

IRON AVAILABILITY BY COASTAL DIATOM *CHAETOCEROS* SP. IN THE SHIZUGAWA BAY, JAPAN

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This study aimed to investigate the spatial distribution of dissolved iron from river to coastal waters and iron bioavailability for coastal phytoplankton. Dissolved iron concentrations and other water quality parameters (e.g., pH, concentrations of dissolved organic carbon and trace metals, etc.) were determined in the Shizugawa Bay and its adjacent rivers, northeast Japan. Coastal dominant diatom (*Chaetoceros* sp.) isolated from the bay was used for incubational assay to examine growth kinetics in a range of iron concentrations. As a result, total dissolved iron concentrations of inland waters (75 ± 80 nM) were substantially higher than those of coastal waters (7.2 ± 4.8 nM). Among inland waters, iron concentrations from anthropogenic waters were relatively higher than those for forested river waters. In the bay, relatively higher concentrations of iron were observed in the inner part. From the growth experiment, half-saturation constant of iron for the growth of *Chaetoceros* sp. was determined to be 1.8 - 3.5 nM. The observed dissolved iron concentrations combined with growth response indicate that growth of *Chaetoceros* sp. is in some cases limited by iron availability. However, this study generally suggests that, while dissolved iron concentration largely decreased from river to coastal waters, terrestrial iron inputs potentially including both natural and anthropogenic sources contribute sufficient growth and iron availability by *Chaetoceros* sp. in the Shizugawa Bay.

Key words: iron, uptake kinetics, phytoplankton, coastal, terrigenous, anthropogenic

I. INTRODUCTION

Terrestrial nutrients loadings contribute to high productivities in coastal areas providing rich ecosystems and abundant fisheries resources. However, eutrophication by excess nutrient loadings from land to the ocean due to human activities have negative impacts on coastal environments, such as occurrences of harmful algal blooms (HABs) and oxygen deficient water. In Japan, HABs by eutrophication due to human activities have caused serious damage to the coastal environments from 1960's [1]. Then, after enforcing regulations for nutrient loads in 1973, eutrophication have been improved resulting om the decrease of red tide events in Japan. On the other hand, low nutrient concentrations have recently been suspected to cause some damage to fisheries, such as the bleaching

of cultured seaweeds (*Pyropia* spp.) in some areas [2, 3]. Thus, in order to preserve the coastal ecosystem with maximum utilization of the coastal environments, such as fisheries and aquaculture, the nutrient dynamics in the coastal regions and the impact of terrestrial loadings on the coastal ecosystems are needed to understand.

Iron (Fe) is an essential micronutrient for marine micro alga [4]. Thus, it limits the algal growth not only in open oceans [5–7] but in coastal areas as well [8] due to the lower solubility of Fe at the circumneutral pH comparing their requirement [9]. Terrestrial iron bound to organic matters, such as humic or fulvic acids is thought to make up most of the bioavailable iron supply to aquatic environments [8, 10, 11]. Although many studies that investigated the dynamics of the river and coastal iron were thought [8, 11–14], the iron dynamics and bioavailabilities from rivers to ocean have hardly been investigated simultaneously. This study aimed to investigate the spatial distribution of dissolved iron from river to coastal waters and iron bioavailability for coastal phytoplankton.

II. MATERIALS AND METHODS

Study site

Shizugawa Bay is a semi enclosed bay with an area of 46.8 km². It is located in northeastern Japan, facing the Pacific Ocean (Fig. 1). Most river mouths that flow into the bay, including the largest one (Hachiman River), are located in the inner part of the bay. The basins of the flowing river are mostly surrounded by mountains covered with coniferous or deciduous broad-leaved forests (>70%). Oysters and seaweeds are abundantly cultured in the entire area of the bay. The tsunami following the 2011 off the Pacific coast of Tohoku earthquake had severe impacts on the bay and most of its coastal flat land area.

Field sampling

Seawater samples were collected from 0 and 10 m layers (if water depth was less than 10 m, samples were collected at 8 m) at three fixed stations located in the inner, middle, and outer part of the Shizugawa Bay (Fig. 1) once every three months from July 2014 to April 2015. River water samples were collected from surface at the downstream of the three main rivers (Hacihman, Oritate, and Mizushiri) once every month from July 2014 to April 2015 (Fig. 1). The sample collection was conducted using acid washed plastic bottles, and basic water quality parameters were measured, including water temperature, pH, and electrical conductivity.

