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Sequential nutrient uptake as a potential mechanism for phytoplankton to maintain high primary productivity and balanced nutrient stoichiometry

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Abstract. We hypothesize that phytoplankton have the sequential nutrient uptake strategy to maintain nutrient stoichiometry and high primary productivity in the water column. According to this hypothesis, phytoplankton take up the most limiting nutrient first until depletion, continue to draw down non-limiting nutrients and then take up the most limiting nutrient rapidly when it is available. These processes would result in the variation of ambient nutrient ratios in the water column around the Redfield ratio. We used highresolution continuous vertical profiles of nutrients, nutrient ratios and on-board ship incubation experiments to test this hypothesis in the Strait of Georgia. At the surface in summer, ambient NO_3^- was depleted with excess PO_4^{3-} and $SiO_4^$ remaining, and as a result, both N: P and N: Si ratios were low. The two ratios increased to about 10:1 and 0.45:1, respectively, at 20 m. Time series of vertical profiles showed that the leftover PO_4^{3-} continued to be removed, resulting in additional phosphorus storage by phytoplankton. The N:P ratios at the nutricline in vertical profiles responded differently to mixing events. Field incubation of seawater samples also demonstrated the sequential uptake of NO_3^- (the most limiting nutrient) and then PO_4^{3-} and SiO_4^{-} (the nonlimiting nutrients). This sequential uptake strategy allows phytoplankton to acquire additional cellular phosphorus and silicon when they are available and wait for nitrogen to become available through frequent mixing of NO_3^- (or pulsed regenerated NH₄). Thus, phytoplankton are able to maintain high productivity and balance nutrient stoichiometry by taking advantage of vigorous mixing regimes with the capacity of the stoichiometric plasticity. To our knowledge, this is the first study to show the in situ dynamics of continuous vertical profiles of N : P and N : Si ratios, which can provide insight into the in situ dynamics of nutrient stoichiometry in the water column and the inference of the transient status of phytoplankton nutrient stoichiometry in the coastal ocean.

1 Introduction

The stoichiometry of the C:N:P Redfield ratio (Redfield, 1958) remains a central tenet in oceanography as it couples ecosystem processes with ocean biogeochemistry, which is driven by physical processes in oceans. The Redfield ratio of C:N:P varies widely across a wide range of environmental conditions. Laboratory cultures of phytoplankton that are in the steady state usually display variable cellular N:P ratios with the nutrient N:P supply ratios (Geider and La Roche, 2002). Recently, Martiny et al. (2013) found strong latitudinal patterns of the elemental ratios, which are closely related to ambient levels of nutrients, by making comparative analysis of elemental ratios of organic matter between different latitudes. Even at a fixed site (the Bermuda Atlantic Time-series Study station in the North Atlantic Ocean), the C: N: P ratio is quite variable (Singh et al., 2015). Four mechanisms have been proposed to explain the variability in C: N: P ratios in marine plankton, as summarized by Weber and Deutsch (2010). The first mechanism emphasizes the relationship between cellular elemental stoichiometry of phytoplankton and ambient nutrient ratios, i.e. the stoichiometry of nutrients in the water column. Based on the average Redfield ratio, this mechanism has been used to infer the most limiting nutrient for phytoplankton and to debate which nutrient (nitrogen or phosphorus) should be managed to control eutrophication effects. The second mechanism suggests that the elemental stoichiometry is taxonomy specific. Diatoms were reported to draw down nutrients with low C:P and N: P ratios (Geider and La Roche, 2002; Elser et al., 2003; Price, 2005), while marine cyanobacteria have higher C:P and N: P ratios (Karl et al., 2001; Bertilsson et al., 2003). Such different uptake ratios of N:P by phytoplankton can influence the magnitude of ocean N fixation (Mills and Arrigo, 2010). Based on the resource allocation theory, the third proposed mechanism is the "growth rate hypothesis", which states that the elemental stoichiometry within a cell is controlled by the biochemical allocation of resources to different growth strategies (Falkowski, 2000; Elser et al., 2003; Klausmeier et al., 2004). Fast-growing cells may have a lower N : P ratio due to a larger allocation to P-rich assembly machinery of ribosomes (Loladze and Elser, 2011), whereas competitive equilibrium favours a greater allocation to P-poor resource acquisition machinery and therefore higher N:P ratios. The fourth mechanism is related to the interference from dead plankton or organic detritus with the measurement of elemental composition of organic matter, and such interference is difficulty to assess due to lack of the measurements of non-living organic matter in oceans and coastal waters. However, the X-ray microanalysis (XRMA) technique was recently used to produce simultaneous quotas of C, N, O, Mg, Si, P and S in single-cell organisms (Segura-Noguera et al., 2016), which will not only help to understand the fourth mechanism but also understand the variability of stoichiometry of phytoplankton in the oceans.

In culture experiments, continuous uptake of non-limiting nutrients has been demonstrated for diatoms under N and Si limitation (Conway et al., 1976; Conway and Harrison, 1977; Harrison et al., 1989). Surge uptake of the limiting nutrient occurs when it is added to the nutrient-starved phytoplankton culture, while the uptake of the non-limiting nutrient is slowed or stopped until the diatom has overcome its nutrient debt. Hence, the sequence of which nutrient is taken up first is directly related to the nutrient status of the phytoplankton. It is difficult to assess the nutritional status of phytoplankton in the field, but the application of laboratory results to the interpretation of vertical nutrient profiles can provide information on their nutritional status. To date, there have been no studies of sequential uptake of nutrients in the field using a series of high-resolution vertical profiles of nutrients and their application to nutritional status of the phytoplankton.

