142.35^{c}±0.71	156.47^{d}±0.46
7	114.21^{a}±0.38	129.74^{b}±0.74	185.09^{c}±0.20	193.28^{d}±0.08
8	143.10^{a}±0.25	162.80^{b}±1.28	220.12^{c}±0.29	234.25^{d}±0.85
9	170.12^{a}±0.36	198.45^{b}±0.95	254.31^{c}±0.61	259.18^{d}±0.13
10	183.82^{a}±0.32	205.82^{b}±0.18	267.24^{d}±0.37	239.54^{c}±0.02
11	175.10^{a}± 0.26	185.72^{b}±0.12	262.95^{d}±0.08	203.56^{c}±0.14
12	144.14^{a}±0.07	159.50^{b}±0.67	234.51^{d}±0.89	169.32^{c}±0.02
13	93.72^{a}±0.29	128.95^{b}±0.29	195.67^{d}±0.30	140.46^{c}±0.01
14	54.15^{a}±0.46	96.45^{b}±0.17	148.34^{d}±0.16	98.63^{c}±0.08

The superscript letters $^{a, b, c}$ in the same column at different times between treatments with different algae density are statistically different ($p<0.05$), (± SEM, standard error of the mean values).

The density of *N. oculata* increased slowly in the first 2 days and grew rapidly from day 3 onwards in all 4 treatments (Table 1). From day 1 to 9, algae densities in the treatments exhibited significant differences ($p<0.05$). At an initial density of $9.5x10^4$ cells mL^{-1}, the algal density gradient was constantly the highest throughout the experiment, followed by $8.5x10^4$, $7.5x10^4$ and $6.5x10^4$. On day 9, the algae with an initial density of $9.5x10^4$ cells mL^{-1} reached the maximum density of 259.18×104 cells mL^{-1}, while in treatments with the initial densities of $8.5x10^4$ and 7.5x104 cells mL^{-1}, the algae exhibited final densities of $254.31×10^4$ cells mL^{-1} and $198.45×10^4$ cells mL^{-1}, respectively. The lowest final algae density in treatment 1 was $170.12×10^4$ cells mL^{-1}. Treatments 1 to 3 all reached maximum densities on day 10, with the density of $183.82×10^4$ cells mL^{-1}, $205.82×10^4$ cells mL^{-1} and $267.24×10^4$ cells mL^{-1}, respectively. From day 10 to the end of the experiment, the algae density gradient in the treatment with the initial density of $8.5×10^4$ cells mL^{-1} was always the highest, followed by the treatments with initial densities of 9.5×104 cells mL^{-1}, $7.5×10^4$ cells mL^{-1} and $6.5×10^4$ cells mL^{-1}. The differences between the algal densities were statistically significant in all 3 treatments ($p<0.05$).

Figure 2 shows that algae started to grow rapidly from day 3 and peaked on days 9 and 10. After reaching its maximum, the density tended to decrease gradually resulting from a progressive turn to the dying stage. Treatment 3 was always the most efficient, followed by treatments 4, 2 and 1 in this order. Treatment 4 increased rapidly and decreased faster than the other 3 treatments, as a result of the high initial density, number of cells involved in cleavage and rapid increase in cell density. This process quickly depletes the nutrient source of the culture medium, also reducing the light received by the cells, which is a limiting factor for the growth of algae. The higher the density of the algae, the faster the obscuration phenomenon, leading to the rapid decline of the algal population.