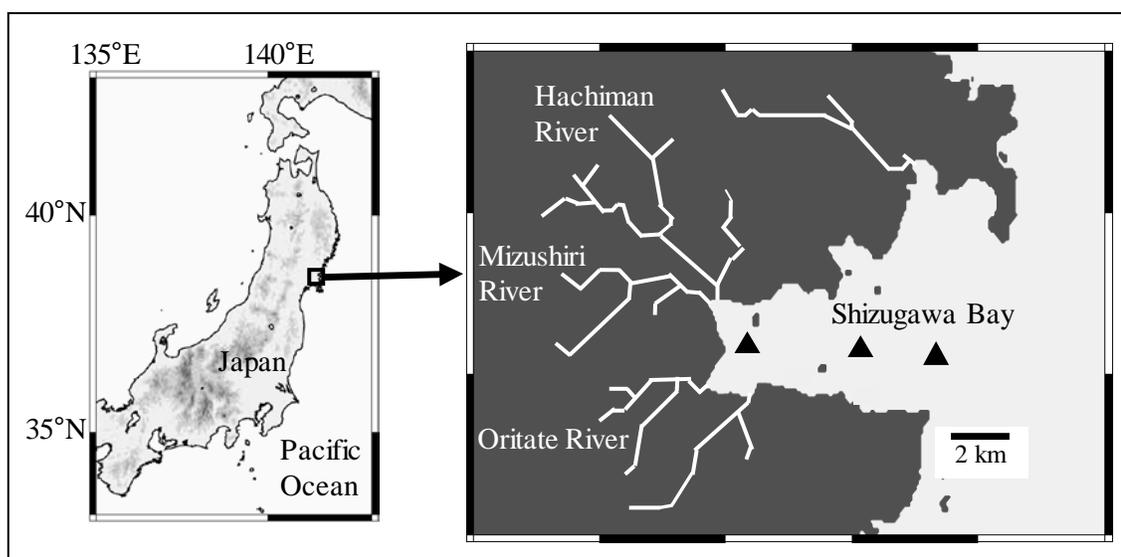


Fig. 1. Sampling location in the Shizugawa Bay, Japan and its main adjacent rivers. ▲ and ◆ indicate the stations at the bay and the rivers (Hachiman, Mizushiri, and Oritate Rivers).

Sample procedure

Collected water samples were filtered through a polytetrafluoroethylene membrane filter with 0.45 μm pore size (Merck Millipore). After filtration, water quality parameters, including salinity and concentrations of Fe, dissolved organic carbon, absorbance and fluorescence spectra were measured. The concentrations of dissolved Fe were determined using an inductively coupled plasma mass spectrometer (ICP-MS; 7700x, Agilent Technologies). Before analysis by ICP-MS, excess salt in the seawater samples were removed with solid phase chelate extraction technique using Nobias CHELATE-PA1 (Hitachi High-Technologies Co.; [15]). Because DOM and the specific UV absorbance (SUVA₂₅₄: ratio of absorbance at a wavelength of 254 nm [A₂₅₄] relative to the dissolved organic carbon concentration [DOC]) has a significant positive correlation with the metal-to-DOC concentration ratio (a parameter defined by the concentration ratio of dissolved trace metals relative to DOC) for all metals studied consists of a range of organic molecules, of which humic substances (HS) are the primary metal-binding ligands for trace metals including copper and iron.³The concentrations of dissolved organic carbon (DOC) and absorbance at a wavelength of 254 nm [A₂₅₄] were determined using a TOC analyzer (TOC-5000, Shimadzu) and a UV-visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) with 1 cm path-length quartz cuvette. All sample analysis were treated with clean techniques.

Iron uptake experiments

Centric diatom *Chaetoceros* sp. was isolated from the station at the inner part of the Shizugawa Bay in April 2015 (Fig. 1). From microscopic observation, this species was found to be most dominant in the bay during the sampling period. This clonal strain was rendered axenic by treatment with AM9 antibiotics [16], which was confirmed by epifluorescence microscopy with 4',6-diamidino-2-phenylindole (DAPI) staining method [17].

