Availability of particulate Fe to phytoplankton in the Sea of Okhotsk

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We investigated availability of particulate iron to marine phytoplankton.

Suspended particulate matter (SPM) collected from the nepheloid layer was used.

Iron in the SPM was available which provided healthy growth of phytoplankton.

Such particulate iron supports biological production around the Kuril Islands.
ABSTRACT

In a shipboard incubation study, we investigated the availability of particulate iron (Fe) to an Fe-starved phytoplankton community through the addition of suspended particulate matter (SPM; > 1 µm) collected from the nepheloid layer in the coastal region of the Sea of Okhotsk. Surface seawater incubations were also conducted at three stations around the Bussol' Strait where the SPM that possibly originated from the coastal nepheloid layer could emerge to the surface mixed layer due to strong vertical mixing around the Kuril Islands. In the SPM-added experiment, the growth rate of phytoplankton was significantly enhanced by the addition of SPM compared to the unamended control. This result clearly indicates that Fe in the SPM collected from the nepheloid layer was available to marine phytoplankton. In addition, phytoplankton particularly coastal diatoms in the nepheloid layer were viable and showed healthy growth. In the surface seawater incubation experiments, phytoplankton growth and nutrient drawdown in unamended control conditions in two of the three stations may be supported by Fe from the particulate fraction (> 0.22 µm), as estimated from stoichiometric calculation. We suggest that the bioavailable particulate Fe in SPM of the coastal region supports biological production and nutrient drawdown even after the depletion of dissolved Fe around the Kuril Islands, where strong vertical mixing occurs.
1. Introduction

Iron (Fe) is the most important micronutrient for marine phytoplankton growth, because of its key role in processes such as photosynthesis and nitrate and nitrite assimilation (Raven et al., 1999). However, the thermodynamically stable oxidation state of Fe in oxic surface seawater is predominantly Fe(III), which has an extremely low solubility (Stumm and Morgan, 1996; Kuma et al., 1996). The Fe in the North Pacific Ocean is mainly derived from continental sources, such as eolian dust, riverine input, and sedimentary Fe from the continental shelf to coastal and oceanic waters (Johnson et al., 1999, 2005; Jickells et al., 2005; Lohan and Bruland, 2006; Nishioka et al., 2007). Moore and Braucher (2008) estimated the global-scale Fe cycle using the Biogeochemical Elemental Cycling ocean model and found that the sedimentary source of Fe contributes as much as 70% of dissolved Fe (D-Fe) in pools in the surface of the northwestern North Pacific. Previous studies indicated that the high Fe water mass possibly related to the sedimentary source would be transported from the western coast of the Sea of Okhotsk to the western North Pacific region via the Kurile Straits (Nishioka et al., 2007; Misumi et al., 2011).

In the Sea of Okhotsk, dense shelf water (DSW) is produced by brine rejection due to sea ice formation during winter, which originates in the region of the Siberian and Sakhalin continental shelves (Shcherbina et al., 2004; Matsuda et al., 2009). The DSW entrains continental sediments during the formation processes and is transported southward along the Sakhalin coast at an intermediate depth (~200–500-m), forming the Okhotsk Sea intermediate water (OSIW) (Fukamachi et al., 2004). Nakatsuka et al. (2004) reported that DSW has high particulate organic matter derived from coastal sediment, which should have high particulate Fe concentrations (e.g., Landing and Bruland, 1987; Elrod et al., 2008). The OSIW flows further southeastward and outflows into the western subarctic Pacific Ocean, through the Kurile Straits with strong vertical diapycnal mixing around the Kurile Islands (Nakamura and Awaji, 2004; Ito et al., 2010, 2011). The Bussol' Strait is a key channel with respect to the exchange of seawater between the Sea of Okhotsk and the Oyashio region (e.g., Yasuda et al., 2002) because the channel is deepest at the Kuril Straits. It can be assumed that the particles in the DSW advect into the surface of the Okhotsk and Oyashio waters via strong water mixing with the OSIW. Intrusion of such bio-active particles into the surface seawater would
affect and play an important role in phytoplankton communities around the region. However, little attention has been paid to the importance of particulate matter on the dynamics of surface phytoplankton communities.

In general, one of the most bioavailable Fe species for photolithoautotrophic phytoplankton is dissolved inorganic Fe (Fe’) (Anderson and Morel, 1982; Morel et al., 2008). There are apparent exceptions to this model in that organic Fe-ligand complexes could be indirectly and/or directly utilized by the cells (Hutchins et al., 1999a; Maldonado and Price, 2001; Shaked et al., 2005; Kustka et al., 2007). In nature, almost all of the dissolved Fe(III) should be bound to organic ligands, which have high and low binding affinities for Fe (Rue and Bruland, 1995). A recent study demonstrated that saccharides are responsible for binding Fe with low affinity in the colloidal size fraction (Hassler et al., 2011). The bioavailability of Fe-saccharide complexes is higher than for Fe binding with high-affinity ligands for eukaryotic phytoplankton (Hassler and Schoemann, 2009; Hassler et al., 2011). There is increasing evidence that the bioavailability of D-Fe should be different depending on the binding affinity of the ligand or differences in the phytoplankton groups and their habitats (Hutchins et al., 1999b; Kuma et al., 2000; Hassler and Schoemann, 2009; Strzepek et al., 2011).

The bioavailability of D-Fe species including the colloidal fraction (between < 200 kDa or 0.025 µm to 0.2 or 0.45 µm; e.g., Nishioka and Takeda, 2000; Nishioka et al., 2001) has been relatively well examined, although the availability of particulate Fe (P-Fe) is largely unknown. According to laboratory experiments (Kuma and Matsunaga, 1995; Yoshida et al., 2006), the surface reactivity or crystalline structure of solid inorganic Fe oxyhydroxide controls the supply of bioavailable D-Fe species for coastal diatoms. In natural conditions, Elrod et al. (2008) and Sugie et al. (2010a) reported that surface chlorophyll-a (Chl-a) concentrations have significant positive correlations with total dissolvable Fe concentrations (T-Fe), suggesting that particulate Fe supports a substantial portion of the ecosystem’s Fe requirements. Using Chl-a and D- and P-Fe concentrations, Nakayama et al. (2010) calculated stoichiometrically that the phytoplankton biomass of intensive spring diatom blooms (> 10 µg Chl-a L⁻¹) in the Oyashio region of the western subarctic Pacific region can be achieved by the utilization of D-Fe and also Fe from the particulate phase. Furthermore, Fitzwater et al.
(2003) measured the T-Fe concentrations in the surface waters, which originated from the benthic boundary layer of the continental shelves during upwelling events that caused a substantial part of the Fe in the particulate fraction to be chemically labile (leachable by 25% acetic acid for 2 h). They also suggested that a small but substantial part of labile particulate Fe contributes to the bloom formation around the upwelling plumes (Fitzwater et al., 2003). Although those previous studies recognize the importance of particulate Fe as a source of bioavailable Fe, the availability of Fe in natural marine particles has not been examined before, except for Fe coming from aeolian dust (e.g., Payten et al., 2009).

In this study, we conducted two types of bioassay experiments to examine the availability of P-Fe for the growth of phytoplankton. First, we examined the availability of Fe associated with marine suspended particulate matters for an Oyashio phytoplankton community through the addition of particulate matters collected in the DSW (SPM\textsubscript{DSW}). Second, surface phytoplankton communities at three stations around the Bussol' Strait were incubated to investigate the importance of P-Fe for the growth of phytoplankton where SPM\textsubscript{DSW} could appear in the surface mixed layer. The availability of the P-Fe in the surface waters was evaluated by stoichiometric calculations using Chl-\(a\) concentration increases and nutrient drawdown data with reported Fe:C and Fe:Chl-\(a\) values. Our results provide the first direct evidence of the availability of Fe in marine suspended particulate matters for natural phytoplankton communities.

2. Methods

2.1. SPM\textsubscript{DSW} addition experiment

Phytoplankton incubations were performed aboard the R/V Professor Khromov during August to September 2007 (Kh-07 cruise). Natural phytoplankton communities used in incubation experiments for the bioavailability of Fe in the SPM\textsubscript{DSW} were collected in the Oyashio region (Stn Oy: 46°00'N 152°30'E; Fig. 1) using an acid-cleaned Teflon-coated 10 L Niskin X sampling bottle (General Oceani cs) attached to a CTD-carousel multi-sampling system (Nishioka et al., 2007, 2011). Hydrographic data (salinity, temperature, and depth) and transmittance were obtained using a CTD (Sea-Bird, Model 9-puls) and transmissometers (Wet Labs, C-Star). Seawater for a natural phytoplankton stock was sieved with 100 µm
acid-cleaned Teflon-mesh to eliminate mesozooplankton and nutrients were added [27 µmol L\(^{-1}\) NO\(_3\), 2 µmol L\(^{-1}\) PO\(_4\) and 47 µmol L\(^{-1}\) Si(OH)\(_4\)] to induce probable Fe-starved conditions for the phytoplankton community before use. Nutrient stock solutions were passed through Chelex-100 ion-exchange resin (Bio-rad) to remove trace metals (Morel et al., 1979). The natural phytoplankton stock was incubated for 6 days in an on-deck incubator. The temperature of the incubator was maintained at near-ambient sea surface temperature (~10°C) by a surface seawater flow-through system. The incubator was covered with a single layer of neutral density screening to achieve approximately 30% of surface irradiance.

