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# **Phytoplankton Community Response to Fe and Temperature Gradients in the NE (SERIES) and NW (SEEDS) Subarctic Pacific Ocean**

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## Abstract

Ship-board iron enrichment bottle experiments were carried out with samples collected at the mesoscale iron fertilization experimental site (SERIES) in the subarctic NE Pacific in the summer of 2002. Samples were collected on Day 14 of the experiment outside the patch that was in a typical HNLC (high nitrate and low chlorophyll) condition. The iron concentration in the incubation bottles ranged from 0.1 to 2.0 nM by adding FeCl<sub>3</sub> solution. The increase in Chl-*a* in the micro (>10 μm) and nano-sized (2-10 μm) fraction was observed as a function of the added iron. Chl-*a* in the pico-sized fraction (0.7-2 μm) showed no increase with time. Nitrate and silicate were exhausted in the Fe-amended bottles, while those in the control bottle remained at the end of incubation. The relative consumption ratio of silicate to nitrate for the control bottles was significantly higher than that for the Fe-amended bottles. As a hyperbolic relation was found between iron concentration and the rate of increase in Chl-*a* (specific growth rate) for the micro and nano-sized fraction, the Monod equation was fit to obtain a maximum growth rate ( $\mu_{\max}$ ) and a half-saturation constant for iron ( $K_{\text{Fe}}$ ). The  $\mu_{\max}$  values were 0.72 and 0.48 d<sup>-1</sup> for the micro and nano-sized fraction, respectively. The

$K_{Fe}$  values were 0.10 and 0.08 nM for the micro and nano-sized fraction, respectively.

The  $\mu_{max}$  agreed with the rate of increase in Chl-*a* observed *in situ* for the mesoscale iron fertilization experiment. The  $\mu_{max}$  value for micro-sized fraction at 12°C was half of that in the western subarctic Pacific Ocean (SEEDS experiment in 2001), indicating the Chl-*a* increase rate (potential growth rate) after iron enrichment was much higher in SEEDS than that in SERIES. The  $K_{Fe}$  values were much lower than that in SEEDS, suggesting that the phytoplankton community in the NE subarctic Pacific Ocean acclimates to a lower ambient Fe concentration. This difference in  $K_{Fe}$  between SERIES (NE) and SEEDS (NW) may reflect the previously suggested gradient in Fe flux to the subarctic Pacific Ocean. A temperature gradient was also applied to investigate the effect of temperature on the growth response of the phytoplankton community. No obvious effect of temperature increase to 16°C was found in SERIES, while  $\mu_{max}$  and  $K_{Fe}$  changed significantly with temperature in SEEDS.

Keywords: Fe concentration, diatoms, nutrients, subarctic Pacific Ocean, phytoplankton size fraction, temperature

## **Introduction**

Mesoscale iron fertilization experiments have demonstrated that phytoplankton growth in the HNLC (high nitrate and low chlorophyll) regions is restricted by the low availability of soluble iron (usually hereafter, Fe) in the surface layer (Martin et al., 1994; Coale et al., 1996; Boyd et al., 2000; Tsuda et al., 2003; Coale et al., 2004). In these mesoscale experiments, phytoplankton biomass and productivity, especially in large-celled phytoplankton such as diatoms, increase several fold after Fe enrichment in the surface mixed layer. However, the magnitude of the drawdown of nutrients and dissolved inorganic carbon, and the increase in autotrophic biomass and productivity are not the same in each of these Fe fertilization experiments.

Two mesoscale Fe fertilization experiments were conducted in the subarctic Pacific Ocean: SEEDS (Subarctic Pacific iron Enrichment and Ecosystem Dynamics Study) in 2001 and SERIES (Subarctic Ecological Response to Iron Enrichment Study) in 2002. The SEEDS experimental site was located in the western subarctic gyre (WSG) and the SERIES site was in the Alaskan Gyre (AG). These sites have ecological and oceanographical similarities such as upper layer temperatures, abundant nutrients and a

low chlorophyll-*a* standing stock (Harrison et al., 1999; 2004).

