

Iron Fertilization with Enhanced Phytoplankton Productivity under Minimal Sulfur Compounds and Grazing Control Analysis in HNLC Region

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The present study investigated quantitatively the significance of HNLC (high-nutrient low-chlorophyll) regions and its grazing control with the improved iron fertilization for climate change. The limitation of iron (Fe) for phytoplankton growth in HNLC regions was confirmed by sulfur compounds (S) such as volcanic ash and hydrogen sulfide (H₂S) in batch cultures, whose chemical sediment of Fe₃S₄ showed 4.06 wt%. The technologies developed for iron fertilization since 1993 till now were not practical to provide sufficient amounts of bioavailable iron due to sedimentary iron sulfides induced by undersea volcanic sulfur compounds. The proposed technology for iron fertilization was improved to enhance the bioavailable iron to phytoplankton by keeping minimal sulfur compounds in HNLC regions. The low productivity of phytoplankton by grazing control in HNLC regions was 6% diatoms whose 52% was grazed by copepods and 42% by krill on the basis of data analysis in 2000 EisenEx Experiment at boundary of Antarctic and African tectonic plates. All of the previous iron fertilization experiments were conducted at volcanic sulfur compounds enriched HNLC regions. The present study revealed that the enhanced phytoplankton productivity in batch culture without sedimentary iron sulfides can be possible only if sulfur compounds are minimal, as is in Shag Rocks (53°S, 42°W) of South Georgia in Scotia Sea in the Southern Ocean.

Keywords

Iron Fertilization, Phytoplankton, Productivity, Grazing Control Analysis, Minimal Sulfur Compounds

1. Introduction

The hypothesis of iron fertilization was speculated by English biologist Joseph Hart in 1934, raised by John Gribbin in 1988, and renewed by American oceanographer John Martin four months later. As reviewed by Dugdale and Wilkerson [1], Barber and Ryther described an area of east of the Galapagos Islands in 1969 and Strickland et al. had interests in waters exhibiting relatively abundant nutrients, but low chlorophyll and low productivity in 1969. Minas et al. had firstly designated the terms of "high nutrient, low chlorophyll (HNLC)" in 1986. Martin's iron enrichment experiments into the amounts of carbon drawn into the seas by algae formed the basis for 14 mesoscale efforts during the last 24 years. Martin and Fitzwater [2] hypothesized in 1988 that primary productivity in HNLC regions was limited by the availability of iron (Fe). Hematite (Fe_2O_3) and goethite (FeO(OH)) in the aeolian dust tend to be associated with fine (0.3 - $1 \mu m$) particles, with long residence times (days) in the atmosphere and thus potentially long transport paths [3]. If Fe reacts with dissolved sulfur (S) under steam heating $(T > 600^{\circ}C)$ conditions [4], the product results in insoluble black ferrous sulfide (FeS), which reacts again with acidic hydrogen sulfide (H₂S) to form pyrite (FeS₂) and hydrogen (H_2) [5]. The existence of HNLC regions was attributed to constant zooplankton grazing over phytoplankton, trace metal toxicity, limitation of iron input, light limitation due to the deep mixed layer. However, none of these factors have been proved conclusively [1] [6]. Woods Hole Oceanographic Institution studied the design for a mesoscale iron enrichment experiment with several questions such as where should the experiment be performed and what would be used as an iron source [7]. No one has yet clearly answered to these questions even if 14 iron enrichment experiments were conducted in HNLC regions. The goal of the present study was to examine empirically the Fe limitations induced by S compounds of volcanic ash and hydrogen sulfide to improve the iron fertilization for the maximal phytoplankton productivity and estimate analytically the grazing control by copepods and krill over diatoms in HNLC regions.

2. Results and Discussion

2.1. Mass Balance of Iron in Global Surface Ocean

In order to differentiate the global ocean into four oceanic regions in terms of accumulation rates of iron (Fe) and sulfur (S), the accumulation rate of Fe in the

ocean,
$$\left(\frac{dFe}{dt}\right)$$
, is given by :

$$\frac{dFe}{dt} = \left(\dot{F}e\right)_{in} - \left(\dot{F}e\right)_{out} + \left(\dot{F}e\right)_{gen} - \left(\dot{F}e\right)_{con} - \left(\dot{F}e\right)_{rxn}$$

where:

 $(\dot{F}e)_{in}$ = the input rate of Fe (nmol·m⁻²·d⁻¹) from desert dust, volcanic ash, rivers, and bottom sediments,

 $(\dot{F}e)_{out}$ = the output rate of Fe during Fe Cycle is negligible due to short term (hours) biological iron uptake,

 $(Fe)_{gen}$ = the generation rate of Fe from vertical mixing, upwelling and biogenic recycling of cellular iron within the ocean [8],

 $(\dot{F}e)_{con}$ = the consumption rate of Fe by phytoplankton assimilation,

 $(\dot{F}e)_{rxn}$ = the removal term for dissolved Fe by scavenging on the sinking particulate matter and chemical reaction rate of Fe with volcanic S compounds as sedimentary FeS and FeS₂.

Oceans are subdivided into four regions based on the amounts of nutrient and chlorophyll; HNLC (high-nutrient, low-chlorophyll), HNHC (high-nutrient, high-chlorophyll), LNLC (low-nutrient, low-chlorophyll), and LNHC (low-nutrient, high-chlorophyll), as shown in Table 1.

It is assumed that $\frac{dFe}{dt} \gg 0$ for HC (high-chlorophyll) regions (as is in HNHC and LNHC) if Fe supplied largely from deserts and upwelling. When $\frac{dFe}{dt} < 0$ and the accumulation rate of volcanic S compounds, $\frac{dS}{dt} \gg 0$ for LC (low-chlorophyll) regions (as is in HNLC and LNLC) are satisfied if Fe is rarely replenished from deserts and subsurface water upwelling while volcanic S compounds are abundant. We present a review of the four oceanic regions listed above is presented here. 1) The southern Omani coast was studied to evaluate HNLC characteristics during the late Southwest Monsoon (Aug.-Sep.) [9]. Fe-replete dusts from the Arabian and Syrian deserts were blocked by high Omani mountains (~3000 m) during the late Southwest Monsoon so that desert dusts could not reach the southern Omani coast, where many volcanoes are active (e.g. 13 volcanoes in Yemen), Saudi Arabia (24 volcanoes), Iran (7 volcanoes), Iraq (1 volcano), India (4 volcanoes), and Pakistan (7 volcanoes). Oxygen minimum zones in the Arabian Sea (not in the open ocean where HNLC water present) forms due to the oxidation of volcanic sulfur compounds of S, SO₂, SO₃, and H_2SO_4 to sulfates (SO₄²⁻) by consuming the dissolved oxygen in waters. Therefore, the contribution of volcanic S compounds to the southern Omani coast during the late Southwest Monsoon is much greater than those of Fe from deserts to satisfy the condition $\frac{dFe}{dt} < 0$, $\frac{dS}{dt} \gg 0$, and $\left|\frac{dFe}{dt}\right| \ll \left|\frac{dS}{dt}\right|$ for HNLC region. 2) Fertilization potential near the Anatahan volcano in the Northern Mariana Islands of western North Pacific Subtropical Gyre showed low concentrations of nitrate (0.042 µM), phosphate (0.003 µM), and iron (0.002 μ M), following the Anatahan eruption leading to the presence of the most oligotrophic LNLC ocean deserts on Earth [10] with chlorophyll-*a* of 0.07 μ g·l⁻¹ [11]. In the western North Pacific Subtropical Gyre there are minimal Asian dust inputs and annually persistent Anatahan volcanic eruptions (2003, 2004, 2005,

