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Environmental effects on growth and biochemical composition of *Nitzschia inconspicua* Grunow

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Abstract

The effects of nitrate and silicate levels, and carbon source on growth, biochemical composition and fatty acid composition of *Nitzschia inconspicua* were investigated using batch cultures. Within the range of silicate levels supplied (8.8–176 μM), no marked variations in growth trend, biochemical composition or fatty acid composition were shown. Biomass at stationary phase, ranging from 64–66 mg ash-free dry weight (AFDW) L^{-1}, and specific growth rate (μ) based on chlorophyll *a* (0.41–0.50 d^{-1}) of the cultures grown within 0.3–3.0 mM $NaNO_3$ were not significantly different. Cultures supplemented with glucose (0.1% w/v), acetate (0.1% w/v) or 5% CO_2 attained higher biomass (85, 85, 97 mg AFDW L^{-1}) than the control which was grown in synthetic seawater and agitated by magnetic stirring. Cells grown at <3.0 mM $NaNO_3$ contained higher carbohydrate contents (14.8–21.5% AFDW) than those grown at 3.0 mM (4.0% AFDW). Lipid content increased at the expense of proteins in cells aerated with 5% CO_2. The dominant fatty acids, 16:0 and 16:1, ranged from 35.7–45.0% and 36.4–45.4% total fatty acids (TFA), respectively, while the relative proportions of 20:4 (n-6) and 20:5 (n-3) ranged from 1.7–5.4% and 3.4–5.9% TFA respectively. Cultures aerated with 5% CO_2 attained the highest biomass (97 mg AFDW L^{-1}) and yield of 20:5 (n-3) (0.34 mg L^{-1}).

Introduction

Some marine diatoms produce significant amounts of long chain polyunsaturated fatty acids (LC-PUFA). Due to the high content of LC-PUFA, especially eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), they are often cultured in hatcheries and used as live feed for prawn and fish larvae (de Pauw & Persoone, 1988). Diatoms from the genera *Skeletonema, Chaetoceros, Thalassiosira, Phaeodactylum* and *Nitzschia* are usually rich in LC-PUFA and useful for live feed.

It is advantageous to grow indigenous isolates in hatcheries as they are more tolerant to local conditions. Renaud et al. (1994) screened twelve Australian microalgal isolates (four diatoms) for LC-PUFA. The two marine diatoms *Nitzschia frustulum* and *N. closterium* contained as much as 23.1 and 15.2% of the total fatty acids as 20:5 (n-3), respectively.

In Malaysia, microalgae used as live feed are usually imported and the biochemical composition has not been characterized in detail. Shamsudin (1992) determined the fatty acid composition of *Chaetoceros calcitrans* and *Skeletonema costatum*, which are commonly used to feed prawn larvae in Malaysia, and found that the proportion of 20:5 (n-3) only ranged from 3–9% of the total fatty acids.

Nitzschia inconspicua is a marine diatom isolated from boat scrapings in Malaysia. The amounts of 20:4 (n-6) and 20:5 (n-3) produced range from 0.6–4.7% and 1.9–8.9% of the total fatty acids respectively (Chu et al., 1994). The proportion of 20:5 (n-3) is highest when the cultures are at the end of the exponential phase.

Changes in the levels of the principal nutrients such as silicon, carbon and nitrogen, can result in marked changes in the biochemical composition and fatty acid composition of diatoms. For example, in *Cyclotella cryptica*, the percentage of carbon partitioned into lipids increases from 27.6% to 54.1% whereas that channelled to carbohydrates decreases from 21.6% to 10.6% after being subjected to silicon starvation (Roessler, 1988). The proportion of 16:1 increases at the expense of 20:5 (n-3) and 22:6 (n-3) in the diatom under silicon starvation. The production of 20:5 (n-3) increases with increasing nitrate level in *Phaeodactylum tricornutum* (Yongmanitchai & Ward, 1991). The content of 20:5 (n-3) in *P. tricornutum* is highest when the cultures are supplemented with CO_2 at 5 mg L^{-1}.

