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NOTE

Nitrogen isotopic fractionation during a simulated diatom spring bloom: importance of N-starvation in controlling fractionation

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ABSTRACT: N isotope fractionation (ε) was first determined during ambient NO_3^- depletion in a simulated diatom spring bloom. After 48 h of N-starvation, NH_4^+ was resupplied to the diatoms in small pulses to simulate grazer-produced N and then ε was determined. Large variations in ε values were observed: from 2.0–3.6 to 14–0‰ during NO_3^- and NH_4^+ uptake, respectively. This is the first study reporting an ε value as low as 0 to 2‰ for NH_4^+ uptake and we suggest that greater N demand after N-starvation may have drastically reduced NH_3 efflux out of the cells. Thus the N status of the phytoplankton and not the ambient NH_4^+ concentration may be the important factor controlling ε, because, when N-starvation increased, ε values for NH_4^+ uptake decreased within 30 h. This study may thus have important implications for interpreting the $\delta^{15}N$ of particulate N in nutrient-depleted regimes in temperate coastal oceans.

KEY WORDS: N isotope fractionation · $^{15}N/^{14}N$ · Nitrate uptake · Ammonium uptake · Diatoms · Spring bloom

The uptake of ammonium by phytoplankton is often responsible for sustaining primary production in the ocean. As a consequence, the N isotope composition ($\delta^{15}N$) of particulate organic N (PON) in the surface oceans may be in part controlled by the $\delta^{15}N$ of NH_4^+ as well as the isotope fractionation that occurs during NH_4^+ uptake and assimilation (overall process defined as incorporation) by phytoplankton. Isotope fractionation is caused by the differential incorporation rates of ^{14}N (^{14}k) and ^{15}N (^{15}k), with ^{14}k typically higher than ^{15}k. As a result, the $\delta^{15}N$ of phytoplankton is lower than the $\delta^{15}N$ of the N source. In this paper, the per mil enrichment factor (ε), e.g. $(^{14}k/^{15}k-1) \times 1000$, is the definition used when referring to isotope fractionation. There are very few estimates of ε for growth on NH_4^+ in the field, mostly due to low ambient NH_4^+ concentrations and a high instrument detection limit. In eutrophic bays and estuaries, ε has been estimated to be 6.5 to 9‰ (Cifuentes et al. 1989, Montoya et al. 1991). It has been hypothesized that under a low NH_4^+ concentration and perhaps N-limiting conditions ε may be close to 0‰ since ε was found to decrease with NH_4^+ concentration (Hoch et al. 1992, Pennock et al. 1996). In the oligotrophic Atlantic subtropical gyre, the low $\delta^{15}N_{PON}$ values of −2 to 2‰ were interpreted to be due to growth on regenerated NH_4^+ and were attributed to an estimated low $\delta^{15}N$ of NH_4^+ (Altabet 1988), implying that ε was essentially 0‰. However, there is little information about the effect of low N availability on ε in the field. Also of importance for our understanding of $\delta^{15}N_{PON}$ in nutrient-depleted regimes is the transition from a NO_3^-– to NH_4^+-based productivity regime, since events that supply new NO_3^- into the euphotic zone via convective mixing are transient and thus often missed.

To gain insight into these issues, we determined $\delta^{15}N_{PON}$ in a laboratory simulation of 2 productivity regimes, e.g. NO_3^- drawdown during a diatom spring bloom and the subsequent supply of NH_4^+ (simulating grazers) to the N-depleted regime, commonly encountered in temperate coastal oceans. Our approach was to simulate a spring bloom in the laboratory by incubating seawater collected in late winter (before the spring bloom occurred), continuously illuminating it and allowing the natural phytoplankton assemblage to deplete the high ambient nitrate concentration. Then, in a simulated post-bloom situation, NH_4^+ was

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resupplied to the collapsed N-starved bloom in 3 pulses to mimic the supply of grazer-produced NH_4^+.