In this study, we used high-resolution continuous vertical profiles of N:P and N:Si ratios to examine how N:Pand N:Si ratios respond to the mixing in a highly dynamic coastal water column as well as the uptake of nutrients. Onboard ship incubation experiments were conducted to support the observations of changes in vertical profiles of N:P and N: Si ratios. We constructed seven conceptual profiles to illustrate how a vertical profile of N: P ratios changes with mixing and uptake of nitrogen and phosphorus, and how they could indicate the nutritional status of the phytoplankton assemblage. The conceptual model also explains how N: P ratios respond to mixing, particularly at the nutriclines (nitracline for NO_3^- , phosphacline for PO_4^{3-} and silicacline for SiO_4^-), and indicates which nutrient (NO₃⁻ or PO₄³⁻) is taken up first in the water column. To our knowledge, this is the first study to show the dynamics of continuous vertical profiles of N:P and N: Si ratios and to examine responses of phytoplankton to the supply of nutrients from water column mixing. We believe that our approach can add a new dimension to examining the in situ dynamics of nutrients in the water column and illustrate the ecological role of phytoplankton stoichiometry in phytoplankton competition for nutrients.

Conceptual model of variability in vertical N : P ratios

The Strait of Georgia (hereafter the Strait) is an inland sea that lies between Vancouver Island and the mainland of British Columbia (LeBlond, 1983). It is an ideal area for studying the interactions between mixing, nutrient vertical profiles and phytoplankton nutrient uptake because of its relatively high biomass, frequent wind mixing and shallow (15 m) photic zone. The Strait is biologically productive, reaching a daily production of up to 5 g C m⁻² d⁻¹ and annual production of up to about > 300 g C m⁻² yr⁻¹ (Harrison et al., 1983, 1991), but inorganic nitrogen is often undetectable in productive seasons in the surface layer. The nutricline sitting within the euphotic zone is often associated with the pycnocline. In the Strait, the ambient N : P ratio of nutrients is ~ 10 : 1, similar to other coastal areas (Hecky and Kilham, 1988).

We illustrate the conceptual model of variability in vertical profiles of N: P ratios based on seven (C0 to C6) vertical profiles that we encountered in our field studies and suggest events that likely occurred to produce these nutrient profiles (Fig. 1).

- **C0:** in winter or after a strong wind speed event, the water column is homogeneously mixed, and NO_3^- and PO_4^{3-} are uniformly distributed in the water column.
- C1: with the onset of stratification, NO_3^- and PO_4^{3-} are taken up within the mixed layer. Assuming that the average nutrient uptake ratio is 16N : 1P, a N : P uptake ratio that is greater than 10 : 1 would decrease the ambient N : Pratio to less than 10 : 1.
- **C2:** the uptake of NO_3^- and PO_4^{3-} proceeds at a N : P ratio greater than 10 : 1 until NO_3^- is just depleted. At this



Figure 1. Conceptual model for changes in vertical profiles of N, P and N : P ratio due to sequential nutrient uptake. C0 to C3 represent a time series of nutrient uptake during bloom development, and C4 to C6 indicate subsequent vertical mixing of nutrients and subsequent uptake. The short horizontal line near the middle of the depth axis indicates the euphotic zone depth. N disappears first at C2, and P is left and continues to be taken up at C3. C4 represents mixing of nutrients into the bottom of the photic zone and phytoplankton have not taken up these nutrients yet. At C5, N is taken up first before P, while at C6, P is taken up first before N.

time, the N : P ratio is near 0 and some PO_4^{3-} remains in the water column.

- C3: the remaining PO_4^{3-} is completely taken up and stored as extra/surplus intracellular PO_4^{3-} .
- **C4:** after cross-pycnocline mixing occurs, the ambient N : P ratio in the newly mixed water should be the same as the ratio in the deep water. As a result, the vertical profile of the N : P ratio will form a right angle on the top part of the nutricline.
- **C5:** depending on how long the phytoplankton are nutrient limited, their response to the mixed limiting nutrient can be different. When N-deficient phytoplankton take up N only, the curve of the N : P ratio parallels the NO_3^- distribution curve, and PO_4^{3-} is left behind in the water column.
- **C6:** on the other hand, if phytoplankton take up PO_4^{3-} before NO_3^{-} (e.g. if phytoplankton were severely N starved, and there is a lag in NO_3^{-} uptake), the N : P ratio would be higher at the nutricline than below (Fig. 1).

Similarly, this conceptual model can be applied to N, $SiO_4^$ and N: Si ratios. The ambient (N: Si) ratio is about 0.5: 1 at 20 m in the Strait, with 20 μ M NO₃⁻ and 40 μ M SiO₄⁻. As the average uptake ratio of N: Si is about 0.7–1 : 1 (equivalent to Si: N = 1.5–1 : 1) (Brzezinski, 1985), the N: Si ratio decreases with depth. SiO₄⁻ is rarely depleted, and therefore the N: Si ratio is mainly determined by the distribution of NO₃⁻. The continuous uptake of SiO₄⁻ without the uptake of NO₃⁻ can be inferred based on the comparison between the gradient of N: Si and the silicacline. For example, a sharper gradient of the N: Si ratio than the silicacline would indicate the continuous uptake of SiO₄⁻ without the uptake of NO₃⁻ as in C5 (Fig. 1)

2 Materials and methods

2.1 Station locations

The transect started from station S2, 8 km beyond the Fraser River mouth and under the influence of the river plume, and extended 108 km northwest to S1 (well beyond the plume) in the Strait of Georgia (Fig. 2). The station numbers are consistent with previous studies (Yin et al., 1997a).

