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Original Article

Environmental modulation of Omega-3 fatty acid biosynthesis in microalgae: Comparative effects of light intensity, temperature, and UV exposure on *Spirulina platensis*, *Chlorella vulgaris*, and *Nannochloropsis oceanica*

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Abstract: Microalgae are increasingly recognized as sustainable biofactories for high-value lipids, particularly omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are vital for human health and industrial applications. This study aimed to evaluate the influence of environmental factors, light intensity, temperature, and ultraviolet (UV) radiation, on omega-3 fatty acid biosynthesis in *Spirulina platensis*, *Chlorella vulgaris*, and *Nannochloropsis oceanica*. The experiment was conducted under controlled laboratory conditions, with light intensities ranging from 10-125 μmol m^{-2} s^{-1}, temperatures from 15-45°C, and UV-B exposure durations of 0-3 hours. Fatty acid methyl esters were extracted and quantified using gas chromatography–mass spectrometry (GC-MS). The results revealed that moderate light (50-75 μmol m^{-2} s^{-1}) and temperature (30-35°C) significantly enhanced EPA and DHA accumulation, while UV exposure led to a pronounced decline in both compounds. *Nannochloropsis oceanica* exhibited the highest productivity, with EPA and DHA peaks of 0.94 and 0.55%, respectively, under optimal conditions, followed by *S. platensis* and *C. vulgaris*. The results confirmed significant effects of all environmental factors on lipid biosynthesis. In conclusion, light and temperature play crucial regulatory roles in omega-3 production, whereas UV radiation inhibits fatty acid synthesis. The findings highlight *N. oceanica* as the most promising species for industrial-scale omega-3 production and provide valuable insights for optimizing microalgal cultivation systems.

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Introduction

Omega-3 fatty acids are long-chain polyunsaturated lipids that are vital for cardiovascular, neurological, and immune function. Despite growing research, the influence of key abiotic factors—namely, light intensity, ultraviolet (UV) exposure, and temperature—on the biosynthesis and accumulation of EPA and DHA across microalgal species remains insufficiently understood. Microalgae have emerged as a vital source of omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), due to their rapid growth, high lipid productivity, and adaptability to different environments (Guschina and Harwood, 2006; Converti et al., 2009). Species such as *Spirulina platensis*, *Chlorella vulgaris*, and *Nannochloropsis oceanica* have been extensively studied for their ability to biosynthesize these valuable PUFAs under different environmental stresses (Ma et al., 2016; Shaikh et al., 2023). Among abiotic factors, light intensity plays a crucial role. Moderate light enhances fatty acid synthesis by increasing photosynthetic efficiency (Sievonen, 1990; Franklin et al., 2002), whereas excessive light induces photoinhibition and oxidative stress (Girotti, 2001; Mumtaz et al., 2022; Zhang et al., 2024). Studies confirmed that an optimal range (40-75 μmol m^{-2} s^{-1}) stimulates lipid accumulation (Yu-Wang et al., 2007; Tang et al., 2022), particularly EPA in *Nannochloropsis* (Liu et al., 2022; Fang et al., 2023).

In contrast, ultraviolet radiation has mostly adverse effects. UV-B induces photo-oxidative damage, lipid peroxidation, and inhibition of biosynthetic enzymes such as desaturases and elongases (Wong et al., 2007;

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Table 1. Comparative summary of optimal environmental conditions and omega-3 fatty acid production in three microalgal species.

Species	Optimal Light (μmol m^{-2} s^{-1})	EPA (%) at Optimal Light	DHA (%) at Optimal Light	Optimal Temperature (°C)	EPA (%) at Optimal Temperature	DHA (%) at Optimal Temperature	UV Duration (hr) for EPA Loss	Final EPA after UV (%)	Final DHA after UV (%)
S. platensis	75	0.93	0.17	35	0.82	0.14	2.0	0.01	0.00
C. vulgaris	50	0.22	0.33	30	0.33	0.23	1.5	0.003	0.002
N. oceanica	75	0.94	0.55	35	0.77	0.33	2.0	0.01	0.00

EPA = Eicosapentaenoic acid; DHA = Docosahexaenoic acid. Values represent mean concentrations (%) determined under controlled laboratory conditions. Optimal light and temperature promoted omega-3 synthesis, while UV exposure significantly reduced fatty acid content.

Gao et al., 2008; Wu et al., 2020; Han et al., 2022). DHA and EPA concentrations decrease with increasing exposure duration across the three aforementioned species (Wang et al., 2023; Gouvea et al., 2008), with *Chlorella* showing a faster decline (Liu et al., 2012). These effects are attributed to ROS accumulation, disruption of chloroplast function, and damage to photosystems (Zucchi and Jr., 2001; Adir et al., 2003; Nixon et al., 2005).