Materials and methods. We collected seawater on March 12, 1997, in Burrard Inlet, British Columbia, Canada, prior to the spring bloom. The ambient nitrate concentration was 23 µM. NH_4^+ and urea were estimated to be between 1 and 2 µM (Harrison et al. 1983). Duplicate cultures (S1 and S2) were grown in carboys containing 16.9 l of seawater, stirred at 60 rpm, incubated at 18°C and at a saturating continuous light intensity of 120 µmol photons m^{-2} s^{-1}. Other culture conditions were as described by Waser et al. (1998a, b). Phytoplankon growth was monitored by *in vivo* fluorescence and phytoplankton species composition by microscopic observation.

The goal of the experimental design of our laboratory simulation was to reproduce the natural conditions of a typical vigorous spring bloom. When the ambient 23 µM of NO_3^- became depleted, the cultures were N-starved for 48 h and then NH_4^+ was resupplied in three 30 µM sequential additions after each previous addition was taken up. Along with the first NH_4^+ addition, HPO_4^{2-} (39 µM), H_4SiO_4 (125 µM), trace metals, vitamins and HCO_3^- (2 mM) were added to ensure that only N was controlling phytoplankton growth during each of the 3 NH_4^+ drawdowns. The relatively high concentrations of NH_4^+ and HPO_4^{2-} compared to those encountered in the field are not inhibitory. NH_4^+ becomes toxic at 250 µM and HPO_4^{2-} at even higher levels (Thomas et al. 1980). The pH increased from 7.8 to 8.8 during NO_3^- drawdown, and it increased slightly from 8.6 to 8.9 during each NH_4^+ drawdown.

Nutrient determinations were as in Waser et al. (1998b). PON samples were collected by vacuum filtration on pre-combusted glass-fiber filters (GF/F) and frozen until analysis. At this point, the samples were dried at 50 to 60°C in an oven and kept in a desiccator until the time of analysis. PON and N isotope determinations were made on an automated CHN analyzer on line with a VG PRISM mass spectrometer. Results are reported in the δ notation: $\delta^{15}N = (R_{sample}/R_{std} - 1) \times 1000$ (‰), where R is the $^{15}N/^{14}N$ ratio and the standard (std) is atmospheric air (0‰). The precision is 0.17‰. The $\delta^{15}N$ of the initial NH_4^+ source is −1.14‰.

The enrichment factor (ε) was determined using the following accumulated product equation (Mariotti et al. 1981) as explained in detail elsewhere (Waser et al. 1998a, b): $\delta^{15}N_{PON} = \delta^{15}N_{DN0} - \varepsilon \times (-f/(1-f)) \times \ln f$, where $\delta^{15}N_{DN0}$ is the $\delta^{15}N$ of the initial N source, f is the fraction of unreacted N source and ε is a positive number (note that $\varepsilon < 0$ in Mariotti et al. 1981). Note that ε is independent of the $\delta^{15}N$ of the N source and that it is assumed that the reaction substrate (NH_4^+ or NO_3^-) → PON is the only reaction occurring and that it is unidirectional. A correction was made to account for the initial concentration of PON in both phases of the simulation (see Waser et al. 1998b).

Results and discussion. The phytoplankton assemblage was dominated by 4 diatoms (*Thalassiosira* sp., *Coscinodiscus* sp., *Chaetoceros* sp., *Skeletonema costatum*) and to a lesser extent by a prymnesiophyte (*Pavlova* sp.). Phytoplankton composition was similar in all the simulated conditions. Growth of the diatom assemblage started immediately after seawater was incubated and continuously illuminated in the laboratory. In 2 d, the 23 µM of ambient nitrate was consumed (Fig. 1A), which is similar to intense spring diatom blooms observed in other areas nearby Burrard Inlet (Takahashi et al. 1977). This was accompanied by variations in $\delta^{15}N_{PON}$ of 2 and 1.5‰ for S1 and S2, respectively (Fig. 1B). The overall isotopic fractionation during nitrate depletion was 2.0 ± 1.4‰ for S1 and 3.6 ± 1.6‰ for S2, respectively. These values compared well with values determined in culture experiments (Needoba 1997, Waser et al. 1998a), where phytoplankton was grown on nitrate as the sole N source and where the culture conditions were very similar to the present ones (continuous light, light intensity of 120 µmol photons m^{-2} s^{-1}, T = 18°C, same stirring speed). The values of 2.0 to 3.6‰ were in the range of values of 2.7 to 6.2‰ determined for 4 (e.g. *Thalas-*

