



## Review

# Using high CO<sub>2</sub> concentrations to culture microalgae for lipid and fatty acid production: Synthesis based on a meta-analysis

Yuan Feng<sup>a</sup>, Jingke Ge<sup>a</sup>, Pau Loke Show<sup>b,c</sup>, Chi Song<sup>a</sup>, Liumiao Wu<sup>a</sup>, Zengling Ma<sup>d</sup>, Guang Gao<sup>a,\*</sup>

<sup>a</sup> State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China

<sup>b</sup> Department of Chemical Engineering, Khalifa University, P.O. Box 127788, Abu Dhabi, United Arab Emirates

<sup>c</sup> Department of Chemical and Environmental Engineering, University of Nottingham Malaysia, Jalan Broga, Selangor, Malaysia

<sup>d</sup> Zhejiang Provincial Key Laboratory for Water Environment and Marine Biological Resources Protection, Wenzhou University, Wenzhou 325035, China

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## ABSTRACT

To gain a comprehensive understanding of cultivating microalgae for lipid and fatty acid production with high CO<sub>2</sub>, this study conducted a meta-analysis based on 757 data sets from 51 papers for the first time. The findings show that high CO<sub>2</sub> concentrations (0.1–30%) generally promote microalgal growth, whereas extreme high CO<sub>2</sub> levels (30–50%) usually exhibit negative effects. High CO<sub>2</sub> levels (0.1–30%) also commonly stimulate cellular lipid accumulation. Therefore, high CO<sub>2</sub> levels (0.1–50%) increase lipid productivity of both freshwater and marine microalgae, particular for Chlorophytina. These elevated CO<sub>2</sub> levels (0.1–30%) reduce saturated fatty acid content of microalgae but enhance the content of unsaturated and polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Advanced molecular editing techniques, e.g., CRISPR-Cas9, can be utilized to improve microalgal tolerance to flue gases that contain hazardous compounds while condition optimization for maximal use of CO<sub>2</sub> in the production of microalgae with high lipid content should also be conducted in future. This research provides crucial insights for designing and optimizing microalgal cultivation with high CO<sub>2</sub> to produce lipid and fatty acids.

## 1. Introduction

The rapid development of heavy industry and the massive application of fossil fuels have led to excessive emission of greenhouse gases – particularly carbon dioxide (CO<sub>2</sub>) since the industrial revolution (Razak et al., 2017). The rapid increase of atmospheric CO<sub>2</sub> has led to an upsurge in global temperatures and associated heatwave, causing significant damage to ecosystems, economic development, and human health (Jacobson et al., 2019). The accelerated increase of atmospheric CO<sub>2</sub> raises the absorption of CO<sub>2</sub> by ocean water, intensifies the acidity of ocean water and leads to ocean acidification (Hopkins et al., 2020). Ocean acidification can pose a threat to calcifiers, including ecologically important corals and coccolithophores (Kroeker et al., 2010). Meanwhile, ocean acidification can stimulate the occurrence of green tides (Young and Gobler, 2016; Feng et al., 2024). Therefore, mitigating and neutralizing CO<sub>2</sub> emissions has become primary goal for global sustainable development since the Paris agreement.

Among various approaches, microalgae cultivation has been

proposed as a promising biological method for capturing CO<sub>2</sub> from the flue gas emissions of thermal power stations (Li et al., 2023; Sharma et al., 2023). Compared with terrestrial plants, microalgae exhibit higher efficiency in capturing carbon dioxide due to their high photosynthetic efficiency. In addition, microalgae cultivation does not require arable land. The cultivation of microalgae using exhaust from coal-fired power plant has emerged as an important research direction for CO<sub>2</sub> biocapture (Ghosh and Kiran, 2017; Wang et al., 2025). Microalgae are ideal for biodiesel production due to their high lipid content, for example, 45–47% for *Nitzschia* sp. (Chisti, 2007). However, the main barrier for biodiesel production using microalgae is the high cost (\$3.88–36.95 L<sup>-1</sup>) compared to fossil fuel (\$0.82 L<sup>-1</sup>) (Branco-Vieira et al., 2020; Gao et al., 2020). In addition to biofuel production, microalgae lipid can be used to generate valuable polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), representing an emerging industry (Jiang et al., 2016; Dubey et al., 2024). CO<sub>2</sub> plays a vital role in regulating microalgal growth by influencing the biosynthesis of essential components (Xu

\* Corresponding author.

E-mail address: [guang.gao@xmu.edu.cn](mailto:guang.gao@xmu.edu.cn) (G. Gao).

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et al., 2022). Increased CO<sub>2</sub> levels can enhance microalgal growth since current CO<sub>2</sub> levels in seawater are limited for microalgal photosynthesis (Kupriyanova et al., 2023). In terms of lipid content, microalgae typically enhance lipid synthesis in response to elevated CO<sub>2</sub> concentrations (Jaiswal et al., 2020). For instance, 5% and 10% CO<sub>2</sub> concentrations enhanced lipid content of *S. costatum* by 70% and 87%, respectively, compared to the ambient condition (0.04% CO<sub>2</sub>) (Wu et al., 2022). A similar trend is observed in fatty acid content. For instance, increasing the CO<sub>2</sub> concentration from 0.04% to 15% led to an increase in total fatty acids content in *Chlorella vulgaris* (Kryvenda et al., 2023). The stimulative effects of elevated CO<sub>2</sub> on lipid and fatty acid biosynthesis can be attributed to two aspects. Firstly, elevated CO<sub>2</sub> can enhance cellular carbon availability that is necessary for lipid biosynthesis of microalgae. Secondly, too high CO<sub>2</sub> concentrations can lead to growth arrest that is in favor for the accumulation of lipid in algal cells (Xie et al., 2022).

Numerous studies have been conducted to investigate the effects of high CO<sub>2</sub> on the growth, lipid, and fatty acids (FAs) production of microalgae. However, different results, including positive effects, neutral and even negative effects of high CO<sub>2</sub>, have been reported (Arudchelvam and Nirmalakhandan, 2012; Jensen et al., 2019). Previous review articles did not conduct quantitative analysis of CO<sub>2</sub> effects. Therefore, the understanding in this field is still fragmented, hindering the use of suitable microalgae and CO<sub>2</sub> levels for optimal lipid and PUFA production. Meta-analysis, a statistical method that synthesizes results from numerous studies on a specific subject, can figure out clear patterns in the relationship between variables and draw conclusions through quantitative analysis. In addition, meta-analysis can identify new research questions and direct further research (Gurevitch et al., 2018). In this study, a meta-analysis was conducted to evaluate the current experimental evidence on the effects of high CO<sub>2</sub> concentrations on the growth, lipid, and fatty acids production of aquatic microalgae, by analyzing 757 experimental data sets from 51 published papers. The current study also offers perspectives and suggestions for future research. The findings in this study contribute new insights into how different CO<sub>2</sub> levels affect growth and lipid production of both freshwater and marine microalgae, providing valuable contributions to the utilization of flue gas for microalgae cultivation for biodiesel with unpurified CO<sub>2</sub> and health care products with purified CO<sub>2</sub> (Daneshvar et al., 2022; Ye et al., 2022).

## 2. Materials and methods

### 2.1. Literature search

In this meta-analysis, data collection, analysis and interpretation were according to PRISMA and Gurevitch et al. (2018). As illustrated in PRISMA Flow Diagram (Fig. S1), target studies were searched through *ISI Web of Science* on Sep 5th, 2023 with keywords: “Carbon dioxide AND microalgae AND (Growth OR Lipid)” and use Scholar Google to cross-check. The initial search yielded 76 published papers that were trimmed down to 51 papers after screening according to the following criteria: (1) the paper was an original research study, not a review, (2) the paper contains a control treatment of atmospheric CO<sub>2</sub> concentration; and (3) experimental results were reported with basic statistical measures, including sample size, mean, and variance for the endpoint measured, etc. Inclusion criteria implies that the studies must contain relevant data on the physiological performance of microalgae while data on macroalgae and insufficient report of results were excluded from this review article. The included studies cover works published in academic journals with considerable impact, although there might be other literature that has been omitted.

### 2.2. Data collection

In this meta-analysis, 51 studies published between 1996 and 2023

were included, representing 67 microalgal species/strains and 36 different concentrations of CO<sub>2</sub> (control group excluded). Relevant information is extracted from the 51 papers retained: data related to the studied microalgae including their habitats, taxonomic classification, CO<sub>2</sub> concentration, exposure time, influencing parameters, as well as relevant data for the control group (i.e., atmospheric CO<sub>2</sub> concentration) and treatment groups. Numerical data presented in tabular form were directly used in this study, while data presented in graphical form were obtained from the corresponding authors or using the analysis software of GetData Graph Digitizer (version 2.24) to extract values from the graphs. The charts from the referenced literature were captured and imported into GetData software. The x and y-axis scales were defined based on the image points, converting the charts into vector format. The necessary data points were then extracted, and the software would display the corresponding data values according to the established coordinates. There might be differences between extracted and real data. It was tested that the data error was <0.5%, suggesting high accuracy of this software. For literature that investigated the cross-effects of multiple factors on microalgal growth and biochemical composition, this study only extracted relevant data related to CO<sub>2</sub> concentration as the influencing factor.

When dealing with a research covering more than one concentration or multiple exposure time, data under different treatments were all included. And when analyzing experiments that reported the same biological response at multiple time points using various metrics, only the most comprehensive metrics were considered for that particular response variable. In cases where the consistency within each response was required, the data were normalized, which involves converting the cell concentrations into the specific growth rate or regarding the cell concentrations at the endpoint as the comparable data.

Referring to Jin et al. (2019) with a slight modification, the wide range of physiological and biochemical responses assessed in the literature were classified into the categories including: (1) growth (e.g., specific growth rate, dry cell weight or cell concentration), (2) lipid content, (3) lipid productivity, (4) fatty acid (e.g., total fatty acid, unsaturated fatty acid, PUFA, SFA), and (5) EPA and DHA.

Data were subdivided into subsets according to (1) taxonomical groups (Bacillariophytina, Chlorophytina, Cyanobacteriota, Myzozoa, Ochrophytina), following the classification provided by AlgaeBase ([www.algaebase.org](http://www.algaebase.org)); (2) habitats (freshwater and marine environment); (3) exposure time (intervals of 1d, 5d, 10d, 15d and > 15d); (4) CO<sub>2</sub> concentrations (intervals of 0.4–1%, 1–10%, 10–30%, 30–50%, indicated as slightly high, moderately high, high, extreme high, respectively). A comprehensive compilation of the extracted data is conducted according to the above subsets (Table S1).