Temperature significantly controls fatty acid profiles. *Spirulina* tolerates higher temperatures (up to 40°C) and shows increased EPA and DHA at 30-35°C (De et al., 1999; Becker, 1993), whereas *Chlorella* and *Nannochloropsis* prefer moderate conditions (Converti et al., 2009; Zhu et al., 2016). High temperatures increase membrane fluidity and activate key lipid biosynthetic pathways (Otles and Pire, 2001; Sayegh and Montagnes, 2011), although extreme temperatures can lead to stress-induced metabolic shifts (Carpenter, 1973; Brouers, 2007).

Based on the above-mentioned background, this study aimed to investigate the influence of environmental factors of light intensity, temperature, and UV exposure on the biosynthesis of omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in *S. platensis*, *C. vulgaris*, and *N. oceanica*.

Materials and Methods

Algal strains and source: Three microalgae species were used in this study: *Spirulina platensis*, *C. vulgaris*, and *N. oceanica*. The strains of *C. vulgaris* and *N. oceanica* were obtained from Carolina Biological Supply (USA) and maintained in Chu-10 and seawater-based media, respectively. *Spirulina platensis* was sourced from the College of Science, Al-Mustansiriya University, Iraq, and cultivated in Zarrouk medium.

Media preparation and experimental conditions: Chu-10 medium for *C. vulgaris* and *N. oceanica* was prepared as described by Chu (1942) and Kassim (1999). The Zarrouk medium for S. platensis was prepared following Zarrouk (1966) and optimized for alkaline conditions (pH 10-11). All media were sterilized by autoclaving at 121°C for 20 minutes at 1.5 bar.

Cultures were incubated in 500 mL Erlenmeyer flasks containing 250 mL of sterile medium. A 16:8 light/dark cycle was maintained using LED light sources. Temperature conditions ranged from 15 to 45°C. Light-intensity treatments ranged from 10 to 125 μmol m^{-2} s^{-1}. UV exposure durations ranged from 0 to 3 hours (UV-B, 280-320 nm). All treatments were conducted in triplicate (Figs. 1-3, Table 2).

Biomass growth measurement: Algal growth was monitored by measuring optical density (OD) at 680 nm for *C. vulgaris* and *N. oceanica*, and 720 nm for *S. platensis*. Measurements were taken daily for 21 days using a spectrophotometer (APEL, Japan). Growth rate (k) was calculated using the formula $k = (\ln(N_t/N_0)) / t$, where N_t = OD at time t, N_0 = initial OD, and t = time (days) (Fogg, 1975).

GC-MS analysis of fatty acids: Extraction and quantification of EPA and DHA were performed using Gas Chromatography–Mass Spectrometry (GC-MS) (Agilent, Germany). Lipids were extracted using the Bligh and Dyer method. Fatty acids were methylated to FAMEs and analyzed. Identification was confirmed using FAMES and LipidBlast spectral libraries. Retention times were 29.10 min for EPA and 34.29 min for DHA. Quantification was based on peak area (%) and spectral match (% similarity).

Table 2. Analysis of variance (ANOVA) and least significant difference (LSD) test results for EPA production under varying light intensity, temperature, and UV exposure.

Environmental Factor	Condition	F-value	p-value	LSD (0.05)	Significant ($p \leq 0.05$)
Light Intensity (µmol m^{-2} s^{-1})	25	1531.000	0.0000	0.031	True
	50	2532.636	0.0000	0.049	True
	75	3724.000	0.0000	0.042	True
	100	900.000	0.0000	0.038	True
	125	50.103	0.0002	0.025	True
Temperature (°C)	20	1399.400	0.0000	0.025	True
	25	1204.000	0.0000	0.022	True
	30	2181.000	0.0000	0.046	True
	35	1471.000	0.0000	0.038	True
	40	1009.000	0.0000	0.041	True
UV Exposure (hours)	0	5113.000	0.0000	0.021	True
	1	1579.000	0.0000	0.049	True
	1.5	763.894	0.0000	0.045	True
	2	8.000	0.0203	0.026	True
	2.5	—	—	0.025	False
	3	—	—	0.026	False

Statistically significant variations ($P \leq 0.05$) were observed for all treatments except UV exposures beyond 2 hours, where no further significant differences occurred due to oxidative inhibition.