2.2 Sampling and data processing

The sampling was designed to investigate the distribution of nutrients $(NO_3^-, PO_4^{3-} \text{ and } SiO_4^-)$ and N : P and N : Si ratios associated with mixing processes during 6-14 August 1991. Data, either at an anchored station for 24 h or along a transect of a few stations within 10h, were used. At each station, a vertical profile (0-25 m) of temperature, salinity, in vivo fluorescence and selected nutrients $(NO_3^- + NO_2^-, PO_4^{3-})$ and SiO_{4}^{-}) was obtained. Only vertical profiles of nutrients are presented in this study. Other data (salinity, temperature and fluorescence) are published elsewhere (Yin et al., 1997a). The vertical profiling system has been described in detail by Jones et al. (1991) and Yin et al. (1995a). Basically, a hose connected to a water pump on deck was attached to the conductivity-temperature-depth (CTD) probe or S4 (InterOcean[®]) which has the dual function of a CTD probe and a current meter. Seawater from the pump was connected into the sampling tubing of an AutoAnalyzer[®] on-board the ship for in situ nutrient measurements, while the CTD probe was lowered slowly into the water at 1 m min^{-1} . Each sampling produced a high-resolution continuous vertical profile of physical and biological parameters, and thus the relationship between these parameters in the water column can be easily recognized. Data from a vertical profile (a datum point every 3 s) were smoothed over 15 s intervals. This smoothing reduced the fluctuations caused by the ship's motion.

2.3 Analysis of nutrients

All nutrients were determined using a Technicon AutoAnalyzer II. Salinity effects on nutrient analyses were tested on-board the ship and were found to be small. Therefore, no correction was made for salinity effects. $NO_3^- + NO_2^-$ and



Figure 2. Map of the Strait of Georgia showing the study area and the sampling stations. Note that the Fraser River is located to the right, with two river channels flowing into the Strait of Georgia.

 PO_4^{3-} were determined following the procedures of Wood et al. (1967) and Hager et al. (1968), respectively. The analysis of SiO_4^- was based on Armstrong et al. (1967) and ammonium analysis followed Parsons et al. (1984). A water sample for particulate organic carbon and nitrogen (POC and PON) was filtered onto a GF/F filter, and POC/PON on the filter were analysed with a Carlo Erba model NA 1500 NCS elemental analyser, using the dry combustion method described by Sharp (1974).

2.4 Field incubation experiments

Incubation experiments of water samples were conducted to examine the disappearance and change in ambient nutrient ratios in the water samples from different cruises in 1989, 1990 and 1991. Niskin bottles (5 L) were used to take seawater samples and the samples were transferred to acid-cleaned carboys (10L). Subsamples of seawater were transferred to transparent polycarbonate flasks (1 L) and placed in Plexiglas tanks. The tanks were kept at the same temperature as the surface water by pumping seawater (from the ship's intake at 3 m) through the tank. At Station S3, on 8 June 1989, water samples taken at 1 and 16 m were incubated in the tanks and wrapped with one and four layers of neutral density screening, respectively, which reduced the light intensity to the corresponding sampling depths (50 and 6% of the surface light). At Station P3, on 11 August 1991, a water sample was incubated under one layer of neutral density screening (about 50% of the surface light). In this incubation experiment, NO_3^- , PO_4^{3-} and SiO_4^- were all added to the sample, yielding final concentrations of 20–30, 2–3 and 20–30 μ M, respectively. At Station S1, on 4 June 1990, a water sample was incubated during the first 28 h (pre-incubation) without the addition of nutrients. After the pre-incubation, during which all nutrients were depleted, nutrients were added in eight treatments: (1) control: no additions, (2) +N: adding NO₃⁻ alone, (3) +P: adding PO₄³⁻ alone, (4) +Si: adding SiO₄⁻ alone, (5) +N+P: adding NO₃⁻ and PO₄³⁻ together, (6) +N+Si: adding NO₃⁻ and SiO₄⁻, (7) +P+Si: adding PO₄³⁻ and SiO₄⁻, and (8) +N+P+Si: adding all three nutrients. The final concentrations of added NO₃⁻, PO₄³⁻ and SiO₄⁻ were 7–8, 1.3–1.6 and 10–12 μ M, respectively. The incubations lasted for 26 h, and subsamples were taken every 1 h during the first 6 h for measurements of fluorescence and nutrients.

Vertical profiles and seawater samples for in situ incubation which were used in this study were collected at different stations and different sampling times. Water column conditions such as salinity, temperature and fluorescence have been described in the listed publications, as shown in Table 1.

3 Results

3.1 Vertical profiles of nutrients and nutrient ratios

At S3, near the edge of the Fraser River plume, the profiles documented changes before (T1) and after wind mixing (T7). At T1, both NO_3^- and PO_4^{3-} were low in the surface layer and N: P ratios were low (<2:1) and increased to ~8:1 at 20 m

Table 1. Water column conditions, such as vertical profiles of salinity, temperature and fluorescence, that can be found in the listed publications for the sampled stations in the Strait of Georgia.

Sampling stations	Dates of field incubation experiments	The studies that described water properties for the Strait of Georgia
S1 (Fig. 9)	4 June 1990	Clifford et al. (1991b)
S3 (Fig. 3)	6–7 August 1991	Yin et al. (1997b)
S3-P4-P6-S1 (Fig. 4)	8 August 1991	Yin et al. (1997b).
S1 (Fig. 5)	8–9 August 1991	Yin et al. (1997b)
P5 (Fig. 6)	10–14 August 1991	Yin et al. (1997b)
P3 (Fig. 7)	11–16 August 1991	Yin et al. (1997b)
S3 (Fig. 8)	8 June 1989	Clifford et al. (1991a)
S1, S2 and S3 (Fig. 10)	20-23 August 1990	Clifford et al. (1991b)



Figure 3. Two vertical profiles (T1 = 12:15 and T7 = 06:15 UTC) in the time series for 6–7 August 1991 of nutrients at S3. (a) NO_3^- , PO_4^{3-} and N : P ratios. (b) SiO_4^- and N : Si.