### 2.3. Data analysis

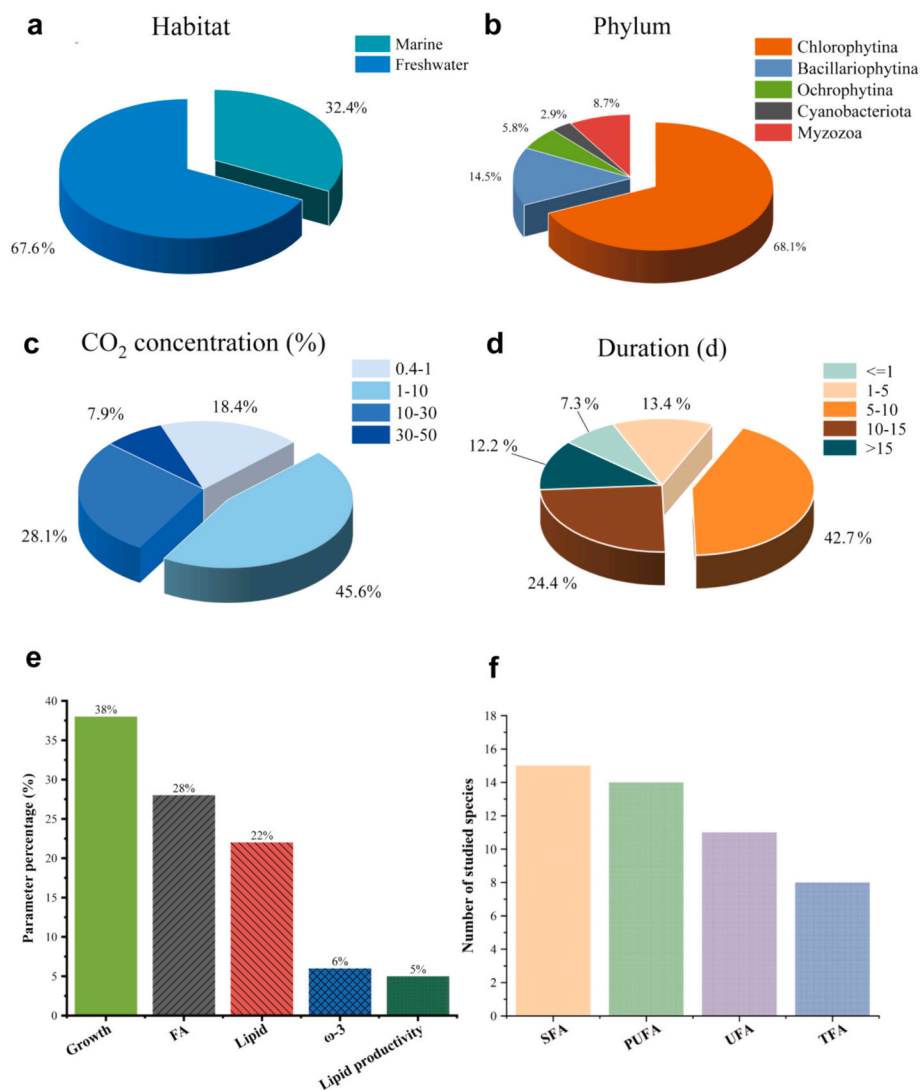
Facing the inconsistent units for CO<sub>2</sub> concentrations across different literature sources, these values were uniformly converted into a representation based on the proportion of CO<sub>2</sub> in the air (volume basis at atmospheric pressure), denoted by the unit “%”. Upon transformation and categorization of the response traits, the values of the effect group were divided by the corresponding control group values, yielding standardized data which facilitates the comparison of data across disparate literary sources. Through the calculation of the logarithm (base 10) of the standardized data, the corresponding magnitudes of the effects were obtained (effect size). A positive effect size indicates a stimulatory influence of high CO<sub>2</sub> concentrations on the given microalgae traits. Conversely, a negative effect size signifies an inhibitory effect of high CO<sub>2</sub> concentrations, while a value of zero suggests the traits remain unaffected by elevated CO<sub>2</sub> levels (Jin et al., 2017). Each of these corresponding effect sizes acts as a data point and was then used in subsequent data analysis to facilitate comparisons among groups.

After converting all collected data into effect sizes, we used the *ggpubr* package in R (version 4.3.2) to compare the effect sizes across

different clusters (e.g., habitats, taxa). In each parameter section, to analyze the differences between two habitats, a homogeneity of error variance test (Levene's test,  $p > 0.05$ ) was conducted ahead for the data from two habitats. If the amphibious habitat data passed the Levene's test, a two-sample  $t$ -test was then performed. Otherwise, a non-parametric statistical test (here Wilcoxon test was adopted) would be conducted. All the related data were considered to conform to a normal distribution. To examine the differences among different subphyla, one-way analysis of variance test (one-way ANOVA,  $p = 0.05$ ) was employed. And following ANOVA analysis of variance, pairwise comparisons between the groups were conducted using the Tukey's post hoc test. Meanwhile, the data of a specific habitat or algal species with sufficient sample size were analyzed by performing linear and quadratic (even cubic) regression, to assess the relationship between effect size and carbon dioxide concentration or exposure time. During the fitting attempts, priority was given to fitting curves with a  $p$ -value  $< 0.050$ . When all regressions had  $p$ -values below 0.050, the fitting with a higher  $R^2$  was favored. If all  $p$ -values were above 0.050, the fit with the smallest  $p$ -value and highest  $R^2$  was prioritized. This analytical approach seeks to explore the relationships between the logarithmic effect sizes of various microalgae traits and the  $CO_2$  concentrations as well as exposure durations. Three decimal places were presented for statistical outcome, two decimal places were used for  $CO_2$  concentrations that is lower than 1%

and only integer part was kept for other percentages.

Challenges encountered in meta-analyses and systematic reviews include issues related to publication bias and research bias. To ensure the robustness of the statistical results, a publication bias assessment was conducted. Q test was conducted using the 'pacman' package in R with original data and extracted the standard error of mean of each corresponding dataset. The outcome showed that the Q value ( $Q = 4,985,364.415$ ) and  $I^2$  value ( $I^2 = 100\%$ ) were very high and  $p$  value ( $p < 0.001$ ) was very low, indicating a high degree of heterogeneity because the thresholds are  $p < 0.05$  and  $I^2 > 70\%$ . Consequently, random-effects model was employed for examination. The Rosenthal's method of fail-safe numbers was employed initially (Rosenthal, 1979), and this method determines the quantity of effect sizes showing no significant effects required to alter the reported significance ( $p$ -value) by the model. Additionally, a funnel plot approach was utilized to illustrate the relationship between study effect sizes and their standard errors. Egger's Test was then employed to examine its symmetry. The outcomes similarly indicated an absence of publication bias ( $p = 0.436$ ).



**Fig. 1.** Features of  $CO_2$  studies on microalgae, involving habitats (a), subphyla (b), concentrations of  $CO_2$  (c), exposure time (d), parameters studied (e) and fatty acid compositions (f). PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid; TFA, total fatty acid.

### 3. Results and discussion

#### 3.1. Data compilation

A basic categorical analysis on the collected data information was conducted as a reference for assessing the major focus of research progress (Fig. 1). In the context of this study, the microalgal community under investigation inhabits two distinct ecological niches, freshwater and marine environments respectively, with freshwater microalgae accounting for 67.6% and marine microalgae comprising 32.4% of the total population (Fig. 1a). This is because extensive research has been conducted on various strains within the *Chlorella* genus of freshwater microalgae. They demonstrate a relatively high tolerance to elevated levels of carbon dioxide, rendering them of significant value in diverse applications.

The microalgal species scrutinized in this study encompass representatives from the subphyla Chlorophytina, Bacillariophytina, Cyanobacteriota, Myzozoa and Ochrophytina, with Chlorophytina and Bacillariophytina (commonly referred to as diatoms) being the most predominant, constituting 68.1% and 14.5% of the total population, respectively, followed by Myzozoa (8.7% of the total) (Fig. 1b). The subphylum Ochrophytina comprises four species of *Nannochloropsis*, whereas Cyanobacteriota only consists of two species (*Nostoc* and *Arthrospira platensis*) in this context. In a comprehensive analysis of techniques and outcomes related to the cultivation of microalgae, it is found that *Chlorella* sp. exhibits exceptional growth rates and enhanced lipid accumulation when exposed to elevated concentrations of CO<sub>2</sub> through aeration (Tang et al., 2011; Chunzhuk et al., 2023), rendering this species to be a hotspot of researches. In such investigations, cyanobacteria commonly demonstrate high sensitivity to elevated CO<sub>2</sub> levels (Kryvenda et al., 2023), thus resulting in comparatively fewer research endeavors on them.

A wide range for CO<sub>2</sub> concentrations was used in previous studies (Fig. 1c), with the lowest concentration being 0.08% for the cultivation of a marine diatom *Phaeodactylum tricorutum* (Wu et al., 2015) and the highest concentration utilized being pure CO<sub>2</sub> on a freshwater green microalga *Chlorella vulgaris* (Huang and Su, 2014). Typically, the control group's carbon dioxide concentration ranges from 0.03% to 0.04%. The data collected for this investigation reveal that the majority of experimental CO<sub>2</sub> concentrations ranges from 1% to 50%. This range covers the proportion of CO<sub>2</sub> in flue gas, since the concentrations of CO<sub>2</sub> from coal-fired power plants' flue gas are usually 10–20% (Fu et al., 2022). Nearly half of the studies (45.6%) used the range of 1–10%, followed by 10–30% (28.1%). The ranges of 0.4–1% and 30–50% constitute 18.4% and 7.9% of the observations respectively.

In terms of culture duration, the range of 5 to 10 days comprises 42.7% of the observations (Fig. 1d). The next most prevalent duration range is from 10 to 15 days, representing 24.4% of the cases followed by the range from 1 to 5 days (13.4%), while experiments exceeding 15 days are relatively rare (accounting for 12.2%). In studies where the sampling time is not specified, the data are not included in the statistical analysis.

In previous CO<sub>2</sub> studies, growth that mainly refers to specific growth rate or dry biomass is the most focused-on parameter, accounting for approximately 38% of the datasets (Fig. 1e). Subsequently, the parameters “FAs (excluding ω3 fatty acids)”, “lipid content”, “total ω3 (DHA, EPA)” and “lipid productivity” are in descending order of prevalence. In addition to physiological indicators, there are several studies in the literature that have investigated the enzyme metabolism and gene responses of microalgae under increased CO<sub>2</sub> concentrations (Wang et al., 2022; Wu et al., 2015). As found in *Chlorella* species, elevated CO<sub>2</sub> can enhance lipid accumulation through fatty acid synthesis, supported by the upregulation of genes involved in triacylglycerol biosynthesis, including glycerol-3-phosphate dehydrogenase and phospholipid-diacylglycerol acyltransferase (Sun et al., 2016; Koh et al., 2023). It was also reported the oxidative pentose phosphate pathway (OPPP) may

participate in the lipid accumulation of *P. tricorutum* cells grown at high CO<sub>2</sub> concentrations (Wu et al., 2015; Wang et al., 2022).