Ship-board Fe addition incubation experiments have been widely used to assess the importance of iron as a factor limiting the growth of phytoplankton in HNLC waters (Boyd et al., 1996; Boyd et al., 1998; Coale et al., 2003; Cochlan et al., 2002; Fitzwater et al., 1996; Martin and Fitzwater, 1988; Timmermans et al., 1998; Zettler et al., 1996). Noiri et al. (2005) conducted ship-board Fe addition experiments during the SEEDS experiments in 2001. They used a Fe concentration gradient similar to the concentration range in the Fe fertilization experiment and found a hyperbolic relationship between Fe concentration and apparent chlorophyll *a* increase rate (growth rate) for the micro- (>10  $\mu\text{m}$ ) and nano-sized (2-10  $\mu\text{m}$ ) fraction. The relationship was well expressed by the Monod equation, giving a maximum growth rate ( $\mu_{\text{max}}$ ) and a half-saturation constant for Fe ( $K_{\text{Fe}}$ ). The  $\mu_{\text{max}}$  value for the micro-sized fraction ( $1.05 \text{ d}^{-1}$ ) was 2.6 times higher than that for the nano-sized fraction ( $0.4 \text{ d}^{-1}$ ). The  $K_{\text{Fe}}$  value for the micro-sized fraction was 0.6 nM. The dissolved Fe concentration (<0.22  $\mu\text{m}$ ) at 5 m on Day 1 after the Fe-fertilization in the SEEDS experiment was 1.76 nM (Nishioka et al., 2003), well above the  $K_{\text{Fe}}$ . However, when the apparent *in situ* growth of phytoplankton ceased on

Day 9, the dissolved Fe concentration decreased to 0.3 nM that was below the  $K_{Fe}$ . Thus, these values are useful parameters judging potential growth and limitation with ambient Fe concentration.

Three major HNLC regions (Southern Ocean, equatorial Pacific and subarctic Pacific Ocean) exhibit a wide range of temperatures in the surface mixed layer (i.e.,  $>25^{\circ}\text{C}$  in the equatorial Pacific and  $<5^{\circ}\text{C}$  in the Southern Ocean). In the subarctic Pacific Ocean this range is from 3 (winter) to  $14^{\circ}\text{C}$  (summer). The growth rate of phytoplankton is a function of temperature, generally increasing with temperature to a certain optimum value (Eppley, 1972, Goldman and Carpenter, 1974; Yoder, 1979). This optimum temperature for growth and dependency on temperature differ among species. Species growing at a higher temperature usually have a higher optimum temperature and vice versa (Suzuki and Takahashi, 1995). Temperature affects not only growth rate, but also cellular C, N and chlorophyll-*a* content (Goldman, 1979, Yoder, 1979; Goldman and Mann, 1980; Montagnes and Franklin, 2001). Iron is one of the most important trace metals for plant metabolism because Fe is required for many proteins and enzymes and reactions associated with them. Thus, temperature and Fe nutrition

have a combined effect on growth and physiology of phytoplankton (Kudo et al., 2000). Only a few degrees change in temperature significantly influences the growth and nitrogen metabolism of phytoplankton (Berges et al., 2002; Kudo et al., 2002, Lomas and Glibert, 1999; Suzuki and Takahashi, 1995).

In this study, we have examined the interactions between iron concentrations and temperature for three different size fractions of phytoplankton in the SERIES experimental site. Because we performed ship-board Fe addition experiments during the SERIES experiment with the same protocol as that in the SEEDS experiment, we were able to compare the response of phytoplankton from the NE and NW Pacific. The comparison of parameters obtained from the two ship-board Fe addition experiments in the northwest and northeast subarctic Pacific Ocean has enabled us to understand how different size fractions growing at different temperatures respond differently to changes in Fe concentration either by natural or artificial Fe addition events.

## **Materials and Methods**

On July 24, 2002, Fe-enrichment bottle incubation experiments were conducted

on board the F.R.V. *Kaiyo Maru* during the SERIES cruise (KY02-3). Sub-surface (5 m) seawater was taken at the SERIES experimental site (50° 20'N, 145° 45'W) during the out patch survey on Day 16 with an X-Niskin sampler attached to a Kevlar wire. Samples were dispensed into acid-washed 1 or 2 L polycarbonate (PC) bottles. An acidified ferric chloride (FeCl<sub>3</sub>) solution was spiked into the PC bottles to attain Fe concentrations ranging from 0.3 to 2.0 nM. The pH of the samples was not affected by the addition of the acidified ferric chloride solution. A control bottle was prepared by adding no Fe. Each incubation condition was conducted singly, but the control was prepared as duplicate bottles. One control bottle was sampled at the same interval as the Fe addition bottles and the other control bottle remained sealed until the end of the incubation. Samples were incubated on board in a tank continuously supplied with surface seawater, or in thermo-controlled water tanks. The incubation experiment was run at the subsurface temperature of 12°C. The incubation experiment at 16°C was conducted in a thermo-controlled tank. The light attenuation by the PC bottles and the plastic bag was 10%. Thus, the light intensity of the on-deck incubation was 90% of incident light. The diurnal variation in the maximum value of incident PAR ranged from

1000 to 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the incubation. The bottles were gently mixed by hand twice a day.

