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Combined application of nitrogen, phosphorus, iron, and silicon improves growth and fatty acid composition in marine epiphytic diatoms

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Introduction: Marine epiphytic diatoms are natural diets for multiple marine invertebrates, including sea cucumbers, sea urchins, etc. They also act as bioindicators in marine waters to assess water quality of coastal ecosystems.

Material and methods: We isolated three strains of *Navicula* and two strains of *Nitzschia* from macroalgal epiphytes. Combined concentrations of four macroelements, five trace metals, and three vitamins in *f/2* medium were optimized for these diatoms using single-factor and orthogonal tests.

Results and discussion: The biomass productivities for 4-L enlarged cultures of the five isolated diatoms in their exclusively optimized *f/2* media all increased by nearly 10-fold relative to *f/2* medium, and that of *Navicula*-1 and *Nitzschia*-2 reached the highest, i.e., 29.52 ± 1.98 and 30.68 ± 2.84 g m⁻² d⁻¹, respectively. Moreover, the relative abundance of C20:5n3 in the exclusively optimized *f/2* medium-cultured *Navicula*-1 notably rose by 24%, and that in *Nitzschia*-2 held stable at > 25%. Thus, *Navicula*-1 and *Nitzschia*-2 were screened out with outstanding growth traits and improved fatty acid compositions at high levels of combined nitrogen, phosphorus, iron, and silicon. The two diatoms have potential for becoming quality diets for marine aquaculture, and ideal candidates for effective nutrient removal.

KEYWORDS

Navicula, *Nitzschia*, macroelements, biomass productivity, fatty acid profile

1 Introduction

Diatoms are pivotal primary producers in aquatic ecosystems and play crucial roles in food webs as well as biogeochemical cycles. They exist in almost all aquatic habitats, including oceans, estuaries, wetlands, lakes, and even certain transient aquatic habitats (Pandey et al., 2018), and are responsible for approximately 20% of global oxygen production. The periphytic diatom cells can be established on varieties of vegetal or mineral substrates. The nature and roughness of the substrates create different species assemblages, including macroalgae and macrophytes, as well as rock, sand, and mud (Desrosiers et al., 2013). In addition, the different host macroalgae are usually adhered by various epiphytic diatoms as a portion of the periphyton assemblages (Almeida and Beltrones, 2008; Burfeid-Castellanos et al., 2021).

Periphytic diatoms have been considered effective ecological bioindicators, mainly due to their short generation times (24 h to several weeks) and their direct and sensitive responses to various biological, chemical, and physical disturbances in ecosystems (Kireta et al., 2012). These diatoms are also widely distributed in marine coastal waters. Marine periphytic diatoms have also been regarded as an emerging marine bioindicator. In mesotrophic and oligotrophic waters, nitrogen and phosphorous enrichment have altered benthic diatom species assemblage (Desrosiers et al., 2013). Their short life span and sessile traits enable them to capture ephemeral environmental variations at sampling sites. Moreover, their sensitivity to multiple environmental factors and nutrient enrichment endows characterizing species in terms of functional categories, e.g., salinity, pH, oxygen demands, nitrogen assimilation, and trophic status (Desrosiers et al., 2013; Han et al., 2023).

Municipal wastewater discharge into nearshore waters has turned into the most frequent practice of ultimate sewage disposal in coastal cities (Otim et al., 2018). However, the concentrations of nutrients, especially nitrogen and phosphorus, in multiple coastal ecosystems have increased by year, accompanied by massive increases in numerous volumes of discharge (Zhang et al., 2020). Various microalgal communities have been found to be abundant in these nearshore waters. They can considerably take in different forms of nitrogen and phosphorus (Meerssche and Pinckney, 2019). Among these pollution-resistant microalgae, the periphytic diatoms attached to macroalgae or stones are highly adaptive to the variable environment. Thus, these diatoms could be further isolated and potentially used for effective nutrient removal in municipal wastewater treatment. Meng et al. (2019) found that the periphytic diatom *Navicula* sp. associated with *Stigeoclonium* sp. and ammonia-oxidizing bacteria enhanced nitrogen and phosphorus removals in the reactors with stronger illumination. However, this study chiefly focused on the nutrient removal performance of the microalgal community, and the nutrient uptake characteristics of the mono-cultured periphytic diatom were still unclear. Currently, very limited studies on growth traits of periphytic diatoms at high levels of nutrients have been reported. They could contribute to the application of nutrient removal using potentially high-quality periphytic diatoms, especially the robust diatoms distributed around municipal wastewater discharge into nearshore waters.

In addition, the periphytic diatoms have been widely used as the essential natural diets for invertebrate marine animals because they are highly rich in polyunsaturated fatty acids (PUFAs), C20:5n3 in particular (Scholz and Liebezeit, 2013; Xie et al., 2017), and these PUFAs are closely bound up with the metamorphosis and development of larvae of invertebrates, e.g., the sea urchin *Paracentrotus lividus* and the red abalone *Haliotis rufescens* (Courtois de Viçose et al., 2012; Sánchez-Saavedra and Núñez-Zarco, 2015; Gomes et al., 2021). Hence, it is necessary to screen periphytic diatoms with high amounts of PUFAs and prominent growth properties. The screened diatoms could serve as an underlying high-quality diet for aquatic animals and be beneficial for developing aquaculture.

Despite the potential uses of the periphytic diatoms in nutrient removal or aquafeeds, only a few reports have described the impacts of culture condition changes in the periphytic diatom growth (Mercado et al., 2004; Yang et al., 2014), especially the requirements for various macro- or micronutrients. Thus, it is crucial to propose nutrient combination strategies for the significant promotion of periphytic diatom growth. In this work, in order to screen the marine epiphytic diatoms with great growth performance, we comprehensively optimized the culture medium, i.e., the general *f/2* medium, for five isolated epiphytic diatoms. In addition, the variations in fatty acid profiles of the two screened diatoms with good growth traits were compared under the control *f/2* and exclusively optimized *f/2* media. This study aims to screen out the potentially fast-growing and nutritional marine epiphytic diatoms. These results could provide valuable insights into the underlying application of pollution-resistant epiphytic diatoms in both aquaculture and wastewater treatment industries.

2 Materials and methods

2.1 Isolation and culture of microalgal strains

Five strains of marine epiphytic diatoms, including three species of *Navicula* and two species of *Nitzschia* (Figures 1, 2), were isolated from macroalgal epiphytes in the Heishijiao intertidal zones of Dalian, China, adjacent to a sewage outlet into the sea. The epiphytes on macroalgae were first scrubbed into the sterile seawater using a banister brush. The collected epiphytes were then filtered through a 300-mesh screen to remove large particles. The filtered diatom concentrates were kept static overnight to make the diatom cells settle onto the bottom of the flasks. After the supernatants were discarded, the bottom diatom mixtures were enriched with *f/2* medium. The enriched diatom cells grown exponentially were further isolated through a combination of multi-methods, including micropipettes, streaking cells across agar plates, and step dilution method. The isolated xenic epiphytic diatoms were sub-cultured in 1-L Erlenmeyer flasks and maintained in *f/2* medium (Harrison and Berges, 2005) at an ambient temperature (15°C–20°C) under a natural light cycle (approximately 10 h of light duration). The diatom biofilms attached to the glass were gently collected and homogenized

using a sterilized banister brush. The diatom concentrates were then transferred to a sterile 10-mL tube and kept static for 5 min to precipitate the clumps. The upper homogenous diatoms were inoculated into 400 mL of *f/2* medium, and 200 mL of *f/2* medium was added into the cultures every 4 days. The subculture lasted for a total of 12 days in 1-L flasks.

2.2 Experimental design and enrichment of microalgae

In this work, the optimized batch cultures (Figure 3) were conducted based on the basic *f/2* medium, including the combined optimization of four macroelements (nitrogen, phosphorus, iron, and silicon) and eight microelements (five trace metal elements and three vitamins), for three species of *Navicula* and two species of *Nitzschia* (Figures 1, 2). Then, three light intensities were also detected to screen out diatoms potentially resistant to high light intensity. The subsequent enlarged cultures for *Navicula* spp. and *Nitzschia* spp. at a 4-L scale were further performed to screen the optimal diatoms with good growth traits in the exclusively optimized *f/2* media. Ultimately, the fatty acid profiles of the screened diatoms in the exclusively optimized *f/2* media were assessed and compared relative to those in the control (*f/2* medium) (Figure 3).

First, the combined concentrations of four macroelements (nitrogen, phosphorus, iron, and silicon) (Yang et al., 2014), five trace metal elements (zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), and cobalt (Co)), and three vitamins (vitamins thiamine (VB₁), cyanocobalamin (VB₁₂), and biotin (VH)) (Figures S1-S10, Tables S1-18) in *f/2* medium were optimized for *Navicula* spp. and *Nitzschia* spp. based on the preliminary single-factor tests and orthogonal tests. Each of the four optimized *f/2* media was further designed for *Navicula* spp. (Table 1) and *Nitzschia* spp. (Table 2) in terms of the growth curves and the maximum growth rates under the above-mentioned optimization conditions. The growth tests for optimization of culture media and illumination (Figure 3, Tables 1, 2) were performed in an illumination incubator (HPG-280B, Donlian Hall, China) using 10-mL glass tubes (13 × 100 mm) sealed with cotton plugs. The diatom cultures were controlled at 20°C under a 12-h light/12-h dark cycle. The light intensity was set at 100 μmol·m⁻²·s⁻¹ for optimization tests of culture media.