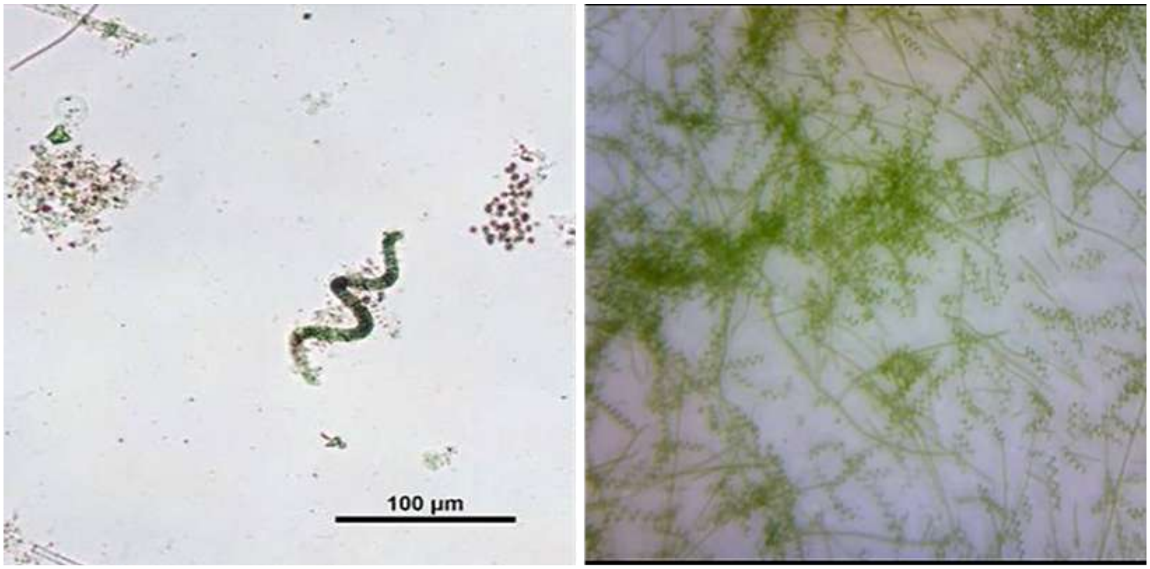

Figure 1. Vegetative cell of the alga *Spirulina platensis* (40x).

Statistical Analysis: All data were analyzed using analysis of variance (ANOVA) at the 95% confidence level, and the least significant difference (LSD) test was used to identify statistically significant differences ($P \leq 0.05$). Results are presented as the mean ± standard deviation (SD) of triplicate measurements.

Results

The results revealed significant differences in EPA and DHA contents among treatments, with *P*-values ranging from 0.028 to 0.031. Based on the results, light intensity significantly affected lipid accumulation ($P \leq 0.05$). The highest EPA yield was observed at 75 µmol m^{-2} s^{-1} for *S. platensis* (0.93%) and *N. oceanica* (0.94%), whereas *C. vulgaris* peaked at 50 µmol m^{-2} s^{-1} (0.22%). Temperature also played a critical role, with optimal EPA production observed at 30-35°C across species. UV exposure caused a marked reduction in omega-3 content, with complete

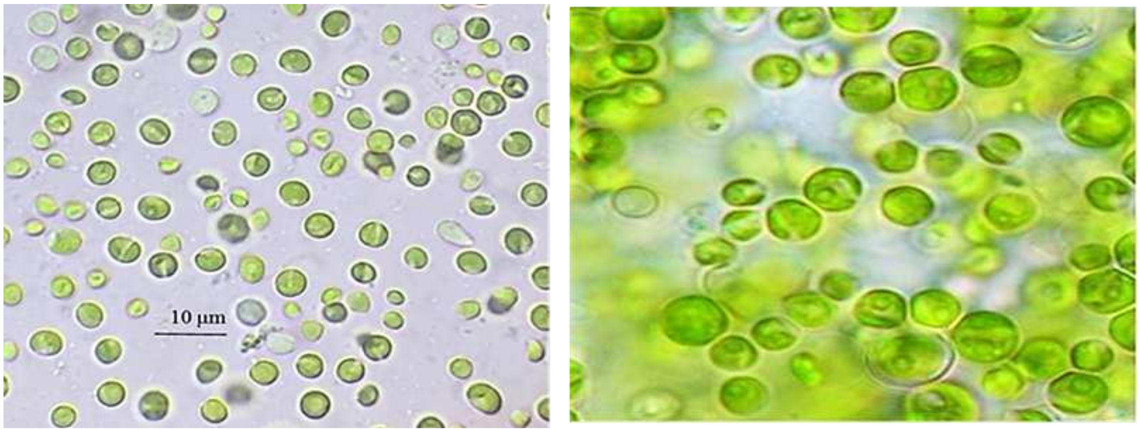

Figure 2. Vegetative cell of the alga *Chlorella terrestris* (40x).