A sub-sample was taken from each bottle at 2-3 day intervals in a laminar-flow clean bench (class 100). Each sub-sample was sub-divided and filtered through a 10  $\mu\text{m}$  Nuclepore, 2  $\mu\text{m}$  Nuclepore filter, or Whatmann GF/F filter, separately. These three filters were used for size-fractionated Chl-*a* measurements. The micro-sized fraction was defined as the retained Chl-*a* on a 10  $\mu\text{m}$  Nuclepore filter. The nano-sized fraction was defined as the difference in Chl-*a* concentration between 2  $\mu\text{m}$  and the 10  $\mu\text{m}$  filters. The pico-sized fraction was defined as the difference between GF/F and 2  $\mu\text{m}$  filters. The filtrate from the GF/F filtration was stored frozen for nutrient analysis.

Chl-*a* was measured with a Turner Designs fluorometer (Model 10-AU) by the method of Holm-Hansen and Riemann (1978) after extracting with 90% acetone.

Nutrients (nitrate, phosphate and silicate) were measured with a Technicon Autoanalyzer II (Grasshoff et al., 1999). The relative standard variation was 5 and 1% for Chl-*a* and nutrients respectively based on five replicate measurements. Samples for Fe(III) were not filtered and they were stored in a refrigerator after adjusting the pH to

3.2 with formic acid buffer. Fe (III) was measured with an automatic Fe(III) analyzer (Kimoto Electric, Ltd), which preconcentrates Fe(III) on an 8-hydroxyquinoline immobilized resin, followed by chemiluminescence detection with luminol (Obata et al., 1993). The measured Fe concentration was defined as total acid-labile Fe (Nishioka et al., 2003).

The specific growth rate was calculated using the increase in Chl-*a* concentration over time and using a linear least squares regression through log-transformed data from the exponential phase (Guillard, 1973). The regression analysis was conducted to select a period of incubation to have a regression coefficient more than 0.99. As grazers were not removed, the specific growth rate was considered equal to net growth rate which potentially includes the grazing loss rate.

Phytoplankton cell counts were performed by inverted light microscopy (micro-sized cells) and epifluorescence microscopy (nano and pico-sized cells).

## **Results**

### *Time course of change in Chl-a and nutrients*

Total Chl-*a* concentration at the beginning of the incubation was 0.92  $\mu\text{g l}^{-1}$  and that for the micro-sized ( $>10 \mu\text{m}$ ), nano-sized (2-10  $\mu\text{m}$ ) and pico-sized (0.7-2  $\mu\text{m}$ ) fraction was 0.44, 0.13 and 0.34  $\mu\text{g l}^{-1}$ , respectively. The micro-sized fraction dominated and made up about 40% of the total Chl-*a* (Fig. 1). The pico-sized Chl-*a* showed no change with time for the control and the Fe-amended bottles at 12 and 16°C. The nano-sized Chl-*a* increased with time and showed a higher Chl-*a* concentration for a higher concentration of amended Fe. The peak concentration of nano-sized Chl-*a* was observed at 150 h for bottles at 16°C, but at 190 h (7.9 days) for those at 12°C. A linear increase in log-transformed Chl-*a* with time (exponential phase) was found between 50 and 150 h while in some bottles it continued until 90 h. The increment of Chl-*a* for nano-sized Chl-*a* was up to 2.0  $\mu\text{g l}^{-1}$  for Fe-amended bottles. The micro-sized Chl-*a* increased exponentially up to 147 h (6.5 days) at a faster rate than the nano-sized Chl-*a*. The increased amounts of Chl-*a* were a function of the amended Fe concentration. The maximum Chl-*a* concentration was observed at 6 and 7  $\mu\text{g l}^{-1}$  for the 2.0 nM Fe-amended bottle at 12 and 16°C, respectively. The micro-sized fraction dominated the total Chl-*a* at more than 70%.