2006, 2007, 2008) to satisfy the criteria of $\frac{dFe}{dt} < 0$, $\frac{dS}{dt} > 0$, and $\left| \frac{dFe}{dt} \right| < \left| \frac{dS}{dt} \right|$ for LNLC region. 3) Benguela upwelling system in Southwestern Africa is one of

	Oceanic Location Major Nutrient Sour			
Region	(Relative Accumulation Rates of Fe and S)	Desert (Fe)	Volcano (Number) (S)	
HNLC	1) Southern Ocean 2) Equatorial Pacific 3) Subarctic Pacific $\left(\frac{dFe}{dt} < 0, \frac{dS}{dt} \gg 0\right)$ $\left(\left \frac{dFe}{dt}\right \ll \left \frac{dS}{dt}\right \right)$	1) Austalian/Patagonian/ Kalahari/Antarctic Polar 2) Gobi/Atacama 3) Gobi	1) Erebus (Antarctica) (19)/ Huaynaputina (Peru) (29)/ Hudson (Chile) (137) 2) Huaynaputina (Peru) (29)/ Hudson (Chile) (137)/ Cotopaxi (Ecuador)(43) Galapagos Islands (Ecuador) (12) 3) Aleutian (40)/ Augustine (USA) (15)/ Kamchatka (Russia)(29)	
LNLC	1) Ryu Kyu/Izu-Bonin Arc 2) Hawaii 3) western North Pacific Subtropical Gyre/Guam 4) Iceland $\left(\frac{dFe}{dt} < 0, \frac{dS}{dt} > 0\right)$ $\left(\left \frac{dFe}{dt}\right < \left \frac{dS}{dt}\right \right)$	1) Gobi 2) (Gobi) 3) (Gobi) 4) Highlands of volcanic desert	1) Japan (108) 2) Hawaii (15) 3) Anatahan (USA) 4) Iceland (130)	
НNHC	Benguela upwelling system $\left(\frac{dFe}{dt} \gg 0, \frac{dS}{dt} < 0\right)$ $\left(\left \frac{dFe}{dt}\right \gg \left \frac{dS}{dt}\right \right)$	Kalahari, Namib	None active but extinct; Angola(0), Namibia (1), South Africa (4)	
LNHC	1) Pacific coast of Mexico 2) Northeast Pacific 3) Northwest Pacific 4) Northeastern Canada 5) Peruvian coast 6) New Zealand 7) Southern Africa 8) The Antarctic $\left(\frac{dFe}{dt} \gg 0, \frac{dS}{dt} > 0\right)$ $\left(\left \frac{dFe}{dt}\right > \left \frac{dS}{dt}\right \right)$	 1) Chihuahuan, Sonoran, Mojave 2) Gobi, Great Basin 3) Gobi 4) Arctic, Sahara 5) Patagonian, Atacama 6) Great Victoria, Great Sandy, Gibson, Simpson 7) Kalahari, Namib 8) Antarctic, Great Victoria, Great Sandy, Gibson, Simpson, Patagonian, Atacama, Kalahari, Namib 	 Barcena, Socorro and others Augustine (15), Kasatochi, Redoubt (13) Pavlof (40), Cleveland (19) Hokkaido (17), Honshu (46), Greenland, Iceland, Newfoundland Seamounts, Fogo Seamounts Peru (29) and Chile (137) White Island, Kermadec Islands Madagascar (5), Mozambique (1), Tanzania (22), South Africa (4) Erebus (19) 	

 Table 1. Oceanic regions with relative accumulation rates of iron (Fe) from desert and sulfur (S) from volcano.

the most productive fishery areas in the world with sand storms from Kalahari and Namib deserts in winter without any active volcanoes (Table 1).

There are four more regions associated with major fisheries and coastal upwelling Currents-; 1) Canary Current with Sahara desert but no volcanoes; 2) California Current with deserts of Mojave, Colorado and Great Basin but no active volcanoes; 3) Humboldt Current with desert of Atacama but no volcanic fallout due to Chilean volcanic ashes blown to the south and Argentina; and 4) Somali Current with desert of Danakil-Kaisut but no fallout of volcanic ashes since Southwest Monsoon during summer moves three active volcanic ashes from northeastward along with the coastal waters. These five coastal upwelling

regions meet the criteria of $\frac{dFe}{dt} \gg 0$, $\frac{dS}{dt} < 0$, and $\left|\frac{dFe}{dt}\right| \gg \left|\frac{dS}{dt}\right|$ for HNHC regions. 4) Indonesia has 127 active volcanoes but no desert. However, western Australian dusts from Great Victoria, Great Sandy, Gibson, Tanami, and Little Sandy deliver Fe-enriched dusts (7 - 18 wt% Fe compared to the common 3.5 -6.0 wt%) to Indonesian marine waters. Java Sea is surrounded by active volcanoes while the South Java Current flows eastward along the coast during the Northwest Monsoons. Nitrate (0.5 - 1.5 μ M), phosphate (0.05 - 0.4 μ M), silicate (4 - 14 μ M), and chlorophyll-*a* (0.3 - 1.0 μ g·l⁻¹) were monitored in Java Sea [12], where is a major fishing ground in Indonesia. Java Sea of Indonesia meets the criteria: $\frac{dFe}{dt} \gg 0$, $\frac{dS}{dt} > 0$, and $\left|\frac{dFe}{dt}\right| > \left|\frac{dS}{dt}\right|$ for LNHC region. Therefore, the present scheme of categorizing oceans in HNLC, LNLC, HNHC and LNHC regions by using the relative magnitude of the accumulation rates of Fe from deserts and subsurface water upwelling while S from volcanic sulfur compound, as illustrated in Table 1, appears to be reasonable.

2.2. Estimation of Desulfurization Time from Fresh Volcanic Ashes

Iron in volcanic ashes have been reported in a number of complex forms that include Fe_2O_3 , Fe_3O_4 , $FeCl_2$, $FeCl_3$, FeF_2 , FeF_3 , FeS, FeS_2 , $FeSO_4$ and $Fe_2(SO_4)_3$ [13]. Fresh volcanic ash from Japanese Ontake volcano erupted in September 27th, 2014, was sampled a week later after its eruption to determine concentration of sulfur (**Table 2**).

Volcanic ash samples from 5 other volcanic eruptions, as morphologically examined in Figure 1, Mount Baekdu of Korea, Mount St. Helens of Washington, Mount Pinatubo of Philippines, Lombok of Indonesia, and Kasatochi of Alaska, were also collected for S concentration. Since the Fe/S ratios of all the aged volcanic ash samples (Table 2) were far greater (10.8 - 191.8) than that of the fresh Ontake volcanic ash (1.13), it was evident that volcanic sulfur was diminished likely due to weathering, as shown in Figure 2.