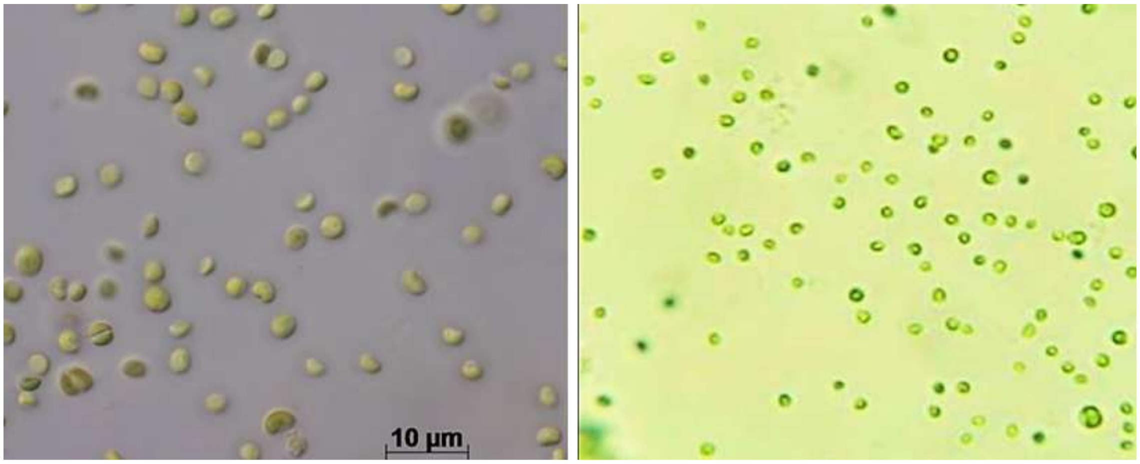

Figure 3. Vegetative cell of the alga *Nannochloropsis oceanica* (40x).

loss of DHA after 2 hours (Table 1).

Growth dynamics: Growth curves exhibited a classic sigmoid pattern across all species under optimal conditions. *Spirulina platensis* entered the exponential phase after day 2, reached the stationary phase on day 17, and entered the death phase after day 22. *Chlorella vulgaris* exhibited faster growth, reaching stability by day 6 and declining after day 12. *Nannochloropsis oceanica* stabilized on day 12 and declined by day 15. The relative growth rate (k) and doubling time varied significantly among species, reflecting species-specific adaptation to environmental stimuli.

Effect of light intensity on omega-3 content: The results confirmed significant variation among the species under each light condition, indicating meaningful differences in lipid responses (Table 2). Visible light intensities between 10-125 µmol m^{-2} s^{-1} influenced EPA and DHA content as follows: In *S. platensis*, the highest EPA content (0.93%) was recorded at 75 µmol m^{-2} s^{-1}, while DHA peaked at 0.17% at the same level. Both declined sharply at 125 µmol m^{-2} s^{-1}. The results indicate that both EPA and DHA production increase with moderate light intensities (50-75 µmol m^{-2} s^{-1}) but decline sharply at higher light levels due to photo-oxidative stress (Fig. 4). *Chlorella vulgaris* achieved maximum DHA content (0.3333%) at 50-75 µmol m^{-2} s^{-1}, whereas EPA peaked at 50 µmol m^{-2} s^{-1} with 0.2233%. Both fatty acids increased with rising light levels up to 50-75 µmol m^{-2} s^{-1}, reaching maximum DHA (0.33%) and EPA (0.22%) yields, followed by a notable decline at higher intensities, suggesting photoinhibition and oxidative stress effects (Fig. 5). *Nannochloropsis oceanica* showed superior productivity, with peak EPA (0.9400%) and DHA (0.5467%) at 75 µmol m^{-2} s^{-1}. The results showed significant variation between light treatments for all species. Both EPA and DHA increased steadily with rising light intensity, reaching maximum yields