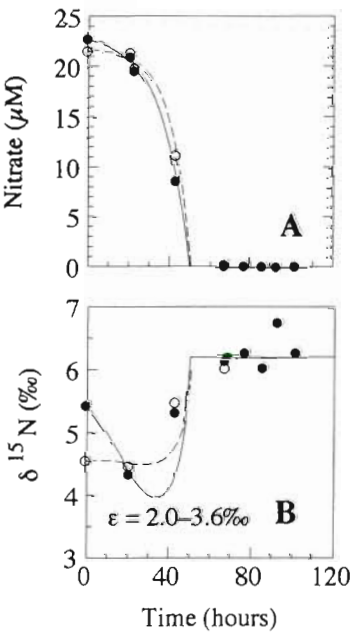

Fig. 1. Growth of an assemblage of natural marine phytoplankton from Burrard Inlet, British Columbia, Canada, in duplicate laboratory cultures S1 (•) and S2 (○), respectively, in a simulated spring bloom experiment. (A) Depletion of the ambient nitrate. (B) Changes in $\delta^{15}N$ of PON during nitrate depletion and at stationary phase. Solid and dashed lines represent the fits of the S1 and S2 data, respectively, using the Rayleigh distillation model

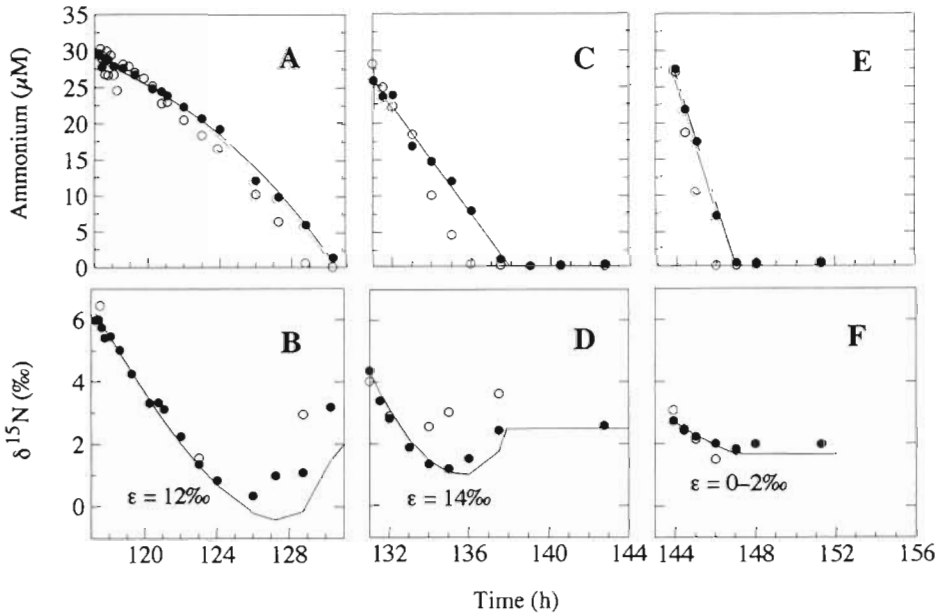

Fig. 2. Growth of the assemblage of natural marine phytoplankton after resupply of NH_4^+ following 48 h of N-starvation in duplicate cultures S1 (•) and S2 (o), respectively. (A) Drawdown of NH_4^+ after the first 30 μM NH_4^+ addition. (B) Changes in $\delta^{15}N$ of PON following the first NH_4^+ addition. (C,D) Second 30 μM NH_4^+ addition. (E,F) Third 30 μM NH_4^+ addition. Solid lines in (B), (D) and (F) represent the fit of the S1 data using the Rayleigh distillation model

siosira, *Chaetoceros*, *Skeletonema*, *Pavlova*) of the 5 dominant genera present in the assemblage (Needoba 1997, Waser et al. 1998a). Finally, the ε values were a little lower than the value of 6‰ for a controlled ecosystem enclosure in Saanich Inlet, BC, Canada (Nakatsuka et al. 1992), and a little lower than the field estimates of 4 and 5‰ for the Northeast Pacific (Goering et al. 1990, Wu et al. 1997).