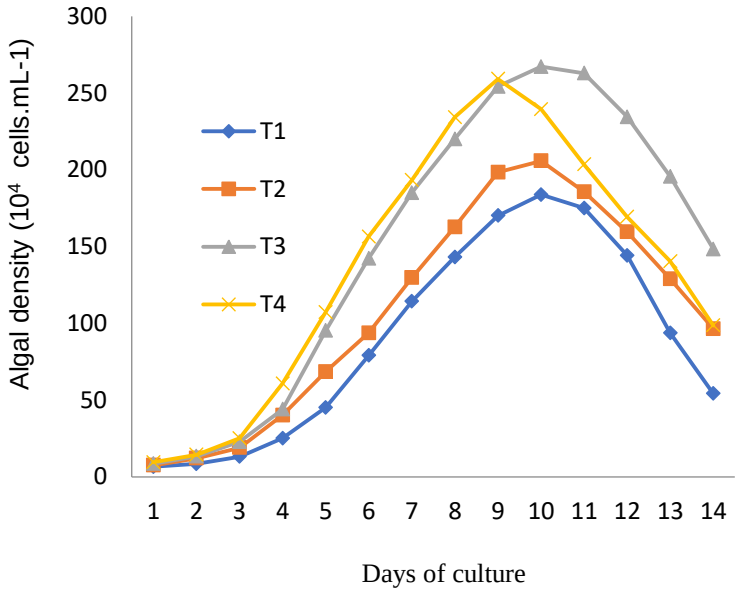

Figure 2. Growth curve of *Nannochloropsis oculata* at different initial densities.

The maximum densities of algae in different treatments differed from each other (Figure 3). Results show that algae culture at an an initial density of $6.5×10^4$ cells mL^{-1} (T1) grew more slowly than under the other treatments and exhibited the lowest maximum density among the treatments, reaching $183.82×10^4$ cells mL^{-1} after 10 days of culture. Algae cultured at the initial density of $9.5×10^4$ cells mL^{-1} reached the maximum density a day earlier than the other 2 treatments, but collapsed very quickly. Therefore, if the intention was to culture as fresh food for the hatchery, the harvesting period was short and the production efficiency was not high enough. In contrast, under the treatments 1

and 2, algae almost simultaneously reached the maximum density on the 10th day of culture, but in the treatment 3, the equilibrium phase was more stable and the collapse was slower than in treatments 1 and 2.

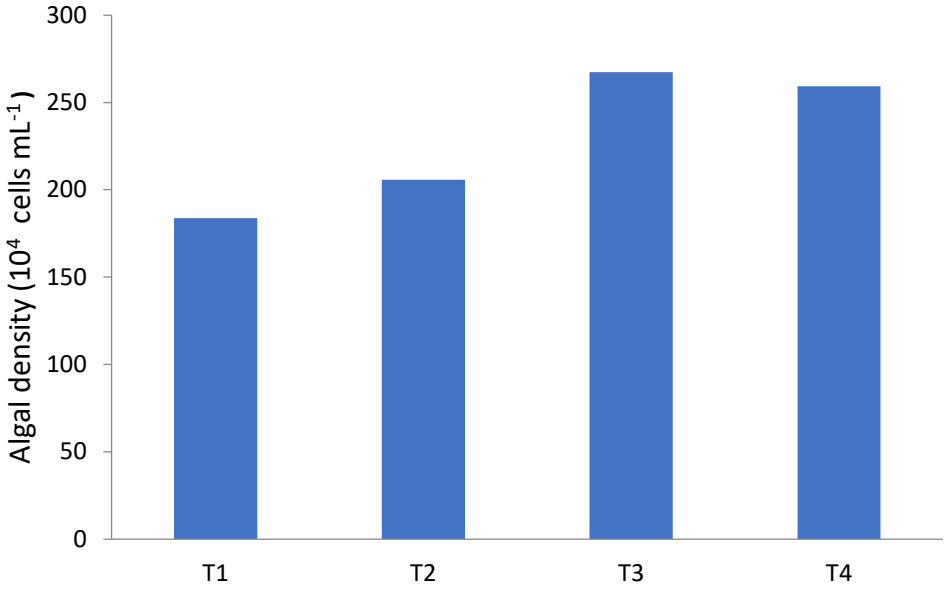

Figure 3. Maximum densities of *Nannochloropsis oculata* at different initial densities.

Research results showed that *N. oculata* cultured at different initial densities exhibited different growth. This observation agrees with previous studies done on the effects of the initial density on the growth of *N. oculata*. Trung et al (2009) studied the effects of initial density and harvest rate on *N. oculata* growth in a continuous water pipeline system. Their results indicate that the initial densities of 8 and 10 million cells mL^{-1} resulted in the highest algal biomass. However, at an initial density of 10 million cells mL^{-1}, algal growth is not stable, does not exhibit a standard growth curve and it only consumed more algal seeds unnecessarily; thus, consequently the initial density of 8 million cells mL^{-1} is recommended by these authors to be the most suitable initial density for algae culture in transparent ducts. Cam et al (2016) observed that the initial density affects the maximum growth rate and time of *N. oculata* population. For experimental initial densities of 5, 10, 20 and 30 million cells mL^{-1}, the maximum growth of a population with the initial densities of 20 and 30 million cells mL^{-1} did not differ significantly, reaching 310 million cells mL^{-1} after 15 days. However, a population of 5 million cells mL^{-1} reached the same maximum density, but the culture time was longer than 3 days (i.e., 18 days of culture) compared with that at the initial stocking density of 20 and 30 million cells mL^{-1}. In the present study, among the 4 experimental initial density levels, initial densities of $8.5x10^4$ and $9.5x10^4$ cells mL^{-1} resulted in the highest maximum algal densities. However, microalgae grew more rapidly and more stably at an initial density of 8.5×10^4 cells mL^{-1} than did the $9.5x10^4$ cells mL^{-1}, reaching a maximum density at day 10.