Second, three light intensities, including 100 μmol·m⁻²·s⁻¹, 200 μmol·m⁻²·s⁻¹, and 350 μmol·m⁻²·s⁻¹, were tested to explore the optimal illumination intensity appropriate for the isolated epiphytic diatoms grown in the exclusively optimized media (Table 3). The optical density (OD₆₈₀, Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA; Wang et al., 1997) of diatom inoculum was

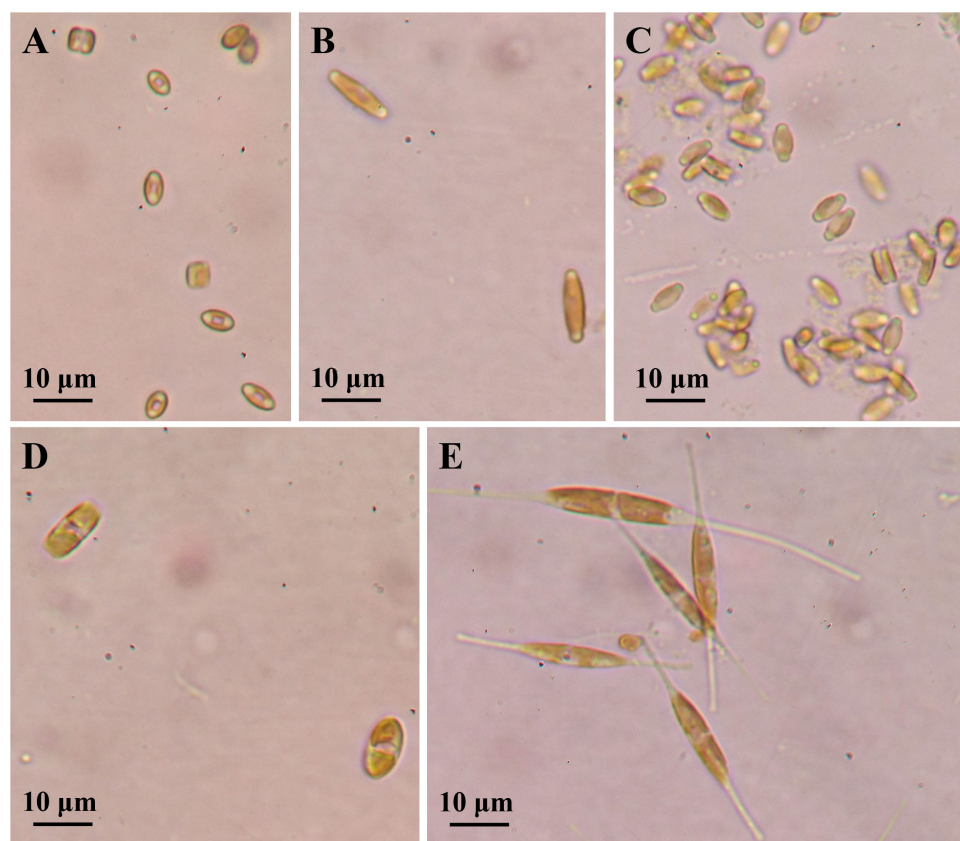


FIGURE 1

Cell morphology of five marine epiphytic diatoms under optical microscope. (A–C) Three species of *Navicula* expressed as *Navicula*-1, *Navicula*-2, and *Navicula*-3. (D, E) Two species of *Nitzschia* expressed as *Nitzschia*-1 and *Nitzschia*-2.

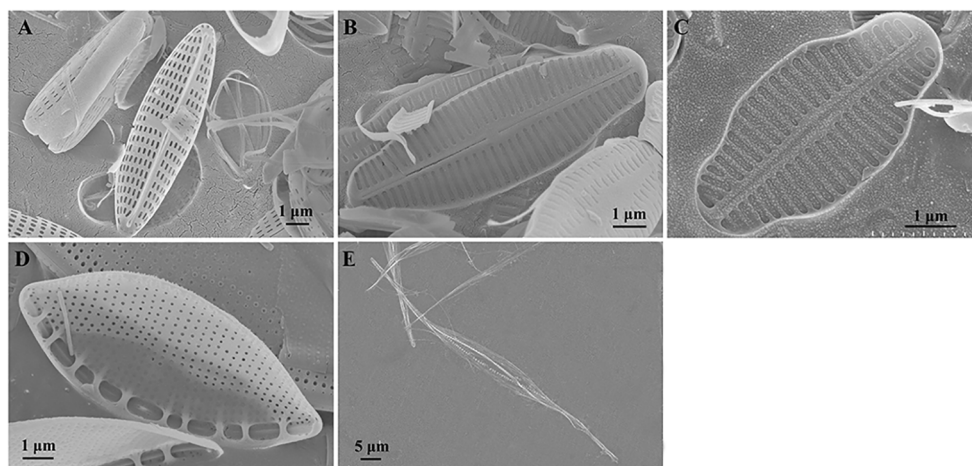


FIGURE 2
Cell morphology of five marine epiphytic diatoms under scanning electron microscopy. (A–C) Three species of *Navicula* expressed as *Navicula*-1, *Navicula*-2, and *Navicula*-3. (D, E) Two species of *Nitzschia* expressed as *Nitzschia*-1 and *Nitzschia*-2.

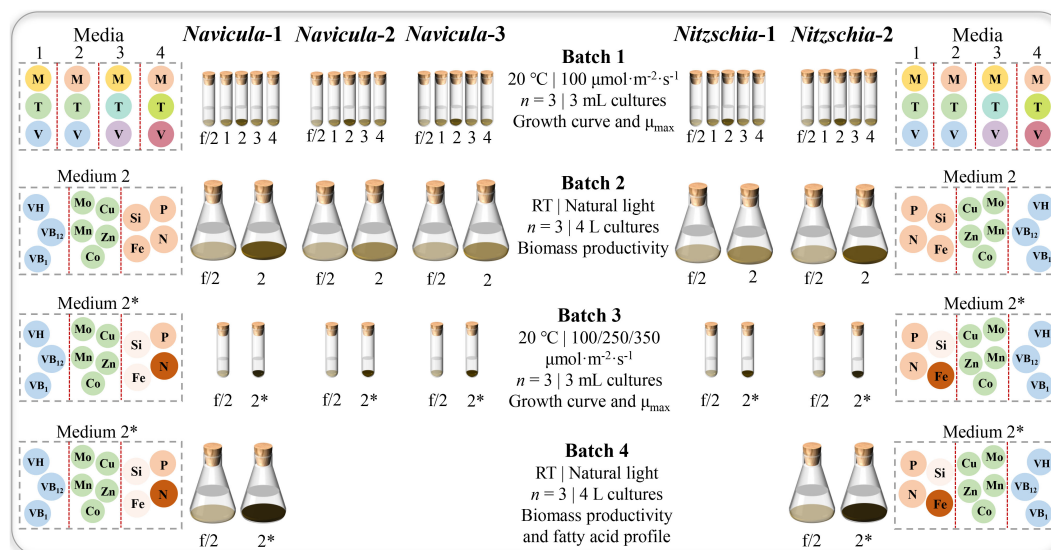


FIGURE 3
Experimental design for optimized cultivation and screening of five marine epiphytic diatoms. Four batches of diatom cultures in 10-mL tubes or 5-L flasks were performed in triplicate. The capital letters M, T, and V indicate macroelements (N, P, Fe, and Si), trace metals (Zn, Cu, Mn, Mo, and Co), and vitamins (VB₁, VB₁₂, and VH), respectively. The different colors of the same capital letter in Media 1, 2, 3, and 4 indicate the different concentrations, while the darker the color of the framed circle for the same element in Media 2 and 2*, the higher the concentration of the element for the same diatom. In addition, the concentration of nitrogen for *Navicula* was increased, and that of silicon for *Navicula* and *Nitzschia* was both reduced in Medium 2* relative to Medium 2; the concentration of iron for *Navicula* was reduced, and that for *Nitzschia* was increased. In Batch 1 of 3-mL cultures, the four optimized f/2 media for *Navicula* spp. and *Nitzschia* spp. (Media 1, 2, 3, and 4 in the figure represent optimized f/2-1, f/2-2, f/2-3, and f/2-4 media, respectively, in Tables 1, 2) were tested in terms of the growth curve, and the maximum specific growth rates, μ_{max} , and the respective optimized f/2-2 media (Tables 1, 2) for *Navicula* spp. and *Nitzschia* spp. were screened out due to the diatoms' good growth properties. In Batch 2 of 4-L cultures, the biomass productivities for five strains of diatoms were evaluated in their respective optimized f/2-2 media (Medium 2 in the figure). Then, the respective optimized f/2-2 media were further optimized for concentrations of nitrogen, phosphorus, and silicon and indicated as the exclusively optimized f/2 media (Medium 2* in the figure) in Table 3. In Batch 3 of 3-mL cultures, the exclusively optimized f/2 media (Medium 2* in the figure) for *Navicula* spp. and *Nitzschia* spp. were used, and three light intensities were set to assess their impacts on growth curve and μ_{max} of five diatoms. Ultimately, *Navicula*-1 and *Nitzschia*-2 both presented more outstanding growth traits than the other diatoms, and their enlarged 4-L cultures in Batch 4 also possessed consistent biomass productivity. The corresponding fatty acid profiles of these two diatoms were then evaluated.

TABLE 1 The four optimized f/2 media designed for three species of *Navicula*.

Components	f/2	Optimized f/2-1	Optimized f/2-2	Optimized f/2-3	Optimized f/2-4
N (mg/L)	12.36	74.16	65.92	74.16	65.92
P (mg/L)	1.14	2.27	2.84	2.27	2.84
Si (mg/L)	2.30	34.51	42.81	34.51	42.81
Fe (mg/L)	0.65	3.33	3.55	3.33	3.55
Zn (µg/L)	6.54	6.54	6.54	19.62	13.08
Cu (µg/L)	2.54	2.54	2.54	2.54	3.18
Mn (µg/L)	49.44	49.44	49.44	2.75	2.20
Mo (µg/L)	2.88	2.88	2.88	0.96	1.92
Co (µg/L)	2.95	2.95	2.95	5.89	11.79
VB ₁ (mg/L)	0.1	0.1	0.1	10.0	7.0
VB ₁₂ (µg/L)	0.5	0.5	0.5	0	2.0
VH (µg/L)	0.5	0.5	0.5	0.5	17.0
N:P mass ratios	10.8	32.7	23.2	32.7	23.2

The compounds of N, P, Si, Fe, Zn, Cu, Mn, Mo, and Co in f/2 medium were NaNO₃, NaH₂PO₄·2H₂O, Na₂SiO₃·9H₂O, FeC₆H₅O₇·5H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O, MnCl₂·4H₂O, NaMoO₄·2H₂O, and CoCl₂·6H₂O, respectively. The concentrations of the above nine components in this table indicate those of the corresponding elements, instead of the compound (e.g., N represents the concentration of N in NaNO₃, rather than the concentration of NaNO₃). The “optimized f/2-2” medium was finally screened out as the most optimal f/2 medium for *Navicula* spp. The component concentrations of the four optimized f/2 media were confirmed based on the preliminary single-factor tests and orthogonal tests (Yang et al., 2014; Figures S1-S10, Tables S1-18).

first adjusted to 0.045 (~1 × 10⁵ ind./mL; Courtois de Viçose et al., 2012) with sterilized seawater, and each 3-mL cell growing exponentially was transferred into the above-mentioned glass tubes following 2 days of static culture for firm attachment to glass. Then, the seawater was refreshed using 3 mL of f/2 medium, and the OD₆₈₀ was monitored on Days 2, 4, 6, 8, and 10. Before measurement, a banister brush was used to completely homogenize

the diatom cells attached to the bottom of the glass tube 10 times, and the homogenates were vortexed for 1 min to disperse the cells and ensure the determination accuracy. A total of 15 biological replicates of diatom cells for each treatment were prepared on Day 0. Each of the three biological replicates was taken every other day to measure the OD₆₈₀, which was discarded following measurement. After the determination of OD₆₈₀, the glass tube

TABLE 2 The four optimized f/2 media designed for two species of *Nitzschia*.

