**Iron availability by coastal diatom *Chaetoceros* sp. in the Shizugawa**

**Bay, Japan**

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Abstract. 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 as 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 loading contributes to the high productivities in the coastal areas providing rich ecosystem and abundant fisheries resources. However, eutrophication by excess nutrients loading from land to the ocean due to human activities causes the negative impacts on costal environments, such as occurrences of harmful algal blooms (HABs) and oxygen deficient water. In Japan, HABs by eutrophication with human activities have caused serious damages to the coastal environments from 1960's [1]. Then, after enforcing the low regulation to the nutrients loads in 1973, eutrophication have been improved with decrease of red tide events in Japan. On the other hand, low nutrients concentrations has been recently considered to cause some fisheries damage, such as bleaching the 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, aquaculture, the nutrients dynamics in the coastal and its impact of the terrestrial loadings on the coastal ecosystem are needed to consider.

Iron (Fe) is an essential micronutrient for marine micro alga [4], but sometimes limits the algal growth in not only open oceans [5–7] but also coastal areas [8], due to the lower solubility of Fe at the circumneutral pH comparing their requirement [9]. Terrestrial iron bound to the organic matters, such as humic or fulvic acids is thought to supply huge bioavailable iron to the aquatic environments [8, 10, 11]. Although many studies investigated the dynamics of the river and coastal iron were investigated well [8, 11–14], the iron bioavailabilities and iron dynamics from rivers to sea have hardly 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 the area of 46.8 km2, and located in the northeastern Japan, facing on the Pacific Ocean (Fig. 1). Most river mouths 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 with the 2011 off the Pacific coast of Tohoku earthquake were attacked the bay and most coastal flat area.

*Field samplimg*

Seawater samples were collected from 0 and 10 m layers (if the water depth was less than 10 m, we collcted the bottom water samples from 8 m) at the three fixed stations located in the inner, middle, and outer part of the Shizugawa Bay (Fig. 1) on every three month from July 2014 to April 2015. River water samples were collected from surface at the downstream of the three main flowing rivers

*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).*

(Hacihman, Oritate, and Mizushiri rivers) into the inner part of the Shizugawa bay on every month from July 2014 to April 2015 (Fig. 1). The sample collection were conducted with acid washed plastic bottles, and basic water quality parameters, including water temperature, pH, and electrical conductivity were measured at the sampling.

*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 studiedconsists 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. From the microscopic observation, this species was found to be most dominant in the bay at the 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 sterved culture was centrifuged at 3,000 g for 10 min, and the pellets were re-suspended to 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 culture medium, 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-wells microplates (IWAKI & Co., LTD.). Finally, 0.1 mL of the iron depleted preculture of *Chaetoceros* sp. was introduced into the medium with initial cell density at 240 cells mL-1. These medium were prepared with triplicate. These procedures were treated with clean techniques in a clean bench. All medium and nutrient solutions filter-sterilized with 0.1 µm filters.

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 their growths were monitored by microscopic cell counts using hemocytometer every other day. As the result the cell counts, log phases at each media were determined, and the specific growth rates (µ [division day-1]) during the log phase were calculated below the equation.

** (1)

Here, t2 and t1 indicate the incubation time (day) of start and end of the log phase, and m2 and m1 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.

** (2)

Here, µ indicate the specific growth rate at each culture media. In this study, µmax was the maximum growth rate of this alga and determined as 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 using river water and seawater samples, respectively (Fig. 2A and 2B). These values were in almost same range, suggesting that the terrestrial Fe transported from river to the Shizugawa Bay was bioavailable for the marine phytoplankton. The dFe concentrations of the river water samples were sufficiently higher than those of seawaters. Thus, transported dFe from rivers to the ocean was considered to contribute to increase in the 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 in the view of the algal growth, and Fe concentrations should be considered to maintain the 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 7.2 ± 4.8 nM (Fig. 3). The surface dFe concentrations were significantly lower than those of bottom waters (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 surface contributes to the iron dynamics in the bay. The dFe concentrations in surface layer of the inner station in Shizugawa Bay (7.0 ± 3.8 nM) were higher than those of 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. This 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 in the inner part of the bay. Significant seasonal changes were not observed both surface and bottom waters in the bay, and the other environmental factors (salinity and Chl a and DOC concentrations) were not shown the any significant correlation with dFe.