Effects of nutrient medium on growth of N. oculata. Currently, there are many different nutritional environments used for algae farming. Depending on the types of algae and water properties, people choose a suitable environment to achieve high biomass with low cost. In the present experiment, 3 different nutrient media were compared. Results of algae growth are presented in Table 2 and Figure 4.

Table 2 shows that in the first 3 days of the experiment, algae growth was significantly different between experimental environments ($p<0.05$). The density of algae increased gradually but slowly, perhaps due to the adaptation to the culture environment. From day 4 onwards, algae cells began to grow rapidly in all treatments and exhibited significant differences ($p<0.05$).

Table 2

Density of algae in different nutrient media

Days	Density (10^4 cells mL^{-1})		
	F/2	*Walne*	*TT3*
1	8.50^{a}±0.02	8.50^{a}±0.00	8.50^{a}±0.01
2	12.52^{c}±0.02	12.36^{b}±0.02	11.92^{a}±0.15
3	20.39^{b}±0.01	20.52^{c}±0.01	19.78^{a}±0.02
4	45.34^{c}±0.06	36.78^{b}±0.08	33.90^{a}±0.02
5	76.02^{c}±0.01	58.12^{b}±0.02	47.58^{a}±0.01
6	130.36^{c}±0.01	98.36^{b}±0.90	71.56^{a}±0.65
7	174.17^{c}±0.02	127.42^{b}±0.85	95.21^{a}±0.02
8	224.71^{c}±0.27	155.92^{b}±0.67	121.56^{a}±1.13
9	286.17^{c}±1.13	188.28^{b}±0.38	146.03^{a}±1.62
10	316.55^{c}±1.19	205.46^{b}±0.74	165.44^{a}±1.26
11	310.49^{c}±0.45	226.35^{b}±0.83	175.63^{a}±1.29
12	304.89^{c}±0.10	248.79^{b}±1.19	195.86^{a}±0.46
13	287.03^{c}±1.70	239.65^{b}±0.97	223.22^{a}±1.48
14	259.12^{c}±0.79	202.32^{b}±0.80	214.98^{a}±0.88
15	230.55^{c}±0.06	197.91^{b}±0.71	183.93^{a}±1.09
16	192.46^{c}±0.08	146.28^{b}±0.37	145.63^{a}±0.96
17	150.12^{c}±0.03	109.82^{b}±0.78	110.65^{a}±0.48

The superscript letters $^{a, b, c}$ in the same column at different times between treatments with different algae density are statistically different ($p<0.05$), (± SEM, standard error of the mean values).

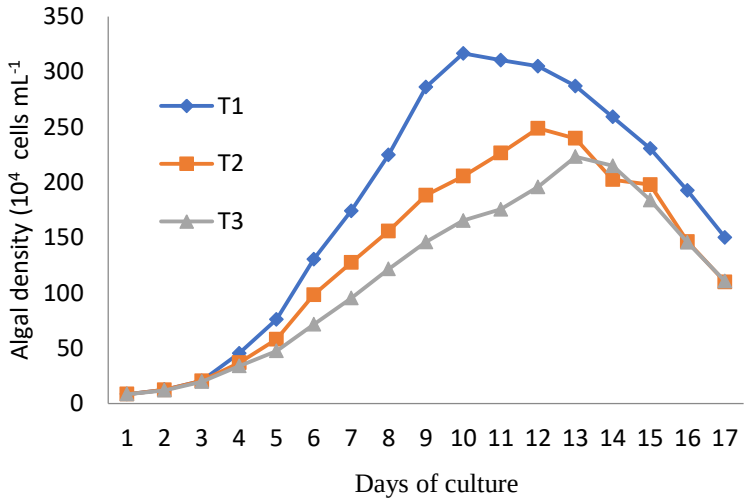

Figure 4. Growth curve of *Nannochloropsis oculata* cultured in different nutrient media.