Components	f/2	Optimized f/2-1	Optimized f/2-2	Optimized f/2-3	Optimized f/2-4
N (mg/L)	12.36	37.08	37.08	37.08	37.08
P (mg/L)	1.14	2.84	2.84	2.84	2.84
Si (mg/L)	2.30	11.50	34.51	11.50	34.51
Fe (mg/L)	0.65	4.00	2.00	4.00	2.00
Zn (µg/L)	6.54	6.54	6.54	0.65	0.65
Cu (µg/L)	2.54	2.54	2.54	1.91	1.91
Mn (µg/L)	49.44	49.44	49.44	27.47	0.00
Mo (µg/L)	2.88	2.88	2.88	6.72	6.72
Co (µg/L)	2.95	2.95	2.95	5.89	2.95
VB ₁ (mg/L)	0.1	0.1	0.1	8.0	10.0
VB ₁₂ (µg/L)	0.5	0.5	0.5	25.0	5.0
VH (µg/L)	0.5	0.5	0.5	5.0	0.5
N:P mass ratios	10.8	13.1	13.1	13.1	13.1

The compounds of N, P, Si, Fe, Zn, Cu, Mn, Mo, and Co in f/2 medium were NaNO₃, NaH₂PO₄·2H₂O, Na₂SiO₃·9H₂O, FeC₆H₅O₇·5H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O, MnCl₂·4H₂O, NaMoO₄·2H₂O, and CoCl₂·6H₂O, respectively. The concentrations of the above nine components in this table indicate those of the corresponding element, instead of the compound (e.g., N represents the concentration of N in NaNO₃, rather than the concentration of NaNO₃). The “optimized f/2-2” medium was finally screened out as the most optimal f/2 medium for *Nitzschia* spp. The component concentrations of the four optimized f/2 media were confirmed based on the preliminary single factor tests and orthogonal tests (Yang et al., 2014; Figures S1-S10, Tables S1-18).

TABLE 3 The exclusively optimized *f/2* media for *Navicula* and *Nitzschia*.

Components	<i>f/2</i>	Exclusively optimized <i>f/2</i>	
		<i>Navicula</i> spp.	<i>Nitzschia</i> spp.
N (mg/L)	12.36	74.16	37.08
P (mg/L)	1.14	2.84	2.84
Si (mg/L)	2.30	34.51	23.01
Fe (mg/L)	0.65	3.33	3.33
Zn (μg/L)	6.54	6.54	6.54
Cu (μg/L)	2.54	2.54	2.54
Mn (μg/L)	49.44	49.44	49.44
Mo (μg/L)	2.88	2.88	2.88
Co (μg/L)	2.95	2.95	2.95
VB ₁ (mg/L)	0.1	0.1	0.1
VB ₁₂ (μg/L)	0.5	0.5	0.5
VH (μg/L)	0.5	0.5	0.5
N:P mass ratios	10.8	26.1	13.1

These two optimized *f/2* media were obtained based on the further optimization of the most optimal *f/2* medium in Tables 1, 2. The concentration of nitrogen for *Navicula* was increased, and that of silicon for *Navicula* and *Nitzschia* was reduced in the exclusively optimized *f/2* medium relative to the optimized *f/2* medium; the concentration of iron for *Navicula* was reduced, and that for *Nitzschia* was increased.

was discarded. The specific growth rate was calculated as $\mu = (\ln OD_2 - \ln OD_1)/(t_2 - t_1)$, where t_1 and t_2 are the beginning and sampling time of the culture period, respectively, and OD_1 and OD_2 are the corresponding optical densities at 680 nm.

Third, to further confirm the significant promotion impacts of the optimized medium on the growth of *Navicula* spp. and *Nitzschia* spp., the enlarged cultures in triplicate were conducted in 5-L flasks. Each 2 L of diatom dilutions with OD_{680} of 0.045 in sterilized *f/2* or optimized *f/2* medium was inoculated into a 5-L flask. After 4 days of initial culture, another 1 L of fresh medium was gently added into the corresponding cultures. The same volume of the above-mentioned medium was re-added following the next 4 days. The enlarged cultures of the isolated epiphytic diatoms were also conducted at an ambient temperature (15°C–20°C) under a natural light cycle, lasting for 12 days. Further, the enlarged cultures for the five diatoms at a 4-L scale were performed in their respective exclusively optimized *f/2* media (Table 3). Prior to harvest, the cultures were gently agitated twice per day. Finally, the fresh diatom cells attached to the glass at the bottom of the 5-L flask were completely collected and homogenized using a long banister brush. The diatom concentrates were centrifuged (4,000 rpm, 10 min, 4°C), and the pellets were weighed to evaluate the growth performance, i.e., the biomass productivity of diatoms (P_d). The biomass productivity was calculated using the fresh weight of the xenic diatom cells, culture days, and surface area of the 5-L flask bottom, as follows:

$$P_d = M/(3.14 \times R^2 \times T),$$

where P_d is the biomass productivity of diatoms, M is the wet weight of harvested diatom cells (g), R is the bottom radius of the 5-L conical flask for culturing diatom cells (m), and T is the culture time (days).

The collected diatom pellets were stored at -80°C until subsequent fatty acid analyses.

2.3 Cell morphology

The normal morphology of the diatom cells was observed and preliminarily characterized under a light microscope (Olympus, Tokyo, Japan) with $\times 400$ magnification. For scanning electron microscopy (SEM) observation, 2 mL of diatom cells was first added with equal volumes of sulfuric acid, followed by boiling for 10–30 min to oxidize the organic matter. The mixture was washed with deionized water several times until a neutral level was reached. The specimens were then collected onto a polycarbonate membrane, attached to aluminum stubs, and sputter-coated with gold (JFC-1100, JEOL, Tokyo, Japan). The samples were observed, and the micrographs were taken under a JSM-6360LV SEM (JEOL, Japan) operated at 15 kV.

2.4 Fatty acid analyses

The fatty acid profiles of diatoms were determined via lipid extraction (Folch et al., 1957) and transesterification (Metcalf and Schmitz, 1961), followed by gas chromatography (GC) analysis for fatty acid methyl esters (FAMES), as previously reported (Yin et al., 2013). In brief, lipids were first extracted using chloroform/methanol (2:1, v/v) with ultrasonication for 15 min and then layered with potassium chloride (0.88%, m/v). The lipid extraction was repeated three times, and the merged lower organic phase was dried under gentle nitrogen flow. The extracted lipids (50–100 mg) were hydrolyzed using 0.5 M KOH–methanol at 70°C for 1 h, and the produced fatty acids were then esterified using methanol under the catalysis of boron trifluoride. FAMES were extracted using petroleum ether and quantified using a Shimadzu GC-2010 fitted with a flame ionization detector (FID) as well as a fused silica capillary column (30 m \times 0.25 mm ID \times 0.3 μm film, FFAP, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China). The temperatures of the inlet and detector were 250°C and 230°C , respectively, and the split ratio was 100:1. The carrier gas was nitrogen with a purity of 99%. The column temperature was programmed from 160°C to 230°C increasing at $2^\circ\text{C}/\text{min}$ and held at 230°C for 5 min. The running time was 40 min. The petroleum ether extracts were condensed under mild nitrogen flow when necessary.

2.5 Statistical analysis

The data were statistically analyzed using SPSS 19.0 and denoted as average \pm standard deviation (SD, $n = 3$). The single-factor tests as well as the orthogonal tests (Tables S1–S4) were performed to evaluate the impacts of the four macroelements (nitrogen, phosphorus, iron, and silicon; Yang et al., 2014), five trace metals (Zn, Cu, Mn, Mo, and Co; Figures S1–S6, Tables S5–S12), and three vitamins (VB₁, VB₁₂, and VH; Figures S7–S10, Tables S13–18) in *f/2* medium on the growth of diatoms. Statistical significance of the difference was determined by one-way analysis of

variance and Tukey's honestly significant difference (HSD) test with a significance level of 0.05 and Student's t-test expressed as * $p < 0.05$ or ** $p < 0.01$.

3 Results

First, the five isolated epiphytic diatoms were characterized under both bright-field and SEM (Figures 1, 2). The two lamellar plastids of *Navicula* spp., including *Navicula*-1, *Navicula*-2, and *Navicula*-3 (Figures 1A–C), were symmetrically distributed on the left and right sides of silica shell, while those of *Nitzschia* spp., including *Nitzschia*-1 and *Nitzschia*-2 (Figures 1D, E), were generally top- and bottom-distributed under bright-field microscopy. In addition, three species of *Navicula* (Figures 2A–C) were characterized by their typical raphes, and two species of *Nitzschia* (Figures 2D, E) had typical canal raphes under SEM.

The results showed that the OD₆₈₀ of *Navicula*-1, *Navicula*-2, *Navicula*-3, and *Nitzschia*-1 under the four optimized f/2 media were all significantly higher ($p < 0.05$) than that under the f/2 medium. The N:P mass ratios in the four optimized f/2 media for *Navicula* spp. increased from 10.8 (f/2 medium) to 23.2 or 32.7, while that for *Nitzschia* spp. also increased to 13.1 (Tables 1, 2). The differences for the OD₆₈₀ of three species of *Navicula* reached up to 2.8–4.0-fold, and those for two species of *Nitzschia* merely reached 1.2–2.5-fold on Day 10 (Figures 4A–D). In particular, *Nitzschia*-2 exhibited no significantly promoted growth when cultured in the four optimized f/2 media for 10 days (Figure 4E). In addition, the

maximum specific growth rates, μ_{\max} of *Navicula* spp. (at Day 6 or 8) occurred later than those of *Nitzschia* spp. (at Day 2) (Figure 5). However, a 2- or 3-fold increase was found in μ_{\max} of three species of *Navicula*, and the increase in μ_{\max} in two species of *Nitzschia* was less than 1.5-fold under the four optimized f/2 media (Figure 5). Moreover, the μ_{\max} of *Nitzschia*-2 was notably higher ($p < 0.05$) than that of the other four epiphytic diatoms in either f/2 or the other four optimized f/2 media (Figure S11). In terms of μ_{\max} , three species of *Navicula*, *Navicula*-1, and *Navicula*-2 were found to be more prominent ($p < 0.05$) than *Navicula*-3 (Figure S11).

