



Effects of Nitrogen and Light Intensity on the Astaxanthin Accumulation in Motile Cells of *Haematococcus pluvialis*

Feng Li^{1,2,3,4}, Minggang Cai^{2,3,4}, Yanqi Wu¹, Qingsheng Lian¹, Zuyuan Qian¹, Jiansen Luo¹, Yulei Zhang¹, Ning Zhang¹, Changling Li^{1*} and Xianghu Huang^{1*}

¹ College of Fisheries, Guangdong Ocean University, Zhanjiang, China, ² Fujian Provincial Key Laboratory for Coastal Ecology and Environmental Studies, Xiamen University, Xiamen, China, ³ Key Laboratory of Marine Chemistry and Applied Technology, Xiamen University, Xiamen, China, ⁴ College of Ocean and Earth Science, Xiamen University, Xiamen, China

OPEN ACCESS

Edited by:

Pengfei Cheng,
Ningbo University, China

Reviewed by:

Fatimah Md Yusoff,
Putra Malaysia University, Malaysia
Jianhua Fan,
East China University of Science and
Technology, China

*Correspondence:

Changling Li
licl@gdou.edu.cn
Xianghu Huang
huangxh@gdou.edu.cn

Specialty section:

This article was submitted to
Marine Fisheries, Aquaculture and
Living Resources,
a section of the journal
Frontiers in Marine Science

Received: 31 March 2022

Accepted: 27 April 2022

Published: 24 May 2022

Citation:

Li F, Cai M, Wu Y, Lian Q, Qian Z,
Luo J, Zhang Y, Zhang N, Li C and
Huang X (2022) Effects of Nitrogen
and Light Intensity on the
Astaxanthin Accumulation in Motile
Cells of *Haematococcus pluvialis*.
Front. Mar. Sci. 9:909237.
doi: 10.3389/fmars.2022.909237

The dietary supplementation of *Haematococcus pluvialis* is a natural, safe, and sustainable method for fish pigmentation. However, astaxanthin-rich *H. pluvialis* cysts have a poor effect in pigmenting salmonid flesh due to their rigid and thick cell wall. *H. pluvialis* thin-walled motile cells have recently attracted attention due to their potential advantages in maintaining compound stability, easy digestion, enhancing the bioavailability of carotenoids, and reducing production costs. This study aimed to investigate the effect of various nitrogen concentrations and light intensities on astaxanthin production in motile cells. We first investigated the effect of four different concentrations of nitrogen on astaxanthin accumulation in motile cells. According to the results, the motile cells had the highest astaxanthin concentration and content under the 0 N condition. Then, we compared the differences in astaxanthin production in motile cells under three different light intensities under 0 N conditions. The results showed that after four days of treatment, the protoplasts of the motile cells in the medium light (ML) group and the high light (HL) group had distinct granularity. The cell mortality rate in the HL group reached more than 15%, which was significantly higher than that in the low light (LL) and ML groups, indicating that high light intensity was not suitable for inducing motile cells to accumulate astaxanthin. There were no significant differences between the LL and ML groups in astaxanthin content, motile cells percentage, and cell mortality rate. Considering these indicators, we recommended inducing motile cells to produce astaxanthin under low light conditions because it is more economical in terms of electricity consumption. This study added to the knowledge that nitrogen and light affects the accumulation of astaxanthin in *H. pluvialis* motile cells. The results would help determine the optimal nitrogen and light conditions in astaxanthin production from motile cells.

Keywords: astaxanthin, *Haematococcus pluvialis*, motile cells, nitrogen starvation, light intensity

1 INTRODUCTION

Farmed fish is globally recognized as one of the healthiest foods (Tacon and Metian, 2013) and plays a significant role in global food security and nutrition strategies. With the continuous increase in the scale of aquaculture, people have higher and higher requirements for farmed fish. Fish flesh color plays an important role in consumer sensory evaluation and sales price (Hart and Colombo, 2022). While flesh color doesn't directly affect flesh quality, consumers often consider it as a sign that fish is fresh and tasty or not (Yesilayer, 2020). Astaxanthin is the main carotenoid responsible for the red or pink pigmentation of most wild salmonids (Nogueira et al., 2021), and accounts for more than 85% of the total carotenoid content in salmonids fresh (Torrissen et al., 1995). Feeding salmonids' diets containing astaxanthin has become the main means of improving their flesh and skin color (Tejera et al., 2007; Kalinowski et al., 2011; Rahman et al., 2016). At present, synthetic astaxanthin occupies more than 95% of the market share astaxanthin (Shah et al., 2016), which is widely used in aquaculture due to its lower production cost (Panis and Garreon, 2016; Li et al., 2020a). However, it has raised consumer concerns about food safety, pollution, and sustainability due to synthetic astaxanthin derived from petrochemicals (Li et al., 2011; Stachowiak and Szulc, 2021). Consumer preference is shifting towards nature astaxanthin (Young et al., 2017; Viera et al., 2018).

Microalgae as fish feed additives have advantages in terms of safe availability of ingredients, ease of cultivation operations, and sustainability of production. *H. pluvialis* could accumulate high levels of astaxanthin (3–5% dry weight) (Lorenz and Cysewski, 2000; Ambati et al., 2014) and is regarded as the most potent natural source of astaxanthin (Bowen et al., 2002). Several studies indicated that dietary supplementation of *H. pluvialis* would improve the color, immune and antioxidant capacity of fish (Moretti et al., 2006; Sheikhzadeh et al., 2012; Li et al., 2018; Yu et al., 2021). However, *H. pluvialis* red cysts typically have thick and less digestible cell walls (Hagen et al., 2002; Shah et al., 2016), which reduce the bioavailability of astaxanthin (Sommer et al., 1991; Choubert et al., 2006). As a result, the tissue pigmentation levels of fish fed *H. pluvialis* were generally lower than those in fish fed synthetic astaxanthin (Choubert et al., 2006; Li et al., 2014). Recent studies suggested that whole-celled *H. pluvialis* with thin cell walls achieves the same efficiency at pigmenting the flesh of rainbow trout as synthetic astaxanthin (Hart and Colombo, 2022). It was attributed to the ease of digestion of *H. pluvialis* thin-walled cells.