The aim of the present study was to investigate the effects on the growth, biochemical composition and fatty acid composition of *N. inconspicua* when the cultures were grown at different levels of silicate and nitrate, and on different carbon sources.

Materials and methods

Culture conditions

The cultures of *Niztschia inconspicua* Grunow were grown in conical flasks (2 L) containing synthetic seawater medium (Chu et al., 1994). Axenic cultures on agar slants of the same medium are deposited in the Algal Culture Collection at the Institute of Advanced Studies, University of Malaya. Exponential phase cultures of 100 mL, with an OD_{700} of 0.1, were inoculated into each flask containing 900 mL medium. The cultures were agitated continuously by magnetic stirring (7 cm Teflon stirrer bars) and placed on shelves illuminated by fluorescent lamps (12:12 h LD, 42 μmol photon m^{-2} s^{-1}).

Effect of silicate levels

Silicate was supplied as sodium metasilicate pentahydrate ($Na_2SiO_3.5H_2O$), with the control containing 8.8 mM Si. An exponential phase culture was placed on the illuminated shelves (without stirring) 24 h before inoculation. After the cells had settled, the supernatant was decanted and cells resuspended by vigorous stirring in medium without silicate, to give an OD_{700} of 0.1. The culture was inoculated into duplicate flasks with medium containing each of the following silicate concentrations: 8.8, 22, 44, 88 and 176 μM. The cultures were harvested for dry weight determination and chemical extraction when they attained the stationary phase on day 17.

Effect of nitrate levels

Pretreatment of the inoculum was as described in the experiment on silicate levels; the settled cells were resuspended in medium without $NaNO_3$, and the OD_{700} was adjusted to 0.1. The cells were inoculated into duplicate flasks containing each of the following $NaNO_3$ concentrations: 0, 0.3, 0.75 and 3.0 (control) mM. Samples (10–20 mL) were withdrawn for growth monitoring. The cultures were harvested by filtration on day 11 for dry weight determination and chemical extraction.

Effect of carbon source

Duplicate cultures were used for each treatment in this experiment. The two organic carbon sources acetate and glucose were autoclaved separately and added to the medium to give a final concentration of 0.1% w/v. A set of duplicate flasks was aerated with 5% CO_2 through airstones. The control was agitated only by magnetic stirring. For growth monitoring, 10–20 mL samples were withdrawn daily. The cultures were harvested by filtration on day 17 (stationary phase) for dry weight determination and chemical extraction.

Growth monitoring

Growth of the cultures was monitored daily by determining the chlorophyll *a* (chl *a*) concentrations. Chlorophyll *a* was extracted in acetone and left in the dark (−20 °C, 24 h) and the concentration determined spectrophotometrically (Strickland and Parsons, 1968). Specific growth rate (μ, d^{-1}) was determined using the following formula:

$$u = \frac{lnN_1 - lnN_0}{t_1 - t_0}$$

where N_1 and N_0 represent chl *a* concentrations at times t_1 and t_0 within the exponential phase.

Dry weight determination and chemical analyses

Cells trapped on glass-fibre filters (Whatman GF/C, 0.45 μm) were rinsed with ammonium formate (HCO_2NH_4) solution (30 g L^{-1}) to remove the salts before determination of dry weight (100 °C, 24 h) and

Table 1. Specific growth rate (μ) based on chl *a* concentration and final biomass[a] of *Nitzschia inconspicua* cultured under different conditions.