The SPM\(_{DSW}\) was collected at 320 m depth east of Sakhalin (Stn DSW: 52°15′N, 144°35′E; Fig. 1) using an *in situ* filtration system (McLane Research Laboratories Inc.). The DSW mass was detected by the anomalous values in low temperature (< ~0°C) with low transmittance around the sigma-\(\tau\) = 26.8 kg m\(^{-3}\) layer as reported previously (Fukamachi et al., 2004; Shcherbina et al., 2004). Approximately 454.5 L of DSW was filtered through a 1.0 µm acid-washed polycarbonate filter (Nuclepore). The water on the filter was flushed by vacuum, and the filter was frozen at ~20°C for ~3 hours. Prior to the experiment, the SPM\(_{DSW}\) on the filter was suspended in 500 mL of 0.2 µm filtered seawater (FSW) which was collected from a depth of 200 m in the Oyashio region during the HK07-1 cruise aboard the R/V Hokko-Maru (January 2007). The FSW contained 40 µmol L\(^{-1}\) NO\(_3^{-}\) plus NO\(_2\), 3.0 µmol L\(^{-1}\) PO\(_4\), 63 µmol L\(^{-1}\) Si(OH)\(_4\) and 0.88 nmol L\(^{-1}\) D-Fe. Six treatments were prepared as follows (Table 1): (1) control: 270 mL of FSW plus 45 mL of Fe-starved natural phytoplankton stock culture prepared above; (2) Fe-added: added inorganic Fe to the control condition to make a final concentration of 5 nmol L\(^{-1}\); (3) DFB (desferrioxamine B): amended with 1 µmol L\(^{-1}\) DFB (Sigma Chem. Co. Ltd.) to the control condition to prevent Fe uptake from the ambient seawater as achieved previously (Wells, 1999; Iwade et al., 2006; Sugie et al. 2010b, 2011); (4) SPM\(_{DSW}\): 250 mL of FSW plus 45 mL of Fe-starved phytoplankton stock plus 20 mL of resuspended SPM\(_{DSW}\) solution as prepared above, i.e., SPM\(_{DSW}\) was concentrated 63.5-fold vs. *in situ* condition; (5) SPM\(_{DSW-Phy}\): 295 mL of FSW plus 20 mL of resuspended SPM\(_{DSW}\) solution without the addition of the Fe-starved phytoplankton stock; and (6) DSW\(_{raw}\): 315 mL of prescreened DSW using 100 µm acid-washed Teflon-mesh collected from 247 m at Stn DSW (Table 1). The SPM\(_{DSW-Phy}\) treatment was intended to examine the viability of the
phytoplankton in the SPM\textsubscript{DSW}, which was once frozen. The DSW\textsubscript{raw} was set to elucidate the viability of phytoplankton in the DSW mass, which mimics their appearance to the sunlit surface waters by tidally induced and strong vertical mixing, such as that observed during winter (Nakamura and Awaji, 2004).

The treatments were incubated in an on-deck incubator as described above. The treatments were prepared in 9-replicate 320 mL polycarbonate bottles in addition to the DSW\textsubscript{raw} treatment, which was prepared in 4-replicate. Three bottles were sacrificed and measured after 1, 3 and 5 days for analysis of macronutrients and Chl-\textsubscript{a} concentrations except for the samples of the DSW\textsubscript{raw} treatment, which were obtained after 3 and 5 days sacrificing two bottles for each day. The concentrations of total dissolvable Fe (unfiltered; T-Fe) and background Fe in the FSW were measured at the beginning of the experiment. The samples for diatom cell densities and species compositions were collected at 0 and 5 days of incubation. Although the abundance of the small flagellates can be expected to be high in the incubation bottles, the large number of particles in the SPM\textsubscript{DSW} and SPM\textsubscript{DSW−Phy} treatments obscured the counts of the flagellates. Therefore, we measured only the diatom abundance using a light microscope. The experimental advantage of counting only diatoms to examine the availability of Fe in the SPM\textsubscript{DSW} is that the diatoms are photolithoautotrophs, but some mixotrophic flagellates are capable of phagotrophic acquisition of P-Fe (Tortell et al., 1996; Maranger et al., 1998). Therefore, we can examine the availability of Fe potentially dissociated from the SPM\textsubscript{DSW}. The Chl-\textsubscript{a} specific growth rate was calculated from the linear regression between the time (day) and the natural log of the Chl-\textsubscript{a} concentrations. For the Chl-\textsubscript{a} specific growth rate calculations, the data from the beginning to the end of the incubations were used for the control, DFB, SPM\textsubscript{DSW−Phy} and DSW\textsubscript{raw} treatments and those from day 1 to the end of the incubations were used for the Fe-added and SPM\textsubscript{DSW} treatments. The growth rate in terms of cell numbers was calculated as follows: growth rate = \(\frac{\ln(N_{d5} / N_{d0})}{5}\), where \(N_{d5}\) and \(N_{d0}\) represent cell numbers at day 5 and the start of the experiment, respectively.

2.2. Surface phytoplankton incubations
Seawater for incubation was collected at three sampling sites located at Stn Oy, in the Bussol' Strait (Stn BS: 46°30'N 151°23.2'E) and in the Sea of Okhotsk (Stn Ok: 47°30'N 150°20.45'E) (Fig. 1). Seawater samples were collected from 10 m depth using acid-cleaned Teflon-coated 10 L Niskin X sampling bottles. Seawater for experiments was sieved with 200 µm acid-cleaned Teflon-mesh to eliminate large mesozooplankton such as *Neocalanus* spp., which dominate in the western subarctic Pacific region (Ikeda et al., 2008). The prescreened seawater sample was homogenized in an acid-washed 20 L polycarbonate tank and then dispensed into acid-cleaned 320 mL polycarbonate bottles. The three treatments were conducted as follows: (1) unamended control; (2) Fe-added media with the addition of 5 nmol L\(^{-1}\) Fe; and (3) Fe-limited media with the addition of 1 µmol L\(^{-1}\) DFB. Fifteen bottles for each treatment were prepared and incubated in an on-deck incubator as described above. Three bottles were sacrificed at 1, 3, 5, 7 and 10 day intervals to measure the Chl-\(a\) concentration, phytoplankton abundance and macronutrient concentrations. The samples for the beginning of the experiment were taken from the residue in the 20 L tank. The Chl-\(a\) specific growth rate was calculated as described above using the Chl-\(a\) data from the beginning of the experiment for the control and the Fe-limited media, and from day 1 for the Fe-added media to the day before the nutrient depletions. The growth rate in terms of cell numbers was calculated using the same method as described in the SPM\(_{DSW}\) addition experiment.

2.3. Sample analysis

For measuring the Chl-\(a\) concentrations, the samples were filtered onto GF/F filters and measured with a Turner Design 10-AU fluorometer (Welschmeyer, 1994) after extraction with \(N, N\)-dimethylformamide (Suzuki and Ishimaru, 1990). The samples for macronutrient analysis during incubation were measured with a QuAAtro continuous flow analyzer (Bran+Luebbe). For Fe analysis, seawater samples were acidified at pH 3.2 with 10 mol L\(^{-1}\) formic acid and 2.4 mol L\(^{-1}\) ammonium formate buffer after the sample collection. The Fe concentration was measured with an automated Fe analyzer (Kimoto Electric) using a combination of an 8-hydroxyquinoline chelating resin concentration and luminol-hydrogen peroxide chemiluminescence detection with a closed flow-through system (Obata et al., 1993).
Unfiltered acidified samples were used to measure the total dissolvable Fe (T-Fe) and the samples passed through a 0.22 µm cartridge filter (Millipore) prior to formic acid and buffer addition were used to measure the dissolved Fe (D-Fe) (detection limit: < 0.05 nmol L\(^{-1}\) in this study). In the surface phytoplankton incubations, the Fe amount in the particulate fraction (P-Fe) was defined as follows: P-Fe = T-Fe – D-Fe. Note that the Fe in particulate form collected using an *in situ* filtration system (> 1.0 µm in diameter) during the SPM\(_{DSW}\) addition experiment is represented as P-Fe\(_{DSW}\). The Fe concentrations were analyzed on-board immediately after the collection of the seawater samples at the beginning of the experiment.

