Nutrient Compositions of Cultured *Thalassiosira rotula* and *Skeletonema costatum* from Jiaozhou Bay

Zhiliang Shen, Yulin Wu, Qun Liu and Yun Yao

Abstract The nutrient compositions of cultured *Thalassiosira rotula* and *Skeletonema costatum* from Jiaozhou Bay were measured. Carbon (C), nitrogen (N), phosphorus (P), and silicon (Si) contents in cell were obvious higher in *T. rotula* than in *S. costatum*, but the percents of N, P, Si contents in cell dry mass in *T. rotula* were lower than those in *S. costatum*. The dry mass concentrations of N, P, Si in *S. costatum* were much higher than those in *T. rotula*, particularly Si, the former was 6.4 times of the latter, showing that *S. costatum* could more assimilate these elements. Especially, *S. costatum* had competitive dominance for assimilation Si, which is beneficial to its becoming a major dominant species in relative short Si of Jiaozhow Bay. There were some differences in numerical value of nutrient ratios both laboratory-cultured phytoplankton and different-sized suspended particulates (mainly phytoplankton) in Jiaozhou Bay, which was caused by the changes of environment. High contents of C, N and relative low P, Si, high N/P ratio (far higher than Redfield value) and low Si/P and Si/N ratios (far lower than Redfield values) in the two diatoms and different-sized suspended particulates were consistent with those in the seawater. Relative short Si in the seawater and phytoplankton showed that Si was possibly affecting phytoplankton growth in Jiaozhou Bay.

Keywords Carbon · Nitrogen · Phosphorus · Silicon composition · Culture *Skeletonema costatum* · *Thalassiosira rotula* · Jiaozhou Bay

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Biological and chemical processes of marine phytoplankton are related to their size to a considerable degree (Ray et al. 2001; Suttle et al. 1991). At present, the ecological study of phytoplankton has advanced into the aspect of particle size distribution and its effects, and element composition of phytoplankton in different-sized fractions is an important study field. Measuring the chemical composition (carbon, nitrogen, Phosphorus, silicon, etc.) of phytoplankton is of important senses to estimate primary production of phytoplankton (Strickland 1960; Conley et al. 1989; Geider and La Roche 2002; Heldal et al. 2003), judgement nutrients limitation (Harrison et al. 1977; Sakshaug and Holm-Hansen 1977; Rhee and Gotham 1980; Brzezinski et al. 1990; Beardall et al. 2001; Claquin et al. 2002; Shen et al. 2006; De La Rocha et al. 2010; Hagstrom et al. 2011), discussion stoichiometric balance between nutrient structure of seawater and nutrient composition of phytoplankton and biogeochemistry of nutrient (Fraga et al. 1998; Heldal et al. 2003; Shen et al. 2006; Hoffmann et al. 2007; Baines et al. 2011). However, so far scientists cannot always separate phytoplankton directly by particulate size from seawater. Measurements of elemental ratios or chemical composition of natural populations of phytoplankton are prone to interference from debris and other microorganisms such as bacteria and microzooplankton that can be collected on the filters used to ample the phytoplankton (Beardall et al. 2001). Therefore, correlative studies in the international concentrated mainly on laboratory- cultured phytoplankton strains (Heldal et al. 2003; Ho et al. 2003; Burkhardt and Riebesell 1997; Verity et al. 1992). Major nutrients studied are carbon, nitrogen, Phosphorus (Menzel and Ryther 1964; Sakshaug and Holm-Hansen 1977; Nøst-Hegseth 1982; Sakshaug et al. 1983; Lirdwitayaprasit et al. 1990; Burkhardt and Riebesell 1997; Geider and La Roche 2002; Vrede et al. 2002; Loebl et al. 2010), less for silicon (Lewin and Guillard 1963; Harrison et al. 1977; Leynaert et al. 1991; Ríos et al. 1998; Marchetti and Harrison 2007; Baines et al. 2011). Researches have been reported in the elemental composition of *Skeletonema costatum,* such as the changes in chemical composition of *S. costatum* under the conditions of nitrate-, phosphate-, and iron-limited growth (Sakshaug and Holm-Hansen 1977) and CO_2 affecting elemental composition (C:N:P) of *S. costatum* (Burkhardt and Riebesell 1997).