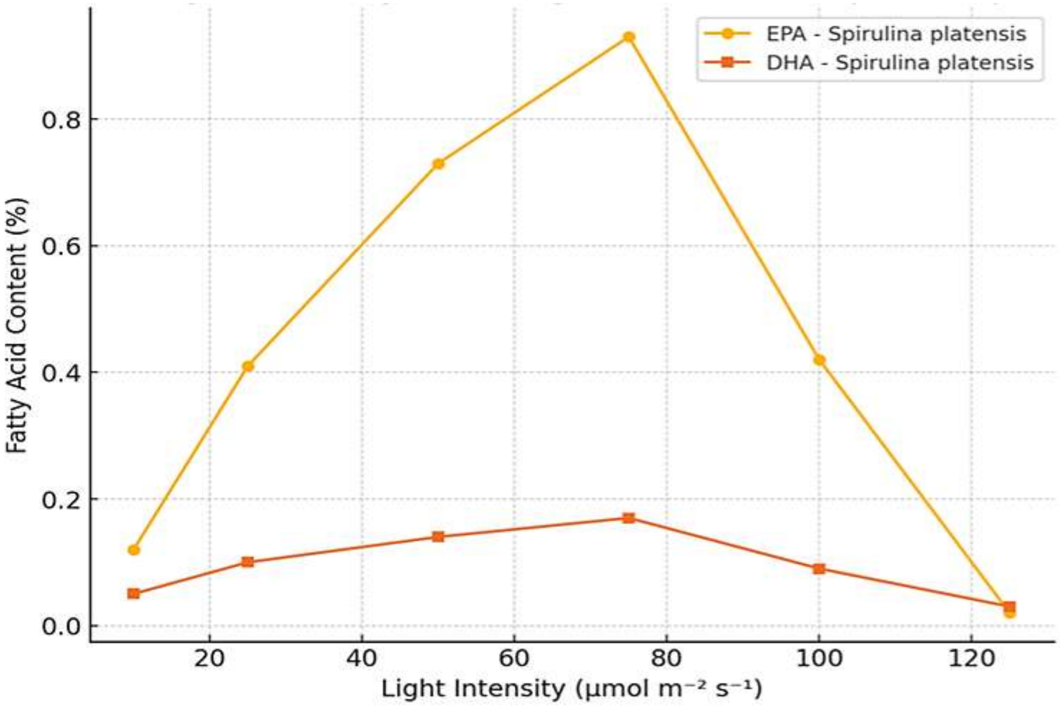

Figure 4. Effect of light intensity on omega-3 fatty acid content in *Spirulina platensis*.

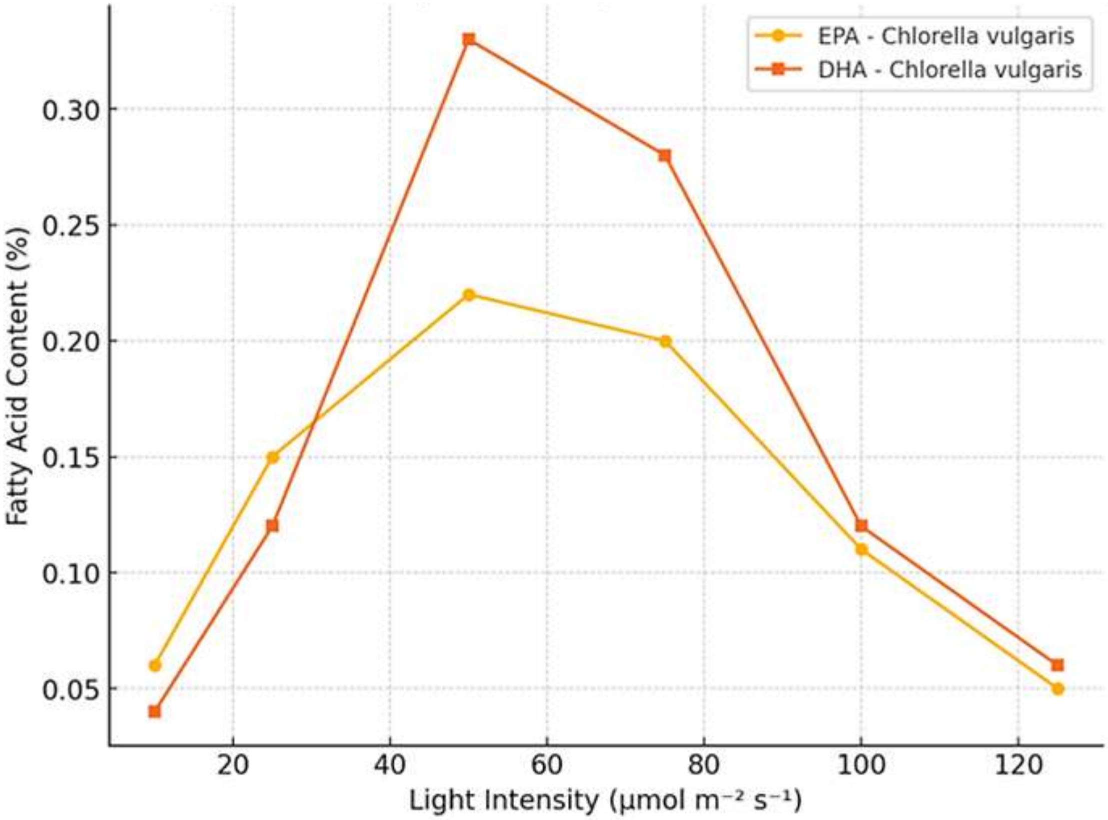

Figure 5. Effect of light intensity on omega-3 fatty acid content in *Chlorella vulgaris*.

(0.94% and 0.55%, respectively) at 75 μmol m^{-2} s^{-1}. Beyond this range, fatty acid synthesis declined markedly, indicating that excessive light intensity induces photo-oxidative inhibition of lipid metabolism (Fig. 6).