There are various types of cells in the *H. pluvialis* life cycle, including motile cells, nonmotile cells, and red cyst cells (Li et al., 2019a), and only motile cells have thin cell walls. More attention has been paid in the past periods to enhancing astaxanthin accumulation in the thick-walled cysts (Boussiba and Vonshak, 1991; Kobayashi et al., 1997; Li et al., 2019b; Fang et al., 2020), but less attention has been paid to motile cells. Previous studies have shown that nitrogen and light intensity play critical roles in the astaxanthin accumulation in *H. pluvialis* (Borowitzka et al., 1991; Fábregas et al., 1998; Orosa et al., 2005). However, little is known about the effects of nitrogen and light intensity on astaxanthin accumulation in motile cells. In this study, we first investigated the

effect of four different concentrations of nitrogen on the accumulation of astaxanthin in motile cells and determined the optimal nitrogen concentration to induce motile cells to produce astaxanthin. The effect of three light intensities on the astaxanthin accumulation in motile cells were then investigated at this nitrogen concentration. The results would help determine the optimal nitrogen and light conditions in the production of astaxanthin from *H. pluvialis* motile cells.

2 MATERIALS AND METHODS

2.1 *H. pluvialis* Strain and Growth Conditions

H. pluvialis CCMA-451 (Genbank accession number MG847145) was acquired from the Center for Collections of Marine Algae of Xiamen University (CCMA). The motile cells were grown photoautotrophically in liquid 3N-BBM (Bold Basal Medium) at 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (side, white light) for 10 days.

2.2 Experimental Design

The first experiment was the effect of different nitrogen concentrations on astaxanthin accumulation in motile cells. Four different nitrogen (NaNO_3) concentrations were tested, including 0 N, 0.5-fold N, 1-fold N (250 mg/L), and 3N. The experiment was carried out under the condition of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity.

For the second experiment, under the conditions of nitrogen concentration screened in experiment 1, motile cells were treated under the conditions of nitrogen concentration screened in experiment 1 with three light intensities: 30, 80, and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, representing a low (LL), medium (ML), and high light (HL), respectively.

The 10-day-old motile cells were collected by centrifugation (3000 rpm, 2 min) for experiments. All experimental groups were performed in 1 L glass columns (inner diameter 5 cm) at an initial optical density of 0.5 (OD_{680}) in $25 \pm 1^\circ\text{C}$ for 4 days, with three parallels per group. The cultures were mixed by continuous bubbling with 1.5% CO_2 gas at 0.5 vvm.

2.3 Analytical Methods

For cell numbers determination, samples were fixed with Lugol's iodine solution first, and then the cell number was counted by a Neubauer improved cell counting chamber under Olympus BX53 light microscope.

Dry weight (DW) is determined daily according to Li et al. (2019a). Briefly, 10 mL algal suspension were filtered by passing through a pre-weighed (m_1) filter paper (47 mm, 1.2 μm , Whatman GF/C). Then dry the filter paper overnight at 90 $^\circ\text{C}$ to a constant weight (m_2). The DW was calculated with Eq. 1.

$$DW = (m_2 - m_1) \times 10^3 / 10 \quad (1)$$

Astaxanthin concentration (AC) is determined by the photometric method (Li et al., 2020b). Briefly, algal cells were collected from a 10 mL sample by centrifugation (3000 rpm, 2 min) and then treated with 5% (w/v) KOH in 30% (v/v) methanol at 75 $^\circ\text{C}$ for 10 min. The remaining pellet was added 25 μL acetic acid first and then extracted

with 5 mL DMSO for 10 min in a water bath at 75 °C. The supernatant was collected after centrifugation (10000 rpm, 5 min). The extraction procedure was repeated several times until the pellet became colorless. Finally, the absorbance of the extract was measured at a wavelength of 492 nm.

$$AC = 4.5 \times A_{492} \times V_a/10 \quad (2)$$

where A_{492} was the absorbance of extracts at 492 nm and V_a was the volume of extracts.

The morphological changes of algal cells were examined daily using Olympus BX53 light microscope and the microphotographs were taken with an Olympus DP74 digital camera. Since the shape of motile cells is irregular, the cell size is characterized by the protoplast length and measured by Olympus application software with an internal reticle scale. No less than 300 cells per group were used for cell size statistics.

All data were subjected to an analysis of variance (ANOVA), and differences at $p \leq 0.05$ were considered statistically significant.

3 RESULTS

3.1 The Effect of Different Nitrogen Concentrations on Astaxanthin Production in Motile Cells

3.1.1 Microalgae Growth

The effect of different nitrogen concentrations on the dry weight is shown in **Figure 1B**. During the first three days of culture, the dry weight development trend of the four groups was the same, and no significant differences were observed. However, on the 4th day of treatment, the dry weight growth of the 0 N group slowed down significantly, while the other three

groups continued to maintain a sustained increase. After four days of cultivation, the maximum dry weight of the 0 N, 0.5 N, 1 N and 3 N groups reached $1.02 \pm 0.08 \text{ g L}^{-1}$, $1.25 \pm 0.02 \text{ g L}^{-1}$, $1.15 \pm 0.02 \text{ g L}^{-1}$ and $1.19 \pm 0.04 \text{ g L}^{-1}$, respectively.