Jiaozhou Bay is seriously affected by human activities. During the last 40 years, nitrogen and Phosphorus concentrations have largely increased in Jiaozhou Bay seawater, especially nitrogen, and silicate concentration may has remained at a relatively lower level. The molar ratio of nitrogen to phosphorus was obviously higher than Redfield value (Redfield et al. 1963) and eutrophication has become increasingly serious, resulting in changes of phytoplankton community structure (Shen 2001; Sun et al. 2002). In order to explore the change rule of phytoplankton community under eutrophication condition, carbon, nitrogen, Phosphorus, silicon compositions, and molar ratios of laboratory-cultured two different-sized phytoplankton common species of *Thalassiosira rotula* and *S. costatum* from Jiaozhou Bay were measured and compared with the nutrient structures of seawater and different-sized suspended particulates in this section.

1 Sampling and Experimental Methods

1.1 Sampling

Investigation was carried out, and nine stations were set up in Jiaozhou Bay in February 2002 (Fig. 1). Surface water samples were collected using a Niskin sampler. Unfiltered water samples were preserved (with 0.3% chloroform) in polyethene bottles and stored in a low temperature ice box (−30 °C) for analyzing nutrients (taking clear superstratum water sample) at the laboratory later. Phytoplankton water samples were collected from surface layer seawater and preserved with neutralized formalin. Phytoplankton were identified and counted under a microscope using a workshop-made Sadgwick-Rafter-like chamber at the laboratory later.

1.2 Laboratory Cultures

T. rotula and *S. costatum* were separated from phytoplankton samples collected in Jiaozhou Bay under a microscope and transferred to 200 mL triangle bottles with 100 mL f/2 medium for culture, respectively. The cultures were carried out at a