(Fig. 3). At T7, higher N : P ratios of 16-20 : 1 occurred due to an increase in NO₃⁻ in the deep water. SiO₄⁻ was ~ 30 μ M at the surface due to input from the Fraser River, and increased to 37 μ M at 20 m (Fig. 3). The N : P ratio curve nearly formed a right angle at the top of the nutriclines at T7 when the gradient of the nitracline was larger than that of the phosphacline. At T1, the N : Si ratio was near zero because NO₃⁻ was near the detection limit, but started to increase along the nitracline at the depth of the SiO₄⁻ minimum. At T7, N : Si increased more rapidly with the nitracline.

A strong wind speed event occurred on 7 August and the water column was mixed (Yin et al., 1997b). We followed

the change in the nutrient profiles and nutrient ratios from S3 near the Fraser River plume to P4 and P6 and well beyond the plume to S1. At S3, N : P ratios in the water column were greater than 7 : 1 when both NO₃⁻ and PO₄³⁻ were high after wind mixing, with N : Si ratios being less than 0.5 : 1 (Fig. 4). As the post-wind bloom of phytoplankton developed along P4–P6 due to the newly supplied nutrients (Yin et al., 1997b), the N : P ratio followed the distribution of NO₃⁻ at P4, and decreased to zero as NO₃⁻ was depleted at the surface at P6 (Fig. 4). It was clear that little PO₄³⁻ was consumed while NO₃⁻ was taken up. At the same time, the silicacline deepened and paralleled the nitracline. At S1, N : P and N : Si ratios formed almost a vertical line. N : P and N : Si ratios were ~ 8 : 1 and 0.5 : 1, respectively, in the deep water (Fig. 4).

The time series (T1, T3, T8 and T11) of 8-9 August captured changes over 1 or 2 days after the wind mixing event at S1 that were well beyond the river plume (Fig. 5). At T1, N : P and N : Si ratios were \sim 9 : 1 and 0.45 : 1, respectively, with NO₃⁻ and PO₄³⁻ being 15 and 1.7 μ M, respectively, at the surface. At T3, the N:P ratio remained constant at $\sim 9:1$, while NO₃⁻ and PO₄³⁻ decreased by 10 and 1.0 μ M, respectively, indicating an uptake N: P ratio of 10:1. In comparison, the N : Si ratio decreased from T1 to T3 when SiO_4^- was $35 \,\mu\text{M}$ at T1 and decreased by > 10 μM at T3, producing an uptake N : Si ratio of \sim 1 : 1. At T8, the N : P ratio followed the NO₃⁻ distribution as NO₃⁻ decreased to $\sim 0 \,\mu\text{M}$ at the surface while PO_4^{3-} was still $\sim 0.5 \,\mu M$. This indicated that NO_3^{-} uptake was more rapid than PO_4^{3-} uptake, and hence NO_3^{-} mainly determined the ambient N:P ratios. The N:Si uptake ratio of ~ 1 : 1 continued until T8. However, at T11, the N : P ratio spiked higher in the top 5-10 m of the nutricline, suggesting a more rapid uptake of PO_4^{3-} relative to NO_3^{-} in the upper portion of the phosphacline (Fig. 5).

Changes in the profiles after the wind event on 7 August were followed over 5 days (10–14 August) at P5, which was still within the influence of the river plume, as evidenced by the higher surface SiO_4^- at the surface (Fig. 6). On 10–11 August, N:P ratios were higher at the surface where the



Figure 4. Vertical profiles at S3 near the Fraser River plume to P4 and P6 finally to S1, which was well beyond the plume (108 km away) on 8 August 1991. (a) NO_3^- , PO_4^{3-} and N : P ratios. (b) SiO_4^- and N : Si ratios.

post-wind induced bloom occurred 2 days earlier, suggesting that uptake of PO_4^{3-} had caught up with uptake of NO_3^- . The right-angle shape of the N : P ratio on 12 August occurred as the nutriclines became sharper due to entrainment of nutrients. By 13 August, more NO_3^- was taken up at depth and the N : P ratio followed the deepening of the nitracline, and PO_4^{3-} was left behind. On 14 August, PO_4^{3-} started to decrease. During 10–14 August, a minimum in SiO_4^- was present at an intermediate depth (5–10 m), coinciding with the top of the nitracline, and the silicacline followed the nitracline below 10 m.



Figure 5. Selected vertical profiles at S1 during the time series (T1, T3, T8 and T11) on 8–9 August 1991. (a) NO_3 , PO_4 and N : P ratios. (b) SiO_4^- and N : Si ratios.

3.2 Changes in nutrient ratios during field incubations

On-deck incubation experiments were used to examine changes in uptake ratios by eliminating any effects due to mixing. Ambient N : P and N : Si ratios were lower at the surface than at depth, indicating higher uptake of NO_3^- at the surface. The indication of a higher uptake ratio of N : P and N : Si was supported by field incubation experiments. During nutrient addition $(NO_3^-, PO_4^{3-} \text{ and SiO}_4^-)$ bioassays on a sample from 1 m at P3, all nutrients decreased as fluorescence increased (Fig. 7). Ambient N : P and N : Si ratios decreased to almost 0.0 after 96 h, indicating more rapid uptake of NO_3^- than uptake of PO_4^{3-} and SiO_4^- . The temporal decline in the N : P and N : Si ratios resembled the temporal progression during a bloom, as illustrated in C0–C3 of the conceptual profiles (Fig. 1) and in the water column (S3, P4, P6) on 8 August (Fig. 4) and during the time se-



Figure 6. Vertical profiles in the time series at P5 during 10-14 August 1991. (a) NO_3^- , PO_4^{3-} and N:P ratios. (b) SiO_4^- and N:Si ratios.

ries at S1 (Fig. 5). During the incubation, both PO_4^{3-} and SiO_4^- continued to be drawn down after NO_3^- became undetectable (Fig. 7). In an earlier incubation experiment, at S3, near the end of the phytoplankton bloom on June 8, PO_4^{3-} was depleted at 1 m, and both NO_3^- and SiO_4^- continued to disappear with $2 \mu M NO_3^-$ and $4 \mu M SiO_4^-$ being taken up. However, for the sample taken at 16 m, PO_4^{3-} (~ 0.5 μM) and SiO_4^- (~ 5 μM) continued to disappear after 1.25 $\mu M NO_3^-$ was depleted after 8 h (Fig. 8).