In the analysis of fatty acids, the majority of research has been focused on PUFA and SFA, with 14, 15 species studied, respectively (Fig. 1f). PUFA are well-known for their positive impact on human health. Consequently, microalgae with high PUFA content are attracting increased attention, not only for their environmental benefits but also for their potential health-related applications (Jiang et al., 2016; Saini and Keum, 2018). Monounsaturated fatty acids followed closely behind, with 11 species investigated. However, there is relatively less research on total fatty acids (FAs). It's worth noting that data regarding total FAs typically refer to their proportion within the cell, while fatty acid data describe their proportion within the total FAs.

#### 3.2. Growth

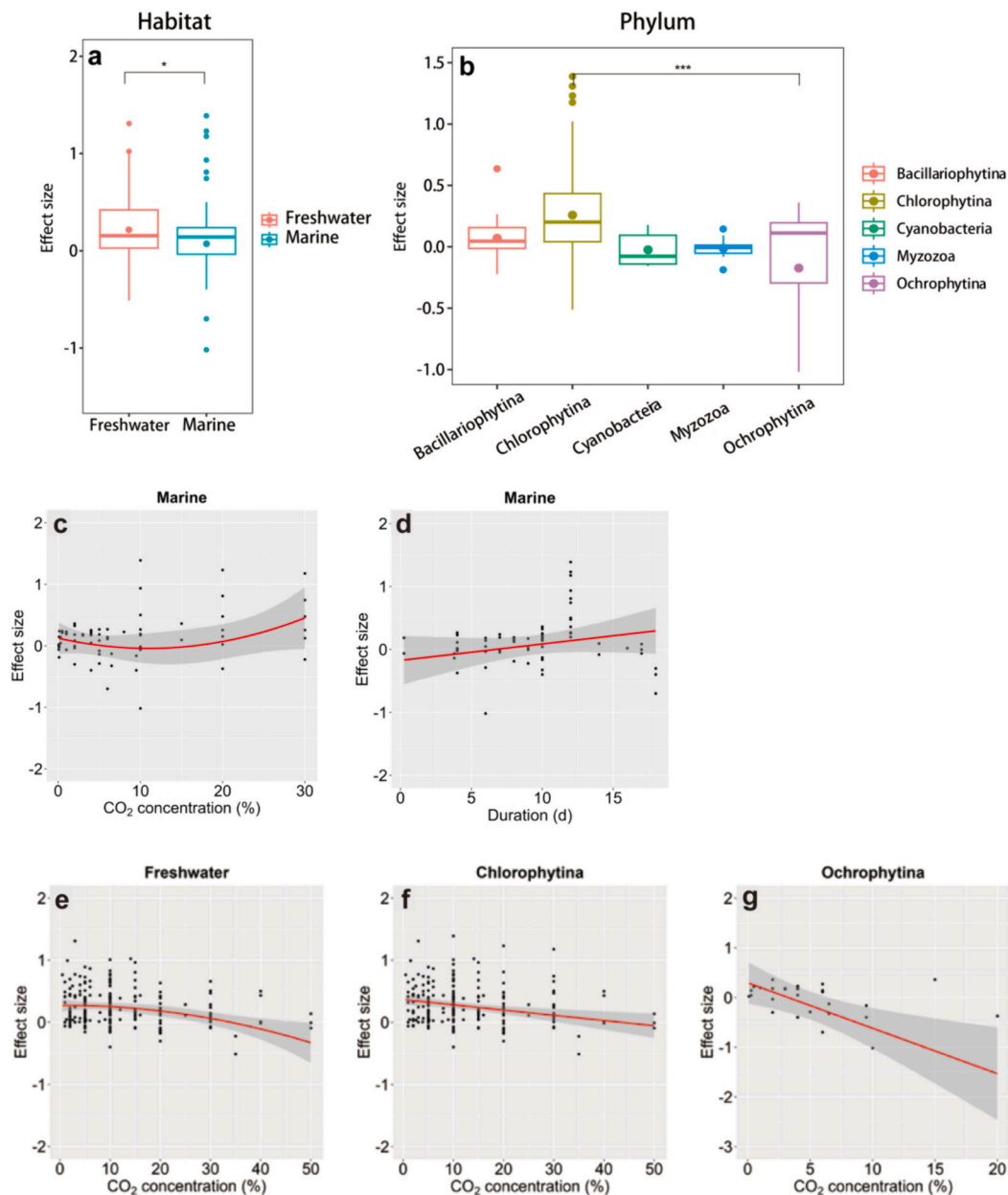
##### 3.2.1. Overall effects of CO<sub>2</sub> on microalgal growth

Despite a few studies showing negative effects of CO<sub>2</sub> concentration on microalgae's growth, overall, high concentrations of CO<sub>2</sub> (0.1–30%) have a positive effect on the growth of both marine and freshwater microalgae, with the effect size for freshwater microalgae is higher than that for marine microalgae (Fig. 2a, Freshwater:  $0.216 \pm 0.382$ ; Marine:  $0.071 \pm 0.663$ ; *t*-test, *p* = 0.022). Such promotion effects can be easily interpreted as elevated CO<sub>2</sub> concentrations can enhance microalgal growth by offering a richer carbon source for photosynthesis and thus stimulate the production of compounds required for growth. This finding was consistent with the conclusions drawn by a previous review article (Singh and Singh, 2014). It is also confirmed that *Chlorella* sp. prioritizes rapid growth in the early stages under high CO<sub>2</sub> conditions by upregulating the Calvin cycle, METE, and bicarbonate transporters (Koh et al., 2023).

Different responses were observed across various subphyla though (Fig. 2b). The high CO<sub>2</sub> concentrations significantly stimulate the growth of microalgae in the Chlorophytina subphylum ( $0.251 \pm 0.399$ ) and negatively affect Ochrophytina ( $-0.173 \pm 0.899$ ) while they have very weak effects on the growth of microalgae in the Bacillariophytina, Cyanobacteriota, Myzozoa ( $0.071 \pm 0.158$ ,  $-0.023 \pm 0.148$ ,  $-0.011 \pm 0.092$ , respectively). The differences across subphyla are not significant (Turkey-Krama HSD, *p* > 0.050) except for difference between Chlorophytina and Ochrophytina (Turkey-Krama HSD, *p* < 0.001) with Chlorophytina showed a significantly higher effect size. These findings indicate that generally high concentrations of CO<sub>2</sub> (1–30%) are beneficial for microalgae growth but only significant in certain subphylum, such as Chlorophytina. In comparison to freshwater algae, the growth of marine microalgae show more diverse responses to high CO<sub>2</sub> since the data points for marine microalgae are more dispersed. The significant differences among different phyla are understandable as it was suggested that CO<sub>2</sub> tolerance and photoautotrophic CO<sub>2</sub> fixation capacities greatly vary among different species by previous review articles (Lim et al., 2021; Peilun et al., 2023). One of the causes leading to these differences is that some of the strains have undergone acclimation or mutation (Peilun et al., 2023), thereby giving them high tolerance to elevated CO<sub>2</sub>. Another reason is that different microalgae species exhibit varying abilities to utilize different forms of inorganic carbon. For instance, *Spirulina* prefers aqueous bicarbonate over gaseous CO<sub>2</sub> (Mehtar et al., 2019), while certain *Chlorella* strains without external carbonic anhydrase favor gaseous CO<sub>2</sub> over liquid bicarbonate or carbonate (Satoh et al., 2001).

##### 3.2.2. Effects of CO<sub>2</sub> concentration and culture duration on microalgal growth

The effect size for the growth of marine microalgae exhibits a correlation with CO<sub>2</sub> concentration, as the effect size first declines and then climbs up with the tipping point around 10% CO<sub>2</sub> (Fig. 2c, quadratic regression, adjusted R<sup>2</sup> = 0.108, *p* = 0.006, see Table S2 for all regression equations), but shows no significant correlation with culture



**Fig. 2.** CO<sub>2</sub> effect size for growth of microalgae from different habitats (a, 290 data analyzed) and taxonomic groups (b, 290 data analyzed), CO<sub>2</sub> effect size for microalgal growth versus CO<sub>2</sub> concentration or culture duration in marine (c & d, 77 & 75 data analyzed respectively) and versus CO<sub>2</sub> concentration broken down by freshwater (e, 213 data analyzed), Chlorophytina and Ochrophytina (f & g, 213 & 31 data analyzed respectively). \*, \*\*\*represent significant difference levels of  $p < 0.05$ ,  $p < 0.001$ , respectively.

duration (Fig. 2d, linear regression, adjusted  $R^2 = 0.010$ ,  $p = 0.187$ ). This downward trend in relation with CO<sub>2</sub> concentration is consistent with previous individual studies which show that the growth rates decrease or even exhibit negative values as CO<sub>2</sub> rises (Huang et al., 2022; Moghimifam et al., 2020). However, the upward trend in this study seems unusual because marine microalgae are commonly intolerant to very high CO<sub>2</sub> and very low pH. The data sets supporting this trend come from two studies involving the *Dunaliella* sp. (Moghimifam et al., 2020). This specie evidently exhibits strong acid tolerance in experiments, allowing it to withstand high CO<sub>2</sub> concentrations. Therefore, this distinctive trait of *Dunaliella* sp. contributes to the overall upward trajectory in the statistical curve.