The relationship between S content and the elapsed time was modeled by the first order decay [14] as $(\ln \frac{C_A}{C_{AO}} = -kt)$, and the resulted rate constant (*k*) of 0.1392 y·r⁻¹ from the slope of **Figure 2**. This rate constant was used to estimate a time necessary for fresh volcanic ash to release S in the surface of the earth, us-

ing the equation of $\{t = \frac{\ln\left(\frac{1}{29531}\right)}{-0.1392 \text{ yr}^{-1}}\}$, by assuming all four volcanic ash from



Figure 1. Morphology of volcanic samples by scanning electron microscopy (SEM). The numbers indicated the year of volcanic eruption with the magnification folds (×). (a) Japanese Ontake (erupted in 2004) (×3000); (b) Alaska Kasatochi (2008) (×2000); (c) Indonesian Lombok (1994) (×2000); (d) Philippines Pinatubo (1991) (×1000); (e) Washington St. Helens (1980) (×1000); (f) Korean Baekdu (969) (×1000).



Figure 2. The first order decay of volcanic sulfur to estimate the elapsed time of 74 years

 $\{t = \frac{\ln\left(\frac{1}{29531}\right)}{-0.1392 \text{ yr}^{-1}}\}, \text{ for desulfurization of the volcanogenic sulfur available from various}$

volcanic ashes (data from Table 2) since the volcanic eruption.

Volcano	Eruption (year)	Fe (mg/kg)	C _A S (mg/kg)	Fe/S	$C_A/C_{AO}^{\ b}$	lnC _A /C _{AO}	Time ^c (yr)
Mount Ontake, Japan	2014	33,312	29,531	1.13	1	0	0
Kasatochi, Alaska	2008	157,942	14,572	10.8	0.493	-0.70	6
Lombok, Indonesia	1994	60,961	701	87.0	0.0240	-3.74	20
Mount Pinatubo, Philippines	1991	73,857	385	191.8	0.0130	-4.33	23
Mount St. Helens, Washington	1980	23,235	955	24.3	0.0323	-3.43	34
Volcanic stone							
Mount Baekdu ^a	969	24,368	191	127.6	-	-	-
Soil							
Tongyoung, Korea	-	26,014	1350	19.3	-	-	-

Table 2. Characteristics of volcanic ashes, volcanic stone, and soil around the world.

Note: ^aVolcanic stone not ash; ^bC_{AO} was taken from Ontake; and ^cTime elapsed since 2014 of Ontake eruption. Concentrations of sulfur and iron were measured using an inductively coupled plasma (ICP) (Optima 5300DV, Perkin Elmer).

Mount St. Helens, Mount Pinatubo, Lombok, and Kasatochi, had the same initial sulfur concentration of the fresh Ontake volcanic ash. Based on the present data set in **Figure 2**, 74 years was required to release S from the fresh volcanic ash left on the oxygenated surface of the earth.

2.3. Phytoplankton Productivity with Iron (Fe) and Volcanic Ash

The effects of Fe and volcanic ash upon the growth of phytoplankton were experimentally examined under aerobic culture of Chlorella vulgaris with various JM media; with its own Fe, mixture of 75% clay and 25% volcanic ash, fresh 100% volcanic ash, without its own Fe. The growth curves for 4 similarity experiments of 4 ecosystems (Table 1) of HNHC (high nutrient, high chlorophyll), HNLC (high nutrient, low chlorophyll), LNHC (low nutrient, high chlorophyll), LNLC (low nutrient, high chlorophyll) were shown in Figure 3 after three reproducible measurements in six days batch cultures expressed by negligible error bar for the standard deviation at each point. 150 ml JM medium without Fe was used as the base medium for the case of HNLC while 0.1 g of Japanese Ontake volcanic ash (100% volcanic ash) was added to JM medium without Fe for the case of LNLC. A mixture of 0.075 g of Tongyoung clay and 0.025 g of Ontake volcanic ash, was added to 150 ml JM medium without Fe for the case of LNHC while JM medium with its own Fe was used for the case of HNHC. The 100% volcanic ash (V100) showed the low growth of phytoplankton, similar to LNLC regions. The base medium without Fe showed the lowest growth, similar to HNLC regions. The present similarity experiments were in good agreement with the phytoplankton productivity of oceanic regions in the sequence of HNHC > LNHC > LNLC > HNLC in Table 1. The present results of bottle experiments showed similar growth patterns of "with iron" (+Fe) and "without iron" (-Fe) in Martin [6].



Figure 3. Similarity experiments of phytoplankton productivity under aerobic culture of *Chlorella vulgaris* with various JM media; with its own Fe (+Fe, -+-) HNHC, mixture of 75% clay and 25% volcanic ash (C75, ---) LNHC, fresh 100% volcanic ash (V100, - \blacktriangle -) LNLC, without its own Fe (-Fe, - \bigcirc -) HNLC. Standard deviation was expressed by each error bar for three measurements with excellent reproducibility. The 100% volcanic ash (V100) showed the low growth of phytoplankton, similar to LNLC regions. The base medium without Fe showed the lowest growth, similar to HNLC regions. The present similarity experiments were in good agreement with the phytoplankton productivity of oceanic regions in the sequence of HNHC > LNHC > LNLC > HNLC in **Table 1**. The present results of bottle experiments showed similar growth patterns of "with iron" (+Fe) and "without iron" (-Fe) in Martin [6].

2.4. Reaction with Iron and Sulfur Compounds

S compounds (S, SO₂, SO₃, H₂S, H₂SO₄, sulfates) induce bio-available Fe²⁺_(aq) toward rapid black iron sulfide (FeS) ($\Delta G_r^{\circ} = -1207.719 \text{ kJ} \cdot \text{mol}^{-1}$ between FeOOH and H₂S) and slow pyrite (FeS₂) sedimentations ($\Delta G_r^{\circ} = -30.7648$ between FeS and H₂S, and -30 kJ·mol⁻¹ between FeS and S°) [15] in HNLC regions [16] without releasing Fe²⁺_(aq) to phytoplankton during pyrite (FeS₂) formation, except for the iron- and sulfur-oxidizing bacteria such as *Acidithiobacillus ferrooxidans*, *Alicyclobacillus* and *Sulfobacillu*, living in pyrite deposits to metabolize ferrous iron and sulfur and producing sulfuric acid [17]. Therefore, HNLC regions are big reservoirs of S compounds from volcanic eruptions to induce sedimentary FeS and FeS₂ with Fe-limited oceans. The more H₂S is available from S compounds, the more sedimentation occurs in the forms of FeS and FeS₂ as shown in **Figure 4**. Therefore, the volcanic eruption enhances the formation of FeS and FeS₂ to be Fe-limited condition of "LC" (low-chlorophyll).

