Article

The Contribution of Small Phytoplankton Communities to the Total Dissolved Inorganic Nitrogen Assimilation Rates in the East/Japan Sea: An Experimental Evaluation

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Abstract: As a part of Korean-Russian joint expeditions in the East/Japan Sea during 2012 and 2015, a set of total and small (<2 µm) phytoplankton NO₃[−] and NH₄⁺ uptake rate estimations were carried out. The study aimed to assess the spatio-temporal variations in dissolved inorganic nitrogen (DIN) assimilation by the total and small phytoplankton. The results show that the total NO₃[−] uptake rates during 2012 varied between 0.001 and 0.150 µmol NL^{−1}h^{−1} (mean ± SD = 0.034 ± 0.033) and that the total NH₄⁺ uptake rates ranged between 0.002 and 0.707 µmol NL^{−1}h^{−1} (mean ± SD = 0.200 ± 0.158). The total uptake rates during 2015 were ranged from 0.003 to 0.530 (mean ± S.D. = 0.117 ± 0.120 µmol NL^{−1}h^{−1}) for NO₃[−] and from 0.008 to 1.17 (mean ± S.D. = 0.199 ± 0.266 NL^{−1}h^{−1}) for NH₄⁺. The small phytoplankton NO₃[−] and NH₄⁺ uptake rates during 2015 ranged between 0.001 and 0.164 (mean ± S.D. = 0.033 ± 0.036) µmol NL^{−1}h^{−1} and 0.010–0.304 (mean ± S.D. = 0.101 ± 0.073) µmol NL^{−1}h^{−1}, respectively. Small phytoplankton's contribution to the total depth-integrated NO₃[−] and NH₄⁺ uptake rates ranged from 10.24 to 59.36% and from 30.21 to 68.55%, respectively. The significant negative relationship observed between the depth-integrated total NO₃[−] and NH₄⁺ uptake rates and small phytoplankton contributions indicates a possible decline in the DIN assimilation rates under small phytoplankton dominance. The results from the present study highlight the possibility of a reduction in the total DIN assimilation process in the East/Japan Sea when small phytoplankton dominate under strong thermal stratification due to sea surface warming. The present study's findings agree with the model projections, which suggested a decline in primary production in the global warming scenario.

Keywords: East/Japan Sea; small phytoplankton; nitrogen uptake; global warming

1. Introduction

The East/Japan Sea is a small, semi-enclosed marginal sea of the Pacific Ocean with boundaries shared by Korea, Japan, and Russia; however, there is no direct water mass exchange with Pacific Ocean waters [1–3]. The biological and physico-chemical processes in the East/Japan Sea are mainly controlled by the inward and outward flows of straits from neighboring seas. The northern part of the East/Japan Sea remains cold throughout the year due to the cold Liman current, whereas the southern part is warm due to the Tsushima warm current [1–3]. Subsequently, the East/Japan Sea is highly dynamic with frequent eddies, subpolar fronts, and coastal upwellings [4–6]. Thus, there are many hotspots in the East/Japan Sea that are critical areas of high primary production and subsequent high fish abundance [5–10].

Previous studies suggest that the East/Japan Sea possesses higher primary production ($246.8 \text{ g C m}^{-2} \text{ y}^{-1}$) rate than the open waters in various global oceans ($55 \text{ g C m}^{-2} \text{ y}^{-1}$ to $102 \text{ g C m}^{-2} \text{ y}^{-1}$) [10]. The higher primary production is likely because the East/Japan Sea receives or possesses sufficient nutrients in the euphotic water column through various physico-chemical processes. A significant set of spatio-temporal assessments of nutrient concentrations and physical processes has already been conducted in the East/Japan Sea. The influence of physicochemical properties on primary production in the East/Japan Sea has also been widely investigated in the recent past [5–10]. However, the assimilation of inorganic nutrients such as NO_3^- and NH_4^+ and the factors influencing nitrogen cycling in the East/Japan Sea is understudied. The uptake efficiency of inorganic nutrients by phytoplankton is one of the significant parts of sustainable primary production. Like primary production, inorganic nutrient uptake is also highly dependent on various factors, such as water temperature, salinity, nutrient availability, and chlorophyll *a* (Chl-*a*) concentration [11]. Apart from these parameters, DIN uptake is highly dependent on the types of phytoplankton species and their abundances [12].

Recent observational and modeling studies from various global oceans suggest that warming oceanic environments can alter phytoplankton size distribution [11–17]. These studies indicate that there will be an increase in small phytoplankton abundance and a considerable decrease in large phytoplankton population abundance. In addition to the rise in small phytoplankton abundance, small phytoplankton contributions toward total C and DIN uptakes were also reported to be enhanced [11–17].