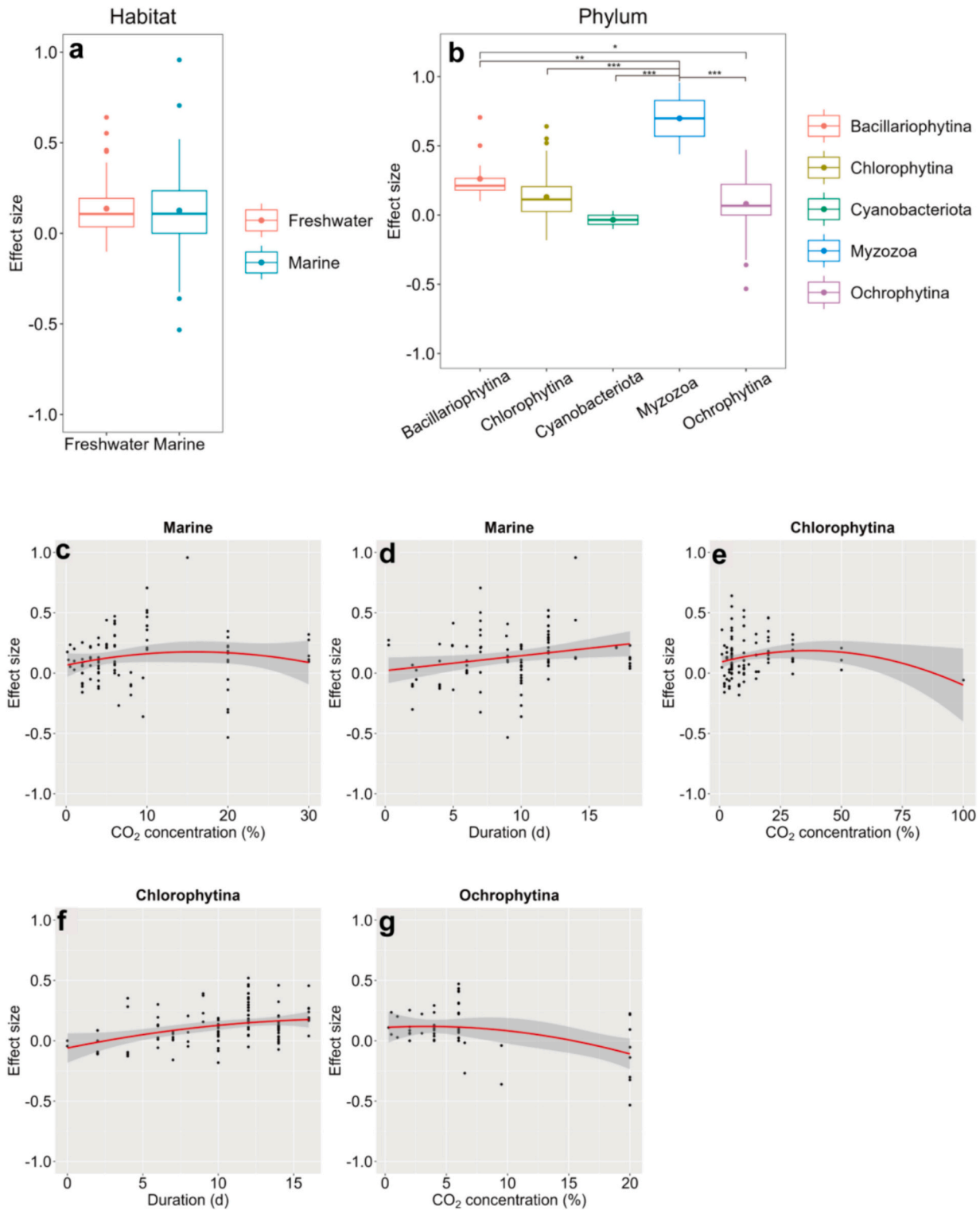
In the case of freshwater microalgae, there is a negative relation between effect size for their growth and CO<sub>2</sub> concentration; effect size slowly decreases with CO<sub>2</sub> concentration till 20% and drops sharply

afterwards, as depicted in Fig. 2e (quadratic regression, adjusted  $R^2 = 0.055$ ,  $p < 0.001$ ). There was no significant correlation between the effect size for the growth of freshwater microalgae and the culture time either ( $p = 0.324$ ) (Fig. S2).

It is important to note that, irrespective of their habitats, microalgal growth and biomass experience a boost when exposed to high CO<sub>2</sub> concentrations below 30%, exhibiting a similar trend with previous study where increasing CO<sub>2</sub> levels increased growth of *C. vulgaris* (de Jesus and Maciel Filho, 2017). However, once the CO<sub>2</sub> concentration exceeds 30%, it has a detrimental effect on microalgal growth. This suggests that CO<sub>2</sub> concentrations at or above this threshold are generally excessive for these microalgae. Although CO<sub>2</sub> serves as the substrate for photosynthetic carbon fixation, higher concentrations of CO<sub>2</sub> (40%) consistently could lead to temporary inhibition of photosynthesis and the growth of algal cells (Satoh et al., 2004; Yang et al., 2020).

When CO<sub>2</sub> concentration becomes extremely high, medium pH can be very low. For instance, seawater pH can be as low as 5.6–6.5 when bubbling >5% CO<sub>2</sub>, and many microalgae species cannot grow well in a such low pH (Su et al., 2023). The acidic environments can have a detrimental effect on photosynthesis and growth of microalgae because some key enzymes involved in CCMs and Calvin cycle can be deactivated

in such acidic conditions (Zhu and Huang, 2017). Some researchers concluded that the inhibition caused by CO<sub>2</sub> was only induced by the decrease in pH because their results showed that controlling the extracellular pH could negate the inhibitory effect on growth in high CO<sub>2</sub> environments (Liu et al., 2019). Furthermore, microalgal transcriptomes have shown significant differences in gene expression patterns of growth



**Fig. 3.** CO<sub>2</sub> effect size for lipid content of microalgae in different habitats (a, 180 data analyzed) and taxonomic groups (b, 180 data analyzed); CO<sub>2</sub> effect size for microalgae lipid content in marine (c & d, 97 & 91 data analyzed respectively), subphyla Chlorophytina (e & f, 109 & 107 data analyzed respectively) and Ochrophytina (g, 53 data analyzed) versus CO<sub>2</sub> concentration or culture time. \*, \*\*, \*\*\* represent significant difference levels of  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ , respectively.

inhibition between solely caused by pH and by CO<sub>2</sub>, indicating that increased CO<sub>2</sub> and decreased pH can play different roles in microalgal growth (Choi et al., 2021).

In relation with CO<sub>2</sub> concentration, only data sets for the subphyla Chlorophytina and Ochrophytina are enough for statistical analysis of effect size. The effect size for growth shows a cubic regression for Chlorophytina (Fig. 2f, adjusted R<sup>2</sup> = 0.052, *p* = 0.003) while a linear regression for Ochrophytina (Fig. 2g, adjusted R<sup>2</sup> = 0.211, *p* = 0.005). High CO<sub>2</sub> concentrations below 20% significantly stimulate the growth of Chlorophytina and afterwards the inhibit effect becomes rather pronounced. In contrast, high CO<sub>2</sub> significantly reduces the growth of Ochrophytina and the effect size becomes larger with the rising CO<sub>2</sub> concentration. No relationship between effect size and culture time is detected either for Chlorophytina (*p* = 0.245) or for Ochrophytina (*p* = 0.454) (Fig. S2). These results show differential response of different microalgae subphyla to high CO<sub>2</sub> concentrations.

### 3.3. Lipid

#### 3.3.1. Overall effects of CO<sub>2</sub> on lipid content of microalgae

In the present meta-analysis, the effect sizes for lipid content in both marine and freshwater microalgae are >0 (Fig. 3a), which indicates that high CO<sub>2</sub> concentrations (1–30%) promote lipid synthesis in both marine and freshwater microalgae. Lipid synthesis depends on carbon skeletons and ATP generated via photosynthesis. Elevated CO<sub>2</sub> levels can thereby boost lipid production by increasing photosynthetic rates of microalgae (Wu et al., 2022). Meanwhile, microalgae usually preferentially synthesize lipids over proteins and carbohydrates under stressful environments as lipids are a cost-effective means for cell's recovery from stress (Rodolfi et al., 2009; Jaiswal et al., 2020). This also explains the increased lipid content under high CO<sub>2</sub> conditions because decreased pH can be stressful for intracellular metabolic activities. Relevant enzymatic and genetic response studies indicate that elevated CO<sub>2</sub> levels (10%) may drive carbon flow by increasing the flux of CO<sub>2</sub> to acetyl-CoA in microalgal chloroplasts, leading to greater lipid accumulation in *C. sorokiniana* (Sun et al., 2016). Transcriptomic analysis also indicated that PDAT and GPDH, which represent the initial and final steps of TAG synthesis, are likely to be pivotal in lipid synthesis under high CO<sub>2</sub> conditions (Koh et al., 2023).

The effect size between marine and freshwater microalgae is not significant (Wilcoxon's test, Freshwater: 0.136 ± 0.1439, Marine: 0.126 ± 0.2221, *p* = 0.818). Among all the microalgal groups studied, all subphyla show positive responses to the high CO<sub>2</sub> concentrations (0.1–30%) except for Cyanobacteriota (Fig. 3b). The data of Cyanobacteriota mainly come from the species *A. platensis*, which is not a typical lipid source except when a two-stage cultivation method with nitrogen starvation is applied at the second stage to stimulate lipid synthesis (Chunzhuk et al., 2023). Notably, the stimulatory effect of high CO<sub>2</sub> concentrations on lipid synthesis in Myzozoa microalgae is the most significant and is much stronger compared to the rest subphyla (Tukey–Kramer HSD, all *p* < 0.001). However, it is worth noting that only two datasets from the species *G. membranacea* are available for Myzozoa in the present study. It is evident that aerating with CO<sub>2</sub> has a substantial and direct impact on lipid accumulation in *G. membranacea* (Mohsenpour and Willoughby, 2016), while whether other species from Myzozoa are also sensitive to high CO<sub>2</sub> needs further study. The stimulatory effects of high CO<sub>2</sub> on lipid synthesis in other microalgal groups are relatively weak and show no significant differences except for the comparison between Bacillariophytina and Ochrophytina (Tukey–Kramer HSD, *p* = 0.006). The subphylum Bacillariophytina also shows a high sensitivity to high CO<sub>2</sub> concentrations with effect size of 0.262 ± 0.164 in average. Lipids make up a significant portion of carbon in diatoms, and the initial holographic reconstruction of *Skeletonema marinoi* and *Thalassiosira rotula* cells shows that as much as one-fifth of the cell volume can be filled with chloroplasts containing glycolipids (Botte et al., 2017). In high CO<sub>2</sub> environments, diatom cells seem to allocate the

surplus carbon toward lipid production (Hennon et al., 2015).

#### 3.3.2. Effects of CO<sub>2</sub> concentration and culture duration on lipid content of microalgae in different habitats

The effect size for lipid content in marine microalgae shows a rising tendency when the CO<sub>2</sub> concentrations are below 15% and then slowly declines as the CO<sub>2</sub> concentration continues to rise, although this pattern is not statistically significant (Fig. 3c, quadratic regression, adjusted R<sup>2</sup> = 0.019, *p* = 0.150). The effect size for lipid content in marine microalgae increases with culture time (from null to positive) (Fig. 3d, linear regression, adjusted R<sup>2</sup> = 0.045, *p* = 0.025). There is no significant correlation between the effect size for lipid content in freshwater microalgae and either CO<sub>2</sub> concentration (*p* = 0.379) or culture time (*p* = 0.187) (Fig. S2).

The elevated concentration of CO<sub>2</sub> supplies a significant carbon source for lipid biosynthesis of microalgae (Xu et al., 2022). Lipids are preferentially synthesized in stressful environments because it is a cost-effective strategy for cell reconstruction following stress (Rodolfi et al., 2009). In addition, growth arrest under high CO<sub>2</sub> concentrations is another reason that leads to increased lipid accumulation. This phenomenon has been found in the diatom *Skeletonema costatum* and the growth was inhibited by 5% CO<sub>2</sub> while the lipid content was enhanced (Xie et al., 2022). And the decreasing trend when CO<sub>2</sub> concentration exceeds a certain value may be due to the negative effect of decreased pH on lipid synthesis. The upward trend in relationship with exposure time in marine species may suggest the acclimation process of algal cells to acidification. Most studies have demonstrated the promotion of lipid accumulation in microalgae in the case of short-term acidification (Patil and Kaliwal, 2017; Sabia et al., 2018). For instance, in *C. vulgaris*, short-term acidification of 15 days results in the intracellular accumulation of acetyl-CoA and lipids (Jose and Suraishkumar, 2016).