Iron sulfate heptahydrate (FeSO₄·7H₂O) has been used in the iron fertilization experiments as main source of iron due to its high solubility in water. Each 25 gram was dissolved in two bottles with 1 liter distilled water; one was bubbled with H₂S gas (50 ppm, 2 LPM, 30 minutes) while another was kept as blank. **Figure 5** showed the pale green color (left) as the control without H₂S bubbling while the dark black color (right) as the experiment with H₂S bubbling. The sedimentary materials were filtered to analyze by X-ray diffractometer (XRD)



Figure 4. Pathways of Fe from aeolian dust and S from volcanic eruption prior to being consumed by phytoplankton in HNLC regions. Hydrogen sulfide (H_2S) is produced by 4 routes; volcanic gas (0.04% - 0.68%), sulfur oxidizing bacteria such as *Beggiatoa*, sulfate reducing bacteria (SRB) such as *Desulfovibrio desulfuricans*, and decomposed microorganism.



Figure 5. Before (left) and after (right) H_2S gas (50 ppm, 2 LPM, 30 minutes) bubbling into distilled water dissolved with FeSO₄.7H₂O.

(Model Dmax2500/PC) for the presence of iron sulfides in form of Greigite-Fe₃S₄ among sulfur and iron oxide, as shown in **Figure 6**. Quantitative analysis of **Figure 6** implied that the crystallinity of Fe₃S₄ constituted 4.06 wt% of total sedimentary materials among amorphous peaks, as shown in **Figure 7**. It was thus evident that H₂S binds Fe to sediment in black iron sulfide and deprive Fe from the growth of phytoplankton, as shown in **Figure 4** and **Figure 7**. For the improved iron fertilization, it was necessary to avoid the locations with volcanoes for the least sedimentary iron sulfide.







Figure 7. Quantitative analysis of Fe_3S_4 in XRD peaks of 4.06 wt % induced by the chemical reaction between dissolved FeSO_4 .7H₂O and H₂S gas bubbling in distilled water.

2.5. Phytoplankton Productivity with H₂S

The effect of S compounds upon the growth of phytoplankton was experimentally examined. H_2S generated by the decomposed white of egg was prepared to see its removal of Fe in JM medium with EDTA-Fe as sedimentary iron sulfide (FeS). **Figure 8** showed the growth curves of phytoplankton in JM medium with various volumes of the decomposed egg solution. Due to the addition of the decomposed egg solution producing dissolved hydrogen sulfide (H_2S) to the



Figure 8. Aerobic culture of *Chlorella vulgaris* in JM media with various percent volumes of the decomposed egg solution; with its own Fe and without decomposed egg solution (0%) (JM + 0 ml, -+-) for the case of HNHC, mixture of 140 ml JM and 10 ml decomposed egg solution (7%) (JM + 10 ml, -•-) for the case of LNHC, 120 ml JM and 30 ml decomposed egg solution (21%) (JM + 30 ml, - \blacktriangle -) for the case of LNLC, 110 ml JM and 40 ml decomposed solution (28%) (JM + 40 ml, - \blacklozenge -), and without its own Fe (JM-Fe, -x-) for the case of HNLC.

present JM culture media, cell density in 10⁴ cells/ml was measured (Figure 8) by microscope to avoid the problem of turbidity instead of measuring absorbance at 660 nm (Figure 3). It was evident that the phytoplankton growth was retarded as increasing the volume of decomposed egg solution, generating H₂S to remove Fe from JM medium with EDTA-Fe as sedimentary iron sulfide (FeS), from 0 ml, 10 ml, 30 ml, 40 ml among total balanced 150 ml JM culture media. The highest productivity of phytoplankton was observed when there was no any addition except JM media itself with EDTA-Fe (iron-replenished). As increasing dissolved H₂S amounts by increasing the volume of the decomposed egg solution, dissolved H₂S reacted with Fe of JM media in EDTA-Fe to sediment as iron sulfide. There was a color change from white of egg solution to dark brown color upon addition of JM media with EDTA-Fe to the decomposed egg solution generating H₂S. As the volume of decomposed egg solution was reached to 40 ml (28%), the resultant phytoplankton growth curve was overlapped with the case without its own Fe, which implied that 40 ml decomposed egg solution with dissolved H₂S was good enough to deprive all the Fe of remained 110 ml JM media with EDTA-Fe. Figure 8 clearly indicated that dissolved H₂S from the decomposed egg solution reacted with Fe in JM media to be Fe-limited. The present results of H₂S generating egg solution were in good agreement with the phytoplankton growth curves in similarity experiments in Figure 3. Therefore, HNLC regions might be caused by locking iron (Fe) with sulfur (S) compounds including volcanic ash and hydrogen sulfide (H₂S), available from sub-aerial or underwater active volcanoes to form insoluble iron sulfide for Fe-limited low chlorophyll condition.

2.6. HNLC Regions

Characteristics of HNLC regions were well described by high surface nitrate, low DO, high SO_2 dispersion and Fe-limited profiles in **Figure 9**.

SO₂ concentration in air over the Sierra Negra Volcano in Galapagos Islands during an eruption in October 2005 showed that SO₂ plume (Figure 9(c)) coincides with the surface nitrate (Figure 9(b)), DO at 200 m (Figure 9(d)), and Fe-limited profiles (Figure 9(e)) in the Equatorial Pacific Ocean. It is thus evident that SO_2 dispersion (Figure 9(c)) from sub-aerial volcanoes in tectonic plate boundaries (Figure 9(e) and Figure 9(f)), is linked to not only high nitrate concentration (Figure 9(b)) but also Fe-limitation (Figure 9(e)), which are typical circumstances of HNLC regions. The number of sub-aerial volcanic eruptions and earthquakes are about fifty and millions events every year, respectively, mostly from plate boundaries of 17 major and minor rigid tectonic plates (Figure 9(f)). Volcanoes and earthquakes are generally found where plate boundaries are at the bottom of the oceans; therefore, most volcanic activity is submarine, as seen in black smokers [18] with SO₂ (0.5% - 11.8%) and H₂S (0.04% - 0.68%). Sulfate reducing bacteria (SRB) produce H₂S from sulfate (SO_4^{2-}) under hypoxic condition while iron reducing bacteria produce Fe²⁺ from Fe₂O₃/FeOOH [5]. The resulting chemical reaction occurred around submarine volcanoes is iron sulfides (FeS/FeS₂) particles which eventually sunk at the deep sea and ultimately buried in the sea floor. The Subarctic Pacific Ocean, one of the large HNLC regions, is surrounded by many active volcanoes. In the Aleutian chains, Pavlof has erupted 40 times since 1700's and continue till today; Cleveland erupted 19 times since 1980's and continue today [19] while Mt. Kasatochi erupted most recently in 2008. Augustine volcanoes have erupted 14 times since 230 and more recently in 2005, and volcanoes in the Cook Inlet area (e.g., Katmari, Redoubt, St. Augstine, Crater Peack, Spurr) have erupted every 10 - 35 years during the 20th century [20]. Besides, the heavy yearly rainfall (more than 2000 mm a year) in the Gulf of Alaska may enhance the effective removal of atmospheric SO₂ dispersion from volcanic plume into the Gulf of Alaska. Therefore, the Subarctic Pacific HNLC region appears to be driven by such volcanic eruptions for at least during the last few hundred years. The Southern Ocean is also surrounded by many active volcanoes. Mt. Erebus belonging to the McMurdo Volcanic Group in Ross Island, Antarctica, [21], has erupted continuously since 1972. More than 26 active and extinct volcanoes in Peru and Chile, 50 plus eruptions in Galapagos Islands during the last 200 years, 10 volcanoes with recent eruption of Cotopaxi in Ecuador, surround the South Pacific Ocean and Equatorial Pacific. Sulfur compounds emitted from active volcanoes are carried away by prevailing winds and deposited on the sea surface. Such volcanic sulfur compounds deposit over the ocean area may induce the formation of three different HNLC regions, as can be observed from the dissolved Fe distribution of Figure 9(e) and summarized in Table 3.