Given that the optimization growth tests were conducted in 10-mL tubes, and the diatoms presented notably promoted growth rates ($p < 0.05$) at different levels, the optimized cultivation was then expanded to 5-L flasks for further testing the rapid growth of five diatoms. Then, the respective “optimized f/2-2” medium for *Navicula* spp. and *Nitzschia* spp. (Tables 1, 2) was chosen to assess the enlarged cultures based on the absolute growth rates of five diatoms (Figure 5). The results demonstrated that the biomass productivities of five diatoms under the optimized f/2 media were all significantly improved ($p < 0.05$), i.e., 6–13-fold of those under f/2 medium during 12 days of optimized cultures (Table 4). Moreover, the biomass productivities for three species of *Navicula* were found to be approximately 1.5-fold of those of two species of *Nitzschia* (Table 4).

The results revealed that all the growth performances of five diatoms cultured with the exclusively optimized f/2 medium under three light intensities were much better than those cultured with the f/2 medium (Figures 6; S12). The N:P mass ratios in the exclusively

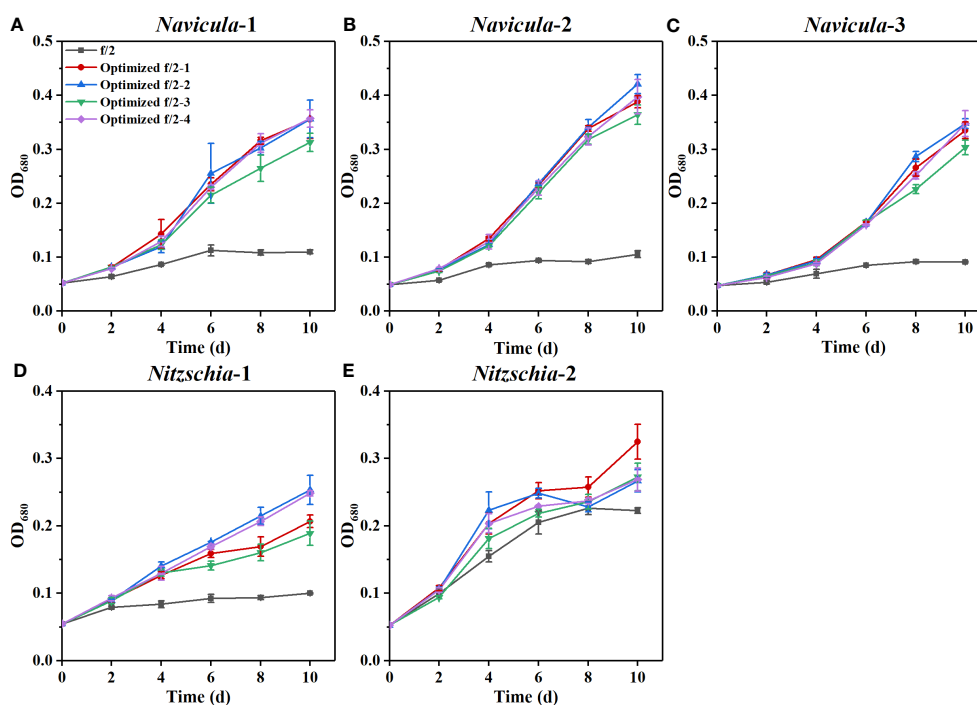


FIGURE 4

Effects of four optimized f/2 media on growth of five marine epiphytic diatoms. The components of the four optimized f/2 media for *Navicula* spp. (A–C) and *Nitzschia* spp. (D, E) are listed in Tables 1, 2, respectively. Values are means of three biological replicates \pm SD ($n = 3$).

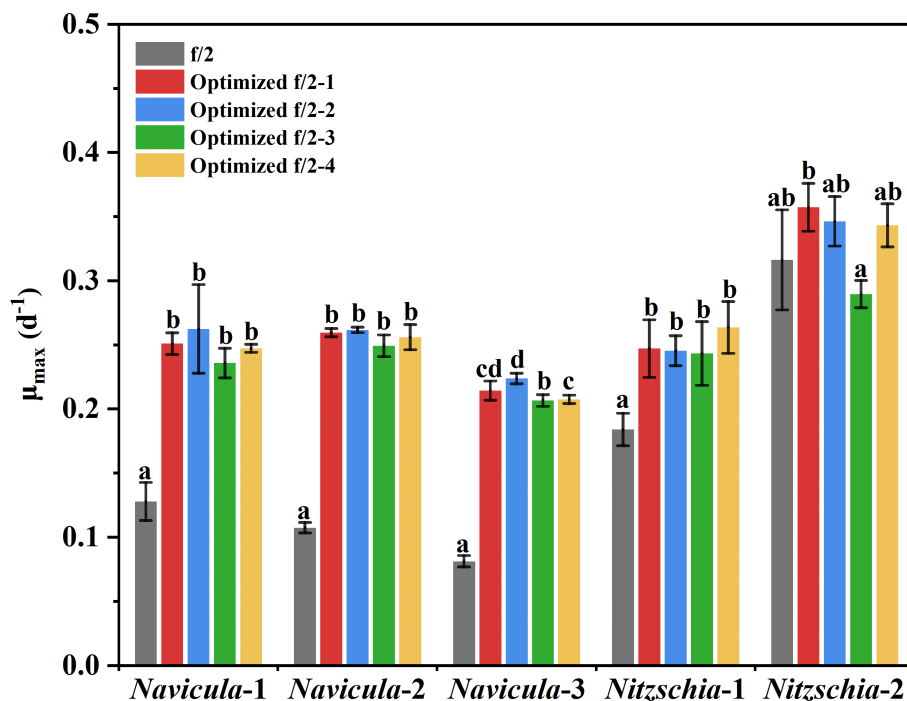


FIGURE 5 The maximum specific growth rates of five epiphytic diatoms in four optimized f/2 media. The μ_{max} indicates the maximum specific growth rates of certain diatoms during certain culture days. The maximum specific growth rates of *Navicula-1*, *Navicula-2*, *Navicula-3*, *Nitzschia-1*, and *Nitzschia-2* occurred on Days 6, 6, 8, 2, and 2, respectively, when they were cultured with f/2 or optimized f/2 media. The different letters labeled for the same diatom indicate the statistically significant difference by Tukey's honestly significant difference (HSD) test. The components of the four optimized f/2 media for *Navicula* spp. and *Nitzschia* spp. are listed in Tables 1, 2, respectively. Values are means of three biological replicates \pm SD ($n = 3$).

optimized f/2 media for *Navicula* spp. and *Nitzschia* spp. were 2.4- and 1.2-fold of those in the f/2 medium, respectively (Table 3). Different light intensities generated no obvious impacts on the growth of the f/2 medium-cultured diatoms, except for *Nitzschia-2*. The growth of *Navicula-2* was found to be significantly inhibited ($p < 0.05$) in the exclusively optimized f/2 medium when the

illumination was increased to $350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, while that of *Navicula-1* and *Navicula-3* was not prominently affected (Figures 6A–C, S12A–C, S13). Differently from *Navicula* spp., two species of *Nitzschia* both presented relatively good growth performance under the high light conditions when cultured with the exclusively optimized f/2 media (Figures 6D, E, S12D, E, S13).

TABLE 4 The biomass productivities of five marine epiphytic diatoms over 12 days of enlarged cultures.

Diatoms	Biomass productivities ($\text{g}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$)		
	f/2	Optimized f/2	Exclusively optimized f/2
<i>Navicula-1</i>	2.82 \pm 0.24 ^c	25.79 \pm 1.92 ^b	29.52 \pm 1.98 ^{ab}
<i>Navicula-2</i>	1.88 \pm 0.12 ^a	25.17 \pm 2.23 ^b	26.51 \pm 1.13 ^a
<i>Navicula-3</i>	2.33 \pm 0.25 ^{bc}	24.56 \pm 2.58 ^b	25.85 \pm 1.72 ^a
<i>Nitzschia-1</i>	2.26 \pm 0.16 ^b	16.58 \pm 0.98 ^a	29.42 \pm 2.56 ^{ab}
<i>Nitzschia-2</i>	2.99 \pm 0.28 ^c	17.19 \pm 1.38 ^a	30.68 \pm 2.84 ^{bc}

The optimized f/2 media for *Navicula* spp. and *Nitzschia* spp. refer to the optimized f/2-2 in Tables 1, 2, and the exclusively optimized f/2 media for the two diatoms refer to those in Table 3. The different letters labeled for the same column indicate the statistically significant difference by Tukey's honestly significant difference (HSD) test. Values are means of three biological replicates \pm SD ($n = 3$).

From the view of the maximum specific growth rates, i.e., μ_{max} , the most outstanding diatom turned out to be *Nitzschia-2*, regardless of whether it was cultured under the high light or in the exclusively optimized medium (Figure 7). The μ_{max} of *Nitzschia-2* was 1.3-2.7 times that of the other four diatoms under three light intensities, though that of the five diatoms was nearly identical in f/2 medium under light of $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In addition, the μ_{max} of *Nitzschia* spp. still occurred 2 days earlier than that of *Navicula* spp. However, the three species of *Navicula* almost could not be differentiated from each other in terms of the μ_{max} under different culture conditions. However, once the light intensity increased to $350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the μ_{max} of *Navicula-2* and *Navicula-3* remarkably decreased ($p < 0.05$) relative to that of *Navicula-1* (Figure 7).