#### 3.3.3. Effects of CO<sub>2</sub> concentration and culture duration on lipid content of microalgae in different subphyla

In the case of Chlorophytina microalgae, no correlation is found between the effect size for lipid content and CO<sub>2</sub> concentration since the data are not widely distributed (Fig. 3e, quadratic regression, adjusted R<sup>2</sup> = 0.004, *p* = 0.307). However, the effect size increased from null to positive with the culture time in Chlorophytina, indicating a potential resilience gained during the culturation (Fig. 3f, quadratic regression, adjusted R<sup>2</sup> = 0.176, *p* < 0.001). As for Ochrophytina microalgae, the effect size for lipid content does not change when CO<sub>2</sub> concentration below 6% and then drops from positive to negative with the increase of CO<sub>2</sub> concentration (Fig. 3g, quadratic regression, adjusted R<sup>2</sup> = 0.237, *p* < 0.001). No apparent relationship is found between the effect size for lipid content and culture time for the data seemingly scatter at random (Fig. S2).

Researches on *Scenedesmus obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2 have demonstrated that even at high concentrations of CO<sub>2</sub> (30–50%), there is an enhancement in lipid accumulation (Tang et al., 2011), which aligns with the present analysis where Chlorophytina species maintain its relatively high lipid production within this range of CO<sub>2</sub> concentration, and continue to increase with extended period of cultivation.

In subphyla Ochrophytina, the negative impact observed at 20% CO<sub>2</sub> concentration is the primary reason for the declining trend in lipid accumulation in this study. These points mainly come from the species *Nannochloropsis salina* that exhibited an inhibited lipid accumulation under excessive CO<sub>2</sub> supply (Chen et al., 2020). The CO<sub>2</sub> concentration within a moderate range could be the most critical factor stimulating lipid accumulation (de Jesus and Maciel Filho, 2017).

### 3.4. Lipid productivity

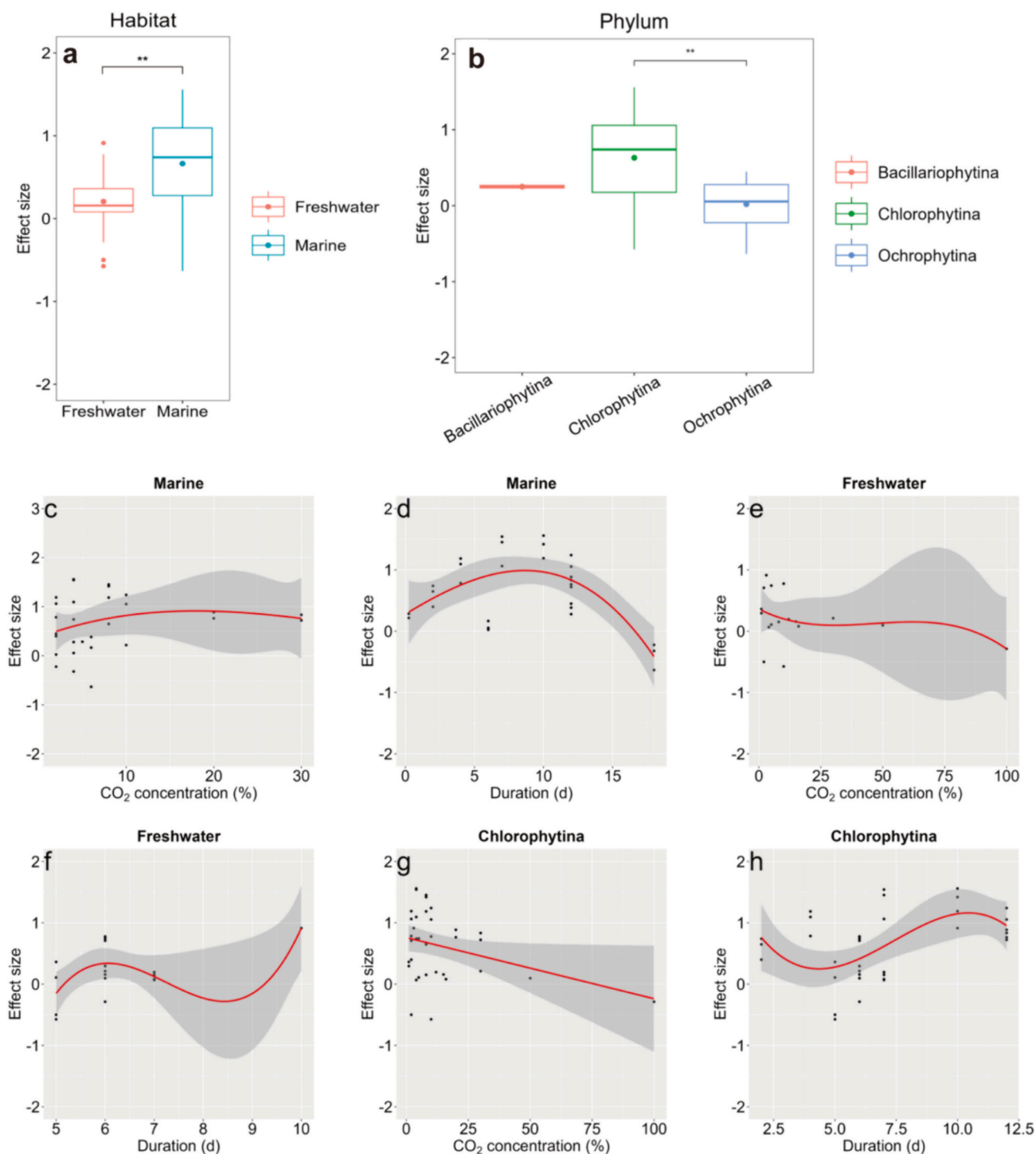
#### 3.4.1. Overall effects of CO<sub>2</sub> on lipid productivity of microalgae

The lipid productivity of microalgae is greatly promoted by high

concentrations of CO<sub>2</sub> (1–30%) regardless of habitat and subphylum as the effect sizes for lipid productivity of microalgae are all >0, and the promotion effect on the lipid productivity of marine microalgae is greatly stronger than that of freshwater microalgae (Fig. 4a, Freshwater: 0.206 ± 0.419, Marine: 0.664 ± 0.580, *t*-test, *p* = 0.007). Likewise, in terms of subphyla, the positive effect is much stronger in Chlorophytina microalgae than Ochrophytina (Fig. 4b, one-way ANOVA, Chlorophytina: 0.631 ± 0.560, Ochrophytina: 0.020 ± 0.355, *p* = 0.008), which is consistent with the earlier analysis related to growth, indicating

that compared to Ochrophytina, Chlorophytina has higher lipid production efficiency under high CO<sub>2</sub> conditions, largely due to their better performance in growth at high CO<sub>2</sub> levels. High CO<sub>2</sub> also has a larger effect size for Bacillariophytina compared to Ochrophytina.

Lipid productivity is a crucial metric for biodiesel production as it combines two important traits: growth rate and lipid content (Griffiths and Harrison, 2009; Gao et al., 2019). According to previous study, in the freshwater diatom *Asterionella formosa*, lipid productivity is notably lower when compared to other diatom species, which could be



**Fig. 4.** CO<sub>2</sub> effect size for microalgal lipid productivity from different habitats (a, 46 data analyzed), taxonomic groups (b, 46 data analyzed); CO<sub>2</sub> effect size for microalgae lipid productivity in marine (c & d, 29 data analyzed), freshwater (e & f, 17 data analyzed) and Chlorophytina (g & h, 35 data analyzed) versus CO<sub>2</sub> concentration or culture duration. \*\* represents a significant difference (*p* < 0.01).



associated with its significantly slower growth rate in comparison to other species (Jensen et al., 2019). However, in the present study the freshwater species do not show significantly lower growth rates compared to marine species at high CO<sub>2</sub> levels. Salinity is one of the primary factors that influence lipid production in microalgae. In previous work *Navicula pelliculosa* cultured in seawater shows higher affinity for CO<sub>2</sub> than that in freshwater (Jensen et al., 2019). Therefore, the increased lipid productivity observed in microalgae cultivated in seawater medium may be attributed more to alterations in carbon conversion, metabolism and lipid synthesis, and salinity could play an important part in such metabolic reprogramming.

### 3.4.2. Lipid productivity in different habitats

In each habitat, the effect size showed no apparent correlation with CO<sub>2</sub> concentration due to the insufficient data on high concentration (Fig. 4c, cubic regression, adjusted R<sup>2</sup> = 0.092, *p* = 0.149; Fig. 4e, cubic regression, adjusted R<sup>2</sup> = 0.2197, *p* = 0.105, marine and freshwater respectively). However, the relationship between CO<sub>2</sub> effect size on lipid productivity and exposure time is noticeable in both habitats. The CO<sub>2</sub> effect size on lipid productivity in marine microalgae initially increases with culture duration and then decreases, with the peak around 9 days (Fig. 4d, cubic regression, adjusted R<sup>2</sup> = 0.223, *p* = 0.025). In terms of freshwater algae, though the effect size regains its climbing trend after its drop between 6 and 8 days (Fig. 4f, cubic regression, adjusted R<sup>2</sup> = 0.560, *p* = 0.003), the correlation could be considered as a quadratic regression with a trend of first uprising and then downward (similar to marine species) if the specific single dataset over 8 days is taken out of consideration. Therefore, the effect size could be considered as increases at first and then decreases below 0 with culture duration extending in both habitats, which is reasonable and could be explained by the microalgae's vulnerability to extreme high CO<sub>2</sub> concentrations that can cause damages to its lipid producing procedure.

It is interesting to find that lipid productivity decreases after 10 days of cultivation. This can be attributed to the changes of growth because microalgal growth shows a similar trend while lipid content slightly increases with extended culture time. Such phenomenon may indicate that the CO<sub>2</sub> effect on growth exceeds that on lipid content, suggesting a potential metabolic shift in the cellular biochemical composition. Although lipids increase, it seems that other biochemical substances contribute more significantly to cell biomass, such as proteins (Gao et al., 2020).

It is worth noticing that the effect size on freshwater species is outstandingly high in ten days of duration. It has been concluded that microalgal lipid production can be affected by culture medium composition and nutrient availability (especially carbon source, nitrogen, and phosphorus) (Metsoviti et al., 2020). The fixed CO<sub>2</sub> may be channeled toward carbohydrates and/or lipids rather than proteins due to the lack of nitrogen, and therefore it is possible that the increased production of macromolecules per unit dry weight enables the cells grown at high CO<sub>2</sub> levels to sustain cell replication for a longer duration, even when nutrients in the medium become limiting (Montoya-Vallejo et al., 2023).