Results showed that at similar periods, the algal density in the TT3 medium was always lower than in the other 2 treatments, Walne and F2 (Figure 4). This may be due to differences in the nutritional composition of the media, although all three media contain the major components such as nitrogen and phosphorus. The nitrogen source of the TT3 medium was the KNO_3 salt, while F/2 and Walne both have nitrogen from the $NaNO_3$ salt. The TT3 medium does not contain other trace elements and vitamins which are necessary for the growth and development of microalgae, which may be the reason for which *N. oculata* had the lowest density in the TT3 medium, compared with F/2 and Walne medium. The phosphorus sources of the three media were the Na and K salts of the H_2PO_4.

In the treatment 1, the algal density reached its maximum on day 10 (316.55×10^4 cells mL^{-1}). Treatments 2 and 3 continued to grow and reached their maximum densities on days 12 (248.79×10^4 cells mL^{-1}) and 13 (223.22×10^4 cells mL^{-1}),

respectively. Thus, algae in the F/2 medium exhibited the highest maximum density, followed by the Walne medium and the TT3 medium. Results show that in the 3 experimental nutrient media, *N. oculata* algae grew better in the F/2 medium, probably due to the different nutritional composition of the environment. TT3 nutrient medium has a simple nutritional profile, lacking trace elements such as Mn, Zn, Cu, Co and vitamins such as B1, B12. Almost all of these trace elements have effects on the algal metabolism, which are required by the enzymatic reactions. Even in very small amounts, a vitamin can promote an increase in the algal biomass. F/2 and Walne medium both contain the same main components, such as $NaNO_3$, NaH_2PO_4, EDTA, $FeCl_3$, and trace elements, such as Cu, Zn, Mn, Co, among others, and these are the essential nutrients for algal growth. Although the nitrogen and phosphorus content of the Walne medium was higher than the F/2 medium, there were more vitamins in the F/2 medium. Also, the Walne medium in the present study had no silicon source from the Na_2SiO_3 salt, while the F/2 medium the Na_2SiO_3 content was 30 mg L^{-1}. Silicon is essential for algal growth, because it is involved in the structure of the cell membranes. The F/2 medium appeared to be the optimal environment for the growth and development of *N. oculata* algae.

Among the three experimental media, F/2 enabled the best growth of *N. oculata*. At uniform initial densities under various media, *N. oculata* in the F/2 medium always reached higher densities, considering the same day of culture. The duration of the equilibrium phase was relatively long and signs of decline also occured slower than in the media Walne and TT3. This result was in line with Giang (2010), regarding the effect of culture conditions on the growth of microalgae *N. oculata* isolated from mangrove forests in Xuan Thuy district, Nam Dinh province in Vietnam. The latter study has observed that the F/2 medium was the most suitable medium for culturing *N. oculata*, due to the prolonged equilibrium phase and slower decline phase than in the other tested media. In the light of the above results, we chose the F/2 medium in the subsequent experiment on the effect of different salinity levels on the growth of the studied microalgae.

Effect of salinity on growth of N. oculata. Salinity is one of the very important factors determining the distribution as well as the growth and development of algae, and influencing algal biomass. Different species of algae are able to adapt to different ranges of salinity. Results of the effect of salinity on the growth of *N. oculata* are shown in Table 3 and Figure 5.