3.1.2 Astaxanthin Production

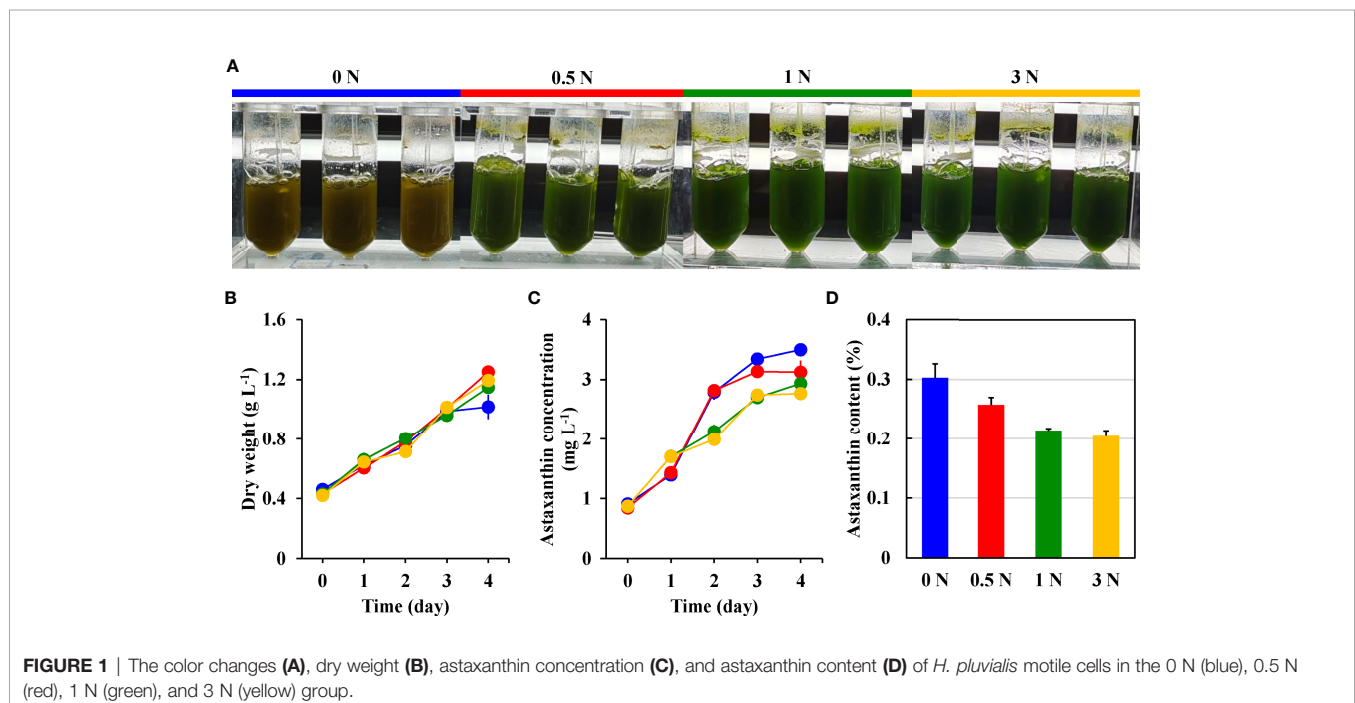
It can be seen from **Figure 1C** that the astaxanthin concentration of the 0 N and 0.5 N groups shows a similar growth trend, and the astaxanthin concentration growth trend of the 1 N and 3 N groups is similar. The 0 N and the 0.5 N groups showed rapid growth at first and then slowed down after one day of treatment, while the astaxanthin concentration of the 1 N group and the 3 N group showed a stable growth trend within four days. After four days of treatment, the astaxanthin concentration in the 0 N group has the highest value of $3.50 \pm 0.10 \text{ mg L}^{-1}$, followed by the 0.5 N group and the 1 N group with the value of $3.12 \pm 0.19 \text{ mg L}^{-1}$ and $2.93 \pm 0.05 \text{ mg L}^{-1}$. The 3 N group had the lowest astaxanthin concentration with a value of $2.76 \pm 0.09 \text{ mg L}^{-1}$.

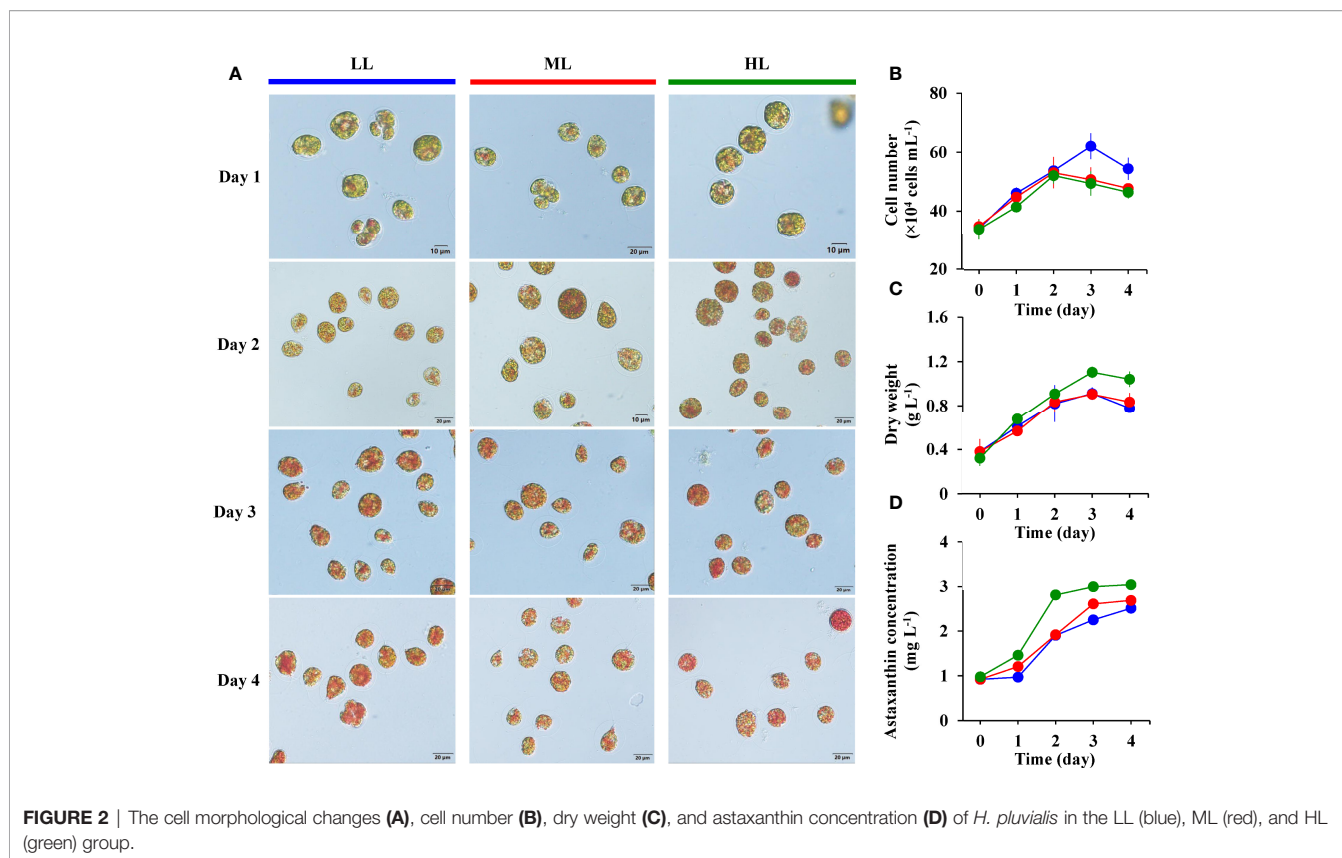
The astaxanthin content of the four cultures at the end of cultivation is calculated, and the results is shown in **Figure 1D**. A trend is observed that the lower the nitrogen concentration, the higher the astaxanthin content. The 0 N group had the highest astaxanthin content at $0.31 \pm 0.02\%$, while the 3N group had the lowest at $0.20 \pm 0.01\%$.