In subphylum Chlorophytina, CO<sub>2</sub> effect size on lipid productivity shows a negative relationship with CO<sub>2</sub> concentration though it is not statistically significant (Fig. 4g, linear regression, adjusted R<sup>2</sup> = 0.082, *p* = 0.052). CO<sub>2</sub> effect size on lipid productivity does not show a clear relationship with culture duration (Fig. 4h, cubic regression, adjusted R<sup>2</sup> = 0.072, *p* = 0.155), suggesting contrast variations in lipid productivity among different species within the Chlorophytina group. The data from other subphyla are insufficient for statistical analysis.

## 3.5. Fatty acid

### 3.5.1. Total fatty acid

Microalgae with a high FA content are favorable to produce high-quality biodiesel, and also have a great potential in the fields of food and feeds (Tang et al., 2011). Thus, evaluating FA content and

composition is a crucial procedure in determining the suitability of algal species to produce biodiesel and dietary supplements. The statistical data on FA largely originate from the subphylum Chlorophytina, and data of other subphyla are not enough for statistical analysis.

The CO<sub>2</sub> effect size for total FA content of Chlorophytina microalgae shows a trend of initially increasing and then slightly decreasing with the increase in CO<sub>2</sub> concentration (adjusted R<sup>2</sup> = 0.502, *p* < 0.001) and culture time (adjusted R<sup>2</sup> = 0.510, *p* < 0.001) (Fig. S2). This trend, especially the initial rising is consistent with the previously analyzed pattern of lipid accumulation. Earlier studies that involved microalgae such as *Coccomyxa* sp., *Desmodesmus* sp., and *Muriella terrestris*, showed a significant improvement in the production of both α-tocopherols and total FAs with a supplementation of 5% CO<sub>2</sub> (Singh et al., 2022). A slight decrease in FA can be found when CO<sub>2</sub> concentrations exceed 40%, which could be the reason for the decrease in lipid accumulation in the Chlorophytina subphylum when the CO<sub>2</sub> levels exceed 50% since they are the vital substrate for lipid synthesis. The abnormally high data points after 10 days of cultivation are attributed to the newly isolate *C. vulgaris* SAG 2606, a member of the Trebouxiophyceae, which exhibited the highest FA production at a CO<sub>2</sub> concentration of 15% compared to the other tested strains (Kryvenda et al., 2023). The stimulative effect of high CO<sub>2</sub> on FA content is consolidated by molecular experiments that have shown that elevated CO<sub>2</sub> (10%) may redirect photosynthetic carbon precursors into FA synthesis pathways, supported by the upregulation of genes involved in TAG biosynthesis (Sun et al., 2016).

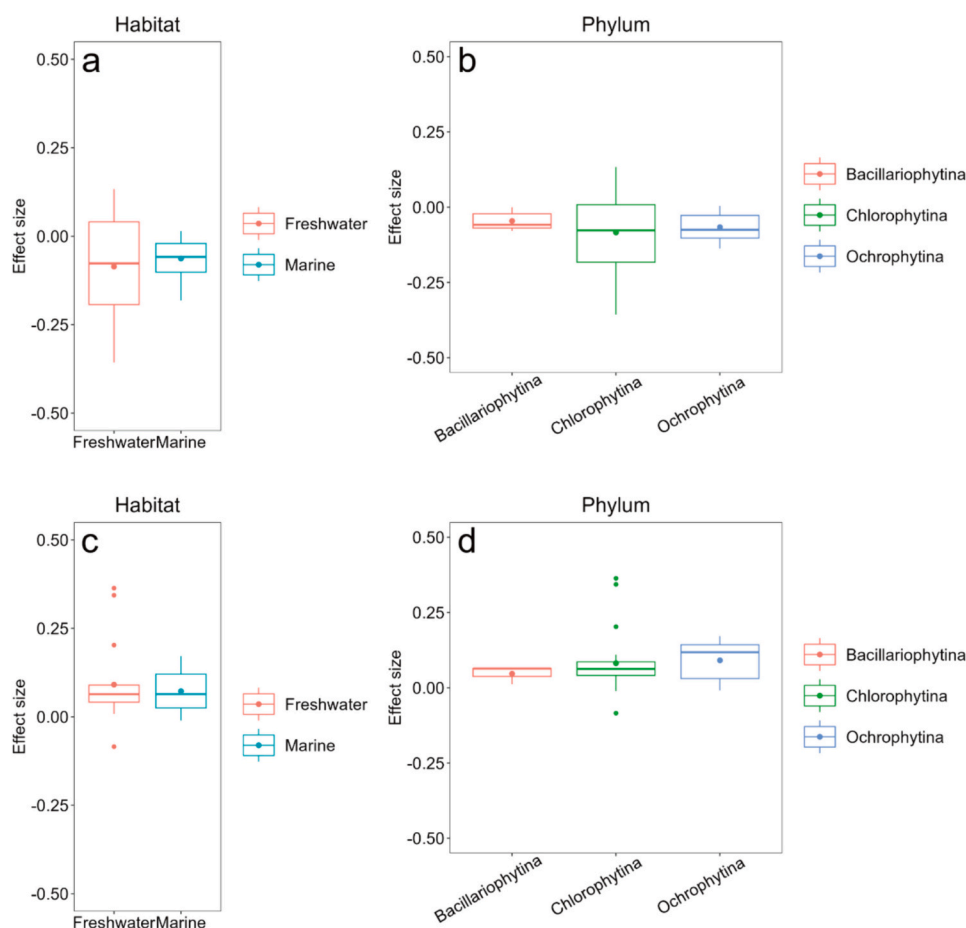
### 3.5.2. Saturated and unsaturated fatty acid

Various concentrations of CO<sub>2</sub> can impact the thylakoid membranes within microalgae cells, resulting in changes in the composition of FAs (Tsuzuki et al., 1990). The degree of unsaturation (DU) is a crucial parameter for evaluating biodiesel product (Sharma and Duraisamy, 2019). Therefore, microalgal SFA and FA were analyzed respectively to explore the potential link between the DU of FA and CO<sub>2</sub> concentration.

Despite previous research suggesting that SFA in *C. vulgaris* increased as CO<sub>2</sub> concentrations rose from 0.2% to 2% (Tsuzuki et al., 1990), statistical findings reveal that, under higher CO<sub>2</sub> concentration ranges (0.1–30%), there is a general suppression of SFA production in microalgae (Fig. 5a & b). Conversely, UFA are almost universally promoted (Fig. 5c & d). And there is no significant difference in the SFA content among microalgae species, regardless of their habitat (Fig. 5a, Wilcoxon's test, Freshwater:  $-0.063 \pm 0.051$ , Marine:  $-0.086 \pm 0.139$ , *p* = 0.539) or subphylum (Fig. 5b, one-way ANOVA, Bacillariophytina:  $-0.046 \pm 0.031$ , Chlorophytina:  $-0.084 \pm 0.125$ , Ochrophytina:  $-0.066 \pm 0.049$ , *p* = 0.661). The same holds true for UFA (Fig. 5c & d, Freshwater:  $0.091 \pm 0.110$ , Marine:  $0.072 \pm 0.059$ , *t*-test, *p* = 0.521 for habitat; one-way ANOVA, Bacillariophytina:  $-0.047 \pm 0.031$ , Chlorophytina:  $-0.082 \pm 0.098$ , Ochrophytina:  $0.091 \pm 0.070$ , *p* = 0.750 for subphylum). Based on the FA profile results, microalgae cells cultured at high CO<sub>2</sub> concentrations generally have reduced SFA content but enhanced UFA content.

There is no clear relationship between SFA content and CO<sub>2</sub> concentration or cultivation time, whether in marine algae or within the Chlorophytina group of microalgae (Fig. S2). Especially within the Chlorophytina subphylum, there is a wide variation in the data, making it difficult to discern any discernible patterns. This wide variation may be related to different gas fluxes used in different studies (Table S3). Gas flow affect CO<sub>2</sub> mass transfer, as well as the interaction between cells and nutrients in seawater (Song et al., 2014). Therefore, it is possible that different gas flow rates can lead to varying microalgal SFA content through CO<sub>2</sub> and nutrients supply.

Similar to SFA, there is no significant relationship between UFA content and CO<sub>2</sub> concentration or exposure time in either habitat, except for CO<sub>2</sub> effect size of UFA content in freshwater species that shows a significant decreasing trend with culture time (Fig. S2). Considering the rather stable state of SFA under extended culture time, the observed



**Fig. 5.** CO<sub>2</sub> effect size for SFA content of microalgae from different habitats (a, 43 data analyzed), taxonomic groups (b, 43 data analyzed); CO<sub>2</sub> effect size for UFA content of microalgae from different habitats (c, 37 data analyzed) and taxonomic groups (d, 37 data analyzed). SFA, saturated fatty acid; UFA, unsaturated fatty acid.

decline here may indicate an impact of high CO<sub>2</sub> on DU in microalgae with extended time.

Previous studies focusing on single microalgae species show high CO<sub>2</sub> can decrease SFA content but increase UFA content (Tsuzuki et al., 1990). The present study reinforces this conclusion through meta-analyzing a large number of datasets. The reason for this finding could be due to increased desaturation enzyme activity stimulated by high CO<sub>2</sub> concentrations, which leads to the transformation from SFAs to UFAs (Vargas et al., 1998; Tang et al., 2011).