Nitrate, phosphate and silicate concentrations at the beginning of the incubation were 9.8, 1.12 and 12.3  $\mu\text{M}$ , respectively (Fig. 2). Nitrate and silicate were exhausted in the Fe-amended bottles at 12°C, while those in the control bottle also decreased, but remained at 2 and 3  $\mu\text{M}$  for nitrate and silicate respectively at 190 h. Phosphate decreased both in the Fe-amended bottles and control bottle at 12°C, but remained at 0.1  $\mu\text{M}$  for 2.0 nM Fe and 0.4  $\mu\text{M}$  for the control at 190 h.

The relative consumption ratio of nitrate to phosphate was about 11-11.5, regardless of whether the bottles were enriched with Fe or not (Fig. 3). Phosphate remained at 0.2-0.3  $\mu\text{M}$  (x-intercept in Fig. 3) when nitrate depletion was observed. The consumption ratio of silicate to nitrate (Fig. 4, Table 1) was  $1.11 \pm 0.06$  for the Fe-amended bottles and 1.39 for the control at 12°C. The ratio for the control was significantly higher than that for the Fe amended bottles (*t*-test,  $p < 0.05$ ). The ratio of silicate to nitrate at 16°C for the control was 1.45, significantly higher (*t*-test,  $p < 0.05$ ) than for the Fe-amended bottles ( $1.07 \pm 0.09$ ). The y-intercept for the control was a slightly negative value, while that for the Fe-amended bottles was a positive value, indicating silicate remained in the Fe-amended bottles when nitrate was depleted.

### *Phytoplankton species composition*

Diatoms were dominant in the incubation bottles in the micro-sized fraction at 12 and 16°C (Table 2). *Pseudo-nitzschia* spp. (mostly *P. turgidula*) increased in the Fe-added bottles while *Fragilariopsis* spp. was abundant in the control bottles. In the nano-sized fraction, the abundance of *Emiliana huxleyi* increased >10 times in the Fe-added bottles. *Synechococcus* sp. was a major species in the pico-sized fraction and showed a slight increase in the Fe-added bottles.

### *Growth rate vs Fe concentration*

No apparent growth was found for the pico-sized fraction in the control and Fe amended-bottles, but apparent specific growth rate for the micro- and nano-sized fraction showed a hyperbolic increase with the increase in Fe concentration at each incubation temperature (Fig. 5). Each data set fit to the Monod type equation  $\{\mu = (C_{Fe} \times \mu_{max}) / (K_{Fe} + C_{Fe})\}$  well ( $r > 0.9$ ), where  $C_{Fe}$  is the Fe concentration in the bottle,  $\mu_{max}$  is the maximum growth rate, and  $K_{Fe}$  is the half-saturation constant for Fe. The total acid-labile Fe concentration in the control bottles measured on the last day of incubation was 0.1 nM for 12 and 16°C. Thus, the Fe concentration in each bottle was recalculated

by summing the spiked amount and the Fe concentration in the control bottles. The Fe concentration in the un-opened control was 0.15 and 0.09 nM for the 12 and 16°C incubation respectively, similar to that in the subsampled control (0.1 nM), confirming no obvious contamination during the incubation and subsampling. The specific growth rate was higher for the micro-sized fraction than for the nano-sized fraction at the same Fe concentration, except for < 0.3 nM Fe at 16°C where nano-sized growth rates were higher than the micro-sized fraction (Table 3, Fig. 5). The  $\mu_{\max}$  was 0.72 and 0.48 d<sup>-1</sup> for micro-sized and nano-sized fraction respectively at 12°C. The  $K_{\text{Fe}}$  was 0.10 and 0.08 nM for micro-sized and nano-sized fraction respectively at 12°C. The  $\mu_{\max}$  values at 16°C were similar to those at 12°C (0.69 and 0.45 d<sup>-1</sup> for micro-sized and nano-sized fraction). The  $K_{\text{Fe}}$  for Fe was 0.19 and 0.03 nM for micro-sized and nano-sized fraction respectively at 16°C.