Treatment	μ (d^{-1})	Biomass	
		mg DW L^{-1}	mg AFDW L^{-1}
Silicate (μM)			
8.8	0.28	145	95
22	0.31	140	97
44	0.33	150	92
88 (Control)	0.31	145	93
176	0.24	146	91
$NaNO_3$ (mM)			
0	0.35	48	31
0.3	0.42	103	65
0.75	0.50	100	64
3.0 (control)	0.41	101	66
Carbon source			
Control[b]	0.27	125	79
Acetate	0.31	132	85
Glucose	0.32	146	85
5%CO_2	0.37	152	97

Abbreviations: DW: dry weight; AFDW: Ash-free dry weight
[a] The cultures were harvested on day 17 (stationary phase) except those grown at different $NaNO_3$ levels, which were harvested on day 11.
[b] The control was continuously agitated with magnetic stirring.

ash (450 °C, 5 h). Separate filtered cells were used for chemical extraction. Lipids were extracted using MeOH-$CHCl_3$-H_2O (2:1:0.8) and determined gravimetrically according to the method described by Bligh and Dyer (1959). The lipids were transesterified in 1 N sodium methoxide (60 °C, 20 min) (Christie, 1989) and the fatty acid methyl esters were analysed by gas chromatography as described in Chu et al. (1994). Proteins were extracted with 0.5 N NaOH (80 °C, 20 min) and the concentrations determined by the dye-binding method (Bradford, 1976). Carbohydrates were extracted in 2 N HCl (80 °C, 20 min) and the concentrations determined by the phenol-sulfuric acid method (Kochert, 1978).

Results

Growth trends

Silicate levels studied did not significantly affect growth of *N. inconspicua* (Table 1). At the time of harvesting, nitrate was depleted in the cultures grown at 0.3 mM $NaNO_3$ while only 8.6 mg L^{-1} remained in the cultures grown at 0.75 mM $NaNO_3$. Excess nitrate (99.3 mg L^{-1}) was detected in the control. The cultures grown at 0.75 mM $NaNO_3$ produced the highest growth rate. In comparison to the cultures grown on other carbon sources, those aerated with 5% CO_2 attained the highest biomass based on ash-free dry weight (AFDW). The cultures grew for three days in the medium without $NaNO_3$ after which they remained at stationary phase until the end of the study.

Biochemical composition

The contents of lipids, carbohydrates, proteins and ash ranged from 12.5–20.5%, 3.0–14.0%, 10.8–18.8% and 31.5–41.8% dry weight respectively (Table 2). Cells grown within the range of silicate studied did not exhibit marked differences in biochemical composition. With increasing $NaNO_3$ levels, carbohydrate content decreased whereas lipid content remained almost constant. A marked decrease in protein content was observed in cells grown without $NaNO_3$. Cells aerated with 5% CO_2 contained highest amount of lipids (32.0% AFDW) but lowest amount of proteins (15.6% AFDW). Cells supplemented with carbon contained more than double the amount of carbohydrates than the control.

Fatty acid composition

No marked changes in the total content and composition of fatty acids were observed when silicate levels varied from 8.8 to 176 μM (Table 3). Total fatty acid content decreased significantly in cells grown without $NaNO_3$ (Table 4). The nitrogen-starved cells contained highest percentages of 14:0 and 20:5 (n-3). More 20:4 (n-6) was produced under nitrogen sufficiency than nitrogen starvation. The content of 18:3 (n-3) increased with increasing $NaNO_3$ levels. Cells aerated with 5% CO_2 contained the highest quantity of total fatty acids based on AFDW (Table 5). Under such conditions, the proportion of 18:3 (n-3) increased at the expense of 14:0. The percentages of 20:4 (n-6) and 20:5 (n-3) were higher in carbon-supplemented cells than the control.

A comparison of the contents (in mg g^{-1} AFDW) and yields (mg L^{-1}) of 20:4 (n-6) and 20:5 (n-3) under the different culture conditions is shown in Table 6. Cultures aerated with 5% CO_2 produced the highest

Table 2. Biochemical composition of *Nitzschia inconspicua* grown under different culture conditions (mean ± standard deviation, n=3)[a].