Figure 7. Time course of duplicate in vivo fluorescence, NO_3^- , PO_4^{3-} and SiO_4^- , and N:P and N:Si ratios during an in situ incubation of a water sample taken from 1 m at P3 on 11 August 1991 (11:45 UTC). NO_3^- , PO_4^{3-} and SiO_4^- were added to the water sample at T=0 before the incubation.

The water sample at S1 on 4 June was incubated for 30 h without an addition of nutrients (Fig. 9a). The initially low NO_3^- and PO_4^{3-} remained near depletion levels during the incubation, but ambient SiO_4^- decreased from 9 to <1 μ M (Fig. 9a), which was an additional $8 \mu M \operatorname{SiO}_4^-$ taken up in excess in relation to N and P. At the end of 30 h, nutrients were added (Fig. 9b). Both ambient NO_3^- and PO_4^{3-} rapidly disappeared during the first 6 h, while ambient SiO_4^- decreased little (Fig. 9b), indicating a sequential uptake of NO_3^- and PO_4^{3-} since 8 µM SiO₄⁻ was previously taken up as shown in Fig. 9a. The ambient N: P ratio decreased faster in the samples with a single addition of NO_3^- or PO_4^{3-} alone $(+\mathrm{N}\,/\,+\mathrm{P})$ than that with additions of NO_3^- and PO_4^{3-} together (+N+P) (Fig. 9c), suggesting an interaction between the uptake of NO_3^- and PO_4^{3-} . The accumulative uptake ratio of NO_3^- to PO_4^{3-} increased with time, especially when only a single nutrient was present. The ratio of ambient N: Si decreased with



Figure 8. Time course of NO_3^- , PO_4^{3-} and SiO_4^- during the field incubation of water samples taken at Station S3 on 8 June 1989. (a) Sample taken at 1 m and incubated under one layer of screening; (b) sample taken at 16 m and incubated under four layers of screening.

time, and the accumulative uptake ratio of N:Si exceeded 3:1 in the presence of PO_4^{3-} (Fig. 9c).

4 Discussion

The Strait is highly productive, reaching up to $2700 \text{ mg Cm}^{-2} \text{ d}^{-1}$ in August (Yin et al., 1997b). This is due to pulsed nutrient supplies and multiple phytoplankton blooms in the shallow photic zone interacting with wind events (Yin et al., 1997b) and fluctuations in river discharge (Yin et al., 1997a, 1995c). Our results revealed sequential nutrient uptake as a potential mechanism to optimize nutrient uptake efficiency and generate high primary productivity of phytoplankton by taking advantage of pulsed nutrients in this highly dynamic, relatively shallow photic zone.

4.1 Responses of N : P and N : Si ratios to vertical mixing and uptake of nutrients

A vertical profile of N: P and N: Si ratios represents a snapshot of the mixing and the uptake of N, P and Si by phytoplankton in the water column. The depletion zone of the most limiting nutrient in the euphotic zone ends at a depth where the uptake of nutrients just balances the upward flux of nutrients through the nutricline, as indicated in C3 in the conceptual profiles (Fig. 1). Different responses of nutrient uptake to pulsed nutrients by mixing appeared to depend on the previous stability of the water column, the depth of the euphotic zone and nutritional status of phytoplankton. Our observations spanned all seven conceptual profiles (Fig. 1) and indicated the dynamic processes influencing the sequence of nutrient uptake. The change in the profiles of the N:P ratio from S3 to P6 (Fig. 4) displayed the spring bloom-like progression, as illustrated in conceptual profiles of CO-C3 (Fig. 1) after the wind mixing event. Various responses illustrated in the conceptual profiles C4, C5 and C6 (Fig. 1) were observed in the observations, including the right angle in the N: P ratio (T7 - Fig. 3; P5, 12 August - Fig. 6), parallel lines between the nitracline and the N : P ratio curve on 12 August, (Fig. 6), and a spike in the N : P ratio curve at T11 at S1 due to continued uptake of PO_4^{3-} , with NO_3^- being depleted during the time period from T1 to T8 (Fig. 5), which was frequently observed on 10 August at P5 (Fig. 6). The recycling of nutrients in different preferences, such as faster P regeneration than N, which in turn is recycled faster than Si, also contributes to the variability of nutrient ratios as shown above.

4.2 Sequential nutrient uptake for balanced stoichiometry and nutritional optimization

Phytoplankton can take advantage of the dynamic mixing regimes and optimize their growth rates by taking up nutrients sequentially. The disappearance of nutrients during the incubation resembled the temporal progression of a bloom as illustrated in C0–C3 of the conceptual profiles (Fig. 1) and in the water column (S3, P4, P6; Fig. 4), or during the time series at S1 (Fig. 5).