Figure 9. Global distribution maps associated with HNLC regions: (a) Locations of iron fertilization till the present detailed in **Table 4** [22]; (b) Surface ocean nitrate concentrations [23]; (c) Sulfur dioxide concentration over the Sierra Negra Volcano, Galapagos Islands during an eruption in October 2005 [24]; (d) Concentration of dissolved oxygen [25]; (e) Surface ocean iron-concentrations, location of Fe-limited HNLC regions and sub-aerially active volcanoes in subduction zones and other tectonic settings [13]; and (f) Major tectonic plates on earth [26].

2.7. Previous 14 Iron Fertilization Experiments

The previous 14 iron enrichment experiments on mesoscale size since IronEx I of 1993 till Haida Gwaii of 2012 (Table 3) have commonly shown short-term impacts on the sequestration of atmospheric CO_2 due to their inappropriate locations near or on the streamlines of high SO_2 (Figure 9(c)), low DO (Figure 9(d)), near active volcanoes (Figure 9(e) and Table 3), and tectonic plate boundaries (Figure 9(f) and Table 3). It was thus very important to choose the location of the iron fertilization, not too close to the source of sulfur (S) compounds such as volcanoes, earthquakes, and boundaries of tectonic plates. A

SINK (HNLC)	CARRIER		SOURCE					
				Fe				
	Current	Wind	Volcano (Number)	Tectonic Plate		Desert		
Southern Ocean	Antarctic Circumpolar, East Wind Drift	Westerly, Polar Jet Stream	Erebus (Antarctica) (19),	Antarctic/	Surrounded	Australian/ Patagonian/ Kalahari/ Antarctic Polar		
			Huaynaputina (Peru) (29),	Pacific/Scotia/				
			Hudson (Chile) (137)	African/Australian				
	Peru (Humboldt), South Equatorial	Equatorial	Galapagos Islands (12),		Surrounded	Gobi/Atacama		
Equatorial Pacific			Huaynaputina (Peru) (29),	Pacific/Cocos/ Nazca/South				
			Hudson (Chile) (137),	American				
			Cotopaxi (Ecuador) (43)					
Subarctic Pacific	Alaska, Kamchatka, Kuroshio, Subarctic, North Pacific	Aleutian Low	Aleutian (40),	Desifie (Olthesteld)				
		Polar Jet Stream	Kasatochi, Redoubt (USA) (13), Kamchatka (Russia) (29)	North American	Blocked	Gobi		

Table 3. Sink, carriers and sources of sulfur (S) compounds and iron (Fe) in HNLC regions.

global distribution of oceanic dissolved oxygen (DO) (**Figure 9(d**)) showed that DO in the Equatorial Pacific was low while that of the Southern Ocean was high. Since SO₂ reacts with DO and becomes H₂SO₄ and sulfates with consumption of DO, the distribution map of DO or O₂ (**Figure 9(d**)) is then the opposite as that of SO₂ (**Figure 9(c**)). That is, if a region contains low DO, then such a region has high SO₂ due to the neighboring active volcanoes. Therefore, SO₂ emissions from Galapagos Islands (**Figure 9(c**)), mountains of Peru, Ecuador and Chile showed long dispersing tails same patterns as those of nitrate (**Figure 9(b**)) and Fe-limited HNLC regions (**Figure 9(e**)) with low profiles of DO (**Figure 9(d**)) in the Equatorial Pacific. Since the Southern Ocean flows very fast ($v = 4 \text{ km} \cdot \text{h}^{-1}$), its mass flow rate ($C_A \cdot Q_A$) enriched with fresh air of 20.9% O₂ (C_A) by westerly winds and Polar Jet Stream contains abundant DO due to rapid mixing of water by Antarctic Circumpolar Current.

Most of SO₂ (C_A) from yearly continuous volcanic eruptions at Mt. Erebus of the Antarctic are quickly dissipated at the deep ocean of the Southern Ocean due to its enormous amount of oceanic volumetric flow rate ($1.3 \times 10^8 \text{ m}^3 \cdot \text{sec}^{-1}$, Q_A) of water causing much higher mass flow rate ($\dot{m}_A = C_A \cdot Q_A$) of SO₂ dissolution and subsequently locking volcanic sulfur compounds, as observed in the oxygen minimum zones (~1100 m) of the abundant H₂S pool (~150 µM) [16]. Since the Southern Ocean is analogous to the system of continuously stirred tank reactor (CSTR) with high revolution agitator ($v = 4 \text{ km} \cdot \text{h}^{-1}$) to have a uniform distribution of volcanic sulfur compounds throughout the Antarctic Circumpolar Current (ACC) with limited leakage to outside, the concentrations of nitrate (**Figure 9(b**)) and DO (**Figure 9(d**)) are the highest among 3 HNLC regions.

Although the aeolian dust over continents carry abundant iron (Fe) (3.5 - 6.0

wt% or 7.0 - 18.0 wt% clay in Tasmania of Australia) to HNLC regions, most of them are sedimented out from the upper surface waters as insoluble iron sulfides (FeS/FeS₂) with reaction of sulfur compounds (S, SO₂, SO₃, H₂S, H₂SO₄, sulfates) in the ocean, available from volcanic eruptions either in submarine or in air, while only negligible amounts of iron $(1 \times 10^{-8} \text{ \%}) \{=(4 \times 10^{-6} \text{ ppm}/3.5 \times 10^{4} \text{ m})\}$ ppm) \times 100)} are available in HNLC regions, assuming 4 \times 10⁻⁶ ppm Fe (0.07 nmol L⁻¹) [22] out of 3.5 % (= 3.5×10^4 ppm) Fe supply by aeolian dust. The present study suggests that HNLC regions (Figure 9(b) and Table 3) could be simultaneously formed due to the following reasons; 1) Blocked (Southern Ocean and Subarctic Pacific) or surrounded (Equatorial Pacific) by tectonic plates (Figure 9(f)), whose boundaries emit volcanic sulfur compounds (Figure 9(c)) to form iron sulfides. The iron sulfide particles are subsequently sunk to the deep waters (Figure 9(e)), 2) Located in the downwind or stream of volcanoes (Figure 9(c)), 3) Located far from continents to take river/bottom sediments except the Subarctic Pacific, 4) Mimic aeolian dust inputs ($4 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ for Southern Ocean, 7 for Equatorial Pacific, and 14 for Subarctic Pacific) [11], and 5) Isolated (Southern Ocean and Subarctic Pacific) from neighboring Oceans by currents and winds, as summarized in Table 3. The locking and eventual removal of Fe by volcanic S compounds may have resulted in nutrient-enriched but Fe-limited HNLC regions while other oceanic regions maintain high chlorophyll with less accumulation of major nutrients (N, P, Si).