Treatment	Lipid		Carbohydrate		Protein		Ash
	%DW	%AFDW	%DW	%AFDW	%DW	%AFDW	%DW
Silicate (μM)							
8.8	14.5 ± 2.2	22.0 ± 3.3	8.4 ± 0	12.7 ± 0	16.0 ± 0.3	24.2 ± 0.5	34.2 ± 0.5
22	12.8 ± 1.8	18.6 ± 2.6	6.3 ± 0.5	9.1 ± 0.7	16.2 ± 0.3	23.5 ± 0.4	31.5 ± 0.6
44	12.4 ± 0.7	20.3 ± 1.1	6.8 ± 0	11.1 ± 0	15.7 ± 1.4	25.7 ± 2.3	38.8 ± 0.8
88 (Control)	11.7 ± 2.1	18.2 ± 3.3	5.6 ± 0	8.8 ± 0	15.5 ± 0.5	24.2 ± 0.8	35.8 ± 0.5
176	14.1 ± 4.1	22.6 ± 6.6	6.7 ± 0	10.8 ± 0	16.9 ± 0.9	27.3 ± 1.5	37.6 ± 0.6
$NaNO_3$ (mM)							
0	13.1 ± 1.5	20.2 ± 2.3	14.0 ± 0.8	21.5 ± 1.2	8.0 ± 0.6	12.4 ± 1.0	35.3 ± 0.7
0.3	15.8 ± 1.5	25.1 ± 2.4	10.1 ± 0.7	16.0 ± 1.1	17.6 ± 0.7	27.9 ± 1.1	37.0 ± 0.6
0.75	13.5 ± 0.8	21.1 ± 1.3	9.5 ± 0.3	14.8 ± 0.5	18.8 ± 1.3	29.4 ± 2.0	36.5 ± 0.9
3.0 (Control)	16.3 ± 1.3	25.1 ± 2.0	4.0 ± 0.5	6.2 ± 0.8	18.3 ± 0.3	28.2 ± 0.5	34.8 ± 0.8
Carbon source							
Control[b]	12.5 ± 1.7	19.8 ± 2.7	3.0 ± 0.5	4.7 ± 0.8	16.0 ± 2.0	25.4 ± 3.2	36.8 ± 1.2
Acetate	13.7 ± 0.4	21.4 ± 0.6	7.3 ± 1.2	11.4 ± 1.9	15.9 ± 1.8	24.8 ± 2.8	35.6 ± 0.8
Glucose	13.6 ± 0.1	23.4 ± 0.2	7.2 ± 1.0	12.4 ± 1.7	15.7 ± 1.3	27.1 ± 2.2	41.8 ± 0.9
5%CO_2	20.5 ± 0.1	32.0 ± 0.2	7.6 ± 0.8	11.9 ± 1.3	10.0 ± 0.5	15.6 ± 0.8	36.4 ± 0.8

Abbreviations: DW: dry weight; AFDW: Ash-free dry weight

[a] The cultures were harvested on day 17 (stationary phase) except those grown at different $NaNO_3$ levels, which were harvested on day 11.

[b] The control was continuously agitated with magnetic stirring.

Table 3. Fatty acid composition of *Nitzschia inconspicua* grown at different silicate levels[a].

Fatty acid	Silicate (μM)				
	8.8	22	44	88 (Control)	176
14:0	6.7 ± 0.1	7.8 ± 0.1	6.2 ± 1.0	7.0 ± 0.2	7.3 ± 0.1
16:0	42.2 ± 0.4	41.5 ± 0.2	42.9 ± 1.3	38.9 ± 1.2	41.4 ± 0.3
16:1	39.7 ± 1.0	39.0 ± 0.3	39.0 ± 1.0	40.2 ± 0.2	36.7 ± 0.9
18:1	1.9 ± 0.4	1.3 ± 0.1	1.3 ± 0.3	1.7 ± 0.2	1.6 ± 0.2
18:2	0.8 ± 0.1	0.7 ± 0	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
18:3(n-3)	4.5 ± 1.0	4.6 ± 0	4.4 ± 0.9	5.4 ± 0.2	6.1 ± 0.4
20:4(n-6)	0.8 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	1.3 ± 0.3	1.6 ± 0.2
20:5(n-3)	3.4 ± 0.4	3.9 ± 0	4.5 ± 1.8	4.3 ± 0.3	4.1 ± 0.1
Total fatty acids					
mg g^{-1} lipids	189.3 ± 37.1	186.7 ± 7.8	180.0 ± 18.1	181.3 ± 7.8	182.3 ± 19.6
mg g^{-1} AFDW[b]	58.2 ± 11.4	52.1 ± 2.2	51.8 ± 5.2	54.0 ± 2.3	53.4 ± 5.7