The results demonstrated that the biomass productivities of the five diatoms in the respective exclusively optimized f/2 media were all significantly higher ($p < 0.05$) than those in the optimized f/2-2 media listed in Tables 1, 2. In particular, *Navicula-1* and *Nitzschia-2* exhibited the highest biomass productivities, i.e., $29.52 \pm 1.98 \text{ g}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ and $30.68 \pm 2.84 \text{ g}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$, respectively (Table 4). Overall, *Navicula-1* and *Nitzschia-2* showed greater growth performance among the five marine epiphytic diatoms.

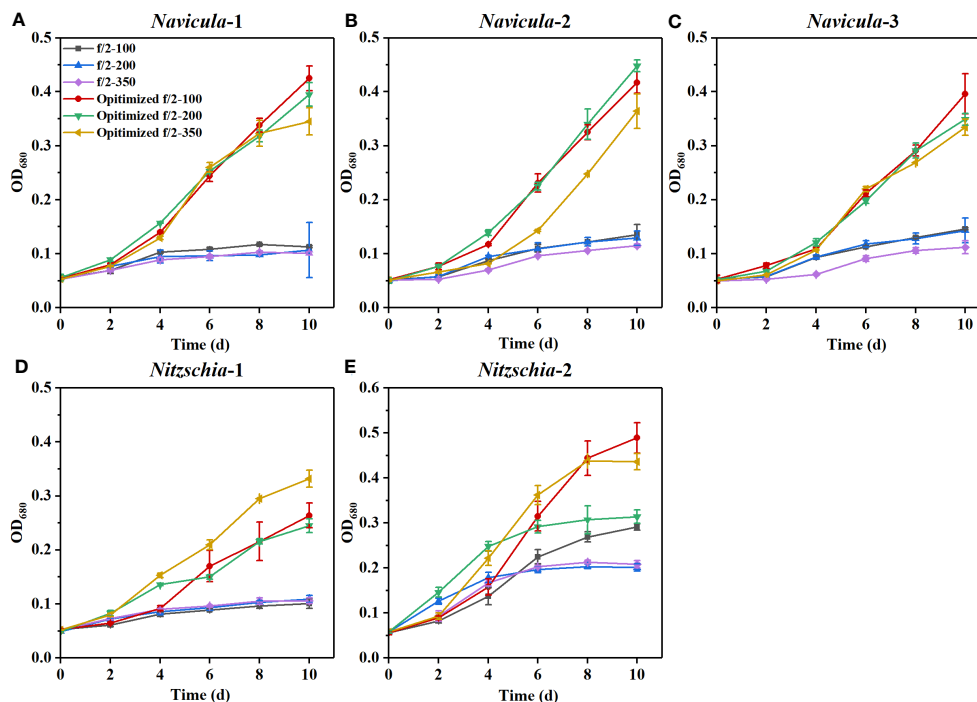


FIGURE 6

Effects of the exclusively optimized $f/2$ media on the growth of five marine epiphytic diatoms under three light intensities. The legends of “optimized $f/2$ ” denote the exclusively optimized $f/2$ media for *Navicula* spp. (A–C) and *Nitzschia* spp. (D, E), which are listed in Table 3. The legends of 100, 200, and 350 behind $f/2$ refer to the light intensities for optimization tests, i.e., 100, 200, and 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. Values are means of three biological replicates \pm SD ($n = 3$).

However, the fatty acid profiles for *Navicula-1* and *Nitzschia-2* cultured with the exclusively optimized $f/2$ media at an enlarged 4-L scale were detected (Figures 8A; S14). It was interesting to note that the relative abundances of C20:5n3 in *Navicula-1* cultured with the exclusively optimized $f/2$ medium were found to notably rise ($p < 0.05$) by 24%, and that of C16:1n7 significantly decreased ($p < 0.05$) by 28% relative to the $f/2$ medium (Figure 8A), though the PUFAs increased without a remarkable difference (Figure S14). In addition, the exclusively optimized $f/2$ medium-cultured *Nitzschia-2* remained stable in the relative abundances of both PUFAs and C20:5n3, and the relative abundance of C16:1n7 was prominently reduced ($p < 0.05$) by 31% (Figures 8B, S14).

4 Discussion

The periphytic diatoms are abundant in nutrient-rich waters and are classified as pollution bioindicators in the diversified environment. This is chiefly because these diatoms can utilize and assimilate large amounts of inorganic and organic nutrients, e.g., nitrogen and phosphorus derived from urban sewage (Kwon et al., 2013). However, periphytic diatoms have also been widely used as the key natural diets for invertebrate marine animals. It is mainly because they are highly rich in polyunsaturated fatty acids, C20:5n3 in particular, and concern the metamorphosis of the invertebrates (Chen, 2007; Sánchez-Saavedra and Núñez-Zarco, 2015; Xie et al., 2017). As such, the isolation and further screening of high-quality

periphytic diatoms are necessary to potentially contribute to wastewater treatment and developing aquaculture industries.

Light drives diatom productivity, and diatoms are well-adapted to utilize flexible intensities and endure variable light exposure, dependent on seasons, latitudes, and depth. Many studies have demonstrated that the growth rates and photosynthesis in various phytoplankton species can be affected by illumination conditions (van Leeuwe et al., 2005; van de Poll et al., 2007). In this study, the enlarged cultures of the five isolated diatoms were performed in 5-L flasks under natural light cycles (approximately 10 h of light duration). The light intensity illuminated on the flasks was often up to nearly 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, especially at noon. Even so, these diatoms mostly grew vibrantly. Although the diatoms generally require not too high light intensity, the isolated epiphytic diatoms in this study might indeed be able to live under high light intensity, e.g., 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This is probably because these isolated indigenous diatoms distributed on the intertidal macroalgae seem to be capable of enduring high light intensity. Thus, three light intensities, including 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, were set to test the tolerance and adaption of the five isolated epiphytic diatoms to high irradiance. However, five strains of diatoms mostly exhibited no significantly promoted growth rates, and even presented inhibited growth rates (Figures 6, 7), when treated with high light intensities (from 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) under either $f/2$ or the exclusively optimized $f/2$ media. It has been shown that certain diatom species in adaption to high light intensities present high growth rates, high photosynthetic

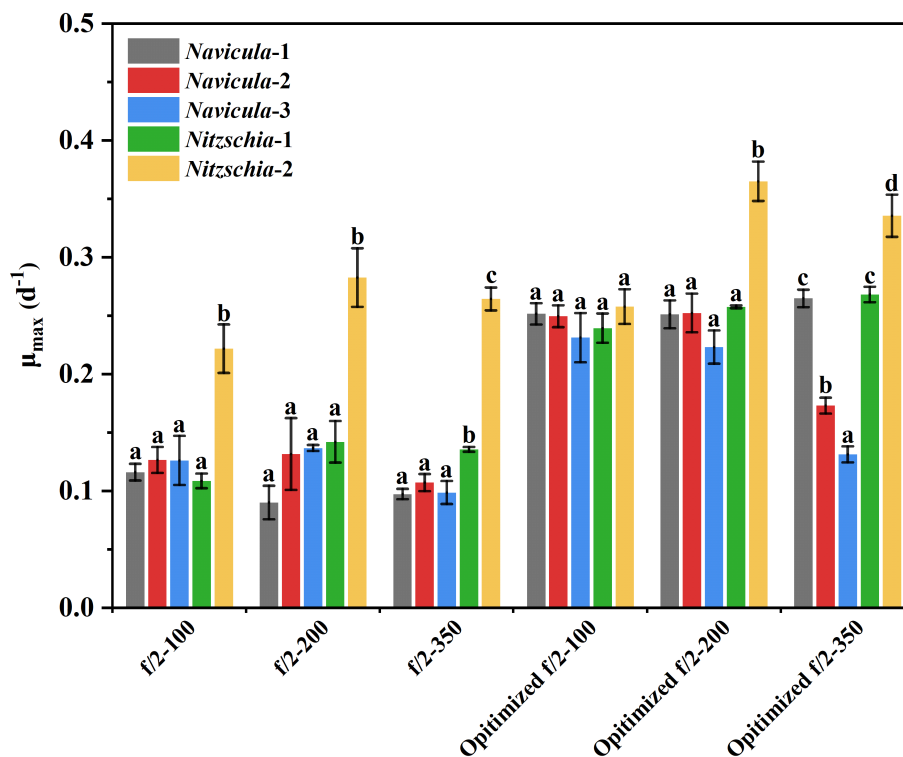


FIGURE 7

Comparison of the maximum specific growth rates of five epiphytic diatoms in *f/2* and the exclusively optimized *f/2* media under three light intensities. The μ_{\max} indicates the maximum specific growth rates of certain diatoms during certain culture days. The maximum specific growth rates of *Navicula-1*, *Navicula-2*, *Navicula-3*, *Nitzschia-1*, and *Nitzschia-2* occurred on Days 6, 6, 6, 4, and 4, respectively, when they were cultured with *f/2* or exclusively optimized *f/2* media. The legends of "optimized *f/2*" denote the exclusively optimized *f/2* media for *Navicula* spp. and *Nitzschia* spp., which are listed in Table 3. The different letters labeled for the same diatom indicate the statistically significant difference by Tukey's honestly significant difference (HSD) test. The components of the exclusively optimized *f/2* media for *Navicula* spp. and *Nitzschia* spp. are listed in Table 3. Values are means of three biological replicates \pm SD ($n = 3$).

activity, high light saturation intensities, and competent responses to photoinhibition under different high irradiance levels (Benjamin et al., 2013; Kvernvik et al., 2020). Given that the five strains of diatoms in this work were isolated from the epiphytes on the macroalgae in the intertidal zone, the habitats that these diatoms inhabit are undergoing variable irradiances, especially in the summer high-temperature extreme days. Thus, they could likely endure high levels of irradiation and have higher biomass productivity under indoor natural light illumination.