Our Fe measurement method was carefully assessed using SAFe (Sampling and Analysis of Fe) cruise reference standard seawater for an inter-comparison study distributed by the Moss Landing Marine Laboratory and University of California Santa Cruz. We measured 0.10 ± 0.010 nmol L\(^{-1}\) (n = 3) and 0.99 ± 0.023 nmol L\(^{-1}\) (n = 3) D-Fe by our method, respectively for concentrations of 0.094 ± 0.008 nmol L\(^{-1}\) (S) and 0.923 ± 0.029 nmol L\(^{-1}\) (D2) D-Fe (www.geotraces.org) for the reference standard seawater. For the phytoplankton cell count, seawater samples were fixed by formalin (1% final volume). An adequate volume of fixed seawater was poured into a settling chamber and allowed to settle for at least 24 hours (Hasle, 1978) for the day 0 sample, and centrifuged approximately 400×g for day 5 samples (Throndsen, 1978) before identification using a phase-contrast inverted microscope and light microscope, respectively. Diatom species were identified according to Hasle and Syvertsen (1997).

3. Results

3.1. SPM\(_{DSW}\) addition experiment

3.1.1. Hydrography

At Stn DSW, cold (< 0°C) and less saline (~33.3) intermediate water was detected around sigma-\(t\) = 26.8 kg m\(^{-3}\) (Fig. 2a, b, c), which was considered as OSIW (Fukamachi et al., 2004). Anomalously low beam transmission, i.e. high abundance of suspended particulate matter was detected below 245 m at Stn DSW (Fig. 2d).

3.1.2. Phytoplankton growth
Chl-α concentrations increased exponentially in the controls, Fe, and SPM<sub>DSW</sub> treatments over the 5-day incubation period, whereas the concentrations decreased with time in the SPM<sub>DSW−Phy</sub> treatment (Fig. 3a). The order of the maximum Chl-α concentrations during the incubation were as follows: SPM<sub>DSW</sub> (60.9 ± 4.5 µg L<sup>−1</sup>) > Fe-added (28.3 ± 3.8 µg L<sup>−1</sup>) > control (18.9 ± 1.0 µg L<sup>−1</sup>) > DFB (5.2 ± 0.2 µg L<sup>−1</sup>) > DSW<sub>raw</sub> (0.99 ± 0.2 µg L<sup>−1</sup>) > SPM<sub>DSW−Phy</sub> (0.14 ± 0.0 µg L<sup>−1</sup>) (mean ± 1 SD of triplicate bottles or mean ± range of duplicate bottles). The Chl-α data in the SPM<sub>DSW−Phy</sub> treatment indicated that most of the phytoplankton on the filter collected from the DSW died during the 3 hour freezing period.

We then offset the Chl-α data for the SPM<sub>DSW</sub> treatment by subtracting the value measured at each data point in the SPM<sub>DSW−Phy</sub> treatment to calculate the specific growth rate. The specific growth rate was highest in the SPM<sub>DSW</sub> (0.80 ± 0.03) (mean ± 95% C.L. of regression), followed by 0.66 ± 0.03 in the Fe-added, 0.59 ± 0.13 in the DSW<sub>raw</sub>, 0.58 ± 0.02 in the control, 0.30 ± 0.02 in the DFB and −0.28 ± 0.09 in the SPM<sub>DSW−Phy</sub> treatment (Fig. 3b). It is notable that the growth rate was the fastest (1.0 ± 0.09) during days 3 to 5 in the DSW<sub>raw</sub> treatment as compared with all treatments.

At the beginning of the experiment, pennate diatom species such as *Pseudo-nitzschia* spp. and *Cylindrotheca closterium* / *Lennoxia faveolata* complex predominated relative to the centric diatoms (we detected mainly *Chaetoceros* spp. subgenus *Phaeoceros*) in the possibly Fe-starved Oyashio phytoplankton community (Table 2). In the treatments without the addition of Oyashio phytoplankton (SPM<sub>DSW−Phy</sub> and DSW<sub>raw</sub>), the centric (mainly *Chaetoceros* spp. subgenus *Hyalochaete* and *Thalassiosira* spp.) and pennate diatoms (mainly *Thalassionema nitzschioides*) dominated, and they contributed equally (Table 2). In addition, *Hyalochaete* spp. resting spores were detected in the DSW<sub>raw</sub> treatment at levels of 80 spores L<sup>−1</sup>. It should be noted that the species in the SPM<sub>DSW</sub> and DSW<sub>raw</sub> treatments were considered as mainly coastal diatoms (Hasle and Syvertsen, 1997). At day 5, abundances of the pennate diatoms were three orders of magnitude higher than those of the centric species in the Oyashio phytoplankton-added treatments, except for the DFB treatment, whereas in the DSW<sub>raw</sub> treatment, *Hyalochaete* spp., *Thalassiosira* spp., *T. nitzschioides*, and, notably, resting spore-forming species such as *Thalassiosira antarctica* ver. *borealis* and *Ditylum brightwellii* were observed. The total diatom abundance at the end of the incubations was the
following order: $SPM_{DSW} > Fe\text{-added} > control > DFB >> SPM_{DSW−Phy} \approx DSW_{raw}$ (Table 2). This order is nearly identical to that of the maximum Chl-$a$ concentrations (Fig. 3a). The net growth rates during the 5 day incubation period were similar between centric and pennate diatoms in the control, $SPM_{DSW}$ and $DSW_{raw}$ treatments, whereas that of the pennate diatoms was faster than that of the centric diatoms in the Fe-added treatment (Table 4). In the DFB-added treatment, the net growth rate of diatoms and flagellates was slower than that in the controls and Fe-added treatments.

3.1.3. Nutrient dynamics

The T-Fe concentrations in the control ($\approx$ DFB) and $SPM_{DSW}$ ($\approx$ $SPM_{DSW−Phy}$) treatments were 0.93 and 47.7 nmol L$^{-1}$, respectively. The concentration of P-Fe$_{DSW}$ ($>1.0$ µm) is derived as an approximation based on the measurement of the concentrated SPM in the experimental bottles. Thus, the P-Fe$_{DSW}$ concentration was 0.83 nmol L$^{-1}$ in the $in situ$ DSW (320 m) mass at Stn DSW. Macronutrient concentrations at the beginning of the experiment were 30.0–48.6 µmol L$^{-1}$ DIN (NO$_3$+NO$_2$+NH$_4^+$), 2.48–3.86 µmol L$^{-1}$ PO$_4$, and 56.7–74.1 µmol L$^{-1}$ Si(OH)$_4$. None of the nutrients were exhausted over the course of the experiment; for example, DIN remained at 31.7, 24.4, 40.0, 12.3, 42.1 and 28.8 µmol L$^{-1}$ in the control, Fe, DFB, $SPM_{DSW}$, $SPM_{DSW−Phy}$ and $DSW_{raw}$ treatments, respectively. During the 5 day incubation, nutrients seemingly increased with time in the $SPM_{DSW−Phy}$ treatment probably due to the dissolution from the added $SPM_{DSW}$ without a substantial biological uptake. Nutrient drawdown in the $SPM_{DSW}$ treatment was offset by subtracting the value measured in the $SPM_{DSW−Phy}$ treatment (Table 3). The order of the amount of nutrient drawdown was the same as that of the increase in Chl-$a$ concentrations (Fig. 3). The Si:N drawdown ratio was higher in the $SPM_{DSW}$ compared to that of the control and Fe-added conditions (Table 3). The Si:P drawdown ratio in the $SPM_{DSW}$ treatment was nearly the same as that of the canonical value for vegetative growing diatoms (Brzezinski, 1985), whereas the values in the control and Fe-added treatments were smaller than that in the $SPM_{DSW}$ treatment. In the DFB treatment, no Si drawdown and low N:P drawdown ratio were measured. The nutrient drawdown in the $DSW_{raw}$ treatment was too small to calculate the drawdown ratios correctly (Table 3).
3.2. Surface phytoplankton incubations

3.2.1. Hydrography

At the beginning of the three experiments, the upper mixed layer depth was approximately 30, 30 and 20 m at Stns Oy, BS and Ok, respectively (Fig. 4), which was estimated from the first downward increase in sigma-$t$ $\geq$ 0.02 m$^{-1}$. The stratifications were weaker at Stns BS and Ok as compared to Stn Oy. At the Stn Oy site, the hydrographic conditions were typical of the Western Subarctic Gyre (WSG) during summer, with less saline waters (~32.6) in the surface, with dichothermal water at around 100 m depth or 26.6 kg m$^{-3}$ sigma-$t$ (e.g., Yasuda et al., 2002) (Fig. 4). Hydrographic conditions at Stns BS and Ok were intermediate between those of the WSG (Stn Oy) and the Okhotsk waters (Stn DSW; Fig. 2). The sigma-$t$ values at Stns BS and Ok were vertically homogenous from ~150 down to 500 m and from ~50 down to 700 m, respectively (data not shown), probably due to the effect of intensive vertical water mixing around the Bussol' Strait. The NO$_3$+NO$_2$ concentrations at 10 m depth used in the incubation experiment were 8.8, 18.9 and 13.8 µmol L$^{-1}$ at Stns Oy, BS and Ok, respectively. The D-Fe concentrations at 10 m were < 0.05, < 0.05 and 0.10 nmol L$^{-1}$, and the T-Fe concentrations were < 0.05, 0.51 and 0.18 nmol L$^{-1}$ at Stns Oy, BS and Ok, respectively.