During the period of NH_4^+ uptake following 48 h of N-starvation, the $\delta^{15}N$ of PON decreased from 6 to 2‰ in both S1 and S2 (Fig. 2). This decrease was mostly due to the uptake of 'new' NH_4^+ with a $\delta^{15}N$ of −1.14‰. More significant is the large decrease in ε values following the third NH_4^+ addition relative to the first and second additions (Fig. 2B,D,F). The ε value was 12 and 14‰ during the first and second NH_4^+ additions and then it dramatically decreased to 0‰ for S1 and 2‰ for S2 in the third addition, respectively. The increase in ε values following the first NH_4^+ addition was expected since ε is much larger for NH_4^+ than for NO_3^- incorporation, possibly due to the different primary enzymes for NH_4^+ and NO_3^- assimilations (Waser et al. 1998a). Nevertheless, the ε values of 12 and 14‰ for the first and second NH_4^+ additions were significantly reduced from the ε values of 17.7 to 19‰ repeatedly found in N-replete conditions (Table 1), suggesting that the N status of the cells as well as the N source (NH_4^+ vs NO_3^-) used for growths have an effect on isotope fractionation. The effect of the N status of cells has been observed previously for diatoms and coccolithophores grown on NO_3^-, NH_4^+ and urea when all the sources were present in the culture medium (Waser et al. 1998b). More comparable with the results of this study is the finding of a large decrease in ε values from 17.7‰ for N-replete cells of *Emiliania huxleyi* to ε values of 8 to 12‰ following NH_4^+ resupply to the N-starved cells (Table 1). This culture was grown on NH_4^+ and in similar conditions to S1 and S2, allowing the comparison to be made.

In the third NH_4^+ addition, ε had the lowest value ever reported in field and culture experiments. This may be related to the longer periods of starvation from 138 to 144 h in S1 and from 136 to 144 h in S2 at the end of the second addition (Fig. 2C). We may speculate that ε may be reduced to 0–2‰ because efflux of ^{15}N-enriched ammonia from the cell may have been significantly reduced due to greater N demand after starvation, assuming isotopic fractionation occurred inside the cell rather than during transport across the cell membrane. Further evidence for the increased N demand in the third addition relative to the first and second addition can be seen in the increased specific uptake rate of NH_4^+ (ρ) in the third addition, e.g. 0.110 to 0.158 h^{-1} (range of ρ calculated for S1 and S2 duplicate cultures) relative to 0.082–0.090 h^{-1} (S1–S2) in the first addition and 0.063–0.095 h^{-1} (S1–S2) in the second addition.

The discrepancy observed between the fit and the data, particularly in the first and second NH_4^+ additions (Fig. 2B,D) indicates that either some loss of NH_3

Table 1. Comparison between field and laboratory culture estimates of N isotope fractionation (ε) for growth of bacteria and phytoplankton on ammonium. $[NH_4^+]_i$: initial NH_4^+ concentration

Species	Location	Condition	ε (‰)	$[NH_4^+]_i$ (μM)	Source
Field					
Diatom assemblage	Burrard Inlet	N-starved	0–14	25–30	This study
Microbial assemblage	Delaware Estuary		10±1.7	5	Hoch et al. (1994)
Skeletonema costatum[a]	Delaware Estuary		9.1±0.8	75	Cifuentes et al. (1989)
	Chesapeake Bay		6.5–8.0	10–20	Montoya et al. (1991)
Laboratory cultures					
Thalassiosira pseudonana		N-replete	19.5±1.0	190	Waser et al. (1998a)
Emiliania huxleyi[b]		N-replete	17.7±0.9	73	Waser unpubl. data
E. huxleyi[b]		N-replete	16–19	35–39	Waser et al. (1998b)
E. huxleyi[b,c]		N-starved	8–12	30–32	Waser unpubl. data
Chaetoceros debilis		N-replete	25.5	31	Waser et al. (1998b)
Skeletonema costatum			8–27	10–80	Pennock al. (1996)
Vibrio harveyi			3.8–26.5	23–182	Hoch et al. (1992)