Although 14 iron fertilization experiments during the last 24 years have been conducted [22] including LOHAFEX in 2009 [27] and Haida in 2012 [11] (**Table 4**), such experiments (**Figure 9(a**)) have never been conducted at location that is free from serial volcanic eruptions in the recent 74 years for the removal of volcanogenic sulfur (see **Figure 2**).

2.8. Improved Iron Fertilization Experiment

The sulfate concentration increased horizontally towards the volcanic source [28]. Vertical profile of O_2 at the southern Omani coast with seasonal HNLC characteristics showed severely O_2 -depleted waters (DO < 0.2 mg·l⁻¹, ~9 μ M O_2) from 170 m to 1000 m depth due to extensive consumption of DO in waters by the volcanic S compounds, while minimum limit of dissolved oxygen content for living fish of about 5 mg·l⁻¹ DO (156 μ M O₂) was available from its oceanic surface to 80 m depth [9]. Thus, the volcanic S compounds deposited on the surface ocean of HNLC regions appear to dissolve their S compounds extensively at the depth range of 170 m and 1000 m where phytoplankton cannot live due to toxic volcanic chemicals (SO₂, H₂S, HCl, HF, H₂SO₄) [29] in waters. In contrast, the concentration of diatom Pseudonitzschia was maximal between 45 to 80 m (or upper 80 m) depth in HNLC region [30]. Therefore, Fe-replete composite should be made using Fe-enriched natural clays (7% - 18% Fe in west Australia) or aged (more than 74 years) desulfurized volcanic ashes while the target sea area should be O₂-enriched upper 80 m layer of the surface ocean with buoyant carriers so as to be assimilated to phytoplankton community.

Experiment	Date	Location	Tectonic Plate	Volcano (Number)	
IronEx I	(Month/Year)	5°S, 90°W	Pacific/Nazca	Galapagos Islands (12)	
IronEx II	June 1995	4°S, 107°W	Pacific/Nazca	near Seamount A at East Pacific Rise	
SOIREE	February 1999	61°S, 140°E	Antarctic/Australian	Erebus (19)	
EisenEx	November 2000	48°S, 21°E	Antarctic/African	Erebus (19)/near Marion at African Plate	
SEEDS I	July 2001	48.5°N, 164.5°E	Pacific/Okhotsk	Kamchatka Peninsula (29)	
SOFEX-N	January 2002	55°S, 172°W	Pacific/Antarctic	Erebus (19)	
SOFEX-S	March 2002	66°S, 172°W	Pacific/Antarctic	Erebus (19)	
SERIES	July 2002	51°N, 144.5°W	Pacific/North American	Augustine (15)	
EIFEX	February 2004	50°S, 2°E	Antarctic/ South American	Erebus (19)/near Bouvet at African Plate	
SAGE	March 2004	46.5°S, 172.5°E	Pacific/Australian	Erebus (19)/near Chatham Islands with volcanic ash from Taupo Volcanic Zone	
FeeP	July 2008	27.6°N, 22.4°W	African/Eurasian	near El Hierro of Canary Islands	
SEEDS II	July 2004	48°N, 166°E	Pacific/Okhotsk	Kamchatka Peninsula (29)	
LOHAFEX	March 2009	48°S,16°E	Antarctic/African	Erebus (19)/near Marion/Bouvet at African Plate	
Haida Gwaii	July 2012	52.7°N, 139.3°W	Pacific/North American	Bowie Seamount	

Table 4. Previous iron fertilizations with location, tectonic plate, and volcano.

Fourteen previous iron enrichment experiments from IronEx I in 1993 to Haida Gwaii in 2012 have been conducted by dumping a few hundred tons of Fe in form of FeSO₄. The previous iron fertilizations (Figure 9(a) and Table 4) were located near subaerial and underwater volcanoes in the divergent boundaries of tectonic plates (Figure 9(c) and Figure 9(e); Table 4). The Ring of Fire is an area in the basin of the Pacific Ocean where a large number of volcanoes and tectonic plate boundaries are present (Figure 9(e) and Figure 9(f)). Therefore, the prospective iron fertilization experiment is recommended to be conducted far from sulfur sources such as volcanoes and boundaries of tectonic plates, not only to minimize the iron consumption by the chemical formation of sedimentary FeS and FeS₂ due to the volcanic S compounds, but also to maximize the availability of dissolved Fe to phytoplankton. The dissolved Fe is engulfed by cyanobacteria (the diazotrophs Trichidesmium and Crocosphaera Watsonii, and the non-diazotrophs Synechococcus and Prochlorococcus) to be grazed by diatoms and subsequently by copepods, krill, and small fish or humpback whale. Humpback whale has been observed in seven HNLC regions of the Subarctic Pacific (Alaska) and the Equatorial Pacific (Galapagos) with five areas in the Southern Ocean (Bransfield Strait, Gerlache Strait, South Georgia, Crozet, Kerguelen), four LNLC regions of Iceland (Snaefellsnes Peninsula), Japan (Zamami), Guam, and Hawaii (Maui), five coastal upwelling HNHC regions (Benguela, Canary, California-Monterey Bay, Humboldt-Galapagos, Somali), and eight hot fishery oceans of LNHC regions in Table 3. However, five locations in the Southern Ocean (Bransfield Strait, Gerlache Strait, South Georgia, Crozet, Kerguelen) were more prospective ones due to their iron availabilities from upwelling and hydrothermal vent [16] with enriched nutrients and high mass flow rate of ACC for wide natural bloom area during warm austral summer [31]. Shag Rocks (53°S, 42°W) of South Georgia in Scotia Sea appeared to be the most prospective location for the following reasons. 1) Not over the tectonic plate boundary, but just outside of the northern Scotia plate [32] and far from volcanoes. 2) Located in the downwind region of near Patagonian Fe-rich desert (800 km distanced) [33] and rocky islands covered with nutrient-enriched ice melt. 3) Cold Weddell Sea Deep Water meets with nutrient warm waters of the Subantarctic upwelling, providing ACC with high flow velocity (~4 km·h⁻¹, v) and the largest current ($125 \times 10^6 \text{ m}^3 \cdot \text{s}^{-1}$, Q) for fast deployment of Fe-replete composite (m_A) or (C_A) over a wide oceanic bloom area (~145,000 km²) [34] with high momentum $(m_A \cdot v)$ and high mass flow rate $(\dot{m}_A = C_A \cdot Q)$, and full depth vertical fields with 100 m plateau and 3500 m deep water of Southern ACC Front [35] to induce nutrient-enriched upwelling, as observed in Crozet Islands and Kerguelen Plateau in the Southern Ocean [36]. 4) High nitrate (22 - 29 µM) and chlorophyll (0.46 - 0.93 $\mu g \cdot \ell^{-1}$) [17] with ACC dominated by diatoms and Weddell Sea Deep Water dominated by coccolithophorids and silicoflagellates passing through, located at the South of the Polar Front dominated by diatoms, and high concentrations of silica for diatoms are available. 5) Located not far (185 km) from South Georgia Island $(170 \times 40 \text{ km})$ which is the unique position inside the Antarctic Convergence yet outside the limit of the yearly sea ice to be home to tens of millions of breeding penguins, 300,000 elephant seals, 3 million fur seals, and 25 species of breeding birds, implying its good location for phytoplankton productivity. 6) Located not far from the used whale station of Grytviken in South Georgia, which had been open in 1904 but closed in 1966 due to diminishing whale population. Since there are still massive elephant seals, fur seals, and king penguins at Grytviken but no humpback whales around, Grytviken can be a good base camp for the iron fertilizing ships and their crew residence not for several weeks, as commonly done in the previous 14 mesoscale iron fertilizations, but for several months or even years to observe the Fe stimulated phytoplankton productivity such as the return of humpback whales as biomarkers for the improved iron fertilization.