In this study, we selected four macroelements (nitrogen, phosphorus, iron, and silicon), five trace metal elements (Zn, Cu, Mn, Mo, and Co), and three vitamins (VB₁, VB₁₂, and VH) mainly because the basal medium for culturing marine diatoms is usually *f/2* medium, and the above 12 elements comprise the whole components. In addition, the pre-cultured epiphytic diatom biofilms in *f/2* medium gradually turned greenish within 7 days. In this case, the diatom cells likely require higher levels of nutrients in this medium; thus, we designed single-factor as well as orthogonal tests for each element in our preliminary experiments (Yang et al., 2014, Figures S1-S10, Tables S1-18). Based on these tests, we further designed four optimized media for *Navicula* spp. and *Nitzschia* spp. (Tables 1, 2). Our results demonstrated that the isolated five strains of epiphytic diatoms were all well-adapted to high concentrations of macronutrients, including nitrogen,

phosphorus, iron, and silicon. It was worth noting that the 12-day enlarged cultures for three species of *Navicula* in the optimized *f/2* media all gradually changed from dark brown to a kind of yellow-green. It was also consistent with the change in colors of the chloroplasts under the light microscope. Hence, we assumed that the macroelement, i.e., nitrogen, was likely insufficient for *Navicula* spp. Additionally, when high levels of silicon stocks in optimized *f/2* media were added into the sterile seawater, a few white precipitates occurred instantly, and the harvested diatom pellets were also covered with a thin layer of white precipitates. Thus, the silicon in both the optimized *f/2* media for these diatoms was presumed to be surplus. Finally, the optimized *f/2-2* media for *Navicula* spp. and *Nitzschia* spp. in Tables 1, 2 were further modified. The nitrogen levels for *Navicula* spp. were elevated, and the silicon levels for the five diatoms were all reduced (Table 3). In addition, in order to distinguish the differences between the nutrient needs of *Navicula* spp. and *Nitzschia* spp., we fine-tuned the concentrations of iron for the two genera to the same level. The two further modified media were called the exclusively optimized media for *Navicula* spp. and *Nitzschia* spp. (Table 3). Specifically, the concentrations of nitrogen, phosphorus, and iron in the exclusively optimized *f/2* media for *Navicula* spp. and *Nitzschia* spp. were 2–6-fold of those in the *f/2* medium. However, the concentrations of silicon for the two genera of diatoms were up to 15- and 10-fold of those in the *f/2* medium

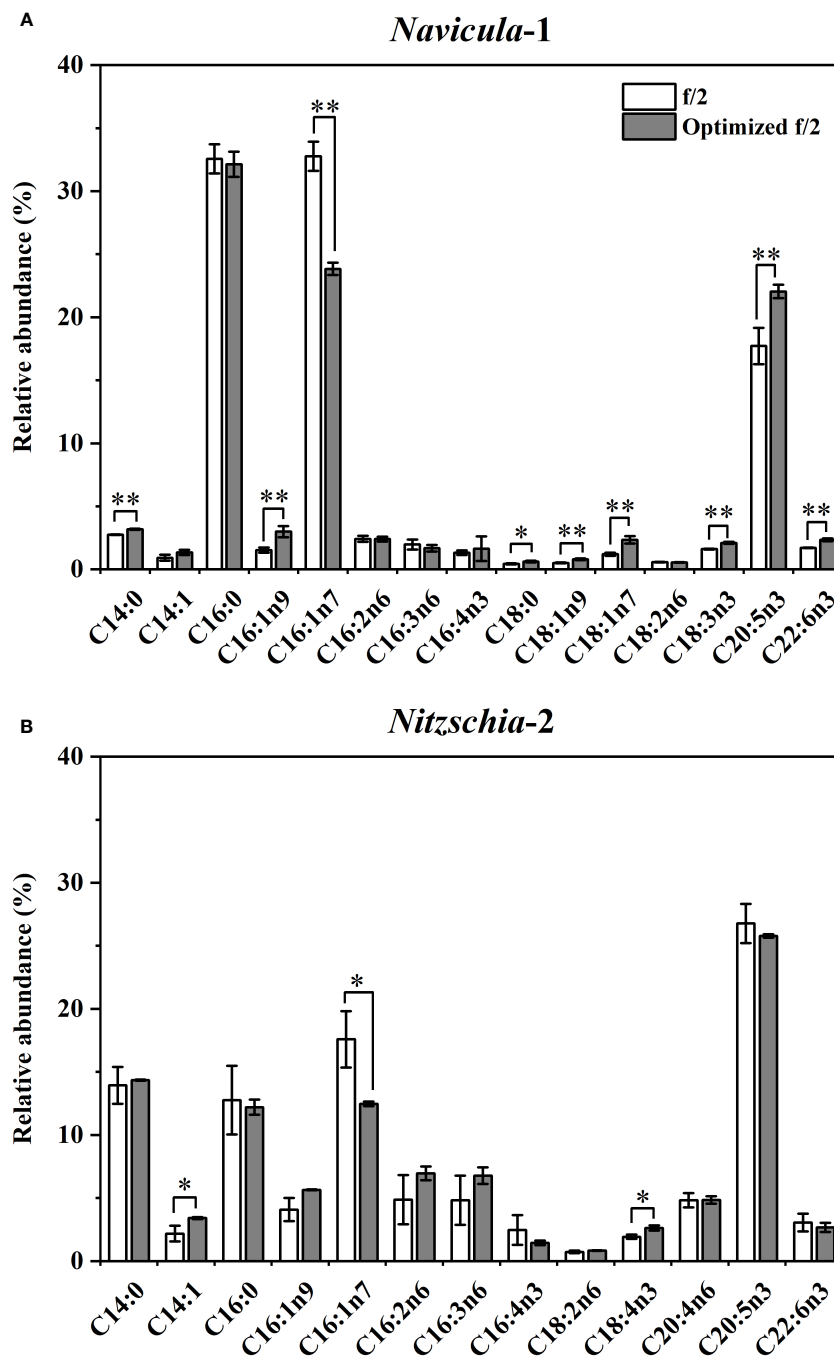


FIGURE 8 Effects of the exclusively optimized *f/2* media on fatty acid profiles of *Navicula*-1 (A) and *Nitzschia*-2 (B). The legends of “optimized *f/2*” denote the exclusively optimized *f/2* media for *Navicula*-1 and *Nitzschia*-2, which are listed in Table 3. Values are means of three biological replicates ± SD (*n* = 3). **p* < 0.05; ***p* < 0.01.

(Table 3). Apart from the equivalent concentrations of phosphorus and iron for the two genera of diatoms, *Navicula* spp. preferred much higher levels of nitrogen (74.16 to 34.51 mg/L) and silicon (37.08 to 23.01 mg/L) relative to *Nitzschia* spp. in the exclusively optimized *f/2* media. Hence, *Navicula* spp. seemed to harbor a higher capability to utilize and assimilate inorganic nitrogen and silicon than *Nitzschia* spp., which will be focused on and further explored in our future study. At the same time, the biomass productivities of these five diatoms in *f/2* medium were found to

increase by 10–14-fold in the exclusively optimized *f/2* media (Table 4). Moreover, the biomass productivities of the three species of *Navicula* in the optimized *f/2*-2 medium notably rose by 5%–14% compared with those in the exclusively optimized *f/2* medium, while those of the two species of *Nitzschia* both significantly rose by more than 77% (Table 4). These results demonstrated that the epiphytic diatoms were indeed more capable of recruiting and storing inorganic nutrients, nitrogen, and silicon in particular.

Kwon et al. (2013) investigated the uptake rates of nitrate and phosphate by four periphytic diatoms, including *Achnanthes* sp., *Amphora* sp., *Navicula* sp., and *Nitzschia* sp. However, *Nitzschia* sp. exhibited the highest specific uptake rates under multiple wavelengths and had been considered a useful species for phytoremediation. In this work, the selective *Navicula*-1 and *Nitzschia*-2 had outstanding growth traits and improved fatty acid profiles under high concentrations of nitrogen. They could also be potentially used for remediating nutrient-rich wastewater. Furthermore, the silicified diatoms require large amounts of silicon for growth together with nitrogen, phosphorus, and carbon, thus connecting the biogeochemical cycles of these elements. Understanding the silicon cycle is essential for deciphering the connected function of marine food webs, biogeochemical cycles, and the biological carbon pump (Tréguer et al., 2021). The fast-growing *Navicula*-1 and *Nitzschia*-2 in this work turned out to be highly adapted to silicon-abundant (34.51 mg/L and 23.01 mg/L) waters, and they were very likely to become favorable candidates for future study of the silicon cycle.

A previous study showed that silicon could become limiting to diatoms after nitrogen and phosphorus enrichment (Carrick, 1988). However, in this study, the silicon concentrations in the exclusively optimized *f/2* media for *Navicula* spp. and *Nitzschia* spp. were 15- and 10-fold, respectively, of those in the control *f/2* medium. In addition, nitrogen and phosphorus concentrations in the exclusively optimized *f/2* media *Navicula* spp. and *Nitzschia* spp. were just 2–6-fold of those in the control *f/2* medium. In general, the Redfield C:N:P ratio of 106:16:1 has been widely considered as a mean composition of the three macroelements in phytoplankton. Nutrient limitation could result in the stoichiometric ratio further away from the Redfield ratio (Spilling et al., 2015). In this case, the limiting nutrients are at the lowest level, and the non-limiting nutrients are utilized as well as over-stored. In this work, the screened optimal N:P mass ratios for *Navicula* spp. and *Nitzschia* spp. in the exclusively optimized *f/2* media turned out to be 26.1:1 and 13.1:1, respectively, and they were both higher than those in the control *f/2* medium (10.8). In particular, the three strains of *Navicula* were found to prefer more levels of nitrogen than the two strains of *Nitzschia*.

Vitamins and trace metals play essential roles in the growth regulation of marine microalgae. Many microalgal species require one or more vitamins as well as trace metals for growth, implying that these nutrients likely serve as limiting resources (Peperzak et al., 2000). It is necessary to make clear the vitamin and trace metal demands of microalgae greatly concerning the primary production of seas and oceans. In the *f/2* medium, the above-mentioned nutrients are all included. The vitamins consist of VB₁, VB₁₂, and VH, and trace metals consist of Zn, Cu, Mn, Mo, and Co. In this study, the growth of five strains of diatoms was found to be not prominently affected by all these nutrients based on the single-factor tests as well as the orthogonal tests (Tables S1–S18, Figures S1–S10). Hence, the concentrations of these seven nutrients, i.e., 0.5–100 µg/L for vitamins and 2.54–49.44 µg/L for trace metals, in the *f/2* medium appeared to be sufficient for favorable growth of the isolated diatoms in this work. Furthermore, *Navicula* spp. and *Nitzschia* spp. were verified to show outstanding growth rates and biomass productivity just in the exclusively optimized *f/2* media, where the concentration of each micronutrient was the same

as that in the *f/2* medium. It was also indicated that the nutritional factors more important for the growth of marine epiphytic diatoms were nitrogen, phosphorus, iron, and silicon, compared with the vitamins and trace metals. Overall, in this study, the growth of the epiphytic diatoms was shown to be greatly affected by nitrogen, phosphorus, iron, and silicon, while the vitamins, trace metals, and the light intensity could just generate minimal impacts on the growth of these diatoms. Indeed, the combined higher concentrations of the above macroelements produced more than 10-fold biomass productivity for either *Navicula* spp. or *Nitzschia* spp. (Table 4), indicating the great potential of these diatoms in nutrient-rich wastewater treatment.