[a]Dominant species in the assemblage of marine phytoplankton
[b]Open ocean clone isolated from the Subarctic Pacific Ocean
[c]Batch culture growth upon ammonium resupply following 48 h of N-starvation

gas or recycling of DON may have occurred during the culture experiment. It seems unlikely that significant amounts of NH_3 were lost via gas exchange because the pH was about 8.6 to 8.9 and remained similar following all 3 additions. The cultures were not bubbled and in the last 2 additions (Fig. 2D,F) the accumulated product equation provided an excellent fit of the data for S1. Thus we suggest that DON recycling caused the discrepancies. In a culture of *Thalassiosira pseudonana* grown on NH_4^+ in conditions similar to S1 and S2, DON uptake and release were observed after NH_4^+ was resupplied to the N-starved culture (Z. Yu unpubl. data). During the surge uptake of 100 μM of NH_4^+, DON concentration varied by 5 to 30% of the initial DON (i.e. 50 μM). Also, DON release increased soon after NH_4^+ was exhausted from the medium and further increased at senescence. This release of DON by healthy cells and senescent cells (due to cell lysis) would also produce departures from the fit, although the cells did not reach senescence until well after 156 h, so cell lysis was not a factor in the second phase of this study (it may have been a factor during the 48 h of N-starvation following nitrate depletion, particularly after 95 h). Overall, DON recycling may explain some of the discrepancies, particularly those observed towards the end of the NH_4^+ depletion (Fig. 2B,D). In the third addition the discrepancy was very small and occurred only after NH_4^+ was consumed from the medium (Fig. 2F). This may in part be due to the increase in PON in the third addition relative to the other additions and/or due to the higher N demand in the third addition which may have reduced DON release. More studies on DON recycling are clearly needed. Finally, we suggest that the discrepancy between the 2 duplicates in the second NH_4^+ addition (Fig. 2D) may also in part be due to the loss of N during storage of the S2 samples (S2 stored longer than S1). Microbial decomposition is known to increase the $\delta^{15}N$ of diatoms in laboratory incubations (Wada 1980) and it may have caused some loss of N. We estimated this loss at a maximum of 15% of PON. This is within the experimental errors, but some loss cannot be ruled out.

In the open ocean, it is difficult to assess the effect of the isotopic composition of NH_4^+ on the $\delta^{15}N$ of PON since the $\delta^{15}N$ of NH_4^+ is seldom measured due to the low NH_4^+ concentrations (i.e. <1–2 μM) and a high instrument detection limit. At present, it is known that, apart from the isotope fractionation that may occur during NH_4^+ incorporation by phytoplankton and heterotrophic bacteria, the $\delta^{15}N$ of NH_4^+ depends mostly on: (1) the $\delta^{15}N$ of the grazers, which is intimately linked to the $\delta^{15}N$ of their food source (Minagawa & Wada 1984), and (2) the 3‰ isotope fractionation during NH_4^+ regeneration by grazers (Checkley & Miller 1989). To some extent it may also depend on the $\delta^{15}N$ of DON and the 3‰ isotope fractionation during bacteria-mediated NH_4^+ regeneration (Hoch et al. 1994). The net effect of all these processes has yet to be quantified, although it is often suggested that $\delta^{15}N$ of NH_4^+ is low and that NH_4^+ regeneration lowers the $\delta^{15}N$ of PON after a bloom (Altabet 1988).