3.2 The Effect of Different Light Intensity on Astaxanthin Production in Motile Cells Under Nitrogen Starvation

3.2.1 The Cell Morphological Changes

As shown in **Figure 2A**, the motile cell morphological changes at different light intensities are observed under the light microscope. The motile cells protoplast was enclosed by a





swollen and gelatinous cell wall layer. After culturing for one day under nitrogen starvation, a small amount of orange-red pigment was observed in the motile cells treated with different light intensities. With the increase of treatment time, the motile cells of the three groups gradually changed from green to red due to astaxanthin accumulation. After being exposed to HL for two days, the gelatinous extracellular matrix outside the protoplasts of some motile cells disappeared, and a few motile cells developed into cysts with thickened cell walls. After four days of treatment, the protoplasts of motile cells in the ML and HL groups had distinct granularity, but the granularity of cells in the LL group was weak.

3.2.2 Microalgae Growth

The effect of light intensity on the cell number of motile cells under nitrogen starvation is shown in **Figure 2B**. During the first two days of culture under nitrogen starvation conditions, the number of cells in the three groups showed an upward trend. The cell number in the ML and HL groups reached the maximum value on the second day, at 5.3×10^5 cells mL⁻¹ and 5.2×10^5 cells mL⁻¹, respectively, and then rapidly decreased until the end. However, the number of cells in the LL group reached a maximum value of 6.2×10^5 cells mL⁻¹ after three days of culture, significantly higher than the ML and HL group.

Figure 2C shows the changes in dry weight in different light intensity groups. Dry weights of the three groups show a similar trend, increasing with culture time, peaking on the third day, and

then decreasing until the end of the 4-day cultivation. After four days of cultivation, the HL group showed the highest dry weight, followed by the ML group, and the LL group had the lowest dry weight. All the maximum dry weight of each group appeared on the third day of culture, and it was 1.11 ± 0.05 g L⁻¹ in the HL group, 0.91 ± 0.02 g L⁻¹ in the ML group, and 0.91 ± 0.06 g L⁻¹ in the LL group (**Table 1**).

3.2.3 Astaxanthin Production

The changes in astaxanthin concentration in different light intensity groups are shown in **Figure 2D**. The concentrations of astaxanthin in the three groups showed an upward trend with the increase of culture time. A significant difference in astaxanthin concentrations among the three groups was observed, with the highest in the HL group, followed by the ML group, and the lowest in the LL group. The maximum astaxanthin concentration values of the three groups all appeared at the end of the 4-day cultivation. As shown in **Table 1**, the maximum astaxanthin concentration of the HL group reached 3.04 ± 0.04 mg L⁻¹, the ML group was 2.69 ± 0.05 mg L⁻¹, and the LL group was 2.51 ± 0.02 mg L⁻¹.

We calculated the astaxanthin content of the three cultures at the end of cultivation. As shown in **Table 1**, no difference in astaxanthin content was observed between LL and ML groups, with values of $0.32 \pm 0.02\%$ and $0.32 \pm 0.04\%$, respectively, while the astaxanthin content in the HL group was lower than that in the other two groups, with a value of $0.29 \pm 0.02\%$.

TABLE 1 | The maximum dry weight and astaxanthin concentration, percentage of motile cells, cell mortality rate, and cell size in the LL, ML, and HL group.

Parameters	LL	ML	HL
The maximum dry weight (g L ⁻¹)	0.91 ± 0.06	0.91 ± 0.02	1.11 ± 0.05
The maximum astaxanthin concentration (mg L ⁻¹)	2.51 ± 0.02	2.69 ± 0.05	3.04 ± 0.04
Astaxanthin content (%)	0.32 ± 0.02	0.32 ± 0.04	0.29 ± 0.02
Percentage of motile cells (%)	87.7 ± 1.6	88.1 ± 1.5	82.8 ± 1.4
Cell mortality rate (%)	10.5 ± 1.6	9.8 ± 1.3	15.1 ± 0.7
Cell size (μm)	21.72 ± 3.82	23.67 ± 4.63	23.22 ± 5.05

3.2.4 Motile Cells Percentage, Cell Mortality Rate, and Cell Size

The motile cells percentage, cell mortality rate, and cell size at the end of the 4-day cultivation were analyzed, and the results are shown in **Table 1**. Among the three groups, the motile cells percentage and cell size in the ML group were 88.1 ± 1.5% and 23.67 ± 4.63 μm, which were higher than those in the LL and HL groups, respectively, while the cell mortality rate in the ML group reached 9.8 ± 1.3%, which was smaller than that in the other two groups. The HL group had the lowest motile cells percentage and highest cell mortality rate among the three groups, with the value of 82.8 ± 1.4% and 15.1 ± 0.7%, respectively. The cell size in the LL group was the lowest among the three groups, with a value of 21.72 ± 3.82 μm.