Nutrient deficiency results from a decrease in the cellular content of the limiting nutrient and continuous uptake of other non-limiting nutrients. Earlier studies found that N limitation results in excess cellular content of P and Si (Conway and Harrison, 1977; Healey, 1985; Berdalet et al., 1996). Some phytoplankton develop enhanced uptake of the limiting nutrients, such as NH₄ and PO₄³⁻, upon its addition after a period of nutrient limitation or starvation, and there is an accompanying shutdown of the non-limiting nutrient (Conway et al., 1976; Conway and Harrison, 1977; McCarthy and Goldman, 1979). A few hours of enhanced N uptake quickly overcome the N debt since the enhanced uptake rate is many times faster than the growth rate (Conway et al., 1976). For



Figure 9. Time course of ambient NO_3^- , PO_4^{3-} and SiO_4^- during the field incubation of a water sample taken at Station S1 on 4 June 1990; (a) pre-incubation: no nutrients were added to the sample during the first 28 h; (b) after pre-incubation, nutrients were added in eight treatments: +N, +P, +Si, +N+P, +N+Si, +P+Si and +N+P+Si, as described in the materials and methods section; (c) ambient nutrient ratios were calculated from measured ambient nutrients during the time course of incubation in panel (b). Ambient +N / +P indicates the ratio of N in +N (the added N alone) over P in +P (the added P alone). Please note that this N : P ratio is not for N and P in the same bottle. Similarly, ambient +N / +Si indicates N in +N over Si in +Si (the added Si alone). The accumulative uptake ratio was directly calculated from the decreasing concentrations over time in panel (b).

example, enhanced uptake of phosphorus could double internal P within 5 min to 4 h depending on the degree of P limitation and the pulsed PO_4^{3-} (Healey, 1973). After the nutrient debt has been overcome by enhanced uptake, the uptake of non-limiting nutrients returns to normal after the cell quota of the limiting nutrient is maximal (Collos, 1986). The sequential uptake of a limiting nutrient and then the uptake of both the non-limiting and limiting nutrients are advantageous to allow phytoplankton to maintain maximum growth rates over several cell generations.

4.3 Significance of sequential uptake of nutrients

There are two essential strategies used by phytoplankton to cope with the limiting nutrient (Collos, 1986). One strategy is the "growth" response, where phytoplankton uptake of the limiting nutrient, and cellular growth are coupled when the limiting nutrient is available. The other strategy is the "storage" response, where phytoplankton have the capability of accumulating large internal nutrient pools, resulting in extensive uncoupling between uptake and growth, and a lag in cell division of up to 24 h following a single addition of the limiting nutrient. The former strategy would have the competitive advantage under frequent pulses of the limiting nutrient, whereas the latter strategy presents an ecological advantage when the nutrient pulsing frequency is lower than cell

division rate. A phytoplankton assemblage can be assumed to contain both strategies in the water column. Phytoplankton species composition in subsurface waters was more or less similar at three stations (S1, S2 and S3), considering a span of 100 km across a large salinity gradient (Clifford et al., 1992). Cryptomonads and Chrysochromulina spp. and Micromonas pusilla were dominant at S2, S3 and S1 in cell density (Clifford et al., 1992). The common diatom species included Chaetoceros spp. and Thalassiosira spp. (Clifford et al., 1992), which are said to use the growth and storage strategies, respectively (Collos, 1986). At Station S2, the chlorophyll maximum at 7 m on 7 August contained 4 times more phytoplankton cells than at the surface (Clifford et al., 1992) and was frequently observed at or associated with the nutricline (Cochlan et al., 1990; Yin et al., 1997a). Phytoplankton there could use either the growth or storage strategy by different species. The storage strategy of non-limiting nutrients would allow phytoplankton to utilize the limiting nutrient when it is available and thus maximize phytoplankton growth by saving the energy expenditure associated with taking up non-limiting nutrients under limiting irradiance. This may explain why there were various modes or patterns of the N: P ratio at the nutricline, which indicates the different strategies of taking up nutrients sequentially based on the nutritional status of phytoplankton. The sequential uptake strategy allows some phytoplankton species to use the storage



Figure 10. Vertical profiles of particulate organic C : N ratios at Stations S2, S3 and S1 along the increasing distance from the river during 20–23 August 1990.

capacity for non-limiting nutrients and other phytoplankton species to use the growth response for the most limiting nutrient when it becomes available by mixing processes.

Sequential uptake of nutrients by phytoplankton can be a fundamental mechanism in maintaining high productivity in the water column where there are frequent mixing events in coastal waters. The sequential uptake strategy largely occurs at the nutriclines near or at the bottom of the photic zone. There is a consistent association between the nutriclines and the chlorophyll maximum in various aquatic environments (Cullen, 2015) and it is also common in the Strait (Harrison et al., 1991). There is a frequent upward flux of nutrients through the nutricline due to entrainment in the Strait (Yin et al., 1995a, b, c) and by internal waves in the open ocean (Pomar et al., 2012). Phytoplankton in the chlorophyll maximum are generally exposed to nutrients, and when these cells are brought up to the surface during entrainment or wind mixing (Yin et al., 1995a) they can quickly photosynthesize (Yin et al., 1995c). When phytoplankton exhaust the most limiting nutrient, their internal nutrient pool decreases and they sink down to the nutriclines, possibly due to the formation of clumps, and take up the abundant nutrients there. Thus, the cycle of sequential uptake of the limiting and then the nonlimiting nutrients may reduce nutrient deficiency in phytoplankton.

Sequential uptake of nutrients can be an important process to maintain the phytoplankton nutrient stoichiometry. Carbon fixation continues after a nutrient becomes deficient (Elrifi and Turpin, 1985; Goldman and Dennett, 1985) and the storage of organic carbon of a higher POC : N ratio is common in phytoplankton (Healey, 1973). When phytoplankton cells with excessive organic carbon, due to limitation of a nutrient, sink from the upper euphotic zone to the nutricline where light becomes limiting, uptake of other nutrients occurs by utilizing stored organic carbon, leading to an increase in the cellular N and P quotas. Thus, the ratios of carbon to other nutrients approach optimum stoichiometry. POC: N ratios at Stations S2 and S3 were observed to be between 6:1 and 7:1 in the water column, even though both ambient NO_3^- and PO_4^{3-} were near detection limits (Fig. 10). In addition, the POC: N ratio was slightly higher than 7:1 (Fig. 10) at Station S1 where nitrogen was more frequently under the detection limit than at Stations S2 and S3. This might suggest the lack of ambient nitrogen limitation on the cellular nutrient stoichiometry. However, using the C: N ratio in particular matter to infer the nutrient limitation has its limitation as particular C: N ratios do not necessarily reflect phytoplankton elemental composition alone, especially in estuarine-influenced waters.