Effect of UV exposure on omega-3 content: UV-B exposure caused a progressive decrease in omega-3 content. All three algal species exhibited the highest

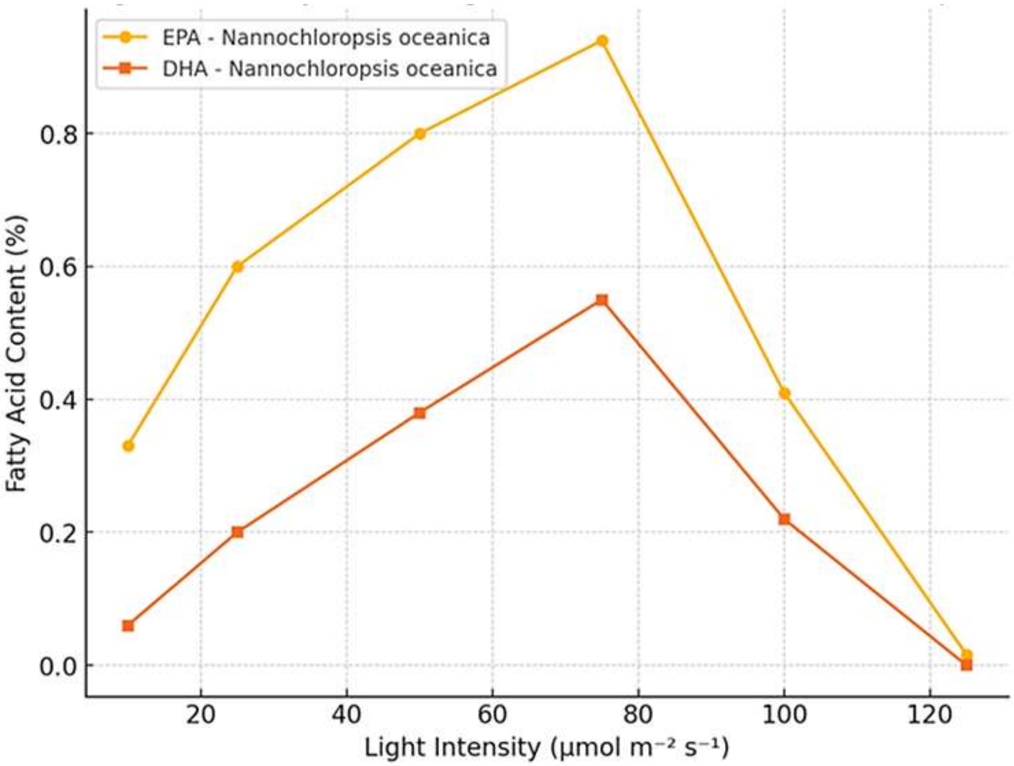

Figure 6. Effect of light intensity on omega-3 fatty acid content in *Nannochloropsis oceanica*.

concentrations of EPA and DHA at 0 hours of UV exposure (control condition). After 1.5 hours of exposure, EPA levels decreased sharply—for instance, reaching as low as 0.0033% in *C. vulgaris*. When exposure exceeded 2 hours, both EPA and DHA became nearly undetectable, indicating severe oxidative damage and inhibition of fatty acid biosynthesis.

Effect of temperature on Omega-3 content: The study results showed a significant temperature effect, validating interspecies variability. Based on the results, *S. platensis* showed thermal tolerance, with EPA and DHA peaking at 35-40°C. *Chlorella vulgaris* favored 30°C, with a DHA content of 0.3333%. In addition, *N. oceanica* exhibited optimal EPA at 30°C (0.7700%) and DHA at 35°C (0.3333%). EPA and DHA contents decreased significantly outside 25-35°C, especially at 15 and 45°C, where omega-3 synthesis was inhibited.

A comparative analysis of the three microalgal species under varying environmental conditions revealed significant differences ($P \leq 0.05$) in their ability to synthesize omega-3 polyunsaturated fatty acids (PUFAs). Figure 7 illustrates the variation in eicosapentaenoic acid (EPA) production (%) among *S. platensis*, *C. vulgaris*, and *N. oceanica* under different environmental conditions—light intensity, temperature, and UV exposure. The results show that both light intensity and temperature significantly enhance EPA accumulation in *N. oceanica* and *S. platensis,* exhibiting the highest yields under optimal light (75 μmol m^{-2} s^{-1}) and temperature (35°C). In contrast, UV exposure caused a pronounced reduction in EPA content across all species, confirming its inhibitory effect on lipid biosynthesis. Figure 8 presents docosahexaenoic acid (DHA) concentrations (%) in *S. platensis*, *C. vulgaris*, and *N. oceanica* under varying environmental conditions. The results show that DHA production is highest under optimal light (50-75 μmol m^{-2} s^{-1}) and moderate temperature (30-35°C) conditions, particularly in *N. oceanica*, followed by *C. vulgaris*. UV exposure significantly suppressed DHA synthesis across all species.