2.9. Data Analysis for Grazing Control in HNLC Regions

The substitution of ferredoxin by flavodoxin, the use of plastocyanin instead of cytochrome bf and a variant stoichiometry of photosynthetic complexes are

notable adaptive strategies to facilitate diatom growth in low-iron condition [37]. Diazotroph *Trichodesmium* required iron to form the enzyme nitrogen (nitrogenase) for fixation of N_2 as much as 465 molar ratio of N/Fe while 5,000 for diatoms in nitrate (nitrate reductase) and 16,000 for flagellates in ammonium (nitrite reductase) [38]. With assumption of 100% Fe by *Trichodesmium* for

N₂-fixation, it was evident that diatom took 9% Fe (= $\frac{465}{5000} \times 100$) and flagellates

took 3% Fe ($=\frac{465}{16000}$ ×100) as illustrated in **Figure 10**. Therefore, diatoms took 109% iron if diatoms (9% Fe) graze *Trichodesmium* (100% Fe) in HNLC regions. Dinoflagellate *Gymnodium breve* had $K_s = 7.2 \mu mol NO_3^- kg^{-1}$ while diatoms had $K_s = 1.3 \mu mol NO_3^- kg^{-1}$, which implied that NO_3^- was mainly consumed by diatoms rather than by dinoflagellates [38]. These observations might be one of reasons why diatoms are dominant at high NO_3^- and low Fe levels in HNLC regions.

Diatoms are important primary producers (>40%) in the ocean with a high sinking velocity of 0.96 m·d⁻¹ for large centric diatoms [39]. Diatoms by cheap (8%) energy requirement with genetically adapted system in HNLC regions [40] are capable of dividing at any point of the diel cycle, which are preferably fed by copepods and krill during the night. The depth of the iron enrichment experiment should be within the top 80 m layer where diatoms [41], krill and humpback reside together while copepods live deeper to escape from krill with inverse relationship between krill and copepods abundances [42]. In the present analysis of the experimental data [43] in 2000 EisenEx Experiment conducted in the Southern Ocean (21°E, 48°S) in **Table 4**, the unsteady state mass balance of diatoms in a controlled patch volume is given by:





$$\frac{\mathrm{d}X_d}{\mathrm{d}t} = \left(\dot{X}_d\right)_{in} - \left(\dot{X}_d\right)_{out} + \mu_d - \left(\mathcal{U}_c + \mathcal{U}_k\right)$$

where $(\dot{X}_d)_{in}$ = diatoms input rate,

 $(\dot{X}_d)_{out}$ = diatoms output rate,

 X_d = concentration of diatoms in patch at time *t* of days since the first rion fertilization,

 μ_d = growth rate of diatoms,

 u_c = diatoms loss rate by copepods,

 u_k = diatoms loss rate by krill.

If diatoms input rate was close to diatoms output rate in a controlled patch volume,

$$\left(\dot{X}_{d}\right)_{in} \approx \left(\dot{X}_{d}\right)_{out}$$
$$\therefore \frac{\mathrm{d}X_{d}}{\mathrm{d}t} = \mu_{d} - \left(\mathcal{U}_{c} + \mathcal{U}_{k}\right)$$

According to the definition [44],

...

$$\mu_d = \frac{1}{X_d} \frac{\mathrm{d}X_d}{\mathrm{d}t} = \frac{\mathrm{d}\ln X_d}{\mathrm{d}t}$$

If assuming that copepods and krill were grown by their grazing diatoms, diatoms loss rates by copepods and krill could be similarly defined as,

$$\mathcal{U}_{c} = \frac{d \ln X_{c}}{dt}$$
$$\mathcal{U}_{k} = \frac{d \ln X_{k}}{dt}$$
$$\frac{d X_{d}}{dt} = \frac{d \ln X_{d}}{dt} - \left(\frac{d \ln X_{c}}{dt} + \frac{d \ln X_{k}}{dt}\right)$$

Thus,

$$\mathrm{d}X_d = \mathrm{d}\ln X_d - \left(\mathrm{d}\ln X_c + \mathrm{d}\ln X_k\right)$$

By integration,

$$\therefore X_d = k + \ln\left(\frac{X_d}{X_c \cdot X_k}\right)$$

where
$$k = X_{do} + \left(\ln \frac{X_{co} \cdot X_{ko}}{X_{do}} \right)$$

 X_d = concentration of diatoms in patch at time *t* of days since the first iron fertilization,

 X_c = concentration of copepods in patch at time *t* of days since the first iron fertilization,

 X_k = concentration of krill in patch at time *t* of days since the first iron fertilization,

k = initial constant,

- X_{do} = initial concentration of diatoms in patch,
- X_{co} = initial concentration of copepods in patch,

 X_{ko} = initial concentration of krill in patch.

There could be 3 special cases at steady state such that the apparent diatoms growth rate was greater, equal and less than grazing loss rates by both of copepods and krill. For simplicity, let's took the second case that the apparent diatoms growth rate was equal to grazing loss rates by copepods and krill as below.

$$\mu_d = \mathcal{U}_c + \mathcal{U}_k$$

$$\frac{d \ln X_d}{dt} = \frac{d \ln X_c}{dt} + \frac{d \ln X_k}{dt}$$

$$\therefore d \ln X_d = d \ln (X_c \cdot X_k)$$

Since there was the inverse relationship between copepods and krill abundances [44], it could be denoted as,

$$X_c = \frac{c_1}{X_k}$$

where c_1 = inversely proportional constant between copepods and krill.

$$\therefore d \ln X_d = d \ln c_1$$

By integration,

$$\ln\!\left(\frac{X_d}{X_{do}}\right) = c_2$$

where c_2 = integration constant

$$X_d = X_{do} \cdot \mathbf{e}^C$$

If we wanted to sequester the atmospheric CO_2 by the efficient growth rate of diatoms with iron enrichment, we had to keep the concentrations of diatoms after the iron fertilization (X_d) to be greater than concentrations of diatoms before the iron fertilization (X_{do}) with the constraint as,

$$\therefore X_d > X_{do} \cdot e^{C_2}$$

If the grazing rate U was slightly modified from previous definition as,

$$\mathcal{U} = F\left(\frac{n}{V}\right)$$

where F = clearance rate (ml·ind⁻¹·h⁻¹),

n = number of grazers (ind),

V = experimental volume (ml) = $S \cdot H$,

S = experimental surface area (m²),

H= upper 150 m of the water column [43].