5 Conclusions

The use of in situ continuous vertical profiles in this study shows a high variability of ambient N : P and N : Si ratios in the water column, suggesting the dynamics of nutrient uptake ratios, as illustrated in the conceptual model of Fig. 1. The incubation experiments demonstrated the sequential uptake of nutrients by phytoplankton, which suggests that deficiency of a nutrient that is based on the ambient nutrient ratio could be transient and overcome by the sequential uptake of the most limiting and non-limiting nutrients. The capacity of sequential uptake of nutrients is an important strategy for phytoplankton to maintain high primary productivity and near-optimum cellular nutrient stoichiometry in the water column. The sequential nutrient uptake strategy also offers another mechanism for the explanation of the variability in the nutrient stoichiometry of phytoplankton in the euphotic zone.

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Data availability. The data set is available upon request from the first author.

Author contributions. Kedong Yin collected data and wrote the manuscript. Hao Liu contributed to the conceptual diagram and the figure design and plots, as well as part of the writing and revision. Paul J. Harrison (deceased) supported the research cruise for collection of data and designed the sampling plan.

Competing interests. The authors declare that they have no conflict of interest.

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References

- Armstrong, F. A. J., Stearns, C. R., and Strickland, J. D. H.: The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer[®] and associated equipment, Deep-Sea Res.-Oceanogr., 14, 381–389, 1967.
- Berdalet, E., Marrasé, C., Estrada, M., Arin, L., and MacLean, M. L.: Microbial community responses to nitrogen- and phosphorusdeficient nutrient inputs: microplankton dynamics and biochemical characterization, J. Plank. Res., 18, 1627–1641, 1996.
- Bertilsson, S., Berglund, O., Karl, D. M., and Chisholm, S. W.: Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea, Limnol. Oceanogr., 48, 1721–1731, 2003.
- Brzezinski, M. A.: The Si: C: N ratio of marine diatoms: interspecific variability and the effect of some environmental variables, J. Phycol., 21, 247–257, 1985.
- Clifford, P. J., Harrison, P. J., St. John, M. A., Yin, K., and Albright, L. J.: Plankton production and nutrient dynamics in the Fraser River plume, 1989, Manuscript Report No. 54, Department of Oceanography, University of British Columbia, Vancouver, BC Canada, 225 pp., 1991a.
- Clifford, P. J., Harrison, P. J., Yin, K., St. John, M. A., and Goldblatt, R. H.: Plankton production and nutrient dynamics in the Fraser River plume, 1990, Manuscript Report No. 58, Department of Oceanography, University of British Columbia, Vancouver, BC Canada, 265 pp., 1991b.
- Cochlan, W. P., Harrison, P. J., Clifford, P. J., and Yin, K.: Observations on double chlorophyll maxima in the vicinity of the Fraser River plume, Strait of Georgia, British Columbia, J. Exp. Mar. Biol. Ecol., 143, 139–146, 1990.

- Collos, Y.: Time-lag algal growth dynamics: biological constraints on primary production in aquatic environments, Mar. Ecol. Prog. Ser., 33, 193–206, 1986.
- Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., Lancelot, C., and Likens, G. E.: Controlling eutrophication: nitrogen and phosphorus, Science, 323, 1014–1015, 2009.
- Conway, H. L. and Harrison, P. J.: Marine diatoms grown in chemostats under silicate or ammonium limitation IV. Transient response of *Chaetoceros debilis*, *Skeletonema costatum* and *Thalassiosira gravida* to a single addition of the limiting nutrient, Mar. Biol., 43, 33–43, 1977.
- Conway, H. L., Harrison, P. J., and Davis, C. O.: Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient, Mar. Biol., 35, 187–199, 1976.
- Cullen, J. J.: Subsurface chlorophyll maximum layers: enduring enigma or mystery solved?, Annu. Rev. Mar. Sci., 7, 207–239, 2015.
- Elrifi, I. R. and Turpin, D. H.: Steady-state luxury consumption and the concept of optimum nutrient ratios: a study with phosphate and nitrate limited *Selenastrum minutum* (Chlorophyte), J. Phycol., 21, 592–602, 1985.
- Elser, J. K., Acharya, M., Kyle, J., Cotner, W., Makino, T., Markow, T., Watts, S., Hobbie, W., Fagan, J., Schade, J., Hood, J., and Sterner, R. W.: Growth rate-stoichiometry couplings in divers biota, Ecol. Lett., 6, 936–943, 2003.
- Falkowski, P. G.: Rationalizing elemental ratios in unicellular algae, J. Phycol., 36, 3–6, 2000.
- Geider, R. J. and La Roche, J.: Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis, Eur. J. Phycol., 37, 1–17, 2002.
- Goldman, J. C. and Dennett, M. R.: Photosynthetic responses of 15 phytoplankton species to ammonium pulsing, Mar. Ecol. Prog. Ser., 20, 259–264, 1985.
- Hager, S. W., Gordon, L. I., and Park, P. K.: A practical manual for the use of the Technicon AutoAnalyzer in seawater nutrient analysis, Technical Report of Department of Oceanography, Oregon State University, Corvallis, 68–33, 1–33, 1968.
- Harrison, P. J., Parsons, T. R., Taylor, F. J. R., and Fulton, J. D.: Review of Biological oceanography of the Strait of Georgia: Pelagic Environment, Can. J. Fish. Aquat. Sci., 40, 1064–1094, 1983.
- Harrison, P. J., Parslow, J. S., and Conway, H. L.: Determination of nutrient uptake kinetics parameters: a comparison of methods, Mar. Ecol. Prog. Ser., 52, 301–312, 1989.
- Harrison, P. J., Clifford, P. J., Cochlan, W. P., Yin, K., St. John, M. A., Thompson, P. A., Sibbald, M. J., and Albright, L. J.: Nutrient and plankton dynamics in the Fraser-river plume, Strait of Georgia, British-Columbia, Mar. Ecol. Prog. Ser., 70, 291–304, 1991.
- Healey, F. P.: Inorganic nutrient uptake and deficiency in algae, CRC Crit. Rev. Microbiol., 3, 69–113, 1973.
- Healey, F. P.: Interacting effects of light and nutrient limitation on growth rate of *Synechococcus linearis* (Cyanophyceae), J. Phycol., 21, 134–146, 1985.
- Hecky, R. E. and Kilham. P.: Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment, Limnol. Oceanogr., 33, 786–822, 1988.