## **Discussion**

In the SERIES experiment, a month long tracking and observation period for the Fe patch was conducted by a three-ship cooperation, CCGS *John P. Tully*, FRV

*Kaiyo-Maru* and RV *El Puma*. Development and decline of the Fe-induced bloom was fully covered by the a-month long observation (Boyd et al., 2004). Prymnesiophytes increased at first after the Fe-fertilization (Marchetti et al., in this volume) that is different from the other mesoscale Fe-fertilization experiments and after more than two weeks of Fe-fertilization diatom bloom was observed.

The initial surface Chl-*a* concentration of  $0.92 \mu\text{g l}^{-1}$  was slightly high compared to  $0.42 \mu\text{g l}^{-1}$  at 5 m depth of the collection site for the incubation sample on Day16. However, the total acid-labile Fe concentration in the control bottle at the beginning was 0.10 nM and total Chl-*a* concentration for the control at 50 h was  $0.85 \mu\text{g l}^{-1}$ . Thus, the slightly high value at the initial value seems to be an artifact during measuring procedure and the initial condition seems to represent the out-patch (HNLC) conditions. In the present study, Chl-*a* was used as a proxy for phytoplankton biomass. Specific growth rates based on the change in Chl-*a* during the exponential phase are net rates due to the potential loss of biomass, primarily grazing. The influence of grazing on the specific growth rate obtained in this study may be significant in the pico and nano-sized fraction because phytoplankton growth in these size fractions is fairly

balanced with grazing loss by micro-zooplankton in the subarctic Pacific Ocean (Liu et al., 2002). The use of Chl-*a* to estimate the specific growth rate was widely accepted in estimating grazing impact of micro-zooplankton on phytoplankton growth (Landry and Hassett, 1982). However, Chl-*a* to C (biomass) ratio may change with time during the incubation because Chl-*a* quota is affected by Fe nutrition. Chl-*a* quota is lower under Fe-limited than that under Fe-replete conditions (Guikema and Sherman, 1983, Greene et al., 1992, Kudo et al., 2000, McKay et al., 1997, van Leeuwe and Stefels, 1998). Phytoplankton assemblages at the beginning of the incubation appeared to be under Fe-limited or stressed condition. After the addition of Fe to the bottles, the physiological condition of phytoplankton may recover with time. Chl-*a* quota for Fe-amended bottles increased with time. However, Chl-*a* for the Fe-amended bottles increased >10 times at the end of the exponential phase, so during this phase the effect of lower Chl-*a* quota for Fe-stressed cells on the growth rate calculation assumed minimal in the present study.

The incubation experiments were conducted at 90% of surface irradiance. The irradiance at 5 m fluctuated from 25 to 50% of the surface irradiance during the SERIES experiment due to the development and decline of the bloom (Saito et al., in this

volume). Thus, the light intensity of the incubation was higher than that at 5 m where the sample was taken. Light inhibition seems not possible because primary productivity at surface irradiance was similar or higher than that at 55 and 33% of the surface irradiance (Kudo unpubl. data). Higher irradiance may influence Fe chemistry and regeneration in the bottle compared with at 5 m condition, enhancing Fe redox cycles and dissolution of Fe colloids. One of objectives in the present study was to compare the physiological response to Fe and temperature between SEEDS (NW) and SERIES (NE). The light condition for the incubation experiments was the same between two experimental sites.

#### *Nutrient consumption*

Nitrate and silicate in the Fe-amended bottles were depleted in 6 days after the Fe addition, but phosphate remained at 0.1-0.3  $\mu\text{M}$ . Nitrate and phosphate were consumed in a ratio of 11:1, while the initial ratio of nitrate to phosphate was 9.1:1, and hence, phosphate remained at the end of the incubation. In the present experiment, the consumption ratio of silicate to nitrate (Table 1) in the control bottles (1.3-1.45:1) was

significantly higher ( $p < 0.01$ ) than that in the Fe-amended bottles (1.07-1.11:1). This ratio has been reported to increase from 1 to 2 when diatoms become physiologically Fe-stressed (Takeda, 1998). An enhanced consumption of silicate over nitrate leading to silicate depletion, was observed in the Fe patch in the SERIES experiment (Marchetti et al., this volume, Saito et al., this volume). The consumption ratio of silicate to nitrate during Day 0-9 was 0.79:1, but increased to 2.6:1 from Day 9 to 17, suggesting physiological stress on the *in situ* phytoplankton assemblages. A high ratio ( $> 2$ ) for the control bottles was not observed, while this ratio increased in the patch at the end of the bloom. The possible explanation for this discrepancy may be attributed to different light regimes between the Fe patch and the ship-board incubation.