However, the fatty acid profiles of microalgae determine their application potential. If the algal cells are abundant in saturated or monounsaturated fatty acids, they are more suitable to become potential raw materials for biofuels (Hu et al., 2008). If they are abundant in PUFAs, they are more appropriate to serve as functional lipids that are beneficial for human health. In common, the fatty acid profiles of diatoms can alter in response to variable nutrient conditions, including nitrogen, phosphorus, iron, and silicon (Jeffryes et al., 2013; Abida et al., 2015; Yang et al., 2017; Cointet et al., 2019; Zhang et al., 2023). In particular, the saturation degree of fatty acids in nutrient-depleted or nutrient-limited algal cells significantly increased, and the lipids could also be hyper-accumulated (Zienkiewicz et al., 2016). However, Feng et al. (2011) found that *Isochrysis zhangjiangensis* (Chrysophyta) accumulated the highest amount of lipids (53%) when cultured in an extremely high nitrate concentration (100-fold of those in *f/2* medium), though the growth of algal cells was suppressed. Nitrogen and phosphorus could affect and even take part in lipid metabolism in microalgae. In diatoms, nitrogen-containing lipids include phosphatidylcholine (PC), phosphatidylethanolamine (PE), and diacylglycerylhydroxymethyl-*N,N,N*-trimethyl-beta-alanine (DGTA); all the phospholipids contain phosphorus; thus, phosphorus is closely related to lipid metabolism (Abida et al., 2015). In addition, iron has been considered one of the most essential trace mineral elements for organisms. It plays crucial roles in nitrogen utilization, chlorophyll biosynthesis, and various biochemical metabolisms in microalgae. As such, iron is a major factor affecting the growth and reproduction of algal cells (Sutak et al., 2020; Nam et al., 2021). In particular, the iron source FeCl₃ added in the culture medium could increase the total lipid content and the PUFA amounts in diatoms, especially C20:5n3 and C22:6n3. Moreover, Fe³⁺ could also affect the expression of crucial genes involved in lipid biosynthesis in diatoms, thus altering lipid accumulation (Govindan et al., 2021). It is commonly accepted that silicon metabolism greatly concerns the frustule formation and influences the cell division in diatoms (Martin-Jezequel et al., 2000). Jeffryes et al. (2013) adopted a bioprocess strategy to enhance lipid production in the diatom *Cyclotella* sp. via controlled silicon addition. Thus, the fatty acid profiles and the lipid content in diatoms could be regulated by the four macroelements, including nitrogen, phosphorus, iron, and silicon. Previous studies demonstrated that nutrient limitation could dramatically alter the fatty acid composition of the diatoms, giving rise to a decline in the number of long-chain PUFAs (Breteler et al., 2005). Furthermore, the diatom feeder, e.g., the copepods, could not reach maturity (Breteler et al., 2005). In contrast, nutrient enrichment

could also change the fatty acid profiles of the epiphytic diatoms, but the relative abundances of PUFAs, C20:5n3, for example, presented an increased trend in this work, which was contrary to the case for nutrient limitation (Breteler et al., 2005). Hence, either eutrophy or nutrient limitation could affect lipid production in diatoms. We explored the impacts of the optimized *f/2* media on the lipid production of these indigenous diatoms. It is mainly because the epiphytic diatoms are potentially applicable as natural diets to feed the echinoderm, e.g., sea cucumbers, in the subsequent work. In addition, the fatty acid components, C20:5n3 in particular, have been largely reported to be crucial for metamorphosis development and robust growth of marine invertebrates (Chen, 2007; Sánchez-Saavedra and Núñez-Zarco, 2015; Xie et al., 2017). The fast-growing *Navicula-1* in this work was rich in saturated and monounsaturated fatty acids (69%–74%), while the relative amounts of PUFAs in *Nitzschia-2* accounted for approximately 50%. In spite of this, the relative amounts of the main polyunsaturated C20:5n3 for both diatoms notably improved or remained stable when these cells were cultured in the exclusively optimized *f/2* media. The result was also consistent with the above-mentioned previous studies (Jeffryes et al., 2013; Abida et al., 2015; Zhang et al., 2023).

In addition, the previous study showed that *Navicula* sp. as a fresh diet could not only enhance growth performance but also improve digestive enzyme activity and immunity in early growth of the sea cucumber, *Apostichopus japonicus* (Xie et al., 2017). Moreover, another diatom, i.e., *Cylindrotheca fusiformis*, was also reported to be a good feed ingredient for juvenile *A. japonicus* (Shi et al., 2013a, Shi et al., 2013b). The appropriate addition of *C. fusiformis* into the feed can enhance the growth of sea cucumbers in farming ponds (Li et al., 2015). In this case, the indigenous epiphytic diatoms isolated in our study were very likely to present growth-promoting effects and essential nutrient supply; thus, they have the potential to become the natural diets for the echinoderms. Overall, the screened *Navicula-1* and *Nitzschia-2* presented good growth traits and optimized fatty acid compositions at high levels of not only nitrogen (3–6-fold of those in *f/2* medium) but also phosphorus (2-fold of those in *f/2* medium), iron (10–15-fold of those in *f/2* medium), and silicon (5-fold of those in *f/2* medium) (Table 3) in this study. The two diatoms are very likely to be applied for remediating nutrient-rich wastewater and further exploitation of fatty acid-based bioproducts.

5 Conclusion

In this study, three strains of *Navicula* and two strains of *Nitzschia* were isolated from macroalgal epiphytes. *Navicula-1* and *Nitzschia-2* were screened out due to their outstanding growth traits under the optimized media with high levels of nitrogen, phosphorus, iron, and silicon. Their biomass productivities at a 4-L enlarged scale in the exclusively optimized *f/2* media both increased by 10-fold relative to the *f/2* medium. Moreover, the fatty acid profiles of the two diatoms were notably improved, and their relative amounts of PUFAs, especially C20:5n3, were significantly elevated or remained stable in the exclusively optimized *f/2* media. Thus, the two screened diatoms have the

potential to become quality diets for marine aquaculture and are ideal candidates for effective nutrient removal.

Data availability statement

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

Author contributions

MY: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. X-YX: Data curation, Investigation, Writing – original draft, Writing – review & editing. H-WH: Investigation, Writing – original draft. W-DZ: Supervision, Writing – review & editing. J-YM: Data curation, Writing – original draft. H-PL: Data curation, Writing – original draft. Q-ZW: Resources, Supervision, Writing – review & editing. XX: Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing. ZG: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1292713/full#supplementary-material>