[a] The cultures were harvested on day 17 (stationary phase).

[b] AFDW: Ash-free dry weight.

yield of 20:5 (n-3) while those grown on acetate produced the highest yield of 20:4 (n-6).

Discussion

Growth trends

Under the present culture conditions, the specific growth rates of *Nitzschia inconspicua* ranged from

Table 4. Fatty acid composition of *Nitzschia inconspicua* grown at different levels of $NaNO_3$[a].

Fatty acid	$NaNO_3$ (mM)			
	0	0.3	0.75	3.0 (Control)
14:0	6.2 ± 0.2	4.2 ± 0.1	4.1 ± 0.2	4.1 ± 0.2
16:0	45.0 ± 0.3	45.0 ± 0.6	39.0 ± 0.4	38.1 ± 0.4
16:1	36.4 ± 0.2	36.8 ± 0.7	38.2 ± 0.7	37.5 ± 0.5
18:1	2.2 ± 0	1.4 ± 0.1	2.2 ± 0.1	2.0 ± 0.1
18:2	0.9 ± 0	0.7 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
18:3(n-3)	0.8 ± 0.1	3.3 ± 0.3	7.2 ± 0.2	7.0 ± 0.2
20:4(n-6)	0.7 ± 1.0	0.9 ± 0.1	1.7 ± 0.1	1.4 ± 0.2
20:5(n-3)	5.9 ± 0	4.3 ± 0.1	3.5 ± 0	4.8 ± 0.1
Total fatty acids				
mg g^{-1} lipids	118.7 ± 15.2	308.0 ± 9.4	248.0 ± 0	232.7 ± 19.0
mg g^{-1} AFDW[b]	24.1 ± 3.1	77.6 ± 2.4	52.1 ± 0	58.9 ± 4.8

[a] Cultures were harvested on day 11.
[b] AFDW: Ash-free dry weight

Table 5. Fatty acid composition of *Nitzschia inconspicua* grown on different carbon sources[a].

Fatty acid	Control[b]	Carbon source		
		Acetate	Glucose	5%CO_2
14:0	4.3 ± 0.2	4.4 ± 0.2	5.2 ± 0.4	2.8 ± 0.2
16:0	43.4 ± 0.5	35.7 ± 0.6	36.4 ± 0.2	36.9 ± 0.3
16:1	42.2 ± 0.6	38.8 ± 0.8	40.4 ± 0.8	45.4 ± 0.3
18:1	1.4 ± 0.1	1.9 ± 0.4	1.9 ± 0.4	1.0 ± 0.1
18:2	1.7 ± 0.3	1.1 ± 0.1	2.3 ± 0.2	1.0 ± 0.1
18:3(n-3)	2.0 ± 0.9	4.5 ± 0.8	4.0 ± 0.9	5.1 ± 0.6
20:4(n-6)	1.2 ± 0.3	3.9 ± 0.2	3.3 ± 0.1	1.7 ± 0.1
20:5(n-3)	4.0 ± 0.7	5.2 ± 0.3	5.6 ± 0.3	4.9 ± 0.1
Total fatty acids				
mg g^{-1} lipids	290.3 ± 4.8	299.7 ± 0	290.3 ± 4.4	224.7 ± 0
mg g^{-1} AFDW[c]	57.4 ± 0.9	63.8 ± 0	67.4 ± 1.0	72.0 ± 0

[a] Cultures were harvested on day 17.
[b] Control agitated continuously with magnetic stirring.
[c] AFDW: Ash-free dry weight.