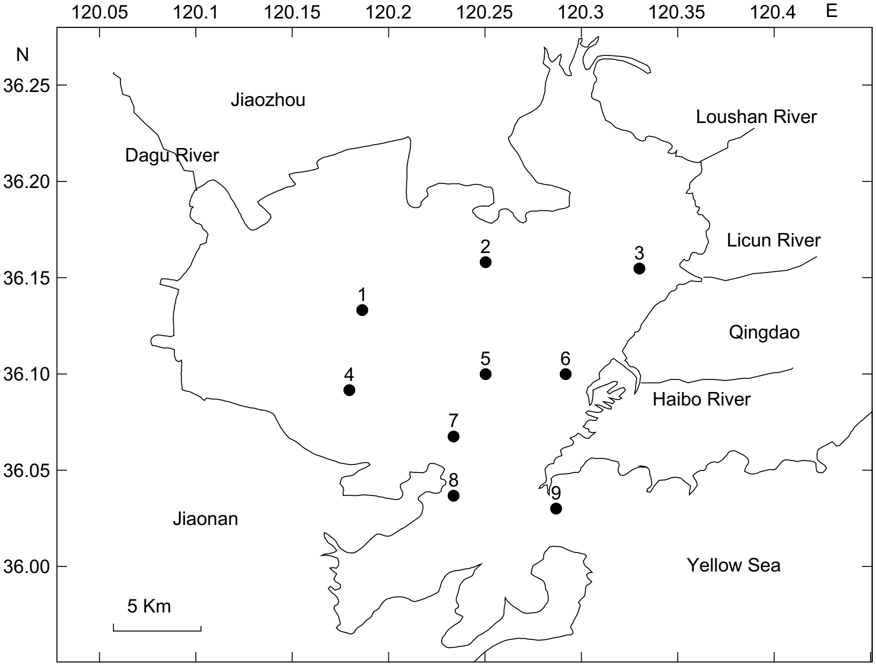

Fig. 1 Station positions

temperature of 18 ± 1 °C, an illumination of 4000LX, and a ratio of light time to dark time equaling 12 h: 12 h. After repeat separations and purifications, extending cultures were lasted 30 days for *T. rotula* and 45 days for *S. costatum,* and then they were counted. Their cell abundances were 35.2×10^3 cells mL^{-1} for *T. rotula* and 480×10^3 cells mL^{-1} for *S. costatum.* Six samples with 2 mL each were taken from every medium. The medium samples for determining phytoplankton carbon and nitrogen were filtered with preignited (450 °C, for 6 h) Whatman GF/D (2.7 μm), and the medium samples for determining phosphorus and silicon were filtered with 2 μm Millipore filter. Four millilitres medium with two shares each were taken and filtered with 0.45 μm Millipore filter for determining the dry mass of the culture cells. All filters were 25 mm in diameter. All filtered membranes and blank membranes immersed filtrates were rinsed with deionized water and stored in a low-temperature icebox for further analysis.

1.3 Analysis and Calculation

Nutrients concentrations were determined by colorimetric methods (Shen et al. 2008). Dissolved inorganic nitrogen (DIN) is equal to the sum of NO_3–N, NO_2–N, and NH_4–N. The sample of phytoplankton P was digested referring to Koroleff's (1976) method and measured with the potassium peroxodisulphate oxidation- colorimetry. Sample membrane or blank membrane, 2 mL of 5% H_2SO_4–$K_2S_2O_8$ solution and 10 mL of distilled water were put into polyfluortetraethylene digestion bottle in order and digested for 1 h at 115 °C in a pressure cooker, then measured by colorimetry. The sample of phytoplankton Si was measured referring to Treguer and Gueneley's (1988) method, putting sample membrane or blank membrane, 10 mL of 5% Na_2CO_3 digestion solution into polyfluortetraethylene digestion bottle in order, then was digested and measured by colorimetry. The samples were determined using a SKALAR Flow Analyzer made in Netherlands. Phytoplankton C and N were measured using a model 240C Element Analyzer. The dry mass of diatom cell was measured using the weight method.

The content of element in cell was calculated as:

$$Q = C \cdot F/D$$

where, Q is content of element in cell (pg cell^{-1}), C is molar concentration of element (μmol L^{-1}), F is conversion coefficient of unit, D is cell density in cultured medium (cell mL^{-1}). The dry mass concentration of element is calculated as:

$$M_1 = C \cdot F/M_2$$

where, M_1 is dry mass concentration of element (mg g^{-1}) and M_2 is dry mass concentration of diatom in cultured medium (mg L^{-1}).

2 Nutrient Concentrations and Structure in Jiaozhou Bay Seawater

Nutrient concentrations and molar ratios in surface seawater in Jiaozhou Bay in February 2002 (Table 1) show that there were high concentration of N and low SiO_3–Si in Jiaozhou Bay and SiO_3–Si concentrations were lower than or equal to the threshold value of diatom growth (2.0 μmol L^{-1}) (Brown and Button 1979; Perry and Eppley 1981; Goldman and Glibert 1983; Nelson and Brzezinski 1990) at station 1 or 2, respectively. Among three forms of inorganic N, NH_4–N was main existent form being 72.4% of DIN and NO_3–N was only 23.6% of DIN, which showed that three forms of inorganic N were not in thermodynamics equilibrium. The ratios of nutrients in average were 27.8 ± 13.6 for DIN/PO_4–P ratio far higher than Redfield value (16) and 7.4 ± 2.6 and 0.32 ± 0.16 for SiO_3-Si/PO_4-P and SiO_3-Si/DIN ratios, respectively, far lower than Redfield value (16 and 1, respectively) (Brzezinski 1985; Redfield et al. 1963), which showed that SiO_3-Si and PO_4–P possibly were potential limiting factor to phytoplankton growth, especially SiO_3–Si (Shen et al. 2006).