References

- Abida, H., Dolch, L. J., Mei, C., Villanova, V., Conte, M., Block, M. A., et al. (2015). Membrane glycerolipid remodeling triggered by nitrogen and phosphorus starvation in *phaeodactylum tricornutum*. *Plant Physiol.* 167, 118–136. doi: 10.1104/pp.114.252395
- Almeida, O. U. H., and Beltrones, D. A. S. (2008). Variations in the structure of epiphytic diatom assemblages in subtropical macroalgae. *Hidrobiologica* 18, 51–61.
- Benjamin, S. C., Anne, J., Torsten, J., Wolfram, W., Maria, M., and Christian, W. (2013). Blue light is essential for high light acclimation and photoprotection in the diatom *Phaeodactylum tricornutum*. *J. Exp. Bot.* 64, 483–493. doi: 10.1093/jxb/ers340
- Breteler, W. C. M. K., Schogt, N., and Rampen, S. (2005). Effect of diatom nutrient limitation on copepod development: role of essential lipids. *Mar. Ecol. Prog. Ser.* 291, 125–133. doi: 10.3354/meps291125
- Burfeid-Castellanos, A. M., Martín-Martín, R. P., Kloster, M., Angulo-Preckler, C., Avila, C., and Beszteri, B. (2021). Epiphytic diatom community structure and richness is determined by macroalgal host and location in the South Shetland Islands (Antarctica). *PLoS One* 16, e0250629. doi: 10.1371/journal.pone.0250629
- Carrick, H. J. (1988). and lowe, RResponse of Lake Michigan benthic algae to *in situ* enrichment with Si, N and P. *L. Can. J. Fish. Aquat. Sci.* 45, 271–279. doi: 10.1139/f88-032
- Chen, Y. (2007). Immobilization of twelve benthic diatom species for long-term storage and as feed for post-larval abalone *Haliotis diversicolor*. *Aquaculture* 263, 97–106. doi: 10.1016/j.aquaculture.2006.12.008
- Cointet, E., Wielgosz-Collin, G., Bougaran, G., Rabesaotra, V., Gonçalves, O., and Méléder, V. (2019). Effects of light and nitrogen availability on photosynthetic efficiency and fatty acid content of three original benthic diatom strains. *PLoS One* 14, e0224701. doi: 10.1371/journal.pone.0224701
- Courtois de Viçosa, G., Porta, A., Viera, M. P., and Fernández-Palacios, H. (2012). and izquierdo, M Effects of density on growth rates of four benthic diatoms and variations in biochemical composition associated with growth phase. *S. J. Appl. Phycol.* 24, 1427–1437. doi: 10.1007/s10811-012-9799-z
- Desrosiers, C., Leflaive, J., Eulin, A., and Ten-Hage, L. (2013). Bioindicators in marine waters: Benthic diatoms as a tool to assess water quality from eutrophic to oligotrophic coastal ecosystems. *Ecol. Indic.* 32, 25–34. doi: 10.1016/j.ecolind.2013.02.021
- Feng, D., Chen, Z., Xue, S., and Zhang, W. (2011). Increased lipid production of the marine oleaginous microalgae *Isochrysis zhangjiangensis* (Chrysochyta) by nitrogen supplement. *Bioresour. Technol.* 102, 6710–6716. doi: 10.1016/j.biortech.2011.04.006
- Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497–509. doi: 10.1016/S0021-9258(18)64849-5
- Gomes, A., Loureno, S., Santos, P. M., Raposo, A., and Pombo, A. (2021). Effects of single and mixed-diatom diets on growth, condition, and survival of larvae of the sea urchin *Paracentrotus lividus* (Lamarck). *Aquacult. Int.* 29, 1069–1090. doi: 10.1007/s10499-021-00676-8
- Govindan, N., Maniam, G. P., Ab. Rahim, M. H., Sulaiman, A. Z., Ajit, A., and Chatsungnoen, T. (2021). Production of renewable lipids by the diatom *Amphora copulata*. *Fermentation* 7, 37. doi: 10.3390/fermentation7010037
- Han, D., Sun, Y., Cong, J., Gao, C., and Wang, G. (2023). Ecological distribution of modern diatom in peatlands in the northern Greater Khingan Mountains and its environmental implications. *Environ. Sci. Pollut. Res.* 30, 36607–36618. doi: 10.1007/s11356-022-24910-9
- Harrison, P. J., and Berges, J. A. (2005). "Chapter 3 – marine culture media," in *Algal culturing techniques*, 1st Edn. Ed. R. A. Andersen (Burlington: Elsevier Academic Press), 21–33.
- Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., et al. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.* 54, 621–639. doi: 10.1111/j.1365-313X.2008.03492.x
- Jeffries, C., Rosenberger, J., and Rorrer, G. L. (2013). Fed-batch cultivation and bioprocess modeling of *Cyclotella* sp. for enhanced fatty acid production by controlled silicon limitation. *Algal Res.* 2, 16–27. doi: 10.1016/j.algal.2012.11.002
- Kireta, A. R., Reavie, E. D., Sgro, G. V., Angradi, T. R., Bolgrien, D. W., Hill, B. H., et al. (2012). Planktonic and periphytic diatoms as indicators of stress on great rivers of the United States: Testing water quality and disturbance models. *Ecol. Indic.* 13, 222–231. doi: 10.1016/j.ecolind.2011.06.006
- Kvernvik, A. C., Rokitta, S. D., Leu, E., Harms, L., Gabrielsen, T. M., Rost, B., et al. (2020). Higher sensitivity towards light stress and ocean acidification in an Arctic sea-ice-associated diatom compared to a pelagic diatom. *New Phytol.* 226, 1708–1724. doi: 10.1111/nph.16501
- Kwon, H. K., Oh, S. J., and Yang, H. S. (2013). Growth and uptake kinetics of nitrate and phosphate by benthic microalgae for phytoremediation of eutrophic coastal sediments. *Bioresour. Technol.* 129, 387–395. doi: 10.1016/j.biortech.2012.11.078
- Li, J., Dong, S., Tian, X., Shi, C., Wang, F., Gao, Q., et al. (2015). Effects of the diatom *Cylindrotheca fusiformis* on the growth of the sea cucumber *Apostichopus japonicus* and water quality in ponds. *Aquac. Int.* 23, 955–965. doi: 10.1007/s10499-014-9854-7
- Martin-Jezequel, V., Hildebrand, M., and Brzezinski, M. A. (2000). Silicon metabolism in diatoms: implications for growth. *J. Phycol.* 36, 821–840. doi: 10.1046/j.1529-8817.2000.00019.x
- Meerssche, E. V., and Pincney, J. L. (2019). Nutrient loading impacts on estuarine phytoplankton size and community composition: Community-based indicators of eutrophication. *Estuaries Coasts.* 42, 504–512. doi: 10.1007/s12237-018-0470-z
- Meng, F., Xi, L., Liu, D., Huang, W., Lei, Z., Zhang, Z., et al. (2019). Effects of light intensity on oxygen distribution, lipid production and biological community of algal-bacterial granules in photo-sequencing batch reactors. *Bioresour. Technol.* 272, 473–481. doi: 10.1016/j.biortech.2018.10.059
- Mercado, J. M., Sánchez-Saavedra, M., del, P., Correa-Reyes, G., Lubián, L., Montero, O., et al. (2004). Blue light effect on growth, light absorption characteristics and photosynthesis of five benthic diatom strains. *Aquat. Bot.* 78, 265–277. doi: 10.1016/j.aquabot.2003.11.004
- Metcalfe, L. D., and Schmitz, A. A. (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.* 33, 363–364. doi: 10.1021/ac60171a016
- Nam, H. I., Shahzad, Z., Dorone, Y., Clowez, S., Zhao, K., and Bouain, N. (2021). Interdependent iron and phosphorus availability controls photosynthesis through retrograde signaling. *Nat. Commun.* 12, 7211. doi: 10.1038/s41467-021-27548-2
- Otim, O., Juma, T., and Savinelli, R. (2018). The effect of a massive wastewater discharge on nearshore ocean chemistry. *Environ. Monit. Assess.* 190, 180. doi: 10.1007/s10661-018-6530-9
- Pandey, L. K., Sharma, Y. C., Park, J., Choi, S., and Han, T. (2018). Evaluating features of periphytic diatom communities as biomonitoring tools in fresh, brackish and marine waters. *Aquat. Toxicol.* 194, 67–77. doi: 10.1016/j.aquatox.2017.11.003
- Peperzak, L., Gieskes, W. W. C., Duin, R., and Colijn, F. (2000). The vitamin B requirement of *Phaeocystis globosa* (Prymnesiophyceae). *J. Plankton Res.* 22, 1529–1537. doi: 10.1093/plankt/22.8.1529
- Sánchez-Saavedra, M. D. P., and Núñez-Zarco, E. (2015). Growth of red abalone, *Haliotis rufescens*, fed cold-stored benthic diatoms. *J. World Aquacult. Soc.* 46, 210–218. doi: 10.1111/jwas.12182
- Scholz, B., and Liebezeit, G. (2013). Biochemical characterisation and fatty acid profiles of 25 benthic marine diatoms isolated from the Solthörn tidal flat (southern North Sea). *J. Appl. Phycol.* 25, 453–465. doi: 10.1007/s10811-012-9879-0
- Shi, C., Dong, S., Pei, S., Wang, F., Tian, X., and Gao, Q. (2013a). Effects of diatom concentration in prepared feeds on growth and energy budget of the sea cucumber *Apostichopus japonicus* (Selenka). *Aquac. Res.* 46, 609–617. doi: 10.1111/are.12206
- Shi, C., Dong, S., Wang, F., Gao, Q., and Tian, X. (2013b). Effects of four fresh microalgae in diet on growth and energy budget of juvenile sea cucumber *Apostichopus japonicus* (Selenka). *Aquaculture* 416–417, 296–301. doi: 10.1016/j.aquaculture.2013.09.050
- Spilling, K., Ylöstalo, P., Simis, S., and Seppälä, J. (2015). Interaction effects of light, temperature and nutrient limitations (N, P and Si) on growth, stoichiometry and photosynthetic parameters of the cold-water diatom *Chaetoceros wighamii*. *PLoS One* 10, e0126308. doi: 10.1371/journal.pone.0126308
- Sutak, R., Camadro, J. M., and Lesuisse, E. (2020). Iron uptake mechanisms in marine phytoplankton. *Front. Microbiol.* 11. doi: 10.3389/fmicb.2020.566691
- Tréguer, P. J., Sutton, J. N., Brzezinski, M., Charette, M. A., Devries, T., Dutkiewicz, S., et al. (2021). Reviews and syntheses: The biogeochemical cycle of silicon in the modern ocean. *Biogeosciences* 18, 1269–1289. doi: 10.5194/BG-18-1269-2021
- van de Poll, W. H., Visser, R. J. W., and Buma, A. G. J. (2007). Acclimation to a dynamic irradiance regime changes excessive irradiance sensitivity of *Emiliania huxleyi* and *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 52, 1430–1438. doi: 10.4319/lo.2007.52.4.1430
- van Leeuwe, M. A., van Sikkelerus, B., Gieskes, W. W. C., and Stefels, J. (2005). Taxon-specific differences in photoacclimation to fluctuating irradiance in an Antarctic diatom and a green flagellate. *Mari. Ecol. Prog. Ser.* 288, 9–19. doi: 10.3354/meps288009
- Wang, Q., Wang, S., Ding, M., Li, M., Shi, R., and Cheng, A. (1997). Studies on culture conditions of benthic diatoms for feeding abalone I. Effects of temperature and light intensity on growth rate. *Chin. J. Oceanol. Limnol.* 15, 296–303. doi: 10.1007/BF02850562
- Xie, X., Zhao, W., Yang, M., Zhao, S., and Wei, J. (2017). Beneficial effects of benthic diatoms on growth and physiological performance in juvenile sea cucumber *Apostichopus japonicus* (Selenka). *Aquacult. Int.* 25, 287–302. doi: 10.1007/s10499-016-0028-7
- Yang, M., Fan, Y., Wu, P., Chu, Y., Shen, P., Xue, S., et al. (2017). An extended approach to quantify triacylglycerol in microalgae by characteristic fatty acids. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01949
- Yang, M., Zhao, W., and Xie, X. (2014). Effects of nitrogen, phosphorus, iron and silicon on growth of five species of marine benthic diatoms. *Acta Ecologica Sinica.* 34, 311–319. doi: 10.1016/j.chnaes.2014.10.003
- Yin, X. W., Min, W. W., Lin, H. J., and Chen, W. (2013). Population dynamics, protein content, and lipid composition of *Brachionus plicatilis* fed artificial macroalgal detritus and *Nannochloropsis* sp. diets. *Aquaculture* 380, 62–69. doi: 10.1016/j.aquaculture.2012.11.018
- Zhang, Z., Peng, R., Xia, X., Liu, P., Chen, S., Xia, R., et al. (2023). Effects of iron on the growth, chlorophyll a, total lipids, and fatty acids of *Chaetoceros lorenzianus*. *J. Appl. Phycol.* 35, 639–647. doi: 10.1007/s10811-023-02912-5

Zhang, X., Qi, M., Chen, L., Wu, T., Zhang, W., Wang, X., et al. (2020). Recent change in nutrient discharge from municipal wastewater in China's coastal cities and implication for nutrient balance in the nearshore waters. *Estuar. Coast. Shelf S.* 242, 106856. doi: 10.1016/j.ecss.2020.106856

Zienkiewicz, K., Du, Z. Y., Ma, W., Vollheyde, K., and Benning, C. (2016). Stress-induced neutral lipid biosynthesis in microalgae-Molecular, cellular and physiological insights. *BBA-Mol. Cell Biol. L.* 1861, 1269–1281. doi: 10.1016/j.bbali.2016.02.008