The East/Japan Sea exhibits highly variable physico-chemical conditions introduced due to seasonal and climatological factors [10,17–21]. A significant number of investigations in the East/Japan Sea observed a remarkable change in small phytoplankton communities' biological characteristics at both local and broad scales due to environmental changes [10,22–24]. However, only a few studies have reported DIN uptake by small phytoplankton, particularly small phytoplankton's contribution in the East/Japan Sea [24]. It is reported that the sea surface temperature (SST) in the East/Japan Sea has drastically increased over several decades [18,19]. The increase in SST has resulted in alterations in the seasonal peak in abundance and community structure of phytoplankton and, subsequently, higher trophic levels in various global oceans (11–16). To understand the seasonal and spatial distributions of primary production, a set of carbon uptake measurements of total and small phytoplankton were conducted during 2012 and 2015 by our parallel study [24]. They reported that the phytoplankton contributions toward the total primary production were significantly lower than those toward the total biomass. Our study presents the DIN uptake rates by total and small phytoplankton during 2012 and 2015. The main objective was to estimate small and large phytoplankton contributions to the total DIN uptake rates. The present study also addresses the temporal and spatial distributions of the DIN uptake rates by phytoplankton in the East/Japan Sea, mainly in the Russian territory, an understudied region concerning DIN assimilation by small and large phytoplankton.

2. Materials and Methods

2.1. Study Area and Sampling

The NO_3^- and NH_4^+ uptake rate measurements were conducted in the East/Japan Sea at 10 (Stations M1, M4, M5, M11, M14, M17, 134-4, 134-6, 134-9, and 136-5) and 9 (Stations M6, M10, M12, M15, 134-2A, 134-5, 143, 149A, and 153A) stations on 13–29 October 2012 and 11 April–2 May 2015, respectively (Figure 1). The Korean-Russian joint cruises onboard R/V *Akademik Oparin* and R/V *Akademik M. A Lavertiev* assisted in the 2012 and 2015 expeditions, respectively. The water samples (10 L) for the various environmental parameters were collected from six light levels (100%, 50%, 30%, 12%, 5%, and 1%) using Niskin bottles attached to the CTD/rosette sampler. Nutrients (NO_3^- and NH_4^+) and Chl-*a* samples were collected from 6 light depths (100%, 50%, 30%, 12%, 5%, and 1% photosynthetically active radiation (PAR)). The underwater PAR sensor was not available during the present study; hence, the light depths were determined based on the euphotic depth derived from the Secchi disk depth. The formula (euphotic depth = $2.81 \times \text{Secchi depth}$) to determine euphotic depth was obtained from [25], where euphotic depth is the depth at which the light percentage decreases to a level of 1% of its surface level. Secchi disc depths were measured at all the productivity-measured stations during the cruise periods using a 30 cm black and white Secchi disk. Before sampling waters, the Secchi disc was lowered by attaching to a rope marked with 0.5 m intervals to disappear from view.

Figure 1. Sampling locations in the East/Japan Sea. The red stars indicate the sampling locations during 2012, and the yellow stars indicate the stations during the 2015 sampling.

Samples (0.5 L) for total Chl-*a* were prepared by filtering them through 25 mm diameter Whatman GF/F (nominal pore size = $0.7 \mu\text{m}$) filters using a low vacuum pressure at less than 5 inHg. For small phytoplankton, the Chl-*a* concentration samples were prepared by subsequent filtration of water samples (1.0 L) through 20- and $2\text{-}\mu\text{m}$ Nucleopore filters (47 mm) and $0.7\text{-}\mu\text{m}$ Whatman

GF/F filters (47 mm) [26,27]. Furthermore, the samples were wrapped in aluminum foil and kept frozen until analysis. The measurement of Chl-*a* concentrations was conducted at Pusan National University (home laboratory), Busan, South Korea, within one month after sampling. Before the measurements, the samples were extracted in 90% acetone, kept at 5 °C for 24 h. After the extraction, the samples were centrifuged at 3500 rpm. The absorbance of the clear supernatant was measured using a Turner Designs model 10-AU fluorometer calibrated with commercially purified Chl-*a* preparations. The method for chlorophyll-*a* calculation followed that of [28].

2.2. Nutrient Measurements

The water samples for nutrient concentrations were collected from the light depths corresponding to the DIN uptake sampling depths using Niskin samplers attached to the CTD device. After collection, the water samples (0.1 L) were filtered through Whatman GF/F filters at a low vacuum pressure less than 5 inHg. The filtrate was stored in airtight bottles at a temperature of −80 °C until the NO₃[−] and NH₄⁺ concentrations analysis. The frozen samples were thawed before analysis and analyzed at the National Institute of Fisheries Science, Korea, using an Auto analyzer (Quattro, Bran+Luebbe, Norderstedt, Germany) within two months after sampling.

2.3. DIN Uptake Rate Measurements

The DIN uptake estimations were conducted using the ¹³C-¹⁵N dual isotope tracer technique. Furthermore, the water samples from each light depth were transferred to polycarbonate incubation bottles (approximately 1.1 L) through a 200 µm net to remove zooplankton to avoid grazing during incubation. The sample bottles were covered with light filtering screens (LEE Filters; [24,29]) corresponding to each light depth. Furthermore, the samples for NO₃[−] and NH₄⁺ uptake were supplied with 98% enriched NO₃[−] (K¹⁵NO₃) and NH₄⁺ (¹⁵NH₄Cl) substrates, respectively, in addition to NaH¹³CO₃ [30–33], and were subjected to deck incubation for 4 hrs in large polycarbonate incubators with running surface seawater under natural light conditions. However, incubation times were longer, as long as 6 hrs, under cloudy weather conditions than under natural light conditions.