Table 3

Density of *Nannochloropsis oculata* at different salinity levels

Days	*Density (10^4 cells mL^{-1})*			
	20‰	*25‰*	*30‰*	*35‰*
1	8.50^{a}±0.01	8.50^{a}±0.03	8.50^{a}±0.06	8.50^{a}±0.01
2	12.32^{a}±0.30	12.79^{b}±0.30	14.47^{d}±0.18	14.03^{c}±0.03
3	22.91^{a}±0.32	23.66^{b}±0.37	27.64^{d}±0.15	26.97^{c}±0.02
4	41.24^{a}±0.73	43.33^{b}±0.50	62.98^{d}±0.33	60.41^{c}±0.58
5	60.23^{a}±0.05	61.74^{b}±0.09	102.98^{d}±0.10	78.32^{c}±0.36
6	77.48^{a}±0.54	83.15^{b}±0.26	149.16^{d}±0.08	102.08^{c}±0.03
7	90.11^{a}±0.57	111.96^{b}±0.63	199.23^{d}±0.77	149.71^{c}±0.77
8	116.05^{a}±1.18	137.42^{b}±0.83	256.09^{d}±0.41	178.69^{c}±0.95
9	129.21^{a}±1.47	154.09^{b}±0.73	294.29^{d}±1.01	247.51^{c}±0.55
10	144.77^{a}±0.26	171.34^{b}±0.94	293.80^{d}±1.00	275.14^{c}±0.32
11	157.77^{a}±0.77	184.85^{b}±0.74	289.39^{d}±0.22	270.20^{c}±0.70
12	171.38^{a}±0.93	207.73^{b}±0.64	268.65^{d}±0.35	255.62^{c}±0.70
13	197.57^{a}±0.64	214.35^{b}±0.55	241.53^{d}±0.37	228.31^{c}±0.83
14	189.08^{a}±1.15	204.79^{b}±0.86	215.90^{d}±0.07	204.52^{b}±0.01
15	174.87^{a}±0.73	184.67^{c}±0.82	186.72^{d}±0.30	175.25^{b}±0.13
16	147.59^{a}±0.26	152.90^{b}±0.01	160.12^{d}±0.01	154.26^{c}±0.21

The superscript letters $^{a, b, c}$ in the same column at different times between treatments with different algae density are statistically different ($p<0.05$), (± SEM, standard error of the mean values).

Microalgae at different salinity levels exhibited different growth patterns (Table 3). After 2 days, algal density started to increase in all 4 treatments, albeit with different patterns between the treatments.

From days 4 to 8, algae began to grow fast in all treatments and significant differences were observed ($p<0.05$) (Table 3). The treatment 3 exhibited the maximum algal density on day 9 (294.29×10^4 cells mL^{-1}). Algae were in equilibrium on day 10 and day 11. From day 12 onwards, algal density declined sharply, after switching to the dying stage. Algae in the treatment 4 peaked more slowly on day 10 with a maximum density of 275.14×10^4 cells mL^{-1} and decreased sharply from day 12 onwards, due to the transition to the decline stage.

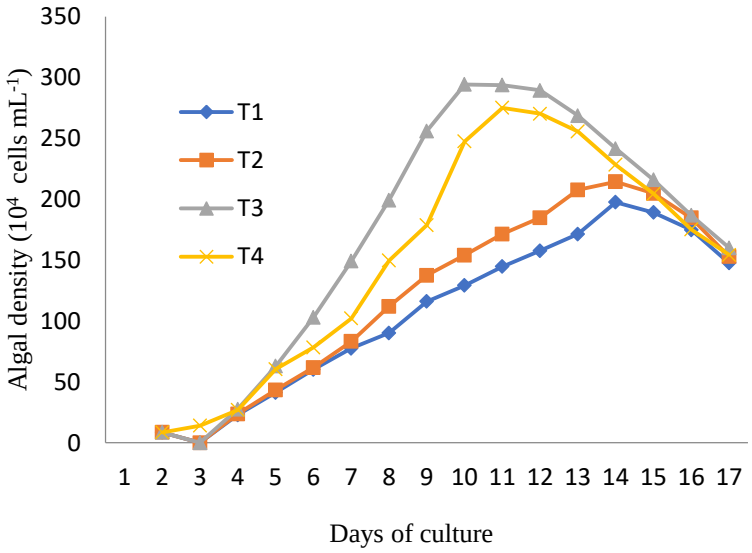

Figure 5. Growth curve of algae at various salinity levels.

For the treatments 1 and 2, the algal density continued to increase until day 13, when the algae under both treatments reached the maximum densities of 197.57×10^4 cells mL^{-1} and 214.35×10^4 cells mL^{-1}, respectively. When the algae transitioned to the dying stage, density quickly decreased.