3.2.2. Phytoplankton growth

At all stations, Fe addition stimulated phytoplankton growth compared to the unamended controls (Fig. 5a, b, c). At the beginning of the experiment, the total diatom abundance was the highest at Stn BS (19,500 cells L$^{-1}$) followed by Stns Ok (12,400 cells L$^{-1}$) and Oy (8,400 cells L$^{-1}$) (Fig. 6). The small pennate diatom *C. closterium / L. faveolata* complex (apical axis: 10–20 µm, transapical axis: <1 µm, equivalent spherical diameter (ESD): ~2–4 µm), was predominant in the bottles at all stations. Other dominant species were also small: *Fragilariopsis* spp. (ESD: 2.5–6 µm) and a narrow group of *Pseudo-nitzschia* spp. (ESD: ~4 µm) (Fig. 6). The abundances of microscopically identifiable small flagellates (ESD: ~3–10 µm) were 3 (Stn BS) to 25 (Stn Ok) times higher than those of the diatoms. During the incubation experiment, small pennate diatoms, especially *C. closterium / L. faveolata*...
complex and *Pseudo-nitzschia* spp., prominently increased in all treatments (Table 4). Interestingly, at Stns Oy and BS, the growth rate of the centric diatoms was not enhanced by the addition of Fe, whereas that of the pennate diatoms and flagellates were faster in the Fe-added treatment at all stations (Table 4). The enhancement effect on the growth rates through the addition of Fe, calculated from cell abundance, was smaller than that due to Chl-\(a\) (Table 4). In the DFB-added treatment, the Chl-\(a\) concentration was unchanged at Stn Oy and increased slightly at Stns BS and Ok. Diatom and flagellate abundance increased after the addition of DFB, although the growth rates were smaller than in the controls (Table 4).

3.2.3. Nutrient dynamics

The NO\(_3^-\)NO\(_2^-\) drawdown reflected the Chl-\(a\) increases; the most rapid drawdown was obtained with the Fe-added treatments, followed by the controls and the DFB treatments (Fig. 5d, e, f). The trends in PO\(_4^3^-\) utilization were the same as those of NO\(_3^-\)NO\(_2^-\) (data not shown). Ammonium utilization varied among the three stations but was statistically insignificant among the experimental treatments; it gradually decreased from 0.54 to ~0.2 \(\mu\)mol L\(^{-1}\) and 0.78 to ~0.2 \(\mu\)mol L\(^{-1}\) at Stn Oy and Ok, respectively, and remained constant at ~0.2 \(\mu\)mol L\(^{-1}\) at Stn BS. The variations in Si(OH)\(_4\) concentrations were almost identical in the controls and Fe added treatments and less utilized in the DFB treatments compared to the other treatments (Fig. 5g, h, i). The Si:N drawdown ratio decreased by the addition of Fe compared to what was observed in the controls at Stns Oy, BS and Ok: 0.12 ± 0.05, 0.42 ± 0.05 and 0.28 ± 0.01 (mean ± 1 SD of triplicate bottles) in Fe-added treatments and 0.45 ± 0.00, 0.78 ± 0.09 and 0.37 ± 0.10 in the controls, respectively. In the DFB treatments, the Si:N ratio was the highest among three treatments ranging from 1.05 (Stn Ok) to 1.53 (Stn Oy).

3.2.4. Effect of Fe manipulation

Four indexes were calculated to examine the Fe nutritional status of phytoplankton (Fig. 7). First, the relative specific growth rate, calculated from the Chl-\(a\) data for the controls \((\mu_{\text{control}})\) and divided by that for the Fe-added treatment \((\mu_{\text{Fe-added}})\), was used as an indicator of apparent bioavailability of all available Fe sources in the control bottles. The \(\mu_{\text{control}}/\mu_{\text{Fe-added}}\)
ratio was significantly smaller at Stn Oy (0.42 ± 0.07) compared to Stn BS (0.66 ± 0.06), whereas the values were not statistically significant between Stns Oy and Ok (0.64 ± 0.12) and Stns BS and Ok (Dunnet t-test, \( p > 0.05 \); Fig. 7a). Second, we calculated \( \Delta \text{Chl-}a \) by subtracting the increase in Chl-\( a \) in the DFB treatment from that of the controls as an index of Fe utilized from the extracellular fraction. It should be noted that phytoplankton such as diatoms acclimated in an Fe-depleted medium with amended DFB-Fe as a sole Fe source can utilize Fe from a DFB-Fe complex via cell surface metalloreductases (Maldonado and Price, 2001; Shaked et al., 2005; Strzepek et al., 2011). This suggests that the utilized extracellular Fe is underestimated due to an overestimation of the Fe in the intracellular fraction. However, the Fe uptake from the DFB-Fe complex is very small, and the Fe taken up by the cells is too small to support Chl-\( a \) synthesis under an extremely high DFB:Fe ratio in seawater (Wells, 1999; Yoshida et al., 2006; Sugie et al., 2011). The \( \Delta \text{Chl-}a \) was smallest at Stn Oy (6.0 ± 0.1 \( \mu g \ \text{L}^{-1} \)) followed by Stn Ok (6.5 ± 1.1 \( \mu g \ \text{L}^{-1} \)) and Stn BS (10.2 ± 0.8 \( \mu g \ \text{L}^{-1} \)) during a 10 day incubation period (Fig. 7b). The third and fourth indices are \( \Delta \text{NO}_3^+\text{NO}_2 \) and \( \Delta \text{NH}_4^+ \), which were calculated by subtracting the \( \text{NO}_3^+\text{NO}_2 \) and \( \text{NH}_4^+ \) drawdown in the DFB treatment from that of the controls (Fig. 7c and 7d). These indices are similar proxies to that of \( \Delta \text{Chl-}a \) as stated above. The trend in \( \Delta \text{NO}_3^+\text{NO}_2 \) was similar to the relative growth rate of \( \mu_{\text{control}}/\mu_{\text{Fe-added}} \) (Fig. 7a) and \( \Delta \text{Chl-}a \) (Fig. 7b); it was higher at Stn BS (12.7 ± 1.2 \( \mu g \ \text{L}^{-1} \)) as compared to Stns Ok (9.3 ± 1.2 \( \mu mo\text{l} \ \text{L}^{-1} \)) and Oy (8.1 ± 0.4 \( \mu mo\text{l} \ \text{L}^{-1} \); Fig. 7c). The \( \Delta \text{NH}_4^+ \) was less than 0.1 \( \mu mo\text{l} \ \text{L}^{-1} \) at all stations (Fig. 7d).

4. Discussion

4.1. Bioavailability of Fe in the SPM

The present study is the first clear demonstration that particulate matter (\( > 1 \mu m \)) collected in the nepheloid layer can supply bioavailable Fe that can promote the growth of phytoplankton such as diatoms. The base FSW, which was collected from a depth of 200 m in the Oyashio region, should contain ample nutrients and other trace elements except for Fe, and measurable nutrients remained in all treatments at the end of the experiment. Furthermore, \( \text{NH}_4^+ \), which potentially promotes the growth of phytoplankton, was constant at ~0.3 \( \mu mo\text{l} \ \text{L}^{-1} \) in the SPM\text{DSW}–Phy treatment throughout the experiment. This suggests that
remineralized elements other than Fe, such as other trace metals and nutrients, did not affect the growth of phytoplankton in the SPM_{DSW}-added treatments (c.f., Coale, 1991). The higher Chl-$a$ concentration and diatom abundance, with slightly faster growth rates in the SPM_{DSW} treatment than the Fe-added treatment, may signify that Fe from the SPM_{DSW} was supplied immediately at a higher concentration than 5 nmol L$^{-1}$ or a higher bioavailability of Fe, compared to added inorganic Fe, a large part of which is considered as solid phase inorganic Fe. However, it is very difficult to compare the bioavailability of Fe in the SPM_{DSW} with that in solid inorganic Fe oxyhydroxide because of the extremely different Fe concentration in the media used in the present study. The rapid growth of phytoplankton in the DSW$_{raw}$ treatment during the 3 to 5 day incubation period partially supports the possibility that P-Fe$_{DSW}$ in the DSW mass was highly bioavailable. It should be noted that it is likely that the high concentration of SPM$_{DSW}$ interferes with the grazing pressure of microzooplankton (Price et al., 1994), which could have enhanced the net growth rate further in the SPM$_{DSW}$ treatment as compared to the Fe-added treatment. In addition, we observed resting spores of *Hyalochaete* spp. at the beginning of the DSW$_{raw}$ treatment, which showed the fastest growth rate among the treatments with a few days of lag period (Fig. 3a). Diatom resting spores are an ecological strategy in order to survive under unfavorable conditions for growth (e.g., Sugie et al., 2010b), and they can germinate rapidly after appearing under favorable conditions (McQuoid and Hobson, 1996). We suggest that the resting spore-forming coastal diatoms contributed to the fast growth of the centric species in the DSW$_{raw}$ treatment with bioavailable Fe sources.