This study suggests that N-starvation may be a cause for the low estimates of ε for NH_4^+ uptake by phytoplankon in the field (Table 1). In batch culture growth of a diatom, low ε values of 8 to 10‰ were determined toward the end of the logarithmic phase, at NH_4^+ concentrations less than 20 μM (Pennock et al. 1996). This is very likely due to the onset of N-limitation in the

batch culture. As first suggested by Fogel & Cifuentes (1993), the decrease in ε with NH_4^+ concentration may be related to an ammonium-scavenging-mechanism. More specifically, we suggest that NH_3 efflux from the cells may have decreased after N-starvation. Our study gives support to this hypothesis and shows that conditions of N availability (e.g. N-replete vs N-starved), and hence the physiological state of the cells, lower ε from 17.7–19.5‰ to as low as 0–2‰ following N-starvation (Table 1). Furthermore, we have evidence that during batch culture growth at an initial NH_4^+ concentration of 30 µM, and thus only a little higher than 20 µM (Pennock et al. 1996), ε values were as elevated as during growth at higher (e.g. 73 and 190 µM) initial NH_4^+ concentrations (Table 1). Thus, the status of the phytoplankton and not the ambient NH_4^+ concentration per se seems to be the important factor controlling ε. It is difficult to say with certainty how these results will apply in the field, where NH_4^+ can be as low as 0 to 100 nM, but our study indicates that the physiological state of the cells is more important than the NH_4^+ concentration. Furthermore, this study suggests that an extra period of N-starvation may act to lower ε values to close to 0‰ and we speculate that it triggered a further reduction of NH_3 efflux from the cells. It may be that this efflux reduction requires time and/or the synthesis of a new compound, perhaps a protein, in which case the reduction might not have occurred after the first and second NH_4^+ additions.

This study has implications for our understanding of $\delta^{15}N_{PON}$. Again, we emphasize that this study suggests that the N status of the phytoplankton is more important than NH_4^+ concentration. Thus to determine the impact of this study in a low NH_4^+ environment, we assume that the ε values found here are applicable to the field. After the spring bloom and before grazers regenerate NH_4^+, phytoplankton can experience severe N-starvation because nitrate has been 'cleaned out' of coastal surface waters by the exponentially growing cells. This study indicates that upon the first pulse of regenerated NH_4^+ (1 µM) to surface waters with a PON concentration ranging from 4 to 8 µM (Nakatsuka et al. 1992), $\delta^{15}N_{PON}$ could very quickly decrease from 6‰ to 3.8 or 4.8‰ (depending on PON) and reach a new steady-state value of 4.4 to 5.1‰ (due to the uptake of NH_4^+ with a low $\delta^{15}N$, possibly as low as −2‰ in the field). If cells experience further short-term N-deprivation, perhaps because regeneration of NH_4^+ is patchy, then upon a second pulse of NH_4^+ the $\delta^{15}N_{PON}$ could decrease linearily from 4.4–5.1 to 3.3–4.4‰. At some point, NH_4^+ regeneration will reach a steady-state and $\delta^{15}N_{PON}$ will then become constant. With this scenario, the $\delta^{15}N_{PON}$ variations observed during the uptake of the first pulse of NH_4^+ are not very large, i.e. at most 2.2‰ with 4 µM of PON, but they are similar to those observed during NO_3^--depletion. Thus, the variations are greatest for larger concentrations of NH_4^+ relative to PON and when NH_4^+/PON falls below 10 to 12% the changes in $\delta^{15}N_{PON}$ due to isotope fractionation can be neglected. Overall, the $\delta^{15}N$ of NH_4^+, the magnitude of the grazer-produced NH_4^+ pulse, and the physiological state of the cells will be important in determining the $\delta^{15}N$ of suspended and sinking PON after the spring bloom.

In summary, this study further emphasizes that N-starvation can drastically reduce ε associated with NH_4^+ incorporation in centric diatoms commonly found in temperate oceans. Moreover, this is the first study to report ε values as low as 0 to 2‰ and it suggests that repeated periods of N-starvation in between NH_4^+ pulses possibly may have produced those low ε values by drastically reducing the efflux of NH_3. Overall, this study provides important information for interpreting time-series of $\delta^{15}N$ of surface PON in nutrient-depleted temperate oceans as well as during transitions from nitrate- to ammonium-based productivity regimes.