3 Distributions of *T. rotula* and *S. costatum* in Jiaozhou Bay

S. costatum was the predominant phytoplankton in Jiaozhou Bay, particularly in winter and summer. *T. rotula* was a phytoplankton common species, but its abundance had not orderliness in seasonal variation and quantity was far lower than *S. costatum.* A phytoplankton peak in quantity was often found in winter (Wu et al. 2004). Twenty-eight species of phytoplankton including 27 species of diatoms and 1 species of *Dictyocha* sp. were identified in February, 2002. The cell abundance of phytoplankton in average was 651.0 cell mL^{-1}, and the dominant species were *S. costatum* and *Thalassiosira nordenskiöldii.* Thereinto, the cell abundance of *S. costatum,* ranged between 185.0 and 822.0 cells mL^{-1} with an average of 427.8 cells mL^{-1} in seawater. The cell abundance of *T. rotula* was between 6.8 and 61.6 cells mL^{-1} with an average of 23.2 cells mL^{-1}. Their ratios in total cell abundance of phytoplankton were as high as 65.7% for *S. costatum* and only 3.6% for *T. rotula.*

4 C, N, P, Si Compositions of *T. rotula* and *S. costatum*

Dry mass and contents of C, N, P, Si in cells of *T. rotula and S. costatum* are indicated in Table 2. The dry mass of each cells of *T. rotula and S. costatum* were 3125.0 and 300.0 pg, respectively, and the former was one order of magnitude higher than the latter which was obviously related to their volumes. *T. rotula* belong to microphytoplankton and *S. costatum* was nanophytoplankton and their diameters are 20–50 and 6–20 μm commonly in Jiaozhou Bay, respectively. C, N, P, Si contents of each

Table 1 Nutrient concentrations (μmol L^{-1}) and their molar ratios in surface seawater in Jiaozhou Bay

Stations	PO_4–P	SiO_3–Si	NO_3–N	NO_2–N	NH_4–N	DIN	N /P	Si/P	Si/N
9	0.35	3.9	1.7	0.55	11.3	13.6	38.9	11.1	0.29
8	0.31	3.1	1.2	0.29	5.1	6.6	21.3	10.0	0.47
7	0.46	4.1	2.4	0.47	8.6	11.5	25.0	8.9	0.36
6	0.54	4.6	5.5	0.81	21.7	28.0	51.9	8.5	0.16
5	0.54	4.5	2.1	0.44	4.9	7.4	13.7	8.3	0.61
4	0.60	3.8	3.0	0.55	5.2	8.8	14.7	6.3	0.43
3	0.41	2.4	3.9	0.57	13.2	17.7	43.2	5.9	0.14
2	0.55	2.0	4.0	0.67	7.8	12.5	22.7	3.6	0.16
1	0.44	1.9	2.9	0.46	4.7	8.1	18.4	4.3	0.23
Averages	0.47	3.40	3.00	0.53	9.20	12.70	27.8	7.4	0.32
	±0.10	±1.1	±1.3	±0.15	±5.6	±6.7	±13.6	±2.6	±0.16

cell of *T. rotula* were 1812.5, 170.5, 12.00, and 4.81 pg, respectively and were 58.0, 5.5, 0.4, 0.2% of the dry mass of cell, respectively. Thereinto, C content exceeds half of the dry weight of cell and was one order of magnitude higher than N content, N content was one order of magnitude higher than P content, and Si content was the lowest. C, N, P, Si contents of each *S. costatum* cell were 97.0, 27.9, 1.63, and 2.95 pg, respectively and were 32.3, 9.3, 0.5, and 1.0% of the dry mass of cell, respectively. Comparing the compositions of C, N, P, and Si of two diatom cells, the various elemental contents in *T. rotula* were notably higher than those in *S. costatum* which was consistent with the high dry mass of the former. There were greater differences in the percent of elemental contents of two diatom cells in the dry mass of cells. The percent of C content in *T. rotula* was much higher than that in *S. costatum*, but the percents of N, P, Si contents in *T. rotula* lower than those in *S. costatum*, particularly Si. Element compositions of two diatom cells showed that the N and P contents in *T. rotula* cell were much higher than those in *S. costatum* cell and the former were 6.1 and 7.4 times the latter, respectively, but C content of the former was 18.7 times that of the latter (the dry mass of the former was only 10.4 times that of the latter). On the basis of cell volume, Sun et al. (1999) estimated the C contents in each cell of *T. rotula* and *S. costatum* in Jiaozhou Bay using the formulas suggested by Mullin et al. (1966), Strathmann (1967), Eppley et al. (1970) and Taguchi (1976), they were 736.2, 579.1, 693.7, and 338.2 pg, respectively for *T. rotula* and 16.9, 15.0, 17.8, and 9.6 pg, respectively for *S. costatum*. N contents in each cells of *T. rotula* and *S. costatum* were estimated using the formulas suggested by Taguchi (1976) being 78.0 and 3.4 pg, respectively (Sun et al. 1999). It showed that considerable differences in C and N contents were found in the two diatom cells which were consistent with the results of this section. However, C and N contents of the two diatom cells determined by the authors were much higher than the results estimated by Sun et al. (1999), which were possibly showed that there was a larger difference between the data measured using chemical method and those calculated by the models. The Si content in each cell of *S. costatum* determined by the authors was a little higher than that previously observed by Harrison et al. (1977) and Paasche (1980) being 2.1 and 2.67 pg, respectively, at 18 °C and under constant light. Brzezinski (1985) showed that there were considerable differences in the elemental compositions for two clones of *S. costatum.* In light-to- dark cycle experiments, the contents of C, N, Si in each cell of the two clones of *S. costatum* were 7.32, 0.91, 1.18 pg, and 66.0, 9.1, 17.08 pg, respectively, and the differences of both were as high as one order of magnitude. The changes of various environmental conditions including light intensity, photoperiod, temperature, nutrient limitation, and species differences can influence significantly nutrient composition of diatoms (Brzezinski 1985).