0.25 to 0.44 d^{-1} and were lower than that for *N. frustulum* (0.75–0.80 d^{-1}) (Renaud & Parry, 1994). The growth of *N. inconspicua* was not affected even though the silicate level was reduced to one-tenth of that in the control. Doubling of the control level of silicate also did not enhance growth. Thus, the range of silicate levels supplied (8.8–172 μM) was probably growth-saturating.

Biomass and μ of *N. inconspicua* were not affected when the $NaNO_3$ level was reduced to one-tenth of the control (0.3 mM). In comparison, biomass of *Phaeodactylum tricornutum* was similar when grown at 8.1–24.2 mM nitrate but decreases when the concentration is reduced to 4.0 mM (Yongmanitchai & Ward, 1991). *N. inconspicua* was able to grow in medium without nitrate for three days. The nutrient was not detected in the medium throughout the study. It was unlikely that the diatom grew on nitrate 'carried over' from the inoculum. The cells probably utilized the stored internal nitrogen as large pools of nitrogen compounds,

Table 6. The amounts and yields of arachidonic acid and eicosapentaenoic acid of *Nitzschia inconspicua* under different culture conditions[a].

Treatment	20:4(n-6)			20:5(n-3)		
	mg g^{-1} DW	mg g^{-1} AFDW	mg L^{-1}	mg g^{-1} DW	mg g^{-1} AFDW	mg L^{-1}
Silicate (μM)						
8.8	0.31	0.47	0.045	1.30	1.98	0.189
22	0.32	0.47	0.045	1.39	2.03	0.195
44	0.25	0.41	0.037	1.43	2.33	0.214
88 (Control)	0.45	0.70	0.065	1.49	2.32	0.215
176	0.53	0.85	0.077	1.37	2.19	0.200
Nitrate (mM)						
0	0.11	0.17	0.005	0.92	1.42	0.044
0.3	0.44	0.70	0.045	2.10	3.34	0.216
0.75	0.56	0.89	0.056	1.16	1.82	0.116
3.0 (Control)	0.54	0.82	0.053	1.84	2.83	0.182
Carbon source						
Control[b]	0.44	0.69	0.055	1.45	2.30	0.181
Acetate	1.60	2.49	0.211	2.14	3.32	0.282
Glucose	1.29	2.22	0.190	2.20	3.77	0.324
5% CO_2	0.78	1.22	0.118	2.24	3.53	0.339

[a] Cultures were harvested on day 17 (stationary phase) except those grown at different $NaNO_3$ levels, which were harvested on day 11.
[b] Control was agitated continuously with magnetic stirring.
Abbreviations: DW: dry weight; AFDW: Ash-free dry weight

including nitrate which is unassimilated, are known to accumulate in diatoms, as shown in *Skeletonema costatum* (Dortch, 1982).

N. inconspicua grew best when aerated with 5% CO_2; however, addition of organic carbon (glucose and acetate) only enhanced growth slightly. The diatom probably lacked an efficient transport system for these organic carbon sources. Organic carbon can be utilized by diatoms under heterotrophic conditions as shown in *N. laevis*, *Navicula incerta* and *N. pelliculosa* which grow well on glucose in the dark (Tan & Johns, 1994). In comparison, *N. angularis* does not take up glucose at significant rates when incubated in the light (Lewin & Hellebust, 1976).

Biochemical composition

Cells of *N. inconspicua* were highly silicified, as indicated by the high amount of ash which ranged from 33.3 to 46.7% dry weight. Ash may make up 27 to 55% dry weight of diatoms (Nalewajko, 1966). The ash content of *N. inconspicua* is much higher than that reported for other species of *Nitzschia*: *N. closterium*, 21.1% dry weight and *N. frustulum* 20.3% dry weight (Renaud et al., 1994). Vigorous stirring is required to keep the diatom cultures in suspension due to the heavily silicified cells.

The proteins and lipids in *N. inconspicua* were distributed in almost equal proportions whereas that of carbohydrates was generally low, except in nitrogen starved cells. In comparison, proteins form the major organic constituent followed by lipids in *N. closterium* and *N. frustulum*, whereas carbohydrates consist less than 10% of their dry weight (Renaud et al., 1994).

Silicate limitation leads to lipid accumulation in *Chaetoceros gracilis*, *Hantzschia* sp. and *Cyclotella* sp. (Taguchi et al., 1987). The range of silicate concentrations used was not limiting for growth, therefore biochemical composition of *N. inconspicua* was not affected.

Nitrogen starvation led to an increase of carbohydrates at the expense of proteins while the lipid content remained almost unchanged in *N. inconspicua*. In contrast, lipid content increases under nitrogen starvation in *N. palea* (Shifrin & Chisholm, 1980).

The exogenous carbon supplied was probably channelled for carbohydrate synthesis in *N. inconspicua*. Aeration with 5% CO_2 led to an increase of the lipid content in *N. inconspicua*, contrasted with trend shown by *Phaeodactylum tricornutum* which produces more proteins instead (Chrismadha & Borowitzka, 1994).

Fatty acid composition

The total fatty acid content of *N. inconspicua* ranged from 15.6 to 48.9 mg g^{-1} dry weight, within the range reported for other species such as *N. closterium* and *N. frustulum* (Renaud et al., 1994). The fatty acids of *N. inconspicua* were dominated by 16:0 and 16:1 irrespective of culture conditions. The dominance of 16:0 and 16:1 has been reported for *N. longissima* and *N. ovalis* (Orcutt & Patterson, 1975). The proportion of 20:5 (n-3) in *N. inconspicua* is lower than that for other species of *Nitzschia*, including *N. angularis* (Kyle et al., 1988), *N. closterium, N. frustulum* (Renaud et al., 1994) and *N. laevis* (Tan & Johns, 1994). The proportions of C18 fatty acids are generally low in *Nitzschia* spp,. except in the heterotroph *N. laevis* (Tan & Johns, 1994).

Nitrogen starvation enhanced total fatty acid content of *N. inconspicua*, and highest yield of 20:5 (n-3) was produced when grown at low $NaNO_3$ level. Such trend contrasted with that shown by *Phaeodactylum tricornutum* where production of 20:5 (n-3) increases with increasing supply of nitrate (Yongmanitchai & Ward, 1991).

The cultures should be aerated to increase the production of 20:5 (n-3) in *N. inconspicua*, contrasted with that shown by *Phaeodactylum tricornutum* (Chrismadha & Borowitzka, 1994).

Potential of Nitzschia inconspicua *as aquaculture feed*

N. inconspicua consists of considerable amounts of lipids and carbohydrates but only low amounts of the 20:4 (n-6) and 20:5 (n– 3). It may not be suitable as a monoalgal diet for maricultured animals. However, it would be useful as a component of mixed algal diet, consisting of other LC-PUFA-rich species such as *Nannochloropsis oculata, Pavlova lutheri* and *Isochrysis* sp. (Dunstan et al., 1993). As the cells have a good settling rate, biomass of *N. inconspicua* may be easily harvested as a live feed. Further manipulative studies should be conducted to increase production of biomass and LC-PUFA.

Conclusion

Silicate level as low as 8.8 μM (one-tenth of the control) did not affect the growth trend, biochemical composition and fatty acid composition of the diatom. Within the range of $NaNO_3$ supplied (0–3.0 mM), the cultures of *N. inconspicua* should be grown at 0.3 mM $NaNO_3$ in order to produce the highest yield of 20:5 (n-3). Cultures should be aerated with 5% CO_2 to increase production of biomass, lipids and 20:5 (n-3).

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References

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248–254.