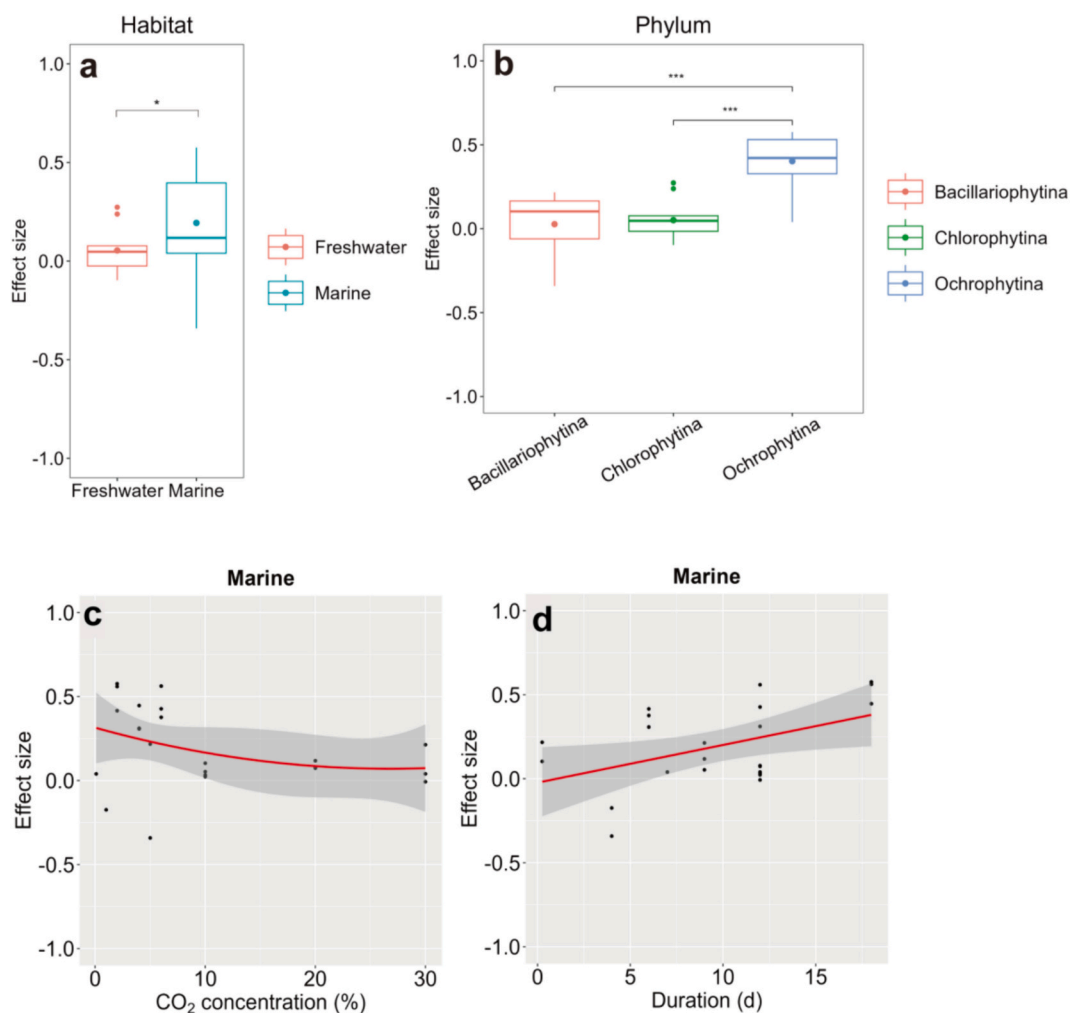
### 3.5.3. Polyunsaturated fatty acid

Based on the previous studies on FA profiles of the microalgae under various CO<sub>2</sub> concentrations, elevated CO<sub>2</sub> seems to promote desaturation pathways, and previous research has shown that elevating the CO<sub>2</sub> concentration during microalgae cultivation tends to boost the accumulation of PUFA within the microalgal cells (Lam et al., 2012). In the present study, PUFA are generally promoted in both freshwater and marine microalgae as well. The benefits for marine-derived PUFA are significantly higher than those from freshwater algae (Fig. 6a, wilcoxon's test, Freshwater:  $0.054 \pm 0.109$ , Marine:  $0.194 \pm 0.240$ ,  $p = 0.013$ ). Analyses of FA composition and lipid class indicate that different genera exhibit unique responses to changes in CO<sub>2</sub> concentration. Likewise, as the representatives of marine microalgae, Ochrophytina algae have significantly larger CO<sub>2</sub> effect size on PUFA content compared to the other two subphyla (Fig. 6b, Turkey-Krama HSD, Bacillariophytina and Chlorophytina, both  $p < 0.001$ ). The data extracted from this subphylum source from *Nannochloropsis*, indicating that this algal species is highly suitable for the production of PUFA.

One possible explanation for this phenomenon is that the increase in CO<sub>2</sub> concentration may result in a relative reduction in O<sub>2</sub> concentration. Such changes could impact enzymatic desaturation (Vargas et al., 1998) and consequently lead to an increase in PUFA content. High CO<sub>2</sub> can also induce the generation of reactive oxygen species (ROS) and lipid peroxidation, which induces an increased production of PUFA (Liang et al., 2006) because these generated PUFA can be utilized as antioxidant agents and repair the damage to the cell membranes by scavenging free radicals. In addition, membrane fluidity can play an essential role in signal-transduction and regulating membrane-associated enzymes activity (Los et al., 2013). The increased PUFA can enhance membrane fluidity, which can modulate enzymes activity and gene-expression pathways inside cells (Los and Murata, 2004; Los et al., 2013), suggesting a strategy that cells respond to high CO<sub>2</sub>.

As a vital proportion of UFA, microalgal PUFA content shows a relatively clear pattern when cultured at high CO<sub>2</sub> levels. In marine microalgae, CO<sub>2</sub> effect size shows a slowly decline trend with CO<sub>2</sub> concentration (Fig. 6c, quadratic regression, adjusted R<sup>2</sup> = 0.202,  $p = 0.040$ ) while linearly increases with culture time (Fig. 6d, linear regression, adjusted R<sup>2</sup> = 0.179,  $p = 0.025$ ). In terms of freshwater algae, no such correlation has been observed between effect size and CO<sub>2</sub> concentration or culture time (Fig. S2).

In Ochrophytina, CO<sub>2</sub> effect size on PUFA content seems to increase with the increase of CO<sub>2</sub> concentration, though not significant with  $p = 0.137$ , and it linearly increases with prolonged culture time (Fig. S2). However, due to the analysis being based on only 10 data points, these results may not be reliable. For Chlorophytina microalgae, there is almost no credible connection between the CO<sub>2</sub> effect size on PUFA and



**Fig. 6.** CO<sub>2</sub> effect size for microalgae PUFA from different habitats (a, 46 data analyzed) and taxonomic groups (b, 46 data analyzed). \*, \*\*\*represent significant difference levels of  $p < 0.05$ ,  $p < 0.001$ , respectively. CO<sub>2</sub> effect size for microalgae PUFA content in marine versus CO<sub>2</sub> concentration (c, 23 data analyzed) or culture duration (d, 23 data analyzed). PUFA, polyunsaturated fatty acid.

CO<sub>2</sub> concentration or the duration of culture (Fig. S2). The previous studies have shown that the PUFA levels decline or increase in different microalgae cultured at high CO<sub>2</sub> concentrations (Tsunami et al., 1990; Riebesell et al., 2000; Fiorini et al., 2010; Torstensson et al., 2013). Our study also reveals a rather genus-specific positive response of PUFA to varying CO<sub>2</sub> availability, especially in subphylum Chlorophytina (Fig. S2).

### 3.6. $\omega$ 3 fatty acids

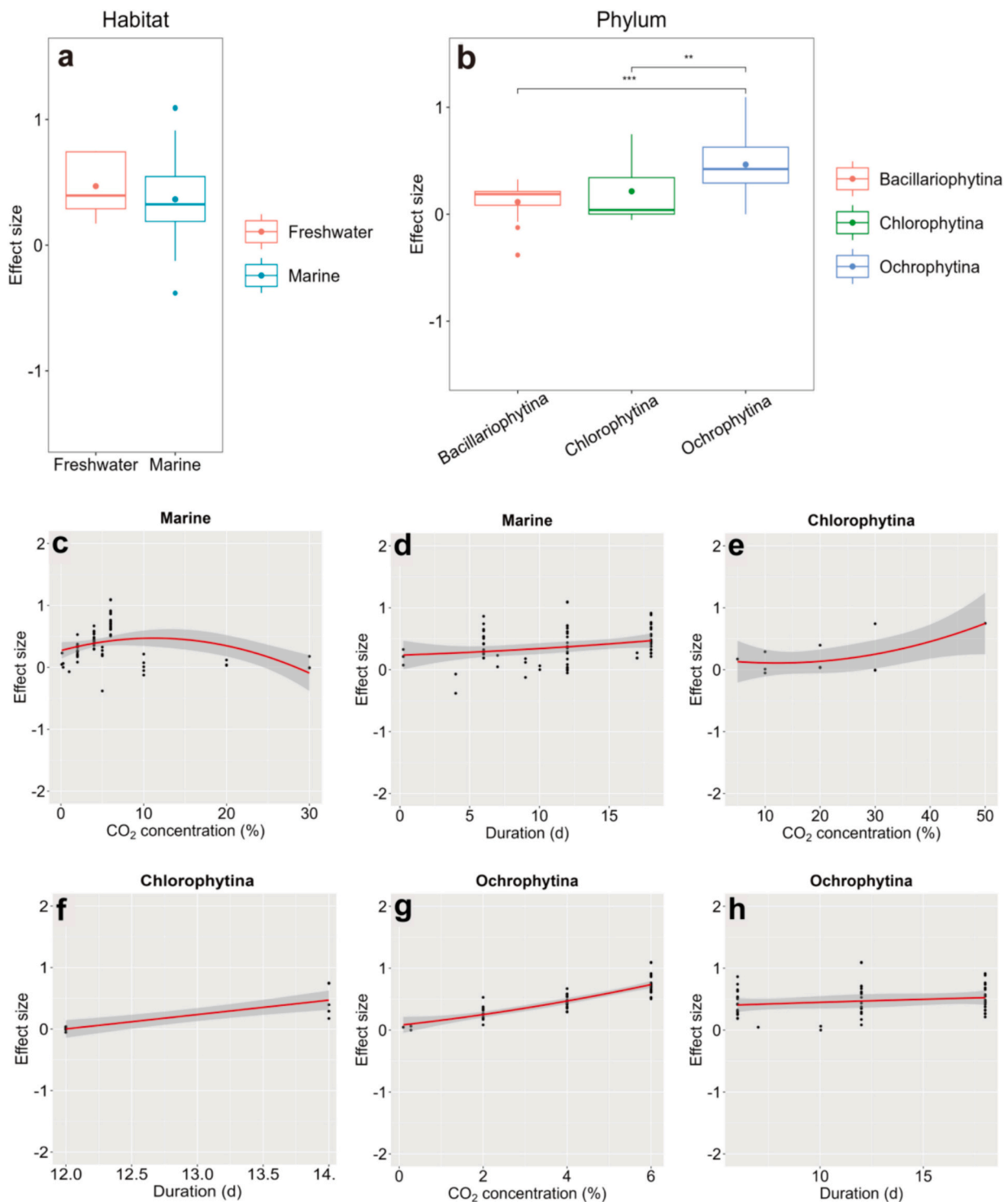
#### 3.6.1. Overall effects of CO<sub>2</sub> on $\omega$ 3 fatty acids

The  $\omega$ 3 fatty acids here mainly refer to the EPA and DHA with C18:3 ( $\omega$ 3) contributing 6 of datasets. High CO<sub>2</sub> concentrations (0.1–30%) do not significantly induce more EPA and DHA in freshwater microalgae than in marine microalgae (Fig. 7a, t-test, Freshwater:  $0.469 \pm 0.264$ , Marine:  $0.365 \pm 0.284$ ,  $p = 0.426$ ). High CO<sub>2</sub> concentrations (0.1–30%) result in largest stimulative effects on microalgae in Ochrophytina with the effect size significantly higher than the other two subphyla (Fig. 7b, Turkey-Krama HSD, Bacillariophytina:  $0.115 \pm 0.189$ , Chlorophytina:  $0.214 \pm 0.297$ , Ochrophytina:  $0.465 \pm 0.247$ ,  $p < 0.001$ ). There's no significant difference on effect size between the subphyla Chlorophytina and Bacillariophytina (Turkey-Krama HSD,  $p = 0.576$ ), with the latter having the least positive effect. The high EPA and DHA contents in Ochrophytina primarily originate from *Nannochloropsis* sp., which is abundant in lipids, including the essential EPA, and has been

demonstrated to increase its EPA content with elevated CO<sub>2</sub> (Shene et al., 2016). The large effect size of DHA in *Nannochloropsis* sp. has also contributed significantly to the differences among various subphyla since it was positively promoted by high CO<sub>2</sub> concentration in subphylum Ochrophytina (Ruangsomboon and Chonudomkul, 2022). The data from Bacillariophytina is exclusively related to EPA, which indicates that in previous research, microalgae's EPA is commonly promoted by high concentrations of CO<sub>2</sub> and is a hotspot across subphyla.