4 DISCUSSION

Astaxanthin accumulation in *H. pluvialis* is normally accompanied by the formation of encystment (Boussiba, 2000). During encystment, a voluminous multilayered cell wall, including trilaminar sheath and secondary wall, is formed with a significantly hardening and thickening (Monstsant et al., 2001; Hagen et al., 2002; Wang et al., 2003). Although the formation of thick cell walls is favorable for the large quality astaxanthin accumulation in *H. pluvialis* (Li et al., 2019a), it is regarded as one of the main factors in reducing the coloration efficiency and bioavailability of astaxanthin when fed to salmonids and other commercial species (Sommer et al., 1991; Young et al., 2017). Previous studies have shown that breaking *H. pluvialis* cysts can improve the astaxanthin deposition rate in trout flesh (Sommer et al., 1991). But, disruption of *H. pluvialis* cyst cell walls often require mechanical, chemical, or enzymatic means, and all of these processes are currently highly energy-intensive and expensive (Tibbetts, 2018). In addition, these processes can decrease the stability of astaxanthin and reduce the shelf life of the compound (Han et al., 2013). In contrast, thin-walled *H. pluvialis* can use whole cells as a source of astaxanthin, which has potential advantages in maintaining compound stability, easy digestion, enhance bioavailability of carotenoids, and reducing production costs.

Astaxanthin is accumulated in *H. pluvialis* under growth-limiting conditions, such as nutrient deprivation, high light, and/or high salinity (Lemoine & Schoefs, 2010; Chekanov et al., 2014; Scibilia et al., 2015; Solovchenko, 2015; Oslan et al., 2021). Nitrogen is one of the essential nutrients that affect cell growth and enzymatic activity of *H. pluvialis* (Zhang et al., 2018). Previous studies showed that nitrogen deficiency inhibited *H. pluvialis* chlorophyll biosynthesis and promoted chlorophyll b degradation, PTOX

activity, and cyclic electron transport (Scibilia et al., 2015). The reduction of chlorophyll content resulted in the limitation of the cell's ability to maintain photosynthetic function (Young and Beardall, 2003). In this case, *H. pluvialis* accumulated large amounts of carbon in the form of carotenoids and lipids (Sun et al., 2018). In the present study, we observed that the lower the nitrogen concentration, the higher the astaxanthin content in motile cells. The results are consistent with previous reports that nitrogen deficiency can stimulate astaxanthin synthesis (Borowitzka et al., 1991; Fábregas et al., 1998).

Light intensity is another one of the most significant factors in the induction of astaxanthin accumulation (Oslan et al., 2021). It can cause oxidative stress and promote the excess of reactive oxygen species in algal cells, thereby activating the biosynthesis of astaxanthin (Han et al., 2012). It has been reported that within the first three days of high light induction, the non-photochemical quenching (NPQ) of *H. pluvialis* cells decreased dramatically, while the chlorophyll a/b ratio increased transiently. In contrast, under the high light combined with nitrogen starvation, cells exhibited a sustained increase in chlorophyll a/b ratio and a rapid rise in NPQ induction. In addition, the PSII quantum yield decreased by 20% under high light conditions, while the Fv/Fm remained stable under the combined induction of high light and nitrogen deficiency (Scibilia et al., 2015). Scibilia et al. (2015) believed that nitrogen starvation enhanced the stress response of *H. pluvialis*, inducing cells to rapidly counteract high-irradiation-induced photooxidative stress, thereby improving the resistance of cells to photo-oxidation. However, motile cells of *H. pluvialis* are sensitive to light intensity, and the PSII core subunits of cells are degraded when exposed to high light conditions (Han et al., 2012). In this study, after four days of culture, the cell mortality rate in the HL group reached more than 15%, which was significantly higher than that in the LL and ML groups, indicating that high light intensity was not suitable for inducing motile cells to accumulate astaxanthin. In addition, no significant differences were observed in astaxanthin content, motile cells percentage, and cell mortality rate between the LL and ML groups. Considering low light is more economical in terms of electricity consumption, we recommend that low light as the light condition for motile cells to accumulate astaxanthin under nitrogen starvation.

We have to point out that the astaxanthin content obtained in the current study was not high. It may account for the short irradiation time. Considering that prolonged induction can lead to an increase in the number of thick-walled cysts, further work investigating the relationship between the induction period and the percentage of motile cells and astaxanthin content is needed with the aim to determine the optimal induction period. In addition,

according to Butler et al. (2018), the intracellular astaxanthin content of *H. pluvialis* motile cells can reach up to 2.7% of dry weight. It is indicated that there is still large improvement room for astaxanthin content in motile cells. Therefore, more effort to optimize induction conditions would be needed for improving astaxanthin production by motile cells. Phosphorus deficiency (Imamoglu et al., 2009) or high salt (Tam et al., 2012) can promote astaxanthin accumulation. However, phosphorus deficiency (Boussiba and Vonshak, 1991; Li et al., 2021) and high salt (Droop, 1955; Li et al., 2021) can induce cyst formation in *H. pluvialis*. Further study on optimizing the astaxanthin production by motile cells should take into account this aspect.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