It has been well documented that the bioavailability of inorganic P-Fe for photolithoautotrophs depends on the surface reactivity of the solid inorganic Fe oxyhydroxide species (Kuma and Matsunaga, 1995; Chen and Wang, 2000; Yoshida et al., 2006). The reactivity of solid inorganic Fe oxyhydroxide decreases with an increase in aging time and temperature and depends on the crystal structure (Kuma and Matsunaga, 1995; Yoshida et al., 2006). The temperature in the DSW is low (< 0°C: e.g. Fukamachi et al., 2004), and hence, the bioavailability of Fe in the SPM$_{DSW}$ may be kept high for a long time if the P-Fe$_{DSW}$ is composed of inorganic substances. Alternatively, the DSW is rich in organic detritus possibly derived from intensive diatom production on the continental shelf of Sakhalin Island (Nakatsuka et al., 2004). Such detritus can release Fe-binding ligands such as humic
substances during particulate remineralization in the water column by bacteria and photodissolution by irradiance in the surface layer (Mayer et al., 2009; Boyd et al., 2010). It has been reported that Fe complexes with humic type ligands are highly bioavailable, similar to inorganic Fe(III) for marine phytoplankton (Chen and Wang, 2008). In other cases, if bioactive Fe can be incorporated into or onto colloidal matrices, and then become enhanced in the particulate phase by the progressive sorption of other organic colloids with cation bridges (Wells, 2002), the Fe in the particles may become bioavailable after bacterial remineralization and/or photodissolution (Boyd et al., 2010). The effect of the short-term freezing of the SPM_{DSW} caused cell death of the phytoplankton, which may also enhance remineralization of the Fe from the SPM_{DSW} because Fe released from the dead phytoplankton potentially is of high bioavailability (Poorvin et al., 2004). In addition, a recent study reported that marine polysaccharides are important Fe-binding ligands in the colloidal fraction and that the Fe bound to polysaccharides is of high bioavailability for phytoplankton (Hasser et al., 2011). Polysaccharides are known as a precursor of transparent exopolymer particles, which are believed to be bridges to create large aggregates (Passow, 2002). These above possibilities allow us to assume that the highly bioavailable Fe could be retained in the particulate phase for a suitable length of time associated with organic matters in the DSW and OSIW. However, the bioavailability of Fe in the SPM_{DSW} may decrease with aging time during transport and with the increase in temperature when it is drawn into the surface mixed layer. Further experiments are needed to determine the accurate chemical state and bioavailability of P-Fe collected from various regions in the western subarctic Pacific with more realistic concentrations added to phytoplankton communities.

4.2. Phytoplankton dynamics and their available Fe sources in the surface waters

The addition of Fe enhanced phytoplankton growth in terms of Chl-\(a\) at all three stations indicating phytoplankton nutritional status was clearly Fe-limited in the control bottles. However, phytoplankton growth measured with cell abundances was consistently faster than that measured with Chl-\(a\) concentrations (Figs. 3, 5 and Tables 2, 4). The difference between the growth rate calculated from the Chl-\(a\) and diatom cell density was caused by the gradual decrease in the intracellular Chl-\(a\) concentration of the phytoplankton
over the incubation period (Sugie et al., 2011). The Chl-a cell quota was also affected due to light conditions, which may differ between in situ and incubation conditions. It should also be noted that bulk Chl-a concentration values reflect the whole community, while cell counts target specific populations. This may also contribute to differences in growth rate determined via Chl-a vs. cell counts.

The Si:N drawdown ratios of the Fe added bottles decreased compared to that of the controls suggesting some contribution from diatoms, especially at Stn Oy, and their recovery from iron-limited conditions, which leads to a high Si:N drawdown ratio (Takeda, 1998; Sugie et al., 2010b). The growth rate of pennate diatoms was enhanced during all experiments, whereas the growth of centric species was promoted in only one (Stn Ok) out of four experiments (Tables 2, 4). The growth rates of centric diatoms, where the stations had a dominance of oceanic Phaeoceros spp., were not enhanced by the addition of Fe. In contrast, the phytoplankton community at Stn Ok had a relatively high abundance of coastal centric diatoms such as Hyalochaete spp. and Thalassiosira spp. compared to the other stations (Fig. 6), which may have contributed to an increase in the growth rate of the centric diatom in response to the addition of Fe. Strezepek and Harrison (2004) reported that oceanic species cannot adapt quickly to rapid changes in external environments due to the trade-off between adaptability vs. the capacity to survive under relatively permanent, Fe-limited conditions. In addition, surge Fe uptake upon Fe addition and subsequent storage using ferritin are considered to be an advantageous strategy of pennate diatom (Marchetti et al., 2009). Our results support previous suggestions that pennate diatoms are better able to increase growth rate with the addition of inorganic Fe in relative to oceanic centric diatoms (Table 4). Ecophysiologic strategies in the Fe utilization of phytoplankton appear to be one of the most important factors controlling phytoplankton dynamics in oceanic Fe-limited regions.

At the beginning of the surface water incubations (i.e., reflecting in situ condition), the Chl-a concentrations were relatively high (0.85 to 1.58 µg L⁻¹) compared to oceanic HNLC regions such as the central subarctic gyres and the equatorial Pacific region (e.g., Price et al., 1994; Nishioka et al., 2003). The net growth rate in the unamended controls calculated from the Chl-a data and diatom cell density ranged from 0.20 to 0.31 d⁻¹ (Fig. 5) and 0.69 to
1.34 d$^{-1}$ (Table 4), respectively. These results indicate that the \textit{in situ} phytoplankton communities were mildly Fe-limited, which may explain the moderately high Chl-$a$ concentrations in the surface waters even under D-Fe depleted conditions. Yoshimura et al. (2010) also reported that the phytoplankton community in the Bussol' Strait in 2006, at virtually the same location as Stn BS in this study, was under a Fe-limited or Fe- and light-co-limited condition. Although D-Fe concentrations were near depletion at the beginning of the incubations, phytoplankton biomass increased in the controls, with the $\mu_{\text{control}}/\mu_{\text{Fe-added}}$ ranging from 0.42 to 0.66. In the DFB treatments, which eliminated almost all external Fe from the bioavailable fraction to phytoplankton, the net Chl-$a$ increase and NO$_3$+NO$_2$ drawdown were less than those in the controls (Fig. 7). It suggests that further growth of phytoplankton in the control bottles was not only supported by Fe in the intracellular fraction but also by the extracellular fractions.

The possible bioavailable Fe source for the phytoplankton was the particulate phase under the D-Fe depleted conditions. We estimated the utilization of intra- and extracellular Fe, which can be distinguished by calculating the increase of Chl-$a$ and the NO$_3$+NO$_2$ and NH$_4^+$ drawdowns in the controls minus that of the DFB-added treatment (hereafter referred to as $\Delta$Chl-$a$, $\Delta$NO$_3$+NO$_2$ and $\Delta$NH$_4^+$, respectively; Fig. 7b, c, d). However, we cannot estimate the extracellular fraction of utilized Fe at Stn Oy due to the fact that both D-Fe and T-Fe concentrations were below the detection limit. The utilized Fe in the extracellular source was potentially underestimated because we did not take into account the Fe uptake from the DFB-Fe complex (e.g., Maldonado and Price, 1999, 2001; Strezepek et al., 2011). However as stated earlier, the Fe uptake and subsequent cell or Chl-$a$ growth should be very small for an extremely high DFB:Fe ratio (1 µmol L$^{-1}$:< 0.05–0.5 nmol L$^{-1}$ (T-Fe)), as observed in previous studies (Wells, 1999; Iwade et al., 2006; Sugie et al., 2011).