For total phytoplankton DIN uptake, 300 mL of each post-incubation sample was filtered onto pre-combusted (450 °C) GF/F filters (25 mm diameter and 0.7 µm pore size). For the small phytoplankton DIN uptake rates, the incubated samples underwent two sets of filtrations. Initially, 600 mL of each incubated sample was passed through 2 µm pore size Nucleopore filters. The filtrate was further filtered through 0.7 µm GF/F filters (25 mm diameter) to collect small phytoplankton. Both sample filters were immediately stored at −80 °C for preservation until mass spectrometric analysis at the stable isotope laboratory of the University of Alaska, Fairbanks, AL, USA. The experiments for small phytoplankton uptake rates were also conducted in parallel during 2012 and 2015; however, most of the data were not presentable for the 2012 sampling due to measurement-related issues. Hence, the small phytoplankton uptake rate data during 2015 are only discussed here. The calculations of DIN uptake rates were performed based on the equations given in Dugdale and Wilkerson [30]:

$$\text{Uptake rate } (\mu\text{mol N l}^{-1} \text{ h}^{-1}) = P * \Delta I_p / (T * (I_0 S_a + I_r S_t) / (S_a + S_t) - I_0) \quad (1)$$

where *P* represents the amount of particulate organic nitrogen in the post-incubation sample, and ΔI_p represents the increase in ¹⁵N atom% in the particulate N during incubation. *S_a* and *S_t* are ambient nutrients and added tracer concentrations, respectively. *I_r* and *I₀* are ¹⁵N atom% of added tracer and natural ¹⁵N atom%, and *T* is the incubation time in hours. This equation assumes no formation of nutrients during incubation, and, therefore, values obtained here are representatives of potential DIN uptake rates.

The measurements were performed two biological replicates, whereas technical replicates were performed only for the standards. Since the particulate organic content in the samples was very low, we could not keep the samples for technical replicates. The isotopic compositions were calculated with

reference to the international standards Pee Dee Belemnite (PDB) and air standards for carbon and nitrogen measurements, respectively. However, there were IAEA lab standards measured at regular intervals during the sample measurements. Uncertainties in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope measurements are 0.1 and 0.3 ‰, respectively. The statistical analysis for the present study used linear regression analysis and Student's *t*-test to evaluate the significance of relationships between various parameters.

3. Results and Discussion

The results from the present study explain spatial and temporal variations in concentrations of NO_3^- , NH_4^+ , Chl-*a*, and DIN uptake rates in the East/Japan Sea during fall in 2012 and early spring in 2015. The temperature distribution in the euphotic zone during 2012 varied between 16.1 and 24.9 °C. The SST during 2012 varied between 16.8 and 24.9 °C, which indicates that vertical mixing existed throughout the euphotic zone (Table 1). During the 2015 sampling, the water column temperatures were lower than those of 2012, and SST varied between 2.58 and 13.6 °C; however, the temperature-depth profile indicated significant vertical mixing (Table 1).

Table 1. Temperature data from the sampling stations during the fall in 2012 and early spring in 2015 sampling in the East/Japan Sea.

Year	Light Level (%)	Temperature (°C)									
		M1	M4	M5	M11	M14	M17	134-4	134-6	134-9	136-5
2012	100	24.9	24.9	24.2	22.2	18.1	19.2	18.4	16.8	21.5	20.1
	50	24.9		24.2		17.4		19.4	16.3	20.8	20.1
	30	23.3	25.2	22.9	21.5	17.5	18.7	18.7	16.1	20.6	19.7
	12	23.2		23.4		17.4		18.3	16.2	21.1	19.9
	5	23.3		23.5		17.1		18.1	16.5	20.8	19.9
	1	23.1	24.7	23.8	21.4	17.3	18.6	18.1	16.5	21.1	19.8
	Light Level (%)	Temperature (°C)									
		M6	M10	M12	M15	134-2A	134-5	143	149A	153-A	
2015	100	2.58	3.59	3.18	4.33	5.97	5.07	7.77	12.1	13.6	
	50	2.53	3.54	3.17	4.34	6.02	5.07	7.69	11.7	13.6	
	30	2.55	3.54	3.03	4.34	5.84	5.07	7.44	11.0	13.0	
	12	2.48	3.47	2.92	4.34	5.73	5.06	7.00	10.5	12.1	
	5	2.29	3.14	2.45	4.30	5.72	5.06	6.46	9.58	11.0	
	1	1.93	2.52	2.04	4.03	5.70	4.92	4.57	9.28	10.1	