Results show that in the treatment 3, algae grew faster and reached a maximum density of 294.29×10^4 cells mL^{-1} at day 9 (Figure 5) while in treatment 4, algae reached the maximum at day 10, with a maximum density of 275.14×10^4 cells mL^{-1}. Algae reached the lowest density in the treatments 1 and 2, both on the 13th day with a maximum density of 197.57×10^4 cells mL^{-1} and 214.35×10^4 cells mL^{-1}, respectively. Thus, *N. oculata* received the maximum density under the treatment 3 gave, followed by the treatment 4; the algal density was the lowest in the treatments 2 and 1. Thus, *N. oculata* algae tended to prefer a higher salinity (30-35‰). During the experiment, *N. oculata* under the treatment 3 always reached a higher density on the same culture day, the maximum density was always higher than the other treatments, the equilibrium phase was longer and transition to the dying stage were slower than those in the 3 treatments 1, 2 and 4. Thus, *N. oculata* should be cultured at 30‰ salinity. This result agreed well with those of Hong (1999) in their study on salinity, light and harvest rate on some biological characteristics, biochemical composition of two algae species *N. oculata* and *Chaetoceros mulleri* in laboratory conditions. Their results show that *N. oculata* grows well at high salinity of 30-35‰ in which the algae produces high nutritional yield and quality. The results of the present study also agrees well with those of Giang (2010) on *N. oculata* isolated from mangrove forests in Xuan Thuy district which showed that the salinity suitable for microalgae *N. oculata* growing is from 30-40‰.

Effect of light intensity on the growth of N. oculata. Light duration, intensity and quality are three decisive factors which influence the growth and development of photosynthetic organisms in general and of algae in particular. In particular, light intensity is considered to be the first factor that directly affects the synthesis of the components in the cell. According to Lavens & Sorgeloos (1996), when the light intensity is too high, photo-oxidation occurs. The reason may be a too strong photosynthesis process in the algae, the algae cells producing an excessive amount of oxygen, inhibiting the growth and possibly inducing toxicity to cells. However, there are also some species of algae that are able to tolerate an intense light intensity, due to their antioxidant enzymes.

The results of the study of the effect of light intensity on the growth of microalgae *N. oculata* are shown in Table 4.

Table 4

Density of *Nannochloropsis oculata* algae at different light intensity levels

Days	*Density (10^4 cells mL^{-1})*			
	2,000 lux	*3,000 lux*	*4,000 lux*	*5,000 lux*
1	8.50^{a}±0.01	8.50^{a}±0.02	8.50^{a}±0.01	8.50^{a}±0.01
2	12.69^{a}±0.15	14.07^{b}±0.08	15.02^{c}±0.04	15.37^{c}±0.12
3	21.14^{a}±0.22	25.49^{b}±0.07	28.64^{c}±0.10	29.64^{d}±0.35
4	35.46^{a}±0.25	47.90^{b}±0.07	51.72^{c}±0.06	61.72^{d}±0.27
5	56.50^{a}±0.14	86.24^{b}±0.08	91.39^{c}±0.03	111.45^{d}±0.02
6	85.98^{a}±0.12	129.56^{b}±0.24	132.23^{c}±0.19	175.09^{d}±0.68
7	120.40^{a}±0.07	188.09^{c}±0.13	170.26^{b}±0.15	226.04^{d}±0.25
8	157.32^{a}±0.06	235.75^{c}±0.03	203.35^{b}±0.08	264.12^{d}±0.12
9	191.76^{a}±0.18	272.21^{d}±0.09	223.12^{b}±0.12	235.12^{c}±0.07
10	217.90^{b}±0.10	283.27^{d}±0.05	235.32^{c}±0.11	209.63^{a}±0.04
11	226.12^{c}±0.20	270.58^{d}±0.10	195.34^{b}±0.06	168.26^{a}±0.17
12	210.27^{c}±0.09	238.64^{d}±0.10	151.78^{b}±0.12	124.12^{a}±0.54
13	175.52^{c}±0.07	192.34^{d}±0.05	113.56^{b}±0.12	82.32^{a}±0.70
14	120.22^{c}±0.08	142.16^{d}±0.05	76.90^{b}±0.01	47.14^{a}±0.15

The superscript letters $^{a, b, c}$ in the same column at different times between treatments with different algae density are statistically different ($p<0.05$), (± SEM, standard error of the mean values).