REFERENCES

- Ambati, R. R., Phang, S. M., Ravi, S., and Aswathanarayana, R. G. (2014). Astaxanthin: Sources, Extraction, Stability, Biological Activities and Its Commercial Applications—A Review. *Mar. Drugs* 12 (1), 128–152. doi: 10.3390/md12010128
- Borowitzka, M. A., Huisman, J. M., and Osborn, A. (1991). Culture of The Astaxanthin-Producing Green Alga *Haematococcus Pluvialis* 1. Effects of Nutrients on Growth and Cell Type. *J. Appl. Phycol.* 3 (4), 295–304. doi: 10.1007/BF02392882
- Boussiba, S. (2000). Carotenogenesis in The Green Alga *Haematococcus Pluvialis*: Cellular Physiology and Stress Response. *Physiol. Plantar.* 108 (2), 111–117. doi: 10.1034/j.1399-3054.2000.108002111.x
- Boussiba, S., and Vonshak, A. (1991). Astaxanthin Accumulation in The Green Alga *Haematococcus Pluvialis*. *Plant Cell Physiol.* 32 (7), 1077–1082. doi: 10.1093/oxfordjournals.pcp.a078171
- Bowen, J., Soutar, C., Serwata, R. D., Lagocki, S., White, D. A., and Davies, S. J. (2002). Utilization of (3S, 3'S) Astaxanthin Acyl Esters in Pigmentation of Rainbow Trout (*Oncorhynchus Mykiss*). *Aquacult. Nutr.* 8 (1), 59–68. doi: 10.1046/j.1365-2095.2002.00190.x
- Butler, T. O., McDougall, G. J., Campbell, R., Stanley, M. S., and Day, J. G. (2018). Media Screening for Obtaining *Haematococcus Pluvialis* Red Motile Macrozooids Rich in Astaxanthin and Fatty Acids. *Biology* 7 (1), 2. doi: 10.3390/biology7010002
- Chekanov, K., Lobakova, E., Selyakh, I., Semenova, L., Sidorov, R., and Solovchenko, A. (2014). Accumulation of Astaxanthin by A New *Haematococcus Pluvialis* Strain BM1 From The White Sea Coastal Rocks (Russia). *Mar. Drugs* 12 (8), 4504–4520. doi: 10.3390/md12084504
- Choubert, G., Mendes-Pinto, M. M., and Morais, R. (2006). Pigmenting Efficacy of Astaxanthin Fed to Rainbow Trout *Oncorhynchus Mykiss*: Effect of Dietary Astaxanthin and Lipid Sources. *Aquaculture* 257 (1-4), 429–436. doi: 10.1016/j.aquaculture.2006.02.055
- Droop, M. R. (1955). Some Factors Governing Encystment in *Haematococcus Pluvialis*. *Arch. Für. Mikrobiol.* 21, 267–272. doi: 10.1007/BF00412349
- Fábregas, J., Dominguez, A., Álvarez, D. G., Lamela, T., and Otero, A. (1998). Induction of Astaxanthin Accumulation by Nitrogen and Magnesium Deficiencies in *Haematococcus Pluvialis*. *Biotechnol. Lett.* 20 (6), 623–626. doi: 10.1023/A:1005322416796
- Fang, L., Zhang, J., Fei, Z., and Wang, M. (2020). Astaxanthin Accumulation Difference Between Non-Motile Cells and Akinetes of *Haematococcus Pluvialis* Was Affected by Pyruvate Metabolism. *Biores. Bioprocess.* 7 (1), 1–12. doi: 10.1186/s40643-019-0293-1
- Hagen, C., Siegmund, S., and Braune, W. (2002). Ultrastructural And Chemical Changes in The Cell Wall of *Haematococcus Pluvialis* (Volvocales, Chlorophyta) During Aplanospore Formation. *Eur. J. Phycol.* 37 (2), 217–226. doi: 10.1017/S0967026202003669
- Han, D., Li, Y., and Hu, Q. (2013). Astaxanthin in Microalgae: Pathways, Functions and Biotechnological Implications. *Algae* 28 (2), 131–147. doi: 10.4490/algae.2013.28.2.131
- Han, D., Wang, J., Sommerfeld, M., and Hu, Q. (2012). Susceptibility And Protective Mechanisms of Motile and Non-Motile Cells of *Haematococcus Pluvialis* (Chlorophyceae) to Photooxidative Stress. *J. Phycol.* 48 (3), 693–705. doi: 10.1111/j.1529-8817.2012.01147.x
- Hart, B., and Colombo, S. M. (2022). Effects of a Novel Weakened Whole-Cell Form of *Haematococcus Pluvialis* on Flesh Pigmentation of Rainbow Trout (*Oncorhynchus Mykiss*) When Compared to Synthetic Astaxanthin. *Aquacult. Res.* 53 (6), 2408–2319. doi: 10.1111/are.15758
- Imamoglu, E., Dalay, M. C., and Sukan, F. V. (2009). Influences of Different Stress Media and High Light Intensities on Accumulation of Astaxanthin in The Green Alga *Haematococcus Pluvialis*. *New Biotechnol.* 26 (3-4), 199–204. doi: 10.1016/j.nbt.2009.08.007
- Kalinowski, C. T., Robaina, L. E., and Izquierdo, M. S. (2011). Effect of Dietary Astaxanthin on The Growth Performance, Lipid Composition and Post-Mortem Skin Colouration of Red Porgy *Pagrus Pagrus*. *Aquacult. Int.* 9 (5), 811–823. doi: 10.1007/s10499-010-9401-0
- Kobayashi, M., Kurimura, Y., and Tsuji, Y. (1997). Light-Independent, Astaxanthin Production by The Green Microalga *Haematococcus Pluvialis* Under Salt Stress. *Biotechnol. Lett.* 19 (6), 507–509. doi: 10.1023/A:1018372900649
- Lemoine, Y., and Schoefs, B. (2010). Secondary Ketocarotenoid Astaxanthin Biosynthesis in Algae: A Multifunctional Response to Stress. *Photosynthesis. Res.* 106 (1), 155–177. doi: 10.1007/s11120-010-9583-3
- Li, F., Cai, M., Lin, M., Huang, X., Wang, J., Ke, H., et al. (2019a). Differences Between Motile and Nonmotile Cells of *Haematococcus Pluvialis* in The Production of Astaxanthin at Different Light Intensities. *Mar. Drugs* 17 (1), 39. doi: 10.3390/md17010039
- Li, F., Cai, M., Lin, M., Huang, X., Wang, J., Ke, H., et al. (2020b). Enhanced Biomass and Astaxanthin Production of *Haematococcus Pluvialis* by A Cell Transformation Strategy With Optimized Initial Biomass Density. *Mar. Drugs* 18 (7), 1–7. doi: 10.3390/md18070341
- Li, F., Cai, M., Lin, M., Huang, X., Wang, J., Zheng, X., et al. (2019b). Accumulation of Astaxanthin Was Improved by The Nonmotile Cells of *Haematococcus Pluvialis*. *BioMed. Res. Int.* 2019, 1–7. doi: 10.1155/2019/8101762
- Li, F., Huang, S., Lu, X., Wang, J., Lin, M., An, Y., et al. (2018). Effects of Dietary Supplementation With Algal Astaxanthin on Growth, Pigmentation, And

AUTHOR CONTRIBUTIONS

FL: data curation, methodology, and writing-original draft. YW: investigations. MC: writing-review and editing. QL: investigations. ZQ: investigations. JL: investigations. YZ: methodology. NZ: methodology. XH: project administration and resources. CL: project administration and resources. All authors contributed to the article and approved the submitted version.