Discussions

The findings of this study demonstrated that environmental modulation, specifically light intensity, temperature, and ultraviolet (UV) radiation, plays a

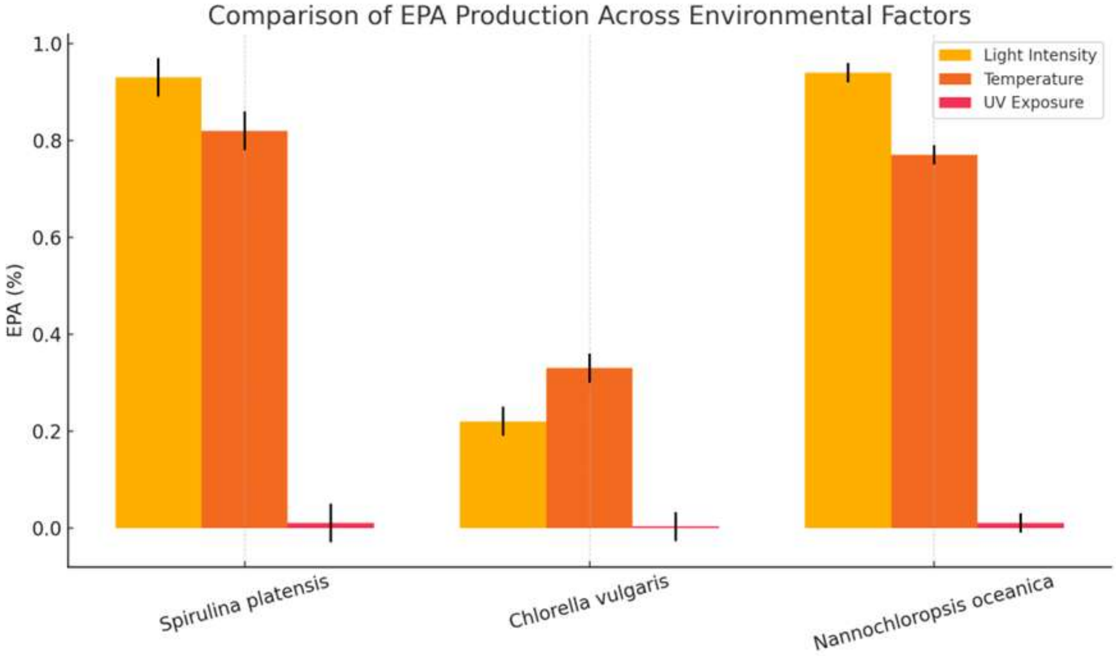

Figure 7. EPA production under varying environmental conditions in the three microalgal species studied.

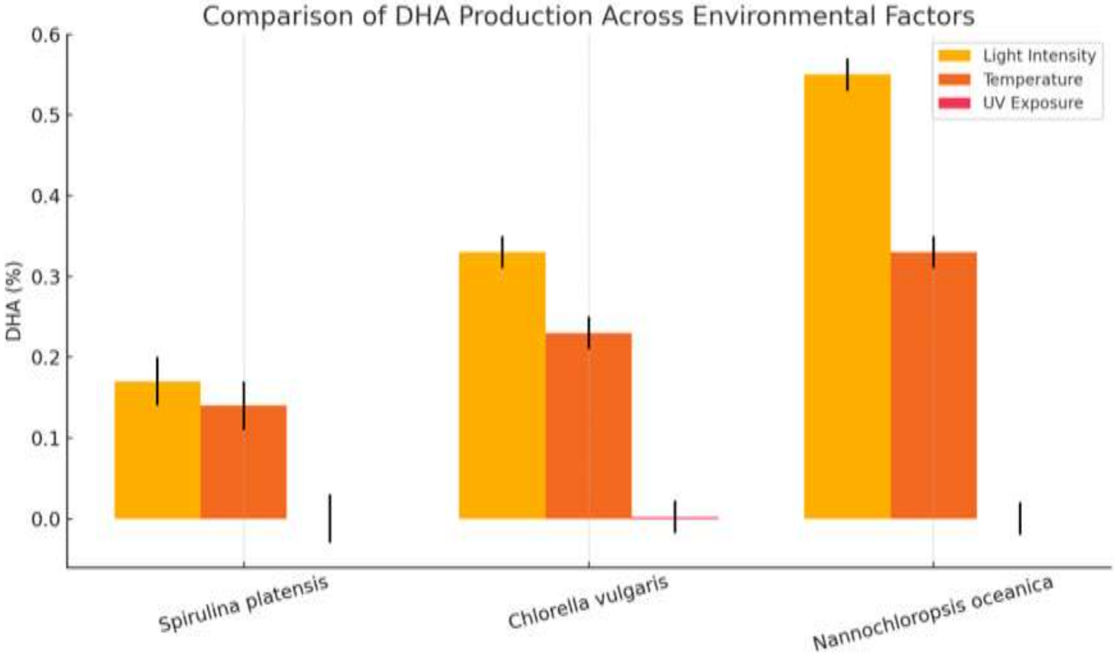

Figure 8. DHA production under varying environmental conditions in the three microalgal species studied.

critical role in regulating the biosynthesis of omega-3 fatty acids (EPA and DHA) in *S. platensis*, *C. vulgaris*, and *N. oceanica*. Abiotic stressors serve as potent metabolic triggers in microalgal systems, influencing lipid accumulation and composition via both physiological and molecular mechanisms (Guschina and Harwood, 2006; Mata et al., 2010).