First, we calculated the potential Fe requirement of the phytoplankton in the control bottles from the extracellular fraction using the $\Delta$Chl-$a$ values during the incubations and reported Fe:Chl-$a$ values. The Fe:Chl-$a$ ratio was calculated from the Fe:C ratio ($\mu$mol:mol) divided by the Chl-$a$:C ratio (g:mol) applied from reported values (Table 5). The Fe:Chl-$a$ value of the Fe-limited oceanic phytoplankton ranged from 4.7 to 108 µmol g$^{-1}$ (Table 5). In this study, phytoplankton communities at Stns BS and Ok were Fe-limited; consequently, we
selected these ratios determined only under Fe-limited conditions. Because the contribution of coastal species was small (~2% at Stn BS and ~8% at Stn Ok), we used the reported values obtained from oceanic phytoplankton species. Although these values were measured through unialgal culture experiments using nano- and micro-sized phytoplankton, we had no information regarding accurate Fe:Chl-α values in the natural phytoplankton community and pico-sized phytoplankton. The reported Fe:C values of the oceanic phytoplankton used in the above calculation was similar to that of natural oceanic phytoplankton in Fe-limited HNLC regions (Table 5). It should be noted that the estimated Fe requirement may be potentially overestimated because the Fe requirements of pico-phytoplankton, which may contribute substantial biomass in the bottles, is believed to be small relative to that of larger phytoplankton (Timmermans et al., 2005). However, the size dependence of the Fe:C ratio is still in debate; Strzepek et al. (2011) reported that the Fe:C ratio increased with a decrease in cell volume. According to the stoichiometric calculation, the phytoplankton community at Stn BS under the D-Fe depleted (< 0.05 nmol L\(^{-1}\)) and P-Fe (0.5 nmol L\(^{-1}\)) conditions required 0.05 to 1.10 nmol L\(^{-1}\) Fe from extracellular sources. The D-Fe (0.10 nmol L\(^{-1}\)) at Stn Ok could support the growth of a phytoplankton community in the control bottles (required 0.03 to 0.71 nmol L\(^{-1}\)) only when applied to the lowest Fe:Chl-α value. The presence of the coastal diatom species would tend to increase Fe demand from the extracellular fraction due to their high Fe requirements compared to the oceanic species (Table 5).

Second, we calculated another index for the community Fe requirement from the extracellular fraction in the controls using the data on ΔNO\(_3^-\)+NO\(_2^-\) and ΔNH\(_4^+\) values. The nitrogen utilizations were converted into carbon biomass using the canonical Redfield ratio (Redfield et al., 1963). The variation in C:N ratio caused by changing the availability of Fe is rather small compared to Fe:C ratios (e.g., Price, 2005; Sugie and Yoshimura, in press). The decrease in Fe requirements of phytoplankton using NH\(_4^+\) relative to NO\(_3^-\) utilization was taken into account according to the theoretical calculations of Raven (1988). The Fe:C ratios of the Fe-limited phytoplankton applied ranged from 0.4 to ~10 µmol mol\(^{-1}\) (Table 5). The Fe:C ratios fall in the minimum to intermediate values of phytoplankton Fe requirements determined for unialgal culture and \textit{in situ} phytoplankton and seston (Table 5). The phytoplankton community required 0.03–0.84 and 0.02–0.62 nmol L\(^{-1}\) Fe at Stns BS and Ok,
respectively, with \(<0.05\) and \(0.10\) nmol L\(^{-1}\) D-Fe and \(\sim0.50\) and \(0.08\) nmol L\(^{-1}\) P-Fe concentrations \textit{in situ}, respectively.

These above estimations suggest that observed phytoplankton growth in control experiments at both Stns BS and Ok could not have been supported by only the D-Fe phase. Fitzwater et al. (2003) reported that \(\sim10\%\) of Fe in the particulate phase was labile (leachable by 25\% acetic acid for 2h) and that labile particulate Fe may contribute to the growth of phytoplankton through Fe remineralization. In addition, a recent model study suggested that the phytoplankton community in the Oyashio region of the western subarctic Pacific compensates their Fe demand from remineralization and/or desorption processes via the particulate phase during the summer (Shigemitsu et al., 2012). Our empirical calculations support the previous measurements (Fitzwater et al., 2003) and estimations (Elrod et al., 2008; Nakayama et al., 2010; Sugie et al., 2010a) that a significant portion of the Fe in the particulate phase is bioavailable. Although some latent uncertainties in the stoichiometric calculation remain, the Fe in the particulate phase is an important Fe source for a phytoplankton community under D-Fe depleted conditions. Previous studies reported that the possible bioavailability of Fe in the particulate phase would change due to the variability of the source of SPM (Fitzwater et al., 2003; Elrod et al., 2008). Note that the source of SPM around the Bussol’ Strait most likely originates from coastal regions of the Sea of Okhotsk (see below), not from the Kurile Islands due to the very narrow continental shelves with steep trenches (Fig. 1). The bioavailability of Fe in the particulate fraction derived from different sources and regions is an important issue to clarify in understanding the variability of Fe bioavailability in coastal and oceanic waters. Furthermore, the remineralization rate for Fe in the various sources of SPM is critically important to better understand the biogeochemical cycle of Fe and other nutrients in the ocean.

4.3. Possible role of SPM\(_{\text{DSW}}\) around the Kuril Strait

The Fe in the SPM\(_{\text{DSW}}\) was apparently bioavailable for phytoplankton, as shown in the present study. Previous field observations and model work indicated that a high Fe water mass would be transported from the OSIW originating from coastal regions of the Sea of Okhotsk to the western North Pacific region via the Bussol' Strait (Nishioka et al., 2007;
Misumi et al., 2011). In the present study, deep vertical mixing was observed at Stns BS and Ok; mixing around the Kuril Straits is a common phenomenon (e.g., Ito et al., 2010, 2011; Yoshimura et al., 2010). Therefore, a substantial portion of Fe, especially P-Fe in the surface near Stns BS and Ok, may be considered to be derived from the OSIW, the residence time of which is considered to be a few years (Yamamoto et al., 2001). The availability of P-Fe in OSIW and DSW would be substantially prolonged, possibly due to the formation of Fe-organic associations as suggested above, and the cold characteristic of the OSIW mass (<1°C) may also support the bioavailability of Fe for a long time (Kuma et al., 2000). Recent studies also indicated that the contribution of lithogenic Fe to the total Fe in the SPM (>1 µm) collected from the OSIW with the same in situ filtration system as the present study decreased from Sakhalin Island toward the Bussol’ Strait (Shigemitsu, unpublished data). This suggests that a substantial part of the P-Fe around the Bussol’ Strait is associated with organic materials, which may be available for phytoplankton as observed in the present study. These above physical and chemical factors potentially enable phytoplankton to utilize bioavailable P-Fe in the surface mixed layer even with the relatively long residence time of the OSIW. In future work, we should examine to what extent the bioavailability of P-Fe is prolonged and the importance of bioavailable P-Fe in the western subarctic Pacific regions where P-Fe contributes to relatively rich environments (Nishioka et al., 2003; Kitayama et al., 2009; Nakayama et al., 2010). In addition, it should be noted that phytoplankton in the DSW mass showed healthy growth (µ = 1.0 d⁻¹), with a lag period of a few days as observed in the DSWraw treatment. The short lag time could stem from the presence of resting spore-forming diatoms such as Hyalochaete spp., T. antarctica ver. borealis and D. brightwellii, which require a few days for germination (c.f., McQuoid and Hobson, 1996). The seed population of coastal diatoms is transported around the Kuril Islands with the southeasterly flow of the OSIW. Yoshimura et al. (2010) reported that coastal diatom species such as Thalassiosira spp. dominated among the diatoms at the end of the incubation experiment conducted near Stn BS in August 2006, when the water column mixed down to ~800 to 1000 m without surface stratification. We also observed an increase in the coastal diatom species, with high net growth rates ranging from 0.78 to 1.1 d⁻¹ when using seawater collected from 50, 200, 500 and 1000 m depths at Stns Oy, BS and Ok for incubation (Sugie, unpublished data). Such
coastal diatom species are clearly different from the summer phytoplankton community in the open western subarctic Pacific regions (Aizawa et al., 2005; Komuro et al., 2005). The complex physical conditions in the Sea of Okhotsk may be able to sufficiently host allochthonous coastal phytoplankton around the Kuril Straits, possibly originating from the pelagic realm of the inner part of the Sea of Okhotsk. Furthermore, nutrients, especially Si(OH)$_4$ dissolved from the SPM$_{DSW}$ as revealed from the data in the SPM$_{DSW}$-Phy treatment, partly enhance nutrient concentrations in the intermediate water. Nevertheless, we still need to quantify the accurate remineralization rate for nutrients dissolved from the SPM$_{DSW}$ with a longer experimental period and low temperatures. If the SPM$_{DSW}$-like particles were transported to the Kuril Straits region and subsequent diapycnal mixing occurred (Nakamura and Awaji, 2004), then bioavailable P-Fe (i.e., P-Fe$_{DSW}$ or SPM$_{DSW}$) and healthy coastal phytoplankton could partly emerge to the sunlit surface waters associated with macronutrients and bioavailable D-Fe. Such bio-active elements and allochthonous phytoplankton populations supplied by mixing events seemingly support high productivity and biodiversity around the Kuril Straits. In addition, the bioavailability of P-Fe for phytoplankton other than diatoms is an important issue because they play a major role in the D-Fe-depleted oceanic regions of the North Pacific Oceans during the summer (e.g., Suzuki et al., 2002).