The strain was maintained with f/2 medium [18], and then 1 mL of the culture was inoculated into 50 mL of 1/10-diluted Aquil* medium [19, 20] with 1/50-diluted Fe (20 nM) and ethylenediaminetetraacetic

acid (EDTA, 200 nM) to introduce the Fe limited condition. The first pre-culture was grown for 7 days until it reached the stationary phase, and then 40 mL of this pre-culture was centrifuged at 3000 g for 10 min. The pellet occupied with algal cells were suspended to 40 mL of the artificial 1/10-diluted Aquil* medium without Fe (< 1 nM) and EDTA, and then inoculated for 2 days to prepare the Fe-starved culture of *Chaetoceros* sp. After the second pre-culture, the iron starved culture was centrifuged at 3,000 g for 10 min, and the pellets were re-suspended in the artificial seawater without any nutrients addition to remove the extracellular Fe. This washing step was conducted twice, and then the algal cells were suspended to the 40 mL of artificial sea water without nutrients. This algal suspension was used for the iron uptake experiments.

As for the culture mediums, 0.4 mL filtrated river water samples were added to 3.5 mL of artificial sea water enriched with 1/10 diluted Aquil* nutrients without Fe and EDTA. Similarly, 3.9 mL of filtrated seawater samples enriched with 1/10 Aquil* nutrients without Fe and EDTA was used for the culture medium. These medium were filter-sterilized with 0.1 µm filters and dispensed into the wells of sterile, plastic, 24-well microplates (IWAKI & Co., LTD.). Finally, 0.1 mL of the iron depleted preculture of *Chaetoceros* sp. was introduced into the medium with an initial cell density of 240 cells mL⁻¹. These procedures were treated with clean techniques in a clean bench. All medium and nutrient solutions filter-sterilized with 0.1 µm filters. Tests for each medium were triplicated.

These algal cultures were incubated at at 15°C, with a 12-h light:12-h dark cycle and a light intensity of 110 – 130 µmol photons m⁻² s⁻¹, and growth was monitored by microscopic cell counts using hemocytometer every other day. Using the cell count data, log phases of at each media were determined, and the specific growth rates (µ [division day⁻¹]) during the log phase were calculated using equation (1) shown below.

$$\mu = \frac{\ln(m_2 - m_1)}{t_2 - t_1} \quad (1)$$

Here, t_2 and t_1 indicate the incubation time (day) of start and end of the log phase, and m_2 and m_1 indicate the cell density at each incubation time. The specific growth rates were the average value from the triplicated cultures at each sample. The obtained iron concentrations and the specific growth rates of medium using the river and sea water samples were fitted to the following Michaelis–Menten equation.

$$\mu = \frac{\mu_{\max} \times [Fe]_T}{K_S + [Fe]_T} \quad (2)$$

Here, μ indicate the specific growth rate at each culture media. In this study, μ_{\max} was the maximum growth rate of this alga and was determined to be 1.2, according to the results of the preliminary test using Aquil* medium enriched saturated Fe (1.0 µM) and EDTA (10 µM). $[Fe]_T$ indicates the dissolved Fe concentration of each water sample measured with ICP-MS. From these data, half-saturation constants (K_S) was finally calculated. Least-square method was used for fitting the data.

III. RESULTS AND DISCUSSION

Bioavailabilities of Fe

The K_s of iron uptake by the *Chaetoceros* sp. was calculated as 1.8 and 3.5 nM with river water and seawater samples, respectively (Fig. 2A and 2B). These values were in almost in the same range,