- Jones, D. M., P. J., Harrison, P. J., Clifford, P. J., Yin, K., and John, M. St.: A computer-based system for the acquisition and display of continuous vertical profiles of temperature, salinity, fluorescence and nutrients, Water Res., 25, 1545–1548, 1991.
- Karl, D. M., Björkman, K. M., Dore, J. E., Fujieki, L., Hebel, D. V., Houlihan, T., Letelier, R. M., and Tupas, L. M.: Ecological nitrogen-to-phosphorus stoichiometry at station aloha, Deep-Sea Res. Pt. II, 48, 1529–1566, 2001.
- Klausmeier, C. A., Litchman, E., Daufresne, T. and Levin, S. A.: Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton, Nature, 429, 171–174, 2004.
- LeBlond, P. H.: The Strait of Georgia: functional anatomy of a coastal sea, Can. J. Fish. Aquat. Sci., 40, 1033–1063, 1983.
- Loladze, I. and Elser, J.: The origins of the Redfield nitrogen-tophosphorus ratio are in a homoeostatic protein-to-rRNA ratio, Ecol. Lett., 14, 244–250, 2011.
- Martiny, A. C., Pham, C. T. A., Primeau, F. W., Vrugt, J. A., Keith Moore, J., Levin, S. A., and Lomas, M. W.: Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter, Nat. Geosci., 6, 279–283, 2013.
- McCarthy, J. J. and Goldman, J. C.: Nitrogenous nutrition of marine phytoplankton in nutrient depleted waters, Science, 203, 670– 672, 1979.
- Mills, M. M., and Arrigo, K. R.: Magnitude of Oceanic Nitrogen Fixation Influenced by the Nutrient Uptake Ratio of Phytoplankton, Nat. Geosci., 3, 412–416, 2010.
- Pomar, L., Morsilli, M., Hallock, P., and Baidenas, B.: Internal waves, an under-explored source of turbulence events in the sedimentary record, Earth-Sci. Rev., 111, 56–81, 2012.
- Price, N. M.: The elemental stoichiometry and composition of an iron-limited diatom, Limnol. Oceanogr., 50, 1159–1171, 2005.
- Redfield, A. C.: The biological control of chemical factors in the environment, Am. Sci., 46, 205-222, 1958.
- Segura-Noguera, M., Blasco, D., and Fortuño, J.-M.: Taxonomic and Environmental Variability in the Elemental Composition and Stoichiometry of Individual Dinoflagellate and Diatom Cells from the NW Mediterranean Sea, PLoS ONE, 11, e0154050, doi:10.1371/journal.pone.0154050, 2016.

- Sharp, J. H.: Improved analysis of particulate organic carbon and nitrogen from seawater, Limnol. Oceanogr., 19, 984–989, 1974.
- Singh, A., Baer, S. E., Riebesell, U., Martiny, A. C., and Lomas, M. W.: C : N : P stoichiometry at the Bermuda Atlantic Time-series Study station in the North Atlantic Ocean, Biogeosciences, 12, 6389–6403, doi:10.5194/bg-12-6389-2015, 2015.
- Weber, T. S. and Deutsch, C.: Ocean nutrient ratios governed by plankton biogeography, Nature, 467, 550–554, 2010.
- Wood, E. D., Armstrong, F. A. J., and Richards, F. A.: Determination of nitrate in sea water by cadmium-copper reduction to nitrite, J. Mar. Biol. Ass. UK, 47, 23–31, 1967.
- Yin, K., Harrison, P. J., Pond, S., and Beamish, R. J.: Entrainment of nitrate in the Fraser River plume and its biological implications, I. Effects of salt wedge, Estuar. Coast. Shelf Sci., 40, 505–528, 1995a.
- Yin, K., Harrison, P. J., Pond, S., and Beamish, R. J.: Entrainment of nitrate in the Fraser River plume and its biological implications, II. Effects of spring vs neap tides and river discharge, Estuar. Coast. Shelf Sci., 40, 529–544, 1995b.
- Yin, K., Harrison, P. J., Pond, S., and Beamish, R. J.: Entrainment of nitrate in the Fraser River plume and its biological implications, III. Effects of winds, Estuar. Coast. Shelf Sci., 40, 545– 558, 1995c.
- Yin, K., Harrison, P. J., and Beamish, R. J.: Effects of a fluctuation in Fraser River discharge of primary production in the central Strait of Georgia, British Columbia, Canada, Can. J. Fish Aquat. Sci., 54, 1015–1024, 1997a.
- Yin, K., Goldblatt, R. H., Harrison, P. J., John, M. A. St., Clifford, P. J., and Beamish, R. J.: Importance of wind and river discharge in influencing nutrient dynamics and phytoplankton production in summer in the central Strait of Georgia, Mar. Ecol. Prog. Ser., 161, 173–183, 1997b.