The distribution of Chl-*a* concentrations in the various plankton size ranges obtained during the present study was previously reported in our parallel research work [24]. Lee et al. [24] noted that the total Chl-*a* concentrations ranged from 0.07 to 1.36 mg m⁻³ (mean ± SD = 0.49 ± 0.29 mg m⁻³) during the 2012 sampling effort. The Chl-*a* concentrations observed during the 2015 sampling effort (0.19–5.65 mg m⁻³; mean ± SD = 2.28 ± 1.47 mg m⁻³) were significantly higher than those of 2012 (data not shown in this paper) [24]. The Chl-*a* values did not show any remarkable spatial distribution during the 2012 sampling. In contrast, stations M15, 134-2A, 143, 149A, and 153-A showed higher Chl-*a* than at rest stations during the 2015 sampling. During both the sampling periods, the Chl-*a* concentrations were distributed evenly throughout the euphotic zone, and no signs of subsurface stratifications or subsurface chlorophyll maximum layers were observed. Nevertheless, there was considerable variability in the Chl-*a* concentrations during the 2015 sampling. Lee et al. [24] also reported, based on the Chl-*a* size distribution pattern, that small phytoplankton was more dominant in the northern region than in the southern part during the cruise in 2012 (mean ± SD = 49.7% ± 16.5%). However, during the 2015 sampling, large phytoplankton was observed to be prominent (47.7–72.5%) at the Russian coast (M15, 134-2A, 143, and 149A). Lee et al. [24] also found that the small phytoplankton contributions to primary productivity based on the Chl-*a* concentrations were similar to the previous

results observed in the East/Japan Sea during summer [34]. There were no significant correlations between primary productivity and Chl-*a* concentration observed by Lee et al. [24]. The results from the present study also could not find any significant relationship between the DIN assimilation rates and the Chl-*a* concentrations during either sampling period in the East/Japan Sea (figure not shown).

Nutrient availability is one of the critical factors that can influence potential DIN uptake [35]. Since no large rivers enter the East/Japan Sea, the significant sources of nutrients are upwelling and ocean currents [4–6]. The NO_3^- concentrations during 2012 varied between 0.004 and 11.65 μM , and, during 2015, they varied between 0.03 and 11.27 μM . Except for station 134-5, all other stations were seen with an elevated NO_3^- concentration at the bottom of the euphotic zone. Interestingly, station 134-5 showed the highest nutrient concentration among the rest of the stations. The elevated and uniformly distributed NO_3^- concentration at this station indicates the presence of upwelling (Figure 2b). Stations 143 and 153A also possessed uniform NO_3^- concentration till 5% light levels from the surface, which indicate the well-mixed water column. Another possible reason for low surface and elevated deep water column nutrient concentrations is the higher consumption of nutrients at surface layers than the dark bottom waters.

In general, the distribution pattern of the NO_3^- concentrations during both sampling periods showed a wide range compared to that of the NH_4^+ concentrations. During the 2012 sampling, station M11 showed a significantly high NH_4^+ concentration at the surface; however, the maximum NH_4^+ concentration was observed at station 134-4 at the 5% and 1% light levels (Figure 2a). NH_4^+ concentrations in the euphotic zone showed relatively high values during 2012, of which the stations, station 134-5, showed the highest value throughout the euphotic zone (Figure 2b).

Overall, the NH_4^+ concentrations during the 2012 sampling were higher than those during the 2015 sampling. There was no considerable difference in the NH_4^+ concentrations throughout the euphotic zone; however, stations (M1, M11, M14, and M17) exhibited considerably higher values near the bottom of the euphotic zone than the surface values during the 2012 sampling. The elevated NH_4^+ concentrations at the bottom of the euphotic zone were also seen during the 2015 sampling (stations; M6, M10, M12, 143, 149A, and 153A) (Figure 2b).

Figure 2. The depth profiles of the nutrient concentrations in the East/Japan Sea and the corresponding light levels for each station during 2012 (a) and 2015 (b). The station numbers are given on corresponding plots.

3.1. Spatiotemporal Distribution of DIN Uptake Rates

Figures 3 and 4 show the depth profiles of the total NO_3^- and NH_4^+ uptake rates estimated from the East/Japan Sea during 2012 and 2015. The results show that the total NO_3^- uptake rates during 2012 varied between 0.001 and 0.150 $\mu\text{mol N L}^{-1}\text{h}^{-1}$ (mean $\pm$ S.D. = 0.034 ± 0.033) and that the total NH_4^+ uptake rates ranged between 0.002 and 0.707 $\mu\text{mol N L}^{-1}\text{h}^{-1}$ (mean $\pm$ S.D. = 0.200 ± 0.158) (Figure 3a).

Figure 3. Depth profiles of total (a) NO_3^- and (b) NH_4^+ uptake rates in the East/Japan Sea during 2012.

Figure 4. Depth profiles of total and small NO_3^- and NH_4^+ uptake rates in the East/Japan Sea during 2015. (a,b) denote the total phytoplankton NO_3^- and NH_4^+ uptake rates, respectively, and the (c) and (d) denote the small phytoplankton NO_3^- and NH_4^+ uptake rates, respectively.

Compared to the other stations, stations M1, M4, and M11 showed relatively higher surface NO_3^- uptake rates, whereas surface NH_4^+ uptakes were higher at M4 and M11. The total NO_3^- uptake rates during 2015 (0.003–0.53; mean $\pm$ SD = $0.12 \pm 0.12 \mu\text{mol N L}^{-1}\text{h}^{-1}$) were relatively higher than those during 2012, with relatively high values at stations M10 and M12 (Figure 4). However, total NH_4^+ uptake rates (0.008–1.17; mean $\pm$ S.D. = $0.22 \pm 0.24 \mu\text{mol N L}^{-1}\text{h}^{-1}$) did not show considerable variation

between the 2012 and 2015 ranges except for station M10 (Figures 3b and 4b). The small NO_3^- and NH_4^+ uptake rates during 2015 ranged between 0.001 and 0.164 (mean $\pm$ S.D. = 0.033 ± 0.036) $\mu\text{mol N l}^{-1}\text{h}^{-1}$ and 0.010 and 0.304 (mean $\pm$ S.D. = 0.101 ± 0.073) $\mu\text{mol N l}^{-1}\text{h}^{-1}$, respectively.