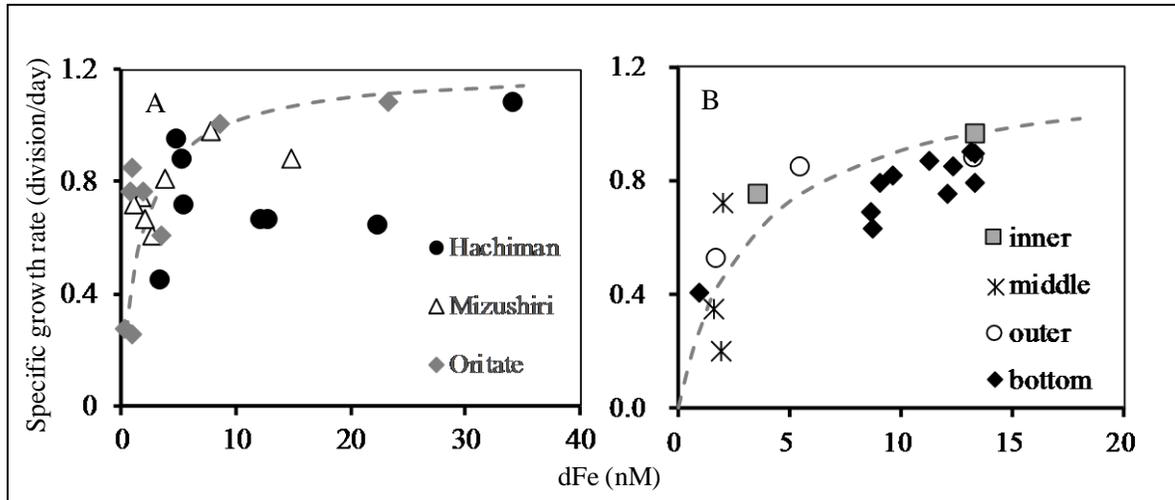


Fig. 2. The relationship between dissolved iron concentrations (dFe) and iron uptake by *Chaetoceros* sp., using the river (A) and sea water (B) samples collected in the Shizugawa Bay and its inflowing rivers. Dashed lines indicated the fitted line of the Michaelis-Menten equation.

suggesting that the terrestrial Fe transported from the rivers to the Shizugawa Bay was bioavailable for the marine phytoplankton. The dFe concentrations of the river water samples were sufficiently higher than those of seawater (Figs. 3 and 4). Thus, transported dFe from rivers to the ocean was considered to contribute to the increase in bioavailable Fe in Shizugawa Bay. On the other hand, dFe concentrations were lower than the K_s values in some cases, especially in the middle areas of the bay through the year, suggesting that iron potentially limits the algal growth in the bay. Thus, distribution of bioavailable Fe was potentially insufficient for algal growth, and Fe concentrations should be considered to maintain sustainable primary production in the bay.

Dynamics of Iron concentration in river and ocean

Total dissolved Fe (dFe) concentrations of the coastal waters in Shizugawa Bay ranged from 0.6 to 13.3 nM with an average of 7.2 ± 4.8 nM (Fig. 3). The dFe concentrations at the water surface were significantly lower than those of water at greater depths (9.5 ± 4.3 nM, t-test, $p < 0.05$), suggesting that iron elution from bottom sediments, Fe decomposition at deeper water, or Fe consumption by phytoplankton at the surface contributes to the iron dynamics in the bay. The dFe concentrations at the surface layer of the inner station in Shizugawa Bay (7.0 ± 3.8 nM) were higher than those at the middle station (1.6 ± 0.4 nM, turkey's test, $p < 0.05$), while no significant difference with the outer station (5.8 ± 4.6 nM) was observed. These dFe concentrations in the inner station were higher than those in the surface oceanic waters (0.42 – 3.53 nM from 5 and 10 m depths; [21]) near the Shizugawa Bay, influenced by the Oyashio and Kuroshio currents. These results indicate that terrestrial dFe loading contributes to the increase in the dFe concentrations in the inner part, but the influence of river input was limited to the inner part of the bay. Significant seasonal changes were not observed at neither

the surface nor bottom waters in the bay, and the other environmental factors (salinity and Chl a and DOC concentrations) did not show any significant correlation with dFe.