Contributors

K.S., J.N., K.K., Y.N.V. and T.N. designed research; K.S., J.N. and T.N. performed research; K.S. and J.N. analyzed data; K.S. wrote the paper and J.N., K.K. and T.N. assisted drafting.

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References


iron connections between desert dust, ocean biogeochemistry, and climate. Science 308, 67–71. doi:10.1126/science.1105959.


northwestern shelf of the Okhotsk Sea: 1. Direct observations of brine rejection. J. 

Shigemitsu, M., Okunishi, T., Nishioka, J., Sumata, H., Hashioka, T., Aita, M.N., Smith, S.L., 
ecosystem model including the iron cycle applied to the Oyashio region, western 

Strzepek, R.F., Harrison, P.J., 2004. Photosynthetic architecture differs in coastal and oceanic 

strategies by Southern Ocean phytoplankton to less iron limitation: Uptake of 
organically complexed iron and reduced cellular iron requirements. Limnol. Oceanogr. 56, 


Sugie, K., Yoshimura, T., in press. Effects of pCO₂ and iron on the elemental composition 
and cell geometry of the marine diatom *Pseudo-nitzschia pseudodelicatissima* 

dynamics during late winter and spring in the Oyashio region of the western subarctic 

Sugie, K., Kuma, K., Fujita, S., Ikeda, T., 2010b. Increase in Si:N drawdown ratio due to 
resting spore formation by spring bloom-forming diatoms under Fe- and N-limited 

Sugie, K., Kuma, K., Fujita, S., Ushizaka, S., Suzuki, K., Ikeda, T., 2011. Importance of 
intracellular Fe pools on growth of marine diatoms by using unialgal cultures and the 
Oyashio region phytoplankton community during spring. J. Oceanogr. 67, 183–196. 


Table and figure captions

Table 1. Experimental design of six treatments to test the bioavailability of particulate Fe collected from dense shelf water (SPM_{DSW}) for the Fe-limited phytoplankton community. Symbols of plus and minus represent the factors added or not added, respectively. *: Not an extra addition, but natural SPM and phytoplankton are present in the DSW_{raw} treatment.

Table 2. Phytoplankton abundance (×10^3 cells L^{-1}) and apparent growth rate (day 0 to 5) of centric and pennate diatoms during the SPM_{DSW} addition experiment.

Table 3. Nutrient drawdown (in µmol L^{-1}) and its ratios during 5 days of the SPM_{DSW} addition experiment. Data represent mean ± 1SD of triplicate incubation bottles. *: Nutrient concentrations and drawdown ratios were offset by subtracting the value dissociated from the SPM_{DSW} measured in the SPM_{DSW−Phy} treatment.

Table 4. Phytoplankton abundance (×10^3 cells L^{-1}) and apparent growth rate (day 0 to 5) of centric and pennate diatoms and flagellates during the surface water incubations.

Table 5. Published elemental composition of the phytoplankton and seston under Fe-limited conditions. The reported values of Fe-starved phytoplankton, cultivated with strong chelator such as desferrioxamine B were excluded. Values with * indicate that extracellularly absorbed iron was not eliminated, whereas the other values represent intracellular elemental compositions. n.d.: no data. †: Fe:Chl-a values with no Chl-a:C measurement were estimated using other reported Chl-a:C values (0.08–0.38 g:mol).
Figure 1. Sampling locations around the Sea of Okhotsk. The gray contours are shown at a 1000 m isobath interval. Abbreviations; DSW: dense shelf water, Ok: Okhotsk, BS: Bussol' Strait, and Oy: Oyashio.

Figure 2. Vertical profiles of (a) temperature, (b) salinity (c) sigma-$t$ and (d) beam transmission at the beginning of the SPM$_{DSW}$ addition experiment at Stn. DSW.

Figure 3. (a) Temporal change in chlorophyll-$a$ concentrations examining Fe bioavailability in the suspended particulate matter collected from DSW (SPM$_{DSW}$) at Stn. DSW. Data represent mean ± 1SD for triplicate or mean ± range for duplicate analysis. The error bars are hidden when smaller than the symbols. (b) Specific growth rate for six treatments. Data represent mean ± 95% C.L. of regression (see method).

Figure 4. Vertical profiles of (a) temperature, (b) salinity and (c) sigma-$t$ at the beginning of the surface phytoplankton incubation experiments.

Figure 5. Temporal changes in (a), (b) and (c) chlorophyll-$a$ (Chl $a$), (d), (e) and (f) NO$_3$+NO$_2$, and (g), (h) and (i) Si(OH)$_4$ at station Oyashio (a, d and g), Bussol' Strait (b, e and h) and Okhotsk (c, f and i). Data represent mean ± 1SD for triplicate incubation bottles.

Figure 6. Diatom species composition and cell densities at the start of the surface phytoplankton incubation experiments at station Oyashio (Oy), Bussol’ Strait (BS) and Okhotsk (Ok).

Figure 7. Comparing phytoplankton response during the surface phytoplankton incubation experiments in terms of (a) the relative growth rate in the unamended control to the Fe-added treatment ($\mu_{control}/\mu_{Fe-added}$), (b) difference in the increased Chl-$a$ between the control and the DFB treatment ([\Delta Chl-$a$]), (c) difference in the NO$_3$+NO$_2$ drawdown between the control and the DFB treatment ([\Delta NO$_3$+NO$_2$]), and (d) difference in $\Delta$NH$_4$\textsuperscript{+} drawdown between the control and the DFB treatment ([\Delta NH$_4$\textsuperscript{+}]). Data represents mean ± 1SD for triplicate incubation bottles.
Table 1. Experimental design of six treatments to test the bioavailability of particulate Fe collected from dense shelf water (SPM$_{DSW}$) for the Fe-limited phytoplankton community. Symbols of plus and minus represent the factors added or not added, respectively. *: Not an extra addition, but natural SPM and phytoplankton are present in the DSW$_{raw}$ treatment.

<table>
<thead>
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<th>Treatment</th>
<th>Phytoplankton</th>
<th>Fe</th>
<th>SPM$_{DSW}$</th>
<th>DFB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Fe</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DFB</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>SPM$_{DSW}$</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>SPM$_{DSW}$−Phy</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DSW$_{raw}$</td>
<td>−*</td>
<td>−</td>
<td>−*</td>
<td>−</td>
</tr>
</tbody>
</table>
Table 2. Phytoplankton abundance (×10³ cells L⁻¹) and apparent growth rate (day 0 to 5) during the SPM_{DSW} addition experiment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Abundance at day 0</th>
<th>Abundance at day 5</th>
<th>Growth rate (d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Centric Pennate</td>
<td>Centric Pennate</td>
<td>Centric Pennate</td>
</tr>
<tr>
<td>Controls</td>
<td>2.6 1350</td>
<td>39 21300</td>
<td>0.54 0.55</td>
</tr>
<tr>
<td>Fe</td>
<td>2.6 1350</td>
<td>40 41400</td>
<td>0.54 0.68</td>
</tr>
<tr>
<td>DFB</td>
<td>2.6 1350</td>
<td>44 1700</td>
<td>0.56 0.05</td>
</tr>
<tr>
<td>SPM_{DSW}</td>
<td>28 1410</td>
<td>173 120000</td>
<td>0.84 0.90</td>
</tr>
<tr>
<td>SPM_{DSW}−Phy</td>
<td>28 56</td>
<td>84 39</td>
<td>0.22 −0.07</td>
</tr>
<tr>
<td>DSW_{raw}</td>
<td>0.32 0.30</td>
<td>52 28</td>
<td>1.0 0.90</td>
</tr>
</tbody>
</table>
Table 3. Nutrient drawdown (in µmol L\(^{-1}\)) and its ratios during 5 day incubation experiment. Data represent mean ± 1SD of triplicate incubation bottles.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ΔDIN</th>
<th>ΔP</th>
<th>ΔSi</th>
<th>ΔSi: ΔN</th>
<th>ΔN: ΔP</th>
<th>ΔSi: ΔP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.4 ± 0.38</td>
<td>0.95 ± 0.16</td>
<td>4.00 ± 1.09</td>
<td>0.28 ± 0.07</td>
<td>15.7 ± 2.94</td>
<td>4.45 ± 1.68</td>
</tr>
<tr>
<td>Fe</td>
<td>21.7 ± 1.04</td>
<td>1.48 ± 0.04</td>
<td>7.55 ± 0.84</td>
<td>0.35 ± 0.02</td>
<td>14.7 ± 0.48</td>
<td>5.10 ± 0.49</td>
</tr>
<tr>
<td>DFB</td>
<td>6.95 ± 0.61</td>
<td>0.69 ± 0.09</td>
<td>−1.15 ± 1.20</td>
<td>−0.18 ± 0.18</td>
<td>10.1 ± 0.48</td>
<td>−1.89 ± 1.82</td>
</tr>
<tr>
<td>SPM(_{DSW})</td>
<td>38.2 ± 1.05*</td>
<td>1.95 ± 0.06*</td>
<td>28.1 ± 1.16*</td>
<td>0.74 ± 0.03*</td>
<td>19.6 ± 0.71*</td>
<td>14.4 ± 0.20*</td>
</tr>
<tr>
<td>SPM(_{DSW})−Phy</td>
<td>−1.92 ± 1.60</td>
<td>−0.17 ± 0.22</td>
<td>−10.9 ± 3.95</td>
<td>9.88 ± 6.36</td>
<td>3.59 ± 24.1</td>
<td>−93.4 ± 313</td>
</tr>
<tr>
<td>DSW(_{raw})</td>
<td>1.18 ± 0.85</td>
<td>0.16 ± 0.13</td>
<td>1.29 ± 2.37</td>
<td>−0.76 ± 2.56</td>
<td>9.42 ± 2.37</td>
<td>−13.2 ± 25.9</td>
</tr>
</tbody>
</table>