Total dissolved Fe (dFe) concentrations of the river waters varied between 3.9 and 341 nM with an average 75 ± 80 nM (Fig. 4). These concentrations were relatively lower comparing to the previous studies in the closer areas. For example, dFe concentrations of the rivers in the same prefecture of this study were reported 0.06 – 2.18 µM in Takagi River, northeast Japan [10] and 0.11 – 5.46 µM, the flowing rivers into the Kesennuma Bay, northeast Japan [12], comparing those in our study. The populations, agricultural fields, and flow length in the basins of the previous researches were much larger and longer than the sampling rivers in this study. Moreover, the dFe concentrations of the Hachiman River, where the populations in the basin is largest, were relatively higher than those in the other sampling rivers (Fig. 4). Therefore, loads or characteristics of river potentially affect the Fe dynamics of river in some cases. Actually, the dFe concentrations of some 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 with highest population in its basin were highest in the sampling rivers, and Fe bioavailabilities of the 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 urban part in Japan such as Tokyo, and thus, Fe loading from urban waters and its bioavailability was 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, or 0.69, respectively, *p* < 0.05; Fig. 5) and [A254] (peason’s simple regression, *r* = 0.79, 0.65, or 0.73, respectively, *p* < 0.05; Fig. 5). [A254] of natural water samples is known to show the strong correlation with the amounts of humic substance and both DOC and [A254] 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 [A254], and thus suggested that humic like substances in the rivers were highly contribute to the transport of bioavailable iron to the sea.



*Conclusion*

The coastal dFe concentraitons 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 not different from those in the seawaters, suggesting the potential contribution of terrestrial iron to the coastal productivity.

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V. REFFERENCES

1. Imai I, Yamaguchi M, Hori Y (2006) Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. Plankt Benthos Res 1:71–84. doi: 10.3800/pbr.1.71

2. Itakura S, Imai I (2014) Economic impacts of harmful algal blooms on fisheries and aquaculture in western Japan – An overview of interannual variability and interspecies comparison. Proc Work Econ Impacts Harmful Algal Bloom Fish Aquac 17–26.

3. Nishikawa T, Tarutani K, Yamamoto T (2010) Nitrate and phosphate uptake kinetics of the harmful diatom Coscinodiscus wailesii, a causative organism in the bleaching of aquacultured Porphyra thalli. Harmful Algae 9:563–567. doi: 10.1016/j.hal.2010.04.007

4. Sunda WG (2012) Feedback interactions between trace metal nutrients and phytoplankton in the ocean. Front Microbiol 3:1–22. doi: 10.3389/fmicb.2012.00204

5. Hutchins D a., Hare CE, Weaver RS, et al (2002) Phytoplankton iron limitation in the Humboldt Current and Peru Upwelling. Limnol Oceanogr 47:997–1011. doi: 10.4319/lo.2002.47.4.0997

6. Coale KH (2004) Southern Ocean Iron Enrichment Experiment: Carbon Cycling in High- and Low-Si Waters. Science (80- ) 304:408–414. doi: 10.1126/science.1089778

7. Martin JH, Coale KH, Johnson KS, et al (1994) Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature 371:123–129.

8. Lewitus AJ, Kawaguchi T, Ditullio GR, Keesee JDM (2004) Iron limitation of phytoplankton in an urbanized vs . forested southeastern U . S . salt marsh estuary. 298:233–254. doi: 10.1016/S0022-0981(03)00361-7

9. Liu X, Millero FJ (1999) The solubility of iron hydroxide in sodium chloride solutions. Geochim Cosmochim Acta 63:3487–3497. doi: 10.1016/S0016-7037(99)00270-7

10. Matsunaga K, Kuma K, Toya K (1998) Riverine input of bioavailable iron supporting phytoplankton growth in Kesennuma Bay. Water Res 32:3436–3442.