#### 3.6.2. Effects of CO<sub>2</sub> concentration and culture duration on $\omega$ 3 fatty acids of microalgae in different habitats

The effect size of EPA and DHA content in marine microalgae first increases and then decreases with rising CO<sub>2</sub> concentration (Fig. 7c, quadratic regression, adjusted R<sup>2</sup> = 0.044,  $p = 0.028$ ), with the peak at 10% CO<sub>2</sub>. The effect size seems to increase with culture time (Fig. 7d, quadratic regression, adjusted R<sup>2</sup> = 0.048,  $p = 0.060$ ). Previous study found that the levels of  $\omega$ 3 fatty acids in *N. oculata* grown at 4% or 6% CO<sub>2</sub> generally showed an upward trend as the cultivation time extended (Ruangsomboon and Chonudomkul, 2022). In the present study, such an increasing trend in these kinds of FA with exposure time is clear as well, which is inspiring for oil extraction or food industry. Research on EPA and DHA in freshwater species cultured at high CO<sub>2</sub> concentrations is relatively limited and cannot be analyzed here, which needs more studies in future.



**Fig. 7.** CO<sub>2</sub> effect size for microalgae ω<sub>3</sub> fatty acid from different habitats (a, 82 data analyzed) and taxonomic groups (b, 82 data analyzed); CO<sub>2</sub> effect size for ω<sub>3</sub> fatty acid content in marine species (c & d, 77 data analyzed) versus CO<sub>2</sub> concentration or culture duration; CO<sub>2</sub> effect size of ω<sub>3</sub> fatty acid content in Chlorophytina (e & f, 11 data analyzed) and Ochrophytina (g & h, 57 data analyzed) versus CO<sub>2</sub> concentration or culture duration. \*\*, \*\*\* represent significant difference levels of  $p < 0.01$ ,  $p < 0.001$ , respectively.

**3.6.3. Effects of CO<sub>2</sub> concentration and culture duration on ω<sub>3</sub> fatty acids of microalgae in different subphyla**

Effect size of ω<sub>3</sub> fatty acid content increases along with CO<sub>2</sub> concentration in subphylum Chlorophytina (Fig. 7e, quadratic regression, adjusted R<sup>2</sup> = 0.454,  $p = 0.036$ ), indicating the stimulative effects of high CO<sub>2</sub> concentrations on ω<sub>3</sub> fatty acid synthesis in Chlorophytina microalgae. However, it is important to mention that only two studies

are included in this analysis, and therefore it is suggested to lay more stress on the study of the EPA and DHA production of Chlorophytina microalgae under high CO<sub>2</sub> concentrations in future. Only two periods of culture time in Chlorophytina are found in this analysis, therefore such relationship is unreliable though significant (Fig. 7f, linear regression, adjusted R<sup>2</sup> = 0.641,  $p = 0.002$ ).

In subphylum Ochrophytina, effect size of EPA and DHA has a

significant positive correlation with CO<sub>2</sub> concentration (Fig. 7g, quadratic regression, adjusted R<sup>2</sup> = 0.786, *p* < 0.001), and the rising rate seems to accelerate with elevated CO<sub>2</sub> concentration. However, research within this subphylum is limited to CO<sub>2</sub> concentrations of up to 6%. Given the upward trend in omega-3 fatty acids, it may be worthwhile to study the response of omega-3 fatty acids in microalgae to higher CO<sub>2</sub> concentrations. The relationship between effect size and culture duration is not clear, (Fig. 7h, linear regression, adjusted R<sup>2</sup> = 0.020, *p* = 0.147).

#### 4. Conclusions and future research needs

##### 4.1. Conclusions

This meta-analysis study shows that high CO<sub>2</sub> (0.1–30%) has larger positive effect on the growth of freshwater microalgae compared to marine microalgae but enhances lipid productivity in marine microalgae more significantly. High CO<sub>2</sub> (0.1–30%) reduce SFA content of both habitats but increase PUFA content, especially in marine microalgae, with Ochrophytina having the largest effect size. High CO<sub>2</sub> (0.1–6%) stimulate ω3 fatty acids in Ochrophytina the most, and the least in Bacillariophytina. Overall, the responses of microalgae to high CO<sub>2</sub> are species and subphylum different. This research contributes significantly to designing microalgae cultivation for high CO<sub>2</sub>-driven lipid and fatty acid production. Meanwhile, meta-analysis, as a tool for research synthesis, indeed has limitations despite its current utility. Insufficient data sample sizes in meta-analysis can yield incomplete and unreliable results, skewing the overall findings due to over- or under-representation of species in the literature (Gurevitch et al., 2018). While such knowledge gaps restrict the generalizability of conclusions drawn from existing literature, they also pinpoint the areas where further research is needed.

##### 4.2. Future research needs

Through in-depth and critical analysis of previous studies and reviewing the latest developments in this field, some suggestions for research needs and future directions were put forward.

Commercial culture of microalgae is usually year-round while most studies of high CO<sub>2</sub> concentrations on growth and lipid production of microalgae are based on short-term culture periods (<15 days). Therefore, future studies should pay more attention to long-term effects of high concentrations of CO<sub>2</sub> because the effects of CO<sub>2</sub> between short-term and long-term may be completely different. In addition, previous studies of culturing microalgae with high CO<sub>2</sub> were conducted indoors. Considering that outdoor culture can effectively reduce the culture cost, outdoor experiments should be carried out in future. One challenge for outdoor culture is the influences of seasonal variation on the performance of microalgal culture. Therefore, robust algal strains that can grow well in fluctuating light and temperature conditions should be screened. Outdoor culture is also very important for reducing the culture cost because it saves the power to supply light and control temperature (Janssen et al., 2022). To reduce cost is always essential for commercial microalgae culture. Future study should pay more attention to enhancing microalgal harvest efficiency with low cost since algal harvest account for a considerable percentage of microalgal culture cost (Vázquez-Romero et al., 2022). Bio-flocculation may be an efficient approach with lost cost to harvest microalgae (Shitanaka et al., 2023). Different methods with different solvents were used to extract and determine lipid and fatty acids of microalgae (Table S3). Although there may be differences in recovery efficiency among these methods, it does not affect the conclusions in the present study because relative values (effect size) rather than absolute values were used. Environmentally friendly and cost-effective solvents are favorable to use in future study. In addition, artificial intelligence (AI) can be used for the optimization, monitoring, and control of production with high CO<sub>2</sub> and harvesting

processes to enhance efficiency and reduce cost (Shitanaka et al., 2024).

The actual availability of dissolved inorganic carbon (DIC) and CO<sub>2</sub> in aquatic phase is vital in affecting growth and lipid production of microalgae. However, most studies did not measure or provide these parameters although medium pH is usually recorded (Table S3). Dependent on cell density and aeration rate, the actual *p*CO<sub>2</sub> in aquatic phase may be different from that in the aerating gas and then it is difficult to know DIC and CO<sub>2</sub> in aquatic phase based on *p*CO<sub>2</sub> and pH. In addition, gas flow rate is an important parameter that can affect mixing of the microalgal culture, the mass transfer of CO<sub>2</sub> and the interaction between cells and nutrients, thus regulating microalgal growth and lipid production (Song et al., 2014). However, some studies did not supply this information (Table S3). Therefore, future CO<sub>2</sub> studies are suggested to measure and provide these parameters.

Due to a limited solubility of CO<sub>2</sub> in water, direct bubbling CO<sub>2</sub> can lead to high CO<sub>2</sub> losses during culture and a low efficiency of CO<sub>2</sub> capture (Klepacz-Smolka et al., 2023). Furthermore, the pH can decrease significantly in response to excess CO<sub>2</sub> supply, which can impose negative effects on algal growth. Therefore, indirect capture that converts CO<sub>2</sub> to bicarbonate through a reversible chemical reaction seems to be advantageous since it can solve the problems of direct capture and the bicarbonate can also be utilized for algae growth (Sharma et al., 2023). A major challenge for implementation indirect capture with open-pond culture is the instability of bicarbonate at high temperatures that can cause the decomposition of bicarbonate and the release CO<sub>2</sub> into the atmosphere, which needs to be solved in future. Most studies used the mixture of pure CO<sub>2</sub> and air to culture microalgae rather in situ flue gas (Table S4). This may be due to the toxic effects of SO<sub>x</sub> and NO<sub>x</sub> in flue gas (Yen et al., 2015). However, purifying flue gas can increase culture cost. Therefore, screening microalgae strains that can grow well in in situ flue gas would be a direction for future study. With the development of systems biology, synthetic biology and bioinformatics, more and more advanced technologies including microalgae genetic engineering can be used to address this problem. For instance, based on the latest genome sequencing (Single Molecule Real-Time Sequencing) bioinformatics techniques, molecular mechanisms that regulate microalgal response to flue gases can be elucidated. Using advanced molecular editing techniques, e.g., CRISPR-Cas9, the relative genes can be silenced or over-expressed to improve microalgal tolerance to flue gases.

Lipid accumulation and growth rate are usually irreconcilable for microalgal culture. Therefore, a two-stage cultivation model is suggested to tackle this issue. In this model, algae are first cultured in optimal conditions during the initial stage to sustain high growth rates. In the second stage, they are exposed to environmental stresses, such as nutrients, temperature, salinity, metal ions, and irradiance, to stimulate lipid accumulation. Facilitating lipid accumulation in algae can be achieved through efficient and sustainable algal flocculation with fungi, and such enrichment can be further enhanced by nitrogen-regulated. Such methods can also result in valuable algae-fungi biomass enriched in UFAs (Shitanaka et al., 2023).

Nutrients, temperature, light intensity, etc., are also very important environmental parameters that can affect growth and lipid synthesis of microalgae. They may have interactive effects with high CO<sub>2</sub> concentrations. Therefore, future studies should investigate the combined effects of these factors and achieve the highest lipid production through collocating these parameters.

#### CRediT authorship contribution statement

**Yuan Feng:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Jingke Ge:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Pau Loke Show:** Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization. **Chi Song:** Writing – review & editing, Methodology, Investigation, Data curation. **Liumiao Wu:** Writing – review & editing,

Methodology, Investigation, Data curation. **Zengling Ma**: Writing – review & editing, Methodology, Conceptualization. **Guang Gao**: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2024.741386>.

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