FUNDING

This research was mainly supported by Program for Scientific Research Start-up Funds of Guangdong Ocean University (grant number 060302022102) and Marine Economic Development Special Fund of Fujian Province (FJHJF-L-2021-5).

- Antioxidant Capacity of The Blood Parrot (*Cichlasoma Citrinellum* × *Cichlasoma Synspilum*). *J. Oceanol. Limnol.* 36 (5), 1851–1859. doi: 10.1007/s00343-019-7172-7
- Li, X., Wang, X., Duan, C., Yi, S., Gao, Z., Xiao, C., et al. (2020a). Biotechnological Production of Astaxanthin From The Microalga *Haematococcus Pluvialis*. *Biotechnol. Adv.* 43, 107602. doi: 10.1016/j.biotechadv.2020.107602
- Li, M., Wu, W., Zhou, P., Xie, F., Zhou, Q., and Mai, K. (2014). Comparison Effect of Dietary Astaxanthin and *Haematococcus Pluvialis* On Growth Performance, Antioxidant Status and Immune Response of Large Yellow Croaker *Pseudosciaena Crocea*. *Aquaculture* 434, 227–232. doi: 10.1016/j.aquaculture.2014.08.022
- Li, F., Zhang, N., Zhang, Y., Lian, Q., Qin, C., Qian, Z., et al. (2021). NaCl Promotes the Efficient Formation of *Haematococcus Pluvialis* Nonmotile Cells Under Phosphorus Deficiency. *Mar. Drugs* 19 (6), 337. doi: 10.3390/md19060337
- Li, J., Zhu, D., Niu, J., Shen, S., and Wang, G. (2011). An Economic Assessment of Astaxanthin Production by Large Scale Cultivation of *Haematococcus Pluvialis*. *Biotechnol. Adv.* 29 (6), 568–574. doi: 10.1016/j.biotechadv.2011.04.001
- Lorenz, R. T., and Cysewski, G. R. (2000). Commercial Potential for *Haematococcus* Microalgae as A Natural Source of Astaxanthin. *Trends Biotechnol.* 18 (4), 160–167. doi: 10.1016/S0167-7799(00)01433-5
- Montsant, A., Zarka, A., and Boussiba, S. (2001). Presence Of a Nonhydrolyzable Biopolymer in The Cell Wall of Vegetative Cells and Astaxanthin-Rich Cysts of *Haematococcus Pluvialis* (Chlorophyceae). *Mar. Biotechnol.* 3 (6), 515–521. doi: 10.1007/s1012601-0051-0
- Moretti, V. M., Mentasti, T., Bellagamba, F., Luzzana, U., Caprino, F., Turchini, G. M., et al. (2006). Determination of Astaxanthin Stereoisomers and Colour Attributes in Flesh of Rainbow Trout (*Oncorhynchus Mykiss*) As A Tool to Distinguish the Dietary Pigmentation Source. *Food Additive. Contaminant.* 23 (11), 1056–1063. doi: 10.1080/02652030600838399
- Nogueira, N., Canada, P., Caboz, J., Andrade, C., and Cordeiro, N. (2021). Effect of Different Levels of Synthetic Astaxanthin on Growth, Skin Color and Lipid Metabolism of Commercial Sized Red Porgy (*Pagrus Pagrus*). *Anim. Feed. Sci. Technol.* 276, 114916. doi: 10.1016/j.anifeeds.2021.114916
- Orosa, M., Franqueira, D., Cid, A., and Abalde, J. (2005). Analysis and Enhancement of Astaxanthin Accumulation in *Haematococcus Pluvialis*. *Biores. Technol.* 96 (3), 373–378. doi: 10.1016/j.biortech.2004.04.006
- Oslan, S. N. H., Shoparwe, N. F., Yusoff, A. H., Rahim, A. A., Chang, C. S., Tan, J. S., et al. (2021). A Review on *Haematococcus Pluvialis* Bioprocess Optimization of Green and Red Stage Culture Conditions for The Production of Natural Astaxanthin. *Biomolecules* 11 (2), 256. doi: 10.3390/biom11020256
- Panis, G., and Carreon, J. R. (2016). Commercial Astaxanthin Production Derived by Green Alga *Haematococcus Pluvialis*: A Microalgae Process Model and A Techno-Economic Assessment All Through Production Line. *Algal. Res.* 18, 175–190. doi: 10.1016/j.algal.2016.06.007
- Rahman, M. M., Khosravi, S., Chang, K. H., and Lee, S. M. (2016). Effects of Dietary Inclusion of Astaxanthin on Growth, Muscle Pigmentation and Antioxidant Capacity of Juvenile Rainbow Trout (*Oncorhynchus Mykiss*). *Prev. Nutr. Food Sci.* 21 (3), 281–288. doi: 10.3746/pnf.2016.21.3.281
- Scibilia, L., Girolomoni, L., Berteotti, S., Alboresi, A., and Ballottari, M. (2015). Photosynthetic Response to Nitrogen Starvation and High Light in *Haematococcus Pluvialis*. *Algal. Res.* 12, 170–181. doi: 10.1016/j.algal.2015.08.024
- Shah, M. M. R., Liang, Y., Chen, J. J., and Daroch, M. (2016). Astaxanthin-Producing Green Microalga *Haematococcus Pluvialis*: From Single Cell to High Value Commercial Products. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00531
- Sheikhzadeh, N., Tayefi-Nasrabadi, H., Khani Oushani, A., and Enferadi, M. H. N. (2012). Effects of *Haematococcus Pluvialis* Supplementation on Antioxidant System and Metabolism in Rainbow Trout (*Oncorhynchus Mykiss*). *Fish. Physiol. Biochem.* 38 (2), 413–419. doi: 10.1007/s10695-011-9519-7