#### *Species composition*

*Pseudo-nitzschia* spp. (mainly *P. turgidula*) were dominant in the micro-sized fraction and increased in the Fe-added bottles at 12 and 16°C, similar to the Fe patch. In the nano-sized fraction, *E. huxleyi* increased  $> 10$  times in the Fe-addition bottles. It is worth mentioning that the incubated seawater was taken outside the patch on Day 16. In the SERIES experiment, *E. huxleyi* dominated in the early phase until Day 10 and then a

diatom bloom composed of *Pseudo-nitzschia* spp. occurred. In the bottle experiment, both species increased coincidentally responding to the Fe enrichment. Thus, other factors such as grazing or light influence the species succession observed in the Fe-patch.

### *Temperature effect*

The  $\mu_{\max}$  values for the micro-sized fraction in this study was  $0.72 \text{ d}^{-1}$  at  $12^{\circ}\text{C}$ , the same temperature as the Fe patch and did not change when temperature was raised to  $16^{\circ}\text{C}$ . No change in the  $\mu_{\max}$  was observed for the nano-sized fraction at the same temperature range. In the SEEDS experiment, the  $\mu_{\max}$  value for the micro-sized fraction increased linearly with temperature from  $5$  to  $13^{\circ}\text{C}$ , but sharply decreased from  $13$  to  $18^{\circ}\text{C}$  (Fig. 6). In addition, a floral shift from diatoms to prymnesiophytes occurred from  $13$  to  $18^{\circ}\text{C}$  in the SEEDS experiment (Noiri et al., 2005) while it was not observed from  $12$  to  $16^{\circ}\text{C}$  in the ship-board experiments in SERIES (this study). The maximum seasonal temperature of the surface at the SEEDS and SERIES site is about  $13$ - $14^{\circ}\text{C}$  (World Ocean Atlas, 2001). The dependency of  $\mu_{\max}$  values on temperature obtained from ship-board Fe addition experiments, agrees with the maximum temperature at the sites. However, the ship-board experiment suggests that an increase in temperature of

several degrees from the present maximum at the sites may cause a floral shift and a change in the composition of the lower trophic levels.

#### *Comparison of growth kinetic parameters for Fe between SEEDS and SERIES*

We performed ship-board Fe addition experiments with subsurface waters at both mesoscale Fe fertilization experimental sites. Thus, the physiological parameters such as the  $\mu_{\max}$  and  $K_{\text{Fe}}$  obtained in our incubation experiments should reflect the characteristics of the *in situ* phytoplankton assemblages.

Mesoscale iron fertilization experiments generally increase Fe concentrations in sub-surface waters to more than 2 nM. This high concentration of Fe seems close to a saturating concentration for Fe uptake, and should be an estimate of the maximum specific growth rate ( $\mu_{\max}$ ). Thus, the  $\mu_{\max}$  value should be a good indicator for comparing the response of natural phytoplankton assemblages to Fe fertilization. The micro-sized fraction, mainly composed of diatoms was, responsible for the main growth response to the Fe-addition at both sites. The  $\mu_{\max}$  in the micro-sized fraction in SERIES (this study) was  $0.7 \text{ d}^{-1}$  at the *in situ* temperature ( $12^{\circ}\text{C}$ ) (Table 3). The  $\mu_{\max}$  in the micro-sized fraction was  $1.05 \text{ d}^{-1}$  in the SEEDS experiment at the *in situ* temperature

(9°C) (Noiri et al., 2005). As the  $\mu_{\max}$  increased with temperature in the SEEDS experiment between 5 and 13°C (Fig. 6), the comparison of the  $\mu_{\max}$  at 12°C between SEEDS and SERIES was essential to eliminate this potential temperature effect on the  $\mu_{\max}$  value. The  $\mu_{\max}$  at 12°C was 1.8 times higher in SEEDS than in SERIES, suggesting the potential growth rate for the micro-sized fraction was significantly higher in the SEEDS site (t-test,  $p < 0.01$ , Fig. 6). The  $\mu_{\max}$  in the nano-sized fraction was almost similar between SEEDS and SERIES at 0.2-0.5 d<sup>-1</sup>. At the *in situ* temperature, the  $\mu_{\max}$  in the micro-sized fraction was 1.8 times higher than in the nano-sized fraction in SEEDS, while the same comparison was 1