Light intensity was the most influential factor in enhancing lipid metabolism, where moderate illumination (50-75 µmol m^{-2} s^{-1}) significantly increased EPA and DHA accumulation in all species. Light intensity played a pivotal role in determining the metabolic performance of each species. The maximum EPA yield was observed at 75 µmol m^{-2} s^{-1} for *S. platensis* (0.93%) and *N. oceanica* (0.94%), whereas *C. vulgaris* achieved its peak EPA (0.22%) and DHA (0.33%) at 50 µmol m^{-2} s^{-1}. This is consistent with the findings of Tang et al. (2022) and

Shaikh et al. (2023), who reported that optimal light conditions stimulate photosynthetic carbon flux toward fatty acid synthesis, whereas excessive irradiance leads to photo-oxidative damage and lipid degradation. The observed reduction at higher intensities (≥125 µmol m^{-2} s^{-1}) likely resulted from overproduction of reactive oxygen species (ROS), which disrupts chloroplast integrity and inactivates enzymes responsible for desaturation (Zhang et al., 2024). Furthermore, the positive relationship between moderate light and omega-3 yield suggests that light intensity regulates the balance between growth and stress metabolism, a crucial factor for photobioreactor optimization (Hu et al., 2008; Mumtaz et al., 2022).

Temperature emerged as a major determinant of omega-3 productivity, with each species exhibiting a distinct thermal preference; beyond this threshold, thermal stress reduced fatty acid accumulation. *Spirulina platensis* and *N. oceanica* exhibited maximal accumulation of EPA and DHA at 30-35°C, reflecting their adaptive mechanisms for maintaining membrane fluidity under thermal stress (Converti et al., 2009; Sayegh and Montagnes, 2011). In the current study, the highest EPA concentrations were recorded at 35°C for *S. platensis* (0.82%), 30°C for *C. vulgaris* (0.33%), and 35°C for *N. oceanica* (0.77%). Similarly, DHA peaked at 0.33% in *N. oceanica* under conditions below 35°C. The increased unsaturated lipid fraction at elevated temperatures indicates activation of key enzymes, such as desaturases and elongases, which are essential for converting precursor fatty acids into long-chain PUFAs. However, beyond the threshold (>40°C), thermal denaturation likely impaired photosynthetic electron transport and lipid stability (Otles and Pire, 2001). Interspecies variability in responses to heat confirms that both genetic and physiological mechanisms govern temperature adaptation.

Exposure to UV-B radiation exerted a profoundly negative effect on fatty acid biosynthesis. *Spirulina platensis* and *N. oceanica* exhibited EPA reductions from 0.93% to 0.01% after 2 hours, while *C. vulgaris* declined to 0.003% after 1.5 hours of exposure. DHA synthesis was nearly eliminated in all species under prolonged UV exposure. The decline in EPA and DHA across species—with a remarkably complete loss after prolonged exposure—can be attributed to ROS-induced oxidative stress, which leads to lipid peroxidation, chlorophyll degradation, and enzyme inhibition (Wong et al., 2007; Gao et al., 2008; Han et al., 2022). *Nannochloropsis oceanica* exhibited slightly higher resilience to UV stress, suggesting superior antioxidant defenses and more efficient repair mechanisms in its photosynthetic machinery. These observations align with previous work showing that UV exposure reduces lipid metabolism through direct damage to desaturase genes and impaired photosystem II activity (Liu et al., 2012; Wu et al., 2020).

Among the three species examined, *N. oceanica* consistently outperformed the others in EPA and DHA productivity under optimal light and temperature conditions, confirming its industrial potential as a sustainable omega-3 source. *Spirulina platensis* also exhibited strong thermal tolerance, underscoring its suitability for biotechnological applications in which temperature fluctuations are common. Conversely, *C. vulgaris* was more sensitive to both UV and high-light conditions, but remains valuable due to its rapid biomass growth rate. These species-specific adaptations underscore the importance of selecting the appropriate algal strain and environmental conditions to maximize PUFA yield (Guedes et al., 2011; Ma et al., 2016). The observed differences also underscore the feasibility of environmental modulation, through controlled photoperiods and temperature regulation, as a cost-effective alternative to genetic modification for enhancing lipid productivity.

Conclusion

Microalgae are increasingly recognized as a sustainable source of high-value bioactive compounds, particularly omega-3 PUFAs such as EPA and DHA, which are essential for human health and industrial applications. Among the three species, *S. platensis*, *C. vulgaris*, and *N. oceanica*, optimal light (50–75 µmol m^{-2} s^{-1}) and temperature (30-35°C) conditions enhance EPA and DHA synthesis, whereas UV-B exposure significantly inhibits lipid accumulation due to oxidative stress. *Nannochloropsis oceanica* emerged as the most promising strain for industrial-scale omega-3 production, followed by *S. platensis* and *C. vulgaris*. The results offer practical insights into optimizing cultivation parameters in photobioreactors for biofuel, nutraceutical, and aquaculture industries.

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