Figures 5 and 6 show the spatial distribution of total depth-integrated NO_3^- and NH_4^+ uptake rates during 2012 and 2015 (total and small). The total depth-integrated uptake rates during 2012 (NO_3^- ; 0.33–2.50, NH_4^+ ; 1.68–22.4 $\text{mmol N m}^{-2}\text{h}^{-1}$) and 2015 (NO_3^- ; 0.15–2.36, NH_4^+ ; 0.39–4.57 $\text{mmol N m}^{-2}\text{h}^{-1}$; Table 2) samplings are given in Table 2. The mean $\pm$ S.D. of the depth-integrated total NO_3^- and NH_4^+ uptake rates were calculated to be 1.19 ± 0.699 and 8.45 ± 6.38 $\text{mmol N m}^{-2}\text{h}^{-1}$ during 2012 and 0.783 ± 0.675 and 1.47 ± 1.30 $\text{mmol N m}^{-2}\text{h}^{-1}$, respectively, during 2015.

Figure 5. Depth integrated total NO_3^- and NH_4^+ uptake rates in the East/Japan Sea during 2012.

Figure 6. The depth-integrated total and small phytoplankton NO_3^- and NH_4^+ uptake rates in the East/Japan Sea during 2015.

Table 2. The depth-integrated total and small phytoplankton uptake rates were obtained during the present study, where uptake rates were given in mmol N m⁻² h⁻¹.

Year	Stn. Name	Latitude (°E)	Longitude (°N)	Euphotic Depth	Total NO ₃ ⁻ Uptake Rate	Small NO ₃ ⁻ Uptake Rate	Contribution (%)	Total NH ₄ ⁺ Uptake Rate	Small NH ₄ ⁺ Uptake Rate	Contribution (%)
2012	M1	37.002	129.999	27	0.750	-	-	6.978	-	-
	M4	36.990	131.232	60	1.214	-	-	16.66	-	-
	M5	37.349	131.459	41	0.850	-	-	6.587	-	-
	M11	40.001	132.333	54	2.365	-	-	22.38	-	-
	M14	41.501	132.337	43	0.726	-	-	8.645	-	-
	M17	42.340	132.335	43	1.142	-	-	8.578	-	-
	134-4	42.335	133.667	46	0.333	-	-	3.457	-	-
	134-6	41.831	134.003	35	2.498	-	-	1.676	-	-
	134-9	40.838	133.997	35	1.036	-	-	5.586	-	-
	136-5	40.653	136.264	54	1.023	-	-	3.957	-	-
2015	M6	37.661	131.944	35	0.873	0.377	43.18	2.381	1.184	49.72
	M10	39.524	132.339	35	1.197	0.495	41.32	4.570	1.381	30.21
	M12	40.513	132.331	33	0.633	0.220	34.71	1.146	0.641	55.93
	M15	41.832	132.334	41	2.363	0.242	10.24	1.351	0.823	60.94
	134-2A	42.255	134.011	43	0.653	0.127	19.39	0.586	0.323	55.20
	134-5	40.990	134.005	43	0.145	0.086	59.36	0.385	0.264	68.55
	143	43.000	135.657	35	0.362	0.089	24.61	0.803	0.456	56.82
	149A	42.518	136.989	35	0.599	0.131	21.86	1.232	0.570	46.25
	153A	44.567	137.673	30	0.227	0.087	38.49	0.792	0.508	64.13

3.2. Factors Influencing DIN Uptake Rates in the East/Japan Sea

Various factors potentially regulate DIN assimilation in marine environments, such as temperature, nutrient availability, and chlorophyll concentrations. The East/Japan Sea is a highly dynamic water body influenced by various water masses and is pivotal for eddies and upwellings [2]. Hence, it is essential to identify the various factors governing the DIN assimilation process in this ecologically crucial marine body. Our study results suggest that the depth-integrated total NO_3^- uptake rates during 2012 were relatively higher than those during 2015, except for station M15. Similarly, depth-integrated NH_4^+ uptake rates during 2015 showed relatively lower values than those during 2012 (Figure 6). One possible explanation for this could be the higher water column temperature during the autumn in the 2012 sampling period than during the spring in 2015 sampling periods due to the differences in the seasons (Table 1). However, most of the NO_3^- uptake rates obtained from the individual light depth analysis during 2012 were relatively lower than those during 2015. Additionally, the individual NO_3^- and NH_4^+ uptake rates from each light depth did not significantly correlate with temperature based on the *t*-test (Figure not shown). Thus, the possibility of temperature as a critical control for DIN uptake rates in the East/Japan Sea during the present study period cannot be projected.