*: Nutrient concentrations and drawdown ratios were offset by subtracting the value dissociated from the SPM\(_{DSW}\) in the SPM\(_{DSW}\)−Phy treatment.
Table 4. Phytoplankton abundance ($\times 10^3$ cells L$^{-1}$) and apparent growth rate (day 0 to 5) during the surface water incubations.

<table>
<thead>
<tr>
<th>Station</th>
<th>Initial Abundance at day 5</th>
<th>Growth rate (d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont.</td>
<td>Fe</td>
</tr>
<tr>
<td>Oy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centric diatoms</td>
<td>0.40</td>
<td>24.8</td>
</tr>
<tr>
<td>Pennate diatoms</td>
<td>1.67</td>
<td>434</td>
</tr>
<tr>
<td>Flagellates</td>
<td>89.7</td>
<td>573</td>
</tr>
<tr>
<td>BS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centric diatoms</td>
<td>1.18</td>
<td>119</td>
</tr>
<tr>
<td>Pennate diatoms</td>
<td>3.90</td>
<td>557</td>
</tr>
<tr>
<td>Flagellates</td>
<td>62.6</td>
<td>2926</td>
</tr>
<tr>
<td>Ok</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centric diatoms</td>
<td>1.44</td>
<td>45.7</td>
</tr>
<tr>
<td>Pennate diatoms</td>
<td>4.16</td>
<td>2080</td>
</tr>
<tr>
<td>Flagellates</td>
<td>17.1</td>
<td>9760</td>
</tr>
</tbody>
</table>
Table 5. Published elemental composition of the phytoplankton and seston under Fe-limited conditions. The reported values of Fe-starved phytoplankton, cultivated with strong chelator such as desferrioxamine B were excluded. Values with * indicate that extracellularly absorbed iron was not eliminated, whereas the other values represent intracellular elemental compositions. n.d.: no data. †: Fe:Chl-α values with no Chl-α:C measurement were estimated using other reported Chl-α:C values (0.08–0.38 g:mol).

<table>
<thead>
<tr>
<th>Data source</th>
<th>Fe:C (µmol:mol)</th>
<th>Chl-α:C (g:mol)</th>
<th>Fe:Chl-α (µmol:g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unialgal culture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diatom</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eucampia antarctica</em> (oceanic)</td>
<td>1.8</td>
<td>n.d.</td>
<td>4.7–23†</td>
<td>Strzepek et al., 2011</td>
</tr>
<tr>
<td><em>Fragilariopsis kerguelensis</em> (oceanic)</td>
<td>4.2</td>
<td>n.d.</td>
<td>10–53†</td>
<td>Strzepek et al., 2011</td>
</tr>
<tr>
<td><em>Probosira inermis</em> (oceanic)</td>
<td>4.5</td>
<td>n.d.</td>
<td>10–53†</td>
<td>Strzepek et al., 2011</td>
</tr>
<tr>
<td><em>Pseudo-nitzschia</em> spp. (oceanic)</td>
<td>2.8–3.7</td>
<td>n.d.</td>
<td>10–35†</td>
<td>Marchetti et al., 2006</td>
</tr>
<tr>
<td><em>Pseudo-nitzschia</em> spp. (coastal)</td>
<td>5.2–11</td>
<td>n.d.</td>
<td>29–65†</td>
<td>Marchetti et al., 2006</td>
</tr>
<tr>
<td><em>Thalassiosira weissflogii</em> (coastal)</td>
<td>10.6</td>
<td>0.08</td>
<td>23</td>
<td>Price, 2005</td>
</tr>
<tr>
<td><em>T. weissflogii</em> (coastal)</td>
<td>13–22</td>
<td>0.11–0.31</td>
<td>63–115</td>
<td>Sunda and Huntsman, 1995</td>
</tr>
<tr>
<td><em>Thalassiosira pseudonana</em> (coastal)</td>
<td>13–25</td>
<td>0.12–0.21</td>
<td>82–123</td>
<td>Sunda and Huntsman, 1995</td>
</tr>
<tr>
<td><em>Thalassiosira oceanica</em> (oceanic)</td>
<td>2.5–7.1</td>
<td>0.17–0.32</td>
<td>11–28</td>
<td>Sunda and Huntsman, 1995</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Emiliania huxleyi</em> (oceanic)</td>
<td>4.1–6.0</td>
<td>0.16–0.38</td>
<td>16–29</td>
<td>Sunda and Huntsman, 1995</td>
</tr>
<tr>
<td><em>Pelagomonas calceolata</em> (oceanic)</td>
<td>4.3–9.6</td>
<td>0.26–0.32</td>
<td>14–32</td>
<td>Sunda and Huntman, 1995</td>
</tr>
<tr>
<td><em>Phaeocystis antarctica</em> (oceanic)</td>
<td>8.6</td>
<td>n.d.</td>
<td>23–108†</td>
<td>Strzepek et al., 2011</td>
</tr>
<tr>
<td><em>Prorocentrum minimum</em> (coastal)</td>
<td>11–20</td>
<td>0.12–0.16</td>
<td>89–129</td>
<td>Sunda and Huntman, 1995</td>
</tr>
<tr>
<td><strong>Natural particle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>6.0–8.7*</td>
<td>n.d.</td>
<td>23–75†</td>
<td>Twining et al., 2004</td>
</tr>
<tr>
<td>Seston</td>
<td>30*</td>
<td>n.d.</td>
<td>79–375†</td>
<td>Martin et al., 1989</td>
</tr>
<tr>
<td>Seston</td>
<td>3.7</td>
<td>n.d.</td>
<td>9.7–46†</td>
<td>Maldonado and Price, 1999</td>
</tr>
</tbody>
</table>
Figure 1

The Sea of Okhotsk
Kamtchatka Peninsula
Amur River
Sakhalin Island
Kurile Islands
Hokkaido

Stn DSW
Stn BS
Stn Oy
Stn Ok
Figure 3

(a) Time (day) vs. Chlorophyll a (µg L$^{-1}$)

- Control
- Fe
- DFB
- DSW$_{raw}$
- SPM$_{DSW}$
- SPM$_{DSW}$-Phy

(b) Specific growth rate (d$^{-1}$)

- Control
- Fe
- DFB
- SPM$_{DSW}$
- SPM$_{DSW}$-Phy
- DSW$_{raw}$
Figure 4

(a) Temperature (°C) vs. Depth (m)
(b) Salinity vs. Depth (m)
(c) Sigma-t (kg m\(^{-3}\)) vs. Depth (m)
Figure 5

(a) Chl a (µg L$^{-1}$) for Stn. Oy
(b) Chl a (µg L$^{-1}$) for Stn. BS
(c) Chl a (µg L$^{-1}$) for Stn. Ok
(d) NO$_3$+NO$_2$ (µmol L$^{-1}$) for Stn. Oy
(e) NO$_3$+NO$_2$ (µmol L$^{-1}$) for Stn. BS
(f) NO$_3$+NO$_2$ (µmol L$^{-1}$) for Stn. Ok
(g) Si(OH)$_4$ (µmol L$^{-1}$) for Stn. Oy
(h) Si(OH)$_4$ (µmol L$^{-1}$) for Stn. BS
(i) Si(OH)$_4$ (µmol L$^{-1}$) for Stn. Ok

Legend:
- Control
- Fe
- DFB

Time (day):
- Stn. Oy
- Stn. BS
- Stn. Ok
Figure 7

(a) Changes in Chl-a (µg L⁻¹)
(b) Changes in [NO₃⁺NO₂⁻] (µmol L⁻¹)
(c) Changes in µcontrol/µFe-added
(d) Changes in [NH₄] (µmol L⁻¹)