From **Table 1** in Manuscript 1 [43], copepods standing stocks were 57,755 for >2 mm and 180,730 for 1 - 2 mm with resultant sum of 238,485 (ind·m⁻²) at 7 days since the first iron fertilization in patch. Thus,

$$\left(\frac{n}{V}\right) = \left\{\frac{(n)}{S}\right\} \left(\frac{1}{H}\right) = \frac{238485 \text{ ind} \cdot \text{m}^{-2}}{150 \text{ m}} = 1590 \text{ ind} \cdot \text{m}^{-3}$$

From **Table 4** in Manuscript 1 [43], clearance rates inside the fertilized patch were given by, *Calanus simillimus* (12.4 in day 8), *Rhincalanus gigas* (7.7 in day 16), and copepods < 2 mm (0.2 in day 7), whose sum was 20.3 (ml·ind⁻¹·h⁻¹).

Therefore, the grazing rate by copepods, U_c , was given by,

$$\mathcal{U}_c = F\left(\frac{n}{V}\right) = \left(20.3 \text{ ml} \cdot \text{ind}^{-1} \cdot \text{h}^{-1}\right) \left(1590 \text{ ind} \cdot \text{m}^{-3}\right) \left(\frac{1 \text{ m}^3}{10^6 \text{ ml}}\right)$$
$$\therefore \mathcal{U}_c = 0.0323 \text{ h}^{-1}$$

From Experiment 2 with dominant diatom *Pseudonitzschia* of **Table 1** in Manuscript 3 [43], the grazing loss rate by krill of *Euphausia superba* from 5.7 [BSi] to 1.7 [BSi] with duration of grazing phase (96 h) and *Calanus propinquus* from 6.5 [BSi] to 1.9 [BSi] with duration of grazing phase (90 h) were, respectively, calculated with the assumption of the first order decay [45] as below:

$$\frac{C}{C_0} = \mathrm{e}^{-\mathcal{U}_k t}$$

where C = free concentration of Biogenic Silica [BS_i] (µmol·l⁻¹).

 C_0 = initial concentration of Biogenic Silica [BS_i] (µmol·l⁻¹).

$$\frac{1.7}{5.7} = e^{-\mathcal{U}_{k_1}(96)}$$
$$\therefore \mathcal{U}_{k_1} = 0.0126 \text{ h}^{-1}$$

for Euphausia superba,

$$\frac{1.9}{6.5} = e^{-\mathcal{U}_{k_2}(90)}$$

$$\therefore \mathcal{U}_{k_2} = 0.0137 \text{ h}^{-1}$$

for Calanus propinquus.

Since diatoms were mainly (70%) ingested by *Euphausia superba* and *Calanus propinquus*, as shown in "BSi ingested %" of **Table 1** in Manuscript 3 [43], the grazing rate by krill, U_k , was given by,

$$\therefore \mathcal{U}_k = \mathcal{U}_{k_1} + \mathcal{U}_{k_2} = 0.0263 \text{ h}^{-1}$$

Since U_c and U_k were determined, the apparent diatoms growth rate was given by,

$$\mu_d = \mathcal{U}_c + \mathcal{U}_k = 0.0323 \text{ h}^{-1} + 0.0263 \text{ h}^{-1} = 0.0586 \text{ h}^{-1}$$

From Figure 2 in Manuscript 1 [43], "diatoms % contribution to abundance in control bottles" were given as a function of days with 5 interpolated data points such that time "(days)" and, "diatoms % contribution to abundance after the first iron fertilization (X_d) " were, (t, X_d) as (2, 55), (7, 65), (8, 62), (11, 75) and (16, 80), respectively. All the diatom % contributions (X_d) were divided by the initial diatom % contribution of 56 (X_{do}) to get (X_d/X_{do}) and $\ln(X_d/X_{do})$ A good linear relationship ($\mathbb{R}^2 = 0.8855$) between $\ln(X_d/X_{do})$ and time (days) was shown in Figure 11 with slope of 0.0893 d⁻¹ or 0.0037 h⁻¹, as the apparent growth rate of diatoms after grazed by copepods and krill, μ_d .



Figure 11. Logarithmic ratio of frequency distribution of the major prey taxa in the control bottles over the course of the experimental days to determine the apparent diatoms growth rate after grazed by copepods and krill, whose data were interpolated and rearranged from **Figure 2** in Manuscript 1 [43] for the present study.

It was summarized as;

1) The apparent growth rate of diatoms $(0.0037 h^{-1})$ and the sum $(0.0586 h^{-1})$ of grazing loss rates by copepods $(0.0323 h^{-1})$ (52%) and krill $(0.0263 h^{-1})$ (42%) gave the overall diatoms growth rate of $0.0623 h^{-1}$ (=0.0037 + 0.0586) equivalent to 1.5 d⁻¹, which was comparable to 1.79 d⁻¹ between day 4 and 7 of the centric diatom of *Chaetocerus debilis* in the western Subarctic Pacific [46],

2) The doubling rate of diatoms after grazed by copepods and krill was 0.13 d^{-1} from dividing (0.0037 h^{-1}) (24 h/d) by ln2, which was comparable to 0.18 - 0.67 d^{-1} of chlorophyll in the Antarctic [6],

3) For the efficient sequestration of atmospheric CO_2 , it was necessary to increase the amount of diatom due to the relatively small apparent growth rate of residual diatom (0.0037 h⁻¹, 6%). To overcome such extreme grazing controls (94%) by copepods and krill, the duration and area of the iron enrichment experiment should be extended to more than 3 months (Dec.-Feb.) when chlorophyll-*a* reached its peak concentration (\geq 0.75 mg·m⁻³) with a large area of phytoplankton blooms (~145,000 km²), as observed in the South Georgia [47] of the Southern Ocean with fast ACC for the high mass flow rate of diatoms stimulated by the Fe-replete buoyant carrier.

3. Conclusion

The present study examined empirically the Fe limitations induced by sulfur compounds of volcanic ash and hydrogen sulfide to improve the iron fertilization for the efficient phytoplankton productivity and estimated analytically the grazing control by copepods and krill over diatoms in HNLC regions. The limitation of Fe for phytoplankton growth in HNLC regions was confirmed by sulfur compounds such as volcanic ash and hydrogen sulfide (H₂S) in batch cultures. Distribution of XRD pattern for the sedimentary materials induced by the chemical reaction between dissolved $FeSO_4$ ·7H₂O and H₂S gas bubbling in dis-

tilled water, showed that the chemical sediment of Fe_3S_4 constituted 4.06 wt% of total sedimentary materials among sulfur, iron oxide and Fe_3S_4 . The low productivity of 6% diatoms was caused by grazing control in HNLC regions with 52% by copepods and 42% by krill on the basis of data analysis in 2000 EisenEx Experiment. Oceans were classified by four characteristic regions (HNLC, LNLC, HNHC and LNHC) based on the relative magnitude of the accumulation rates of Fe from deserts and S compounds from volcanoes. All of the previous iron fertilization experiments were conducted at volcanic sulfur compounds enriched HNLC regions. The present study revealed that the enhanced phytoplankton productivity in batch culture without sedimentary iron sulfides can be possible only if sulfur compounds are minimal, as is in Shag Rocks (53°S, 42°W) of South Georgia in Scotia Sea in the Southern Ocean.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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