Apart from temperature, nutrient availability is also a critical factor that can control primary production and DIN uptake rates. Since various current systems and localized upwellings govern the East/Japan Sea, a deep understanding of nutrient dynamics is a must to identify the factors controlling DIN assimilation rates. Park et al. [3] reported that the frontal zone between the warm Tsushima current and the nutrient-rich cold polar current often serves as a hub for enhanced primary production. Consequently, this zone can reflect the DIN assimilation rates; hence, our present study also reported high depth-integrated NO_3^- and NH_4^+ uptake rates (2012: Stations; M15 and M10 and 2015: Stations; M11 and 134-6; Figures 5 and 6).

Despite having lower nutrient concentrations (Figure 2), the surface waters showed higher NO_3^- and NH_4^+ uptake rates than deep waters. The NO_3^- uptake rates had higher surface values than those in the deeper waters except for stations 134-6 (Figure 3a). The insignificant linear correlations between the nutrient concentrations and their respective substrate uptake rates (figure not shown) suggest that the higher depth-integrated NO_3^- and NH_4^+ uptake rates during the 2012 sampling than during the 2015 sampling cannot be explained by nutrient influence. It suggests that the DIN assimilation rates in the East/Japan Sea during our sampling periods were primarily dependent on light conditions rather than nutrient availability. Our parallel study also reported similar results, where they reported that high primary production rates were mainly found at the surface layers [24].

Another possible reason for the higher depth-integrated NO_3^- and NH_4^+ values in the previous study than the current study could be the depth of the euphotic zone selected for sampling. The euphotic depths observed were different at almost all stations, ranging from 27 to 60 m and 30 to 43 m during 2012 and 2015, respectively. There was no significant correlation observed between euphotic depths and depth-integrated NO_3^- and NH_4^+ uptake rates during our present study based on the *t*-test. For example, stations M11 ($2.37 \text{ mmol N m}^{-2}\text{h}^{-1}$) and 134-6 ($2.50 \text{ mmol N m}^{-2}\text{h}^{-1}$) during 2012 were observed with higher depth-integrated NO_3^- uptake rates; their euphotic depths were 54 and 35 m, respectively. However, the station with the deeper euphotic depth (M4; 60 m) than the rest was observed with a significantly lower NO_3^- uptake rate. Similarly, the station (134-2A) with the deeper euphotic depth than the rest during the 2015 sampling also showed a lower depth-integrated NO_3^- uptake rate ($0.65 \text{ mmol N m}^{-2}\text{h}^{-1}$) (Table 2). Overall, depth-integrated NH_4^+ uptake rates also did not show any significant relationship with euphotic depth. Hence, the variation in the euphotic depths is not a critical factor in determining the NO_3^- and NH_4^+ uptake pattern in the present study area.

3.3. Turnover Times of Nutrients

The turnover time for any nutrient substrate is a significant tool to understand how fast a substrate is consumed during its assimilation when there is no external inflow or outflow of that substrate.

Technically, the turnover time estimation is performed by dividing the substrate concentrations with the corresponding uptake rates. Figures 7 and 8 show the NO_3^- and NH_4^+ turnover times during the 2012 and 2015 sampling periods. The turnover times for NO_3^- and NH_4^+ substrates during the 2012 sampling, when the total phytoplankton communities are the consumers, show that the consumption of available nutrients in the euphotic zone is very fast till 12% light levels. However, the turnover times for NO_3^- were distinctively longer (400–3000 h) than NH_4^+ at depths close to euphotic depth. The low nutrient availability under sufficient light conditions can be the possible reason for the fast nutrient consumption at the surface waters compared to deeper waters with high nutrient concentrations and low light availability. The poor light availability can potentially suppress NO_3^- uptake, whereas NH_4^+ uptake can still be performed, which leads to faster turnover for NH_4^+ at the deep waters than that of NO_3^- .

Figure 7. Depth profiles of (a) NO_3^- and (b) NH_4^+ turnover time by total phytoplankton in the East/Japan Sea during 2012.

Similarly, 2015 sampling also appeared with a faster NH_4^+ turnover time faster than that of NO_3^- both at the water column and bottom of the euphotic zone. The differences in turnover times can be due to the relative preference for NH_4^+ over NO_3^- by the phytoplankton. However, the higher supply of the NO_3^- substrate in the deep waters than the NH_4^+ substrate can also be one reason for longer turnover times for NO_3^- at the deep waters. The results from a set of experiments in a tropical eutrophic estuary in India showed rapid turnover times (3–232 h for NH_4^+ and 7–2419 h for NO_3^-) by the total phytoplankton communities for the DIN substrates despite high nutrient concentrations [34]. On the other note, the turnover times in the Arctic Ocean were found to be long despite the low nutrient concentrations [34]. It is also reported that the inhibition of phytoplankton communities for NO_3^- uptake under sufficient NH_4^+ concentrations is a widely known phenomenon as they prefer the lighter NH_4^+ over the heavier NO_3^- substrate [34–36]. Figure 8b,d show the NO_3^- and NH_4^+ turnover times during 2015 for small phytoplankton communities. Except for the stations 134-2A and 134-5, the small and total phytoplankton turnover times were observed to have a similar range. These two stations showed significantly long turnover times throughout the euphotic zone. The higher NO_3^- concentrations and lower NO_3^- uptake rates by the small phytoplankton communities in the stations 134-2A and 134-5 compared to other stations might be the possible reason. However, such a difference was not observed in the case of NH_4^+ turnover times.