11. Nishioka J, Nakatsuka T, Ono K, et al (2014) Quantitative evaluation of iron transport processes in the Sea of Okhotsk. Prog Oceanogr 126:180–193. doi: 10.1016/j.pocean.2014.04.011

12. Fujii M, Sasaki A, Otono S, et al (2006) Spatial and seasonal distributions of dissloved organic matter and iron Matsushima Bay, Japan. Jounal Japan Soc Water Environ 29:169–176.

13. Kuma K, Katsumoto A, Shiga N, et al (2000) Variation of size-fractionated Fe concentrations and Fe(III) hydroxide solubilities during a spring phytoplankton bloom in Funka Bay (Japan). Mar Chem 71:111–123. doi: 10.1016/S0304-4203(00)00044-X

14. Testa JM, Charette MA, Sholkovitz ER, et al (2002) Dissolved iron cycling in the subterranean estuary of a coastal bay: Waquoit Bay, Massachusetts. Biol Bull 203:255–256.

15. Sohrin Y, Urushihara S, Nakatsuka S, et al (2008) Multielemental Determination of GEOTRACES Key Trace Metals in Seawater by ICPMS after Preconcentration Using an Ethylenediaminetriacetic Acid Chelating Resin. Anal Chem 80:6267–6273. doi: 10.1021/ac800500f

16. Provasoli L, Shiraishi K, Lance JR (1959) NUTRITIONAL IDIOSYNCRASIES OF ARTEMIA AND TIGRIOPUS IN MONOXENIC CULTURE\*. Ann N Y Acad Sci 77:250–261. doi: 10.1111/j.1749-6632.1959.tb36905.x

17. Porter KG, Feig YS (1980) The use of DAPI for identifying aquatic microfloral. Limnol Oceanogr 25:943–948. doi: 10.4319/lo.1980.25.5.0943

18. Guillard RRL (1975) Culture of Phytoplankton for Feeding Marine Invertebrates BT - Culture of Marine Invertebrate Animals: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals Greenport. In: Smith WL, Chanley MH (eds). Springer US, Boston, MA, pp 29–60

19. Harrison PJ, Berges JA (2005) Marine culture media. Algal Cult Tech 21–34.

20. Morel FMM, Rueter JG, Anderson DM, Guillard RRL (1979) AQUIL: A CHEMICALLY DEFINED PHYTOPLANKTON CULTURE MEDIUM FOR TRACE METAL STUDIES12. J Phycol 15:135–141. doi: 10.1111/j.1529-8817.1979.tb02976.x

21. Takata H, Kuma K, Iwade S, et al (2004) Spatial variability of iron in the surface water of the northwestern North Pacific Ocean. Mar Chem 86:139–157. doi: 10.1016/j.marchem.2003.12.007

22. Maloney KO, Morris DP, Moses CO, Osburn CL (2005) The role of iron and dissolved organic carbon in the absorption of ultraviolet radiation in humic lake water. Biogeochemistry 75:393–407. doi: 10.1007/s10533-005-1675-3

23. Weishaar J, Aiken G, Bergamaschi B, et al (2003) Evaluation of specific ultra-violet absorbance as an indicator of the chemical content of dissolved organic carbon. Environ Sci Technol 37:4702–4708. doi: 10.1021/es030360x

24. Xiao Y-H, Sara-Aho T, Hartikainen H, Vähätalo A V. (2013) Contribution of ferric iron to light absorption by chromophoric dissolved organic matter. Limnol Oceanogr 58:653–662. doi: 10.4319/lo.2013.58.2.0653

25. Laglera LM, van den Berg CMG (2009) Evidence for geochemical control of iron by humic substances in seawater. Limnol Oceanogr 54:610–619. doi: 10.4319/lo.2009.54.2.0610

26. Powell RT, Wilson-Finelli a. (2003) Importance of organic Fe complexing ligands in the Mississippi River plume. Estuar Coast Shelf Sci 58:757–763. doi: 10.1016/S0272-7714(03)00182-3

27. Buck KN, Lohan MC, Berger CJM, Bruland KW (2007) Dissolved iron speciation in two distinct river plumes and an estuary: Implications for riverine iron supply. Limnol Oceanogr 52:843–855. doi: 10.4319/lo.2007.52.2.0843