- Solovchenko, A. E. (2015). Recent Breakthroughs in the Biology of Astaxanthin Accumulation by Microalgal Cell. *Photosynthesis. Res.* 125 (3), 437–449. doi: 10.1007/s11120-015-0156-3
- Sommer, T. R., Potts, W. T., and Morrissy, N. M. (1991). Utilization of Microalgal Astaxanthin by Rainbow Trout (*Oncorhynchus Mykiss*). *Aquaculture* 94 (1), 79–88. doi: 10.1016/0044-8486(91)90130-Y
- Stachowiak, B., and Szulc, P. (2021). Astaxanthin for the Food Industry. *Molecules* 26 (9), 26. doi: 10.3390/molecules26092666
- Sun, X. M., Ren, L. J., Zhao, Q. Y., Ji, X. J., and Huang, H. (2018). Microalgae for the Production of Lipid and Carotenoids: A Review With Focus on Stress Regulation and Adaptation. *Biotechnol. Biofuels.* 11 (1), 1–16. doi: 10.1186/s13068-018-1275-9
- Tacon, A. G. J., and Metian, M. (2013). Fish Matters: Importance of Aquatic Foods in Human Nutrition and Global Food Supply. *Rev. Fish. Sci.* 21 (1), 22–38. doi: 10.1080/10641262.2012.753405
- Tam, L. T., Hoang, D. D., Mai, D. T. N., Thu, N. T. H., Anh, H. T. L., and Dang, D. H. (2012). Study on the Effect of Salt Concentration on Growth and Astaxanthin Accumulation of Microalgae *Haematococcus Pluvialis* As the Initial Basis for Two Phase Culture of Astaxanthin Production. *Academia. J. Biol.* 34 (2), 213–223. doi: 10.15625/0866-7160/v34n2.964
- Tejera, N., Cejas, J. R., Rodríguez, C., Bjerkeng, B., Jerez, S., Bolaños, A., et al. (2007). Pigmentation, Carotenoids, Lipid Peroxides and Lipid Composition of Skin of Red Porgy (*Pagrus Pagrus*) Fed Diets Supplemented With Different Astaxanthin Sources. *Aquaculture* 270 (1–4), 218–230. doi: 10.1016/j.aquaculture.2007.01.019
- Tibbetts, S. M. (2018). “The Potential for ‘Next-Generation’, Microalgae-Based Feed Ingredients for Salmonid Aquaculture in Context of The Blue Revolution,” in *Microalgal Biotechnology*. Eds. E. Jacob-Lopes, L. Q. Zepka and M. I. Queiroz, 151–175. London, United Kingdom: IntechOpen. doi: 10.5772/intechopen.73551
- Torrissen, O. J., Christiansen, R., Struksnaes, G., and Estermann, R. (1995). Astaxanthin Deposition in the Flesh of Atlantic Salmon, *Salmo Salar* L, in Relation to Dietary Astaxanthin Concentration and Feeding Period. *Aquacult. Nutr.* 1 (2), 77–84. doi: 10.1111/j.1365-2095.1995.tb00022.x
- Viera, I., Pérez-Gálvez, A., and Roca, M. (2018). Bioaccessibility of Marine Carotenoids. *Mar. Drugs* 16 (10), 397. doi: 10.3390/md16100397
- Wang, B., Zarka, A., Trebst, A., and Boussiba, S. (2003). Astaxanthin Accumulation in *Haematococcus Pluvialis* (Chlorophyceae) As an Active Photoprotective Process Under High Irradiance¹. *J. Phycol.* 39 (6), 1116–1124. doi: 10.1111/j.0022-3646.2003.03-043.x
- Yeşilayer, N. (2020). Comparison of Flesh Colour Assessment Methods for Wild Brown Trout (*Salmo Trutta Macrostigma*), Farmed Rainbow Trout (*Oncorhynchus Mykiss*) and Farmed Atlantic Salmon (*Salmo Salar*). *Pakistan J. Zool.* 52 (3), 1007–1014. doi: 10.17582/journal.pjz/20190520140524
- Young, E. B., and Beardall, J. (2003). Photosynthetic Function in *Dunaliella Tertiolecta* (Chlorophyta) During a Nitrogen Starvation and Recovery Cycle. *J. Phycol.* 39 (5), 897–905. doi: 10.1046/j.1529-8817.2003.03042.x
- Young, A. J., Pritchard, J., White, D., and Davies, S. (2017). Processing of Astaxanthin-Rich *Haematococcus* Cells for Dietary Inclusion and Optimal Pigmentation in Rainbow Trout, *Oncorhynchus Mykiss* L. *Aquacult. Nutr.* 23 (6), 1304–1311. doi: 10.1111/anu.12505
- Yu, W., Lin, H., Yang, Y., Zhou, Q., Chen, H., Huang, X., et al. (2021). Effects of Supplemental Dietary *Haematococcus Pluvialis* on Growth Performance, Antioxidant Capacity, Immune Responses and Resistance to *Vibrio Harveyi* Challenge of Spotted Sea Bass *Lateolabrax Maculatus*. *Aquacult. Nutr.* 27 (2), 355–365. doi: 10.1111/anu.13189
- Zhang, W., Zhou, X., Zhang, Y., Cheng, P., Ma, R., Cheng, W., et al. (2018). Enhancing Astaxanthin Accumulation in *Haematococcus Pluvialis* by Coupled Light Intensity and Nitrogen Starvation in Column Photobioreactors. *J. Microbiol. Biotechnol.* 28 (12), 2019–2028. doi: 10.4014/jmb.1807.07008

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

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Li, Cai, Wu, Lian, Qian, Luo, Zhang, Zhang, Li and Huang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.