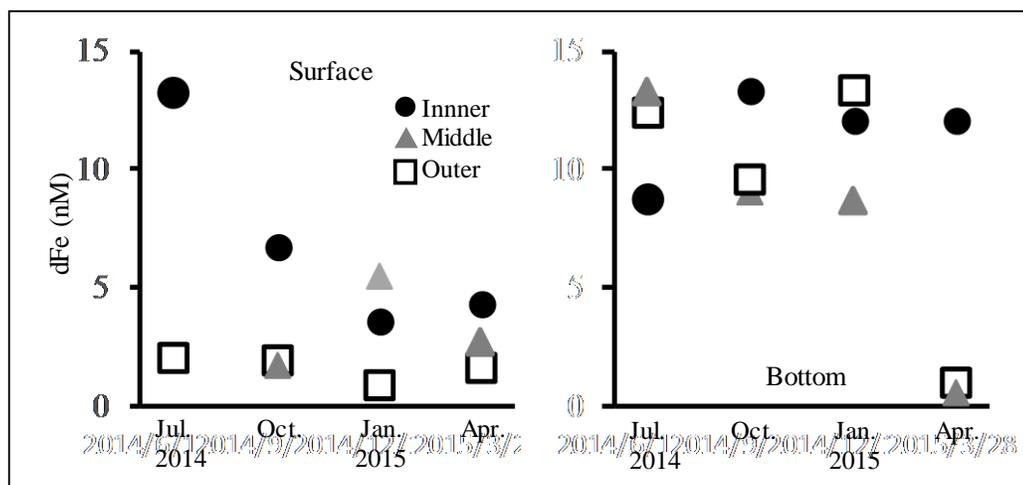


Fig. 3. The seasonal and spatial changes of dissolved iron (dFe) concentrations from surface and bottom layers at the inner (●), middle (▲), and outer stations (□) in the Shizugawa Bay during July, 2014 to April, 2015.

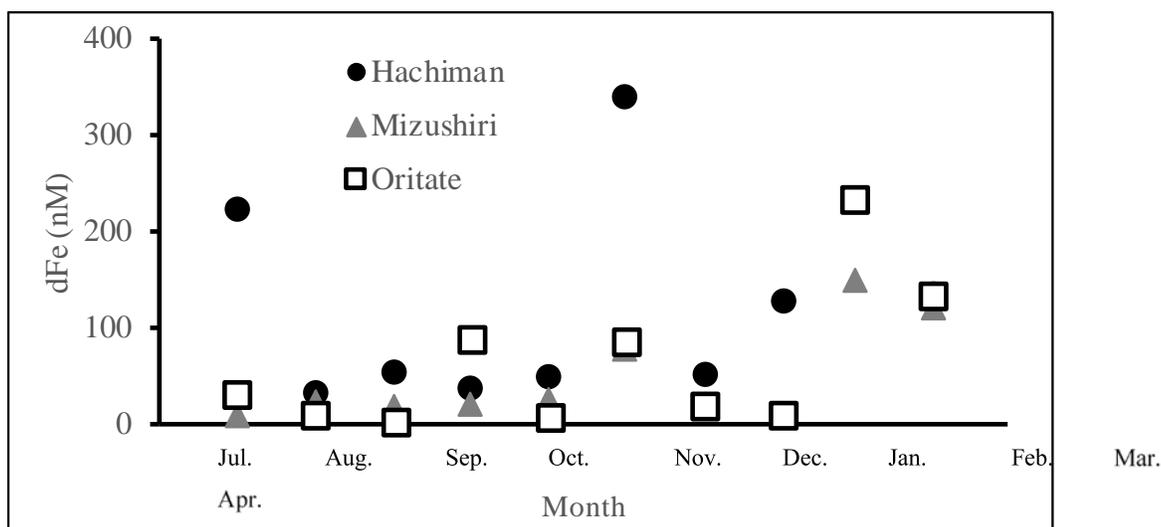


Fig. 4. The seasonal changes of dissolved iron (dFe) concentrations at downstreams of the Hachiman River (●), Oritate Rivwe (▲), and Mizushiri River (□) in the Shizugawa Bay during July, 2014 to April, 2015.

Total dissolved Fe (dFe) concentrations of the river waters varied between 3.9 and 341 nM with an average of 75 ± 80 nM (Fig. 4). These concentrations were relatively lower compared to the previous studies in close-by areas. For example, dFe concentrations of the rivers in the same prefecture of this study were reported to be 0.06 – 2.18 μM in Takagi River [10] and 0.11 – 5.46 μM , the rivers

flowing into the Kesenuma Bay [12], significantly lower than those in our study. The populations, agricultural fields, and flow length in the basins of the previous research were much larger and longer, respectively, than the rivers sampled in this study. Moreover, the dFe concentrations of the Hachiman River, where the population is the largest in the basin, were relatively higher than those in the other sampling rivers (Fig. 4). Therefore, the loads or characteristics of a river may potentially affect the Fe dynamics of river in some cases. In fact, the dFe concentrations of some of the anthropogenic waters collected from a septic tank and paddy fields in the Hachiman River basin (160 - 560 nM) were higher than those of the Hachiman river waters.