Results showed that in the first 3 days, *N. oculata* in all 4 treatments grew slowly, but from day 4 onwards, algae grew faster (Table 4). The algae density exhibited significant differences ($p<0.05$) from day 2 and algae density was always the highest in the treatment 4, reaching the density of 264.12×10^4 cells mL^{-1}, on day 8. Algal density in the treatment 2 was always higher than under the other 3 treatments. On day 10, the algae under the treatments 2 and 3 reached the maximum densities of 283.27×10^4 cells mL^{-1} and 235.32×10^4 cells mL^{-1}, respectively. The treatment 1 reached the maximum density one day later, with a value of 226.12×10^4 cells mL^{-1}. After reaching the maximum density, algae in all 3 treatments tended to a transition to the decline stage and their color turned from dark green to light green or yellow.

Figure 6 shows the density of *N. oculata* at different light intensities. Results showed that the algae in the treatment 1 reached the lowest maximum density, followed by the treatments 3 and 4 and by the highest maximum algae density, reached in the treatment 2. Algae in the treatments 3 and 4 was arranged with high light intensity of 4,000 lux and 5,000 lux, after reaching the maximum density, algae transitioned to the dying stage and declined more abruptly than did the 2 other groups. The probable reason was an increase of the light intensity, accelerating the processes of photosynthesis and cell division and resulting in a rapid increase of algal biomass. However, high density and temperature resulted in a faster decline.

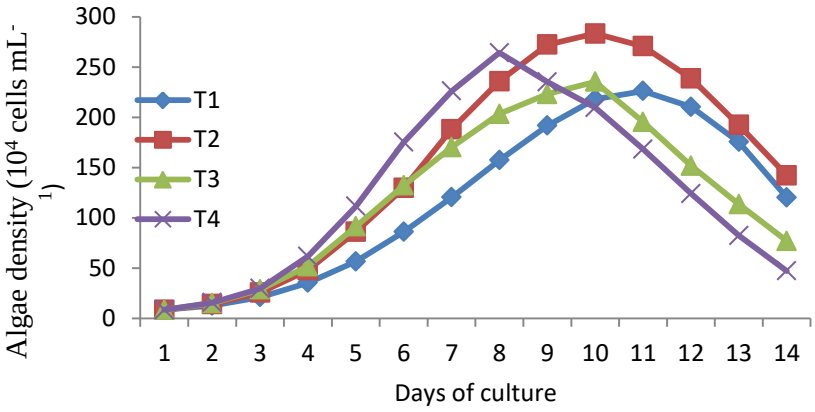

Figure 6. Growth curve of *Nannochloropsis oculata* at different light intensities.

Given that the initial density and nutrient medium were similar in all treatments, at a light intensity of 3,000 lux, *N. oculata* reached the highest maximum density, the equilibrium phase was more stable and the process fading also occurred more slowly than under the three treatments with illumination intensities of 2,000, 4,000 and 5,000 lux. Lavens & Sorgeloos (1996) also showed that the growth of the microalgae *N. oculata* is highly dependent on light intensity and depth, and that a high light intensity is required for outdoor biomass culture; under laboratory conditions, the light intensity required for algal growth ranged between 1,000 and 5,000 lux. Our study also showed that the growth and stability of the algae *N. oculata* was optimal at 3,000 lux, while at 5,000 lux the algae grew rapidly but died quickly.

Spirulina platensi also has a range of light intensity suitable for algae growth similar to *N. oculata*. Tram et al (2018) demonstrated that *S. platensi* collected in the Thanh Hoa (TH) and Binh Thuan (BT) provinces required a light intensity of 4,000 lux and 3,000 lux, respectively. Unlike the two types of algae mentioned above, *Thalassiosira weissflogii* algae species requires a higher light intensity, of 5,000 lux (Cong et al 2014).

Conclusions. The most suitable initial density for growing the microalgae *N. oculata* was 8.5×10^4 cells mL^{-1}. At this density, the algae reached the highest maximum density and the equilibrium phase time was more stable than the other initial densities. F/2 is the ideal nutrient medium to grow the microalgae *N. oculata* that gave the best growth and development results, cultured algae reached maximum density earlier than either the Walne or the TT3 medium. Salinity of 30‰ was the preferred salinity level to grow the microalgae. At light intensity of 3,000 lux, the algae reached the maximum density and could be considered a suitable light threshold to grow the microalgae.

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Conflict of interest. The authors declare no conflict of interest.

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