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LITERATURE CITED

Altabet MA (1988) Variations in nitrogen isotopic composition between sinking and suspended particles: implications for nitrogen cycling and particle transformation in the open ocean. Deep-Sea Res 35:535–554

Checkley DM, Miller CA (1989) Nitrogen isotope fractionation by oceanic zooplankton. Deep-Sea Res 36:1449–1456

Cifuentes LA, Fogel ML, Pennock JR, Sharp JH (1989) Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. Geochim Cosmochim Acta 53:2713–2721

Fogel ML, Cifuentes LA (1993) Isotope fractionation during primary production. In: Engel MH, Macko SA (eds) Organic geochemistry. Plenum Press, New York, p 73–98

Goering J, Alexander V, Haubenstock (1990) Seasonal variability of stable carbon and nitrogen isotopes ratios of organisms in a North Pacific bay. Estuar Coast Shelf Sci 30:239–260

Harrison PJ, Fulton JD, Taylor FJR, Parsons TR (1983) Review of the biological oceanography of the Strait of Georgia: pelagic environment. Can J Fish Aquat Sci 40:1064–1094

Hoch MP, Fogel ML, Kirchman DL (1992) Isotope fractionation associated with ammonium uptake by a marine bacterium. Limnol Oceanogr 37:1447–1459

Hoch MP, Fogel ML, Kirchman DL (1994) Isotope fractionation during ammonium uptake by marine microbial assemblages. Geomicrobiol J 12:113–127

Mariotti A, Germon JC, Hubert P, Kaiser P, Letolle R, Tardieux A, Tardieux P (1981) Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. Plant Soil 62:413–430

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along the food chains: further evidence and relation between d15N and animal age. Geochim Cosmochim Acta 48:1135–1149

Montoya JP, Horrigan SG, McCarthy JJ (1991) Rapid, storm-induced changes in the natural abundance of ^{15}N in a planktonic ecosystem, Chesapeake Bay, USA. Geochim Cosmochim Acta 55:3627–3638

Nakatsuka T, Handa N, Wada E, Wong CS (1992) The dynamic changes of stable isotopic ratios of carbon and nitrogen in suspended and sedimented particulate organic matter during a phytoplankton bloom. J Mar Res 50:267–296

Needoba J (1997) Nitrogen isotope fractionation by four groups of phytoplankton grown on nitrate. BSc thesis, University of British Columbia, Vancouver

Pennock JR, Velinsky DJ, Ludlam JM, Sharp JH (1996) Isotopic fractionation of ammonium and nitrate during uptake by *Skeletonema costatum*: implications for $\delta^{15}N$ dynamics under bloom conditions. Limnol Oceanogr 41:451–459

Takahashi M, Seibert DL, Thomas WH (1977) Occasional blooms of phytoplankton during summer in Saanich Inlet, B.C., Canada. Deep-Sea Res 24: 775–780

Thomas WH, Hastings J, Fujita M (1980) Ammonium input to the sea via large sewage outfalls. Part 2: Effects of ammonium on growth and photosynthesis of southern California phytoplankton cultures. Mar Environ Res 3: 291–296

Wada E (1980) Nitrogen isotope fractionation and its significance in biogeochemical processes occurring in marine environments. In: Goldberg ED, Horibe Y, Saruhashi K (eds) Isotope marine chemistry. Uchida–Rokakuho, Tokyo, p 375–398

Waser NAD, Harrison PJ, Nielsen B, Calvert SE, Turpin DH (1998a) Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium and urea by a marine diatom. Limnol Oceanogr 43:215–224

Waser NAD, Yin K, Yu Z, Tada K, Harrison PJ, Turpin DH, Calvert SE (1998b) Nitrogen isotope fractionation during nitrate, ammonium and urea uptake by marine diatoms and coccolithophores under various conditions of N availability. Mar Ecol Prog Ser 169:29–41

Wu J, Calvert SE, Wong CS (1997) $^{15}N/^{14}N$ isotope ratios in the subarctic northeast Pacific ecosystem: nitrate utilization and trophic structure. Deep-Sea Res 44:287–314

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