Some studies suggested that forested river water supplies more bioavailable Fe than urbanized river water [8]. In this study, however, dFe concentrations of the Hachiman River were the highest among the sampling rivers, and it also had the highest population in the basin. Meanwhile, Fe bioavailabilities for coastal diatoms were not different from river to river. Therefore, the present study suggests that both forested and urbanized river waters are both important for coastal productivity. However, the population density in the basin around the sampling site is much lower than the most urbanized regions in Japan such as Tokyo, and thus, Fe loading from urban waters and its bioavailability is still unclear.

The concentrations of dFe in each river (Hachiman, Mizushiri, or Oritate Rivers) significantly correlated with DOC (peason's simple regression, $r = 0.77, 0.70, \text{ or } 0.69$, respectively, $p < 0.05$; Fig. 5) and $[A_{254}]$ (peason's simple regression, $r = 0.79, 0.65, \text{ or } 0.73$, respectively, $p < 0.05$; Fig. 5). $[A_{254}]$ of natural water samples is known to show the strong correlation with the amounts of humic substance and both DOC and $[A_{254}]$ are reported to affect the dFe dynamics in the river waters [22–24]. Previous studies revealed that humic like organic matters contributes to the iron solubility in natural rivers as a function of iron chelator [25–27]. Similarly, the present study revealed the positive relationships between dFe concentrations, and DOC and $[A_{254}]$, and thus suggested that humic like substances in the rivers were highly contribute to the transport of bioavailable iron to the sea.

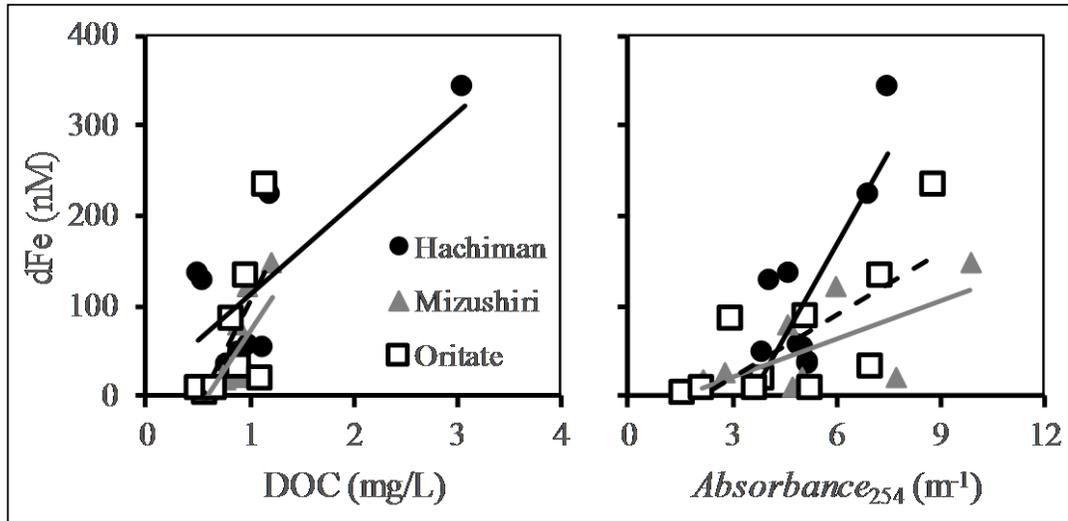


Fig. 5. The relationships between the dissolved Fe (dFe) and dissolved organic carbon concentrations and absorbance of UV (254 nm) light in the river water samples. Black, grey, and dashed lines indicate the regression lines of Hachiman, Mizushiri and Oritate Rivers water samples. DFe and dissolved organic carbon concentrations and absorbance of UV (254 nm) light in the river water samples show the significantly linear relationships ($p < 0.05$) in each river.

Conclusion

The coastal dFe concentrations were mostly higher than the K_s of iron uptake by the dominant diatom *Chaetoceros* sp., whereas the dFe concentrations were insufficient in some cases to maintain the *Chaetoceros* growth, especially in the middle and outer part of the bay, suggesting that Fe potentially limits the algal growth in the Shizugawa Bay. The dFe concentrations in the rivers were much higher than those in the river waters and surface iron concentrations in inner part of the Shizugawa Bay were higher than those in middle and outer part. In addition, the bioavailability of the dFe in the rivers were similar to those in seawater, suggesting the potential contribution of terrestrial iron to the coastal productivity.

IV. ACKNOWLEDEMENT

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