Figure 8. Depth profiles of turnover times of NO_3^- and NH_4^+ substrates by total and small phytoplankton in the East/Japan Sea during 2015. (a,b) denote the NO_3^- turnover times by total and small phytoplankton, respectively, and (c,d) denote the NH_4^+ turnover times by total and small phytoplankton, respectively.

3.4. Contribution of Small Phytoplankton to Total Nitrogen Uptake

Experimental and theoretical investigations on small phytoplankton communities have reported that the small phytoplankton possesses higher nutrient uptake rates per unit of biomass and lower half-saturation constants than large phytoplankton due to their higher surface area to volume ratios [37–39]. Therefore, the lower minimum cellular metabolic requirement assists small phytoplankton to survive in nutrient-poor environments [40,41]. Several investigations were conducted globally on this topic, and they reported that the small phytoplankton contribution to the total annual carbon and DIN assimilations generally varied between 20 and 65% [22–24,42,43]. The MODIS-derived data in the Ulleung Basin in the East/Japan Sea from 2003 to 2012 showed that the annual contribution of small phytoplankton communities varies between 19.6% and 28.4%, with an average of 23.6% (SD = $\pm 8.1\%$) [23], which suggests that the large phytoplankton communities are the major contributors to primary production in the Ulleung Basin.

Basin-wide studies suggest that small phytoplankton contributions mainly control primary production in the Japan Basin and Yamato Basin at 85% and 82%, respectively. In contrast, the Ulleung Basin has a smaller phytoplankton contribution (35%) [34]. Lee et al. [24] (our parallel study) also reported a similar range of small phytoplankton contributions to the total primary production in the Ulleung Basin during 2015. Lee et al. [24] also reported that the contributions of small phytoplankton (<2 μm) to the total carbon uptake rates have significant spatial and temporal variations. They found remarkable differences in small phytoplankton contributions during 2012 (60.6%) and 2015

(34.1%) samplings. Unfortunately, the data for small phytoplankton uptake is unreliable due to lack of enough material for the stable isotope measurements; the contributions to the total NO_3^- and NH_4^+ uptake rates during 2015 were only taken for the present discussion (Table 2).

The contributions of small phytoplankton to the total NO_3^- and NH_4^+ uptake rates during 2015 were 32.6 and 54.2%, respectively, which indicate that small phytoplankton is not the dominant contributor to the total NO_3^- assimilation in the East/Japan Sea. The sampling period during 2015 was before the spring bloom, which is reported to be dominated by large cell diatoms [9,24]. Interestingly, small phytoplankton cells significantly contributed to the NH_4^+ uptake rates in comparison to large phytoplankton cells. Previous studies have reported a clear preference for NO_3^- by large phytoplankton cells [29,44–47], where small cells depend primarily on regenerated nitrogen, such as NH_4^+ , e.g., [36,45–47]. However, large cell phytoplankton is highly dependent on the threshold availability of the NO_3^- substrate [48,49], where large cell dominance was observed when NO_3^- concentrations were above 3.5 μM . A similar dependency of large cell phytoplankton on NO_3^- availability was reported by Tamigneaux et al. [50] in nearshore Baie des Chaleurs, Canada. Interestingly, Wilkerson et al. [51] observed much higher threshold NO_3^- concentrations (10–12 μM) than those above, where the percent contributions by the large cells to PON and Chl-*a* were always more than 50% [52]. In contrast, the present study showed that the station with the highest NO_3^- concentrations (134–5; close to 10 μM) and lowest NH_4^+ concentrations showed high contributions (59% and 69%) of small phytoplankton to the total NO_3^- and NH_4^+ uptake rates, respectively.

Notably, the high NO_3^- concentrations in oligotrophic waters primarily depend on upwelling that brings NO_3^- rich bottom waters to the surface. A few studies were conducted to investigate the alteration in phytoplankton species composition in response to upwelled cold water in the southwestern East/Japan Sea [52]. Kim et al. [52] also reported that the phytoplankton abundance and the proportion of large phytoplankton were higher when there was upwelling-induced nutrient-rich cold water than when there was strong vertical stratification. In general, these studies point toward the influence of nutrient-rich upwelled bottom water on the abundance and composition of phytoplankton communities in the East/Japan Sea. However, a few investigations are available on the C and DIN uptake rates by small and large phytoplankton and their response to vertical mixing due to upwelling in the East/Japan Sea [24]. The present study did not estimate the contribution of bacteria to the total DIN uptake rates; therefore, the role of bacteria in DIN assimilation and dissolved organic nitrogen as a nitrogen source could not be assessed.

3.5. Impact of Small Phytoplankton on the Total DIN Assimilation Rates

Observational and model studies on the impact of increasing small phytoplankton uptake suggest that primary productivity will decrease if small phytoplankton becomes the predominant species [24,53–56]. Since our study did not include species identification and abundance, the discussion on species diversity is limited in this regard. However, a few studies recorded the species identification and abundance from the East/Japan Sea, although the study regions are slightly different from the present study [34,56]. The reported studies suggest that the overall dominant groups could be diatoms with some large spatial variations in our study area during spring, summer, and fall seasons [57]. Prymenesiophytes were the second dominant group during spring and fall seasons, and cyanobacterial abundance was very low over seasons [57].