The dry mass concentrations of C, N, P, Si in *T. rotula* and *S. costatum* listed in Table 3 show that their dry mass concentrations were 580.0, 54.55, 3.85, 1.54 and 323.3, 93.05, 5.43, 9.84 mg g^{-1}, respectively. Similar to C, N, P, Si contents in the cells, there were very high mass concentration of C, higher of N and low P, Si in the two diatoms. But, comparing the two diatoms, considerable difference between the difference in the dry mass concentrations of C, N, P, Si and the difference in C, N, P, Si contents of cells was found. The dry mass concentration of C in *T.*

Table 2 C, N, P, Si contents in each cell of *T.rotula* and *S. costatum*

Diatoms	Cell dry mass	C		N		P		Si	
	pg	pg	%	pg	%	pg	%	pg	%
T. rotula	3125.0	1812.5	58.0	170.5	5.5	12.00	0.4	4.13	0.1
T. rotula		693.7[a]		78.0[b]					
S. costatum	300.0	97.0	32.3	27.9	9.3	1.63	0.5	3.81	1.3
S. costatum		17.8[a]		3.4[b]					

[a] Estimated by Sun et al. (1999) using the formula of Eppley et al. (1970)

[b] Estimated by Sun et al. (1999) using the formula of Taguchi (1976). Percentage indicate proportions of the contents of corresponding elements accounting for the dry mass of cell

Table 3 Dry mass concentrations (mg g^{-1}) and molar ratios of C, N, P, Si in *T. rotula* and *S. costatum*

Diatoms	C	N	P	Si	C/N	N/P	Si/P	Si/N
T. rotula	580.0	54.55	3.85	1.54	12.4	31.4	0.38	0.01
S. costatum	323.3	93.05	5.43	12.70	4.1	38.0	2.6	0.07

rotula was obviously higher than that in *S. costatum*, however, the difference of both was much less than the difference in C contents in cells. As opposed to C, the dry mass concentrations of N, P, Si in *S. costatum* were higher than those in *T. rotula*, which was consistent with the percentage of their contents in cells (Table 3). In considerable degree, the higher N, P, Si concentrations in *S. costatum* reflected that these elements in the seawater could be more assimilated by *S. costatum*, and was favorable for its growth, which was probably related to their small cell size associated with small diffusion boundary layers and large surface area per unit volume (Raven 1986). Particularly for Si, its dry mass concentrations in *S. costatum* were 6.4 times that in *T. rotula* showing that *S. costatum* had competitive dominance for assimilating Si. Previous studies showed that diatoms do not store sufficient Si for new valve formation (Azam 1974; Sullivan 1977; Binder and Chisholm 1980) and must accumulate most of the requisite amount immediately before cell division (Brzezinski 1985). Therefore, small unit of *S. costatum* with competitive dominance for assimilating Si could become a major dominant species under the condition of relatively low concentration of Si in Jiaozhou Bay. The ecological significance of dry mass concentrations of C, N, P, Si is that produce 1 g dry diatom, for *T. rotula,* it would assimilate 54.55 mg N, 3.85 mg P, and 1.54 mg Si (dry mass) from the seawater and simultaneously yield 580.0 mg organic C, but, for *S. costatum*, it would need more N, P, Si, however, organic C yielded was only 55.7% of the *T. rotula* (Table 3).