Chrismadha T, Borowitzka MA (1994) Effect of cell density and irradiance on growth, proximate composition and eicosapenatenoic acid production of *Phaeodactylum tricornutum* grown in a tubular photobioreactor. J. appl. Phycol. 6: 67–74.

Christie WW (1989) Gas Chromatography and Lipids. The Oily Press, Scotland: 69–70.

Chu WL, Phang SM, Goh SH (1994) Studies on the production of useful chemicals, especially fatty acids in the marine diatom *Nitzschia conspicua* Grunow. Hydrobiologia 285: 33–40.

De Pauw N, Persoone G (1988) Micro-algae for aquaculture. In Borowitzka MA, Borowitzka LJ (eds), Micro-algal Biotechnology. Cambridge University Press, Cambridge: 257–287.

Dortch Q (1982) Effect of growth conditions on accumulation of internal nitrate, ammonium, amino acids, and protein in three marine diatoms. J. exp. mar. Biol. Ecol. 61: 243–264.

Dunstan GA, Volkman JK, Barett SM, Garland CD (1993) Changes in the lipid composition and maximisation of the polyunsaturated fatty acid content of three microalgae grown in mass culture. J. appl. Phycol. 5: 71–83.

Kochert AG (1978) Carbohydrate determination by the phenol-sulphuric acid method. In Hellebust JA, Craigie JS (eds), Handbook of Phycological Methods – Physiological and Biochemical Methods. Cambridge University Press, Cambridge: 95–97.

Kyle D, Behrens P, Bingham S, Arnett K, Lieberman D (1988) Microalgae as a source of EPA-containing oils. In Applewhite TH (ed.) Proc. World Conf. Biotech. for Fats and Oils Industry. Am. Oil Chemists' Soc., Champaign, Illinois: 117–121.

Lewin JC, Hellebust JA (1976) Heterotrophic nutrition of the marine pennate diatom *Nitzschia angularis* var. *affinis*. Mar. Biol. 36: 313–320.

Nalewajko C (1966) Dry weight, ash, and volume data for some freshwater planktonic algae. J. Fish. Res. Bd Can. 23: 1285–1288.

Orcutt DM, Patterson GW (1975) Sterol, fatty acid and elemental composition of diatoms grown in chemically defined media. Comp. Biochem. Phyciol. 50B: 579–583.

Renaud SM, Parry DL (1994) Microalgae for use in tropical aquaculture II: Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. J. appl. Phycol. 6: 347–356.

Renaud SM, Parry DL, Thinh LV (1994) Microalgae for use in tropical aquaculture I: Gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. J. appl. Phycol. 6: 337–345.

Roessler PG (1988) Changes in the activities of various lipid and carbohydrate biosynthetic enzymes in the diatom *Cyclotella cryptica* in response to silicon deficiency. Arch. Biochem. Biophys. 267: 521–528.

Shamsudin L (1992) Lipid and fatty acid composition of microalgae used in Malaysian aquaculture as live food for the early stage of penaeid larvae. J. appl. Phycol. 4: 371–378.

Shifrin NS, Chisholm SW (1980) Phytoplankton lipids: environmental influences on production and possible commercial applications. In: Shelef G & Soeder CJ (eds) Algae Biomass: Production and Use. Elsevier/North Holland Biomedical Press, Amsterdam: 627–645.

Strickland JDH, Parsons TR (1968) A practical handbook of seawater analysis. Bull. Fish. Res. Bd Can. 167: 311.

Taguchi S, Hirata JA, Lawa EA (1987) Silicate deficiency and lipid synthesis of marine diatoms. J. Phycol. 23: 260–267.

Tan CK, Johns MR (1994) Eicosapentaenoic acid production by heterotrophic *Nitzschia laevis* in chemostat cultures. In Phang SM, Lee YK, Borowitzka MA, Whitton BA (eds), Proceeding of the First Pacific Conference on Algal Biotechnology, University of Malaya, Kuala Lumpur: 33–38.

Yongmanitchai W, Ward OP (1991) Growth and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. Appl. Envir. Microbiol. 57: 419–425.