Several studies reported that, when large phytoplankton communities dominate, the primary productivity obtained will be higher than when small phytoplankton dominate [58–61]. Similarly, Lee et al. [24] found a significant negative relationship between total primary productivity and the small phytoplankton contribution in the East/Japan Sea. However, few studies have evaluated the influence of small phytoplankton contributions on total DIN uptake rates. Previously, Wilkerson et al. [51] also suggested that, in comparison to the dominance of small cells (<5 μm), the dominance of large phytoplankton cells > 5 μm can potentially lead to higher C and DIN assimilation rates. The present study also observed a significant negative relationship ($R^2 = 0.77$, *t*-test; $p < 0.01$) of total NH_4^+

uptake rates with the increasing small phytoplankton contribution. The total NO_3^- uptake rates also showed a negative correlation of $R^2 = 0.30$ with a p -value < 0.05 (t -test) with the contribution of small phytoplankton (Figure 9). The possible reason for the relatively high significance level in the correlation between the small phytoplankton contribution and the total NH_4^+ than that with NO_3^- uptake rates during the present study could have been due to the dominance of NH_4^+ uptake since NH_4^+ is the most preferred substrate [30].

Figure 9. Relationships between small phytoplankton contributions to $\text{NO}_3^-/\text{NH}_4^+$ uptake rates and total $\text{NO}_3^-/\text{NH}_4^+$ uptake rates in the northeastern East/Japan Sea during 2015. The green and magenta dots indicate depth-integrated total NO_3^- and NH_4^+ uptake rates, respectively. The significance levels for the correlations are obtained from the t -test.

Although the small phytoplankton communities prefer NH_4^+ as the significant substrate, the high contributions of small phytoplankton to the total NH_4^+ uptake rates did not appear to be a supporting element in sustaining the high total NH_4^+ uptake rates in the East/Japan Sea during the present study. The results suggest that, if the small phytoplankton species are the dominant species in the future due to global warming, then the East/Japan Sea may have decreased NH_4^+ assimilation rate compared to the present time. The possibility of a decrease in NO_3^- uptake under a small phytoplankton-dominant environment in the East/Japan Sea cannot be neglected. The main consumers of the NO_3^- substrate are large cell phytoplankton such as diatoms. Specifically, the water column stratification introduced by global warming can lead to small cell phytoplankton dominance. The abundance and uptake of large cell phytoplankton may reduce as the availability of NO_3^- reduce in the absence of upwelling under thermal stratification [10,15,62]. Additionally, small cell phytoplankton preferentially assimilates regenerated nutrient forms such as NH_4^+ , whose availability is not primarily dependent on upwelling. Therefore, total DIN assimilation can be widely impacted by its rates, processes, and fluxes under ongoing warming conditions in the East/Japan Sea.

In general, studies on the abundance, size, and assimilation rates of phytoplankton communities under *in situ* and simulated conditions suggest that global warming phenomena can potentially reduce DIN assimilation rates [10,62]. These studies also suggest that the uptake efficiency of small cell phytoplankton is relatively lower than that of large cell phytoplankton. Hence, there will be a decrease in total primary production and DIN assimilation rates in the global oceans if a condition of small phytoplankton dominance occurs [11]. Since the functioning of the marine food web is entirely dependent on the capability of phytoplankton communities to assimilate carbon and DIN, any alteration that affects their efficiency would significantly redefine the food distribution in the

entire ecosystem. Therefore, it is highly essential to record factors influencing phytoplankton size, abundance, and assimilation rates in global oceans.

4. Conclusions

The present study estimated the DIN uptake rates by total and small phytoplankton ($<2\ \mu\text{m}$) in the East/Japan Sea as a part of the Korea–Russia joint expeditions during 2012 and 2015. The study used the dual isotopic tracer technique to estimate the DIN uptake rates. The primary objectives were to understand the spatiotemporal variations in the DIN assimilation rates by total and small phytoplankton and their contribution to the total DIN uptake rates. The study also aimed to understand the various environmental factors that play vital roles in governing nitrogen dynamics in the East/Japan Sea. There were no significant correlations between environmental parameters and the DIN uptake rates observed during the present study; however, the higher DIN uptake rates in the surface layers than in the bottom waters suggest the potential influence of light conditions. The results show that 10.24 to 59.36% and 30.21 to 68.55% of the total NO_3^- and NH_4^+ uptake rates are contributed by small phytoplankton. The results from the present study found that there is a negative correlation between the depth-integrated NH_4^+ uptake rates and the small phytoplankton contribution, which suggests the adverse effect of increasing the small phytoplankton population as a result of global warming on the nitrogen assimilation process. In general, the results from the present study provide significant information on the potential for reduced DIN assimilation conditions in the East/Japan Sea if the small phytoplankton abundance increases as a result of global warming. Since the East/Japan Sea is an economically and ecologically important ecosystem, it is highly relevant to have a set of monitoring experiments on its potential to assimilate carbon and nitrogen under varying environmental conditions.

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