5 Comparing Nutrient Compositions of *T. rotula* and *S. costatum* with Nutrient Structures of Seawater and Particulates

Nutrient concentrations and their molar ratios in the seawater and different-sized particulates (Shen et al. 2006) are compared with those in laboratory-cultured phytoplankton common species (Table 4), showing that except for N/P ratio close, C/N, Si/P and Si/N ratios in laboratory- cultured *T. rotula* and *S. costatum* were much lower than those in the seawater, and that the C/N ratio in different-sized particulates were between two phytoplankton common species and the Si/P and Si/N ratios were much higher than those in phytoplankton common species, and their N/P ratio was also close. Comparing the particulates with seawater, however, besides C, the contents of N, P, Si and their molar ratios were more close. Because different-sized particulates were mainly composed of phytoplankton, similar nutrient contents and

Table 4 Nutrient concentrations and molar ratios in phytoplankton common species (mg g^{-1}), different-sized particulates (μmol L^{-1}) and seawater (μmol L^{-1})

Element ratios	Seawater	Particulates (μm)[b]			Particulates[b]	Phytoplankton common species	
		20–200	2–20	<2	Total	*T. rotula*	*S. costatum*
C	2200[a]	21.54	26.93	17.36	65.83	580.0	323.3
N	12.70	2.27	3.63	3.02	8.92	54.55	93.05
P	0.47	0.06	0.11	0.03	0.20	3.85	5.43
Si	3.40	0.61	0.53	0.20	1.34	1.32	12.70
N/P	27.8	37.8	33.0	100.7	44.6	31.4	38.0
Si/P	7.4	10.2	4.8	6.7	6.7	0.38	2.6
Si/N	0.32	0.27	0.15	0.07	0.15	0.01	0.07
C/N	173.2[a]	9.5	7.4	5.7	7.4	12.4	4.1

[a] Inorganic C content in the seawater from Shen et al. (1997)
[b] Average values of stations 3, 5, 7, mainly phytoplankton in different sized particulates (Shen et al. 2006)

their ratios both the particulates and seawater reflected an ecological response of phytoplankton to the nutrient structure of seawater to a certain extent (Shen et al. 2006). The difference in numerical value of nutrient ratios both laboratory-cultured phytoplankton and the particulates in Jiaozhou Bay reflected the difference between laboratory-cultured phytoplankton and the phytoplankton in natural seawater, which was obviously caused by natural phytoplankton cultured in the laboratory, showing the importance of environment to phytoplankton growing. However, high C, N contents and low P, Si in the two laboratory-cultured diatoms and different-sized particulates were consistent with those in the seawater. There was also obvious similitude in the molar ratios of elements. Those ratios have large deviation from the mean ratio (DIN/PO_4–P/SiO_3–Si = 16/16/1) of nutrients contained in marine diatom (Brzezinski 1985; Redfield et al. 1963). High N/P ratios were far higher than Redfield values, and low Si/P and Si/N ratios were far lower than Redfield values. It could be suggested that laboratory-cultured phytoplankton were bred under better nutritional condition, however, its nutrient structure characteristics formed long-term in Jiaozhou Bay had been not completely changed. Since the seminal work of Redfield, the elemental composition of phytoplankton, and even the composition of the water in which the organisms are growing, has been used as a potential index of nutrient limitation (Beardall et al. 2001). High contents of C, N and relative low contents P, Si, high N/P ratio and low Si/P and Si/N ratios in phytoplankton showed that P and Si were possibly potential influence on affecting phytoplankton growth, especially Si. In Jiaozhou Bay where Si was relative short, once SiO_3–Si concentration increases in the seawater, it would probably lead to abnormal breeding of diatoms (Yao and Shen 2007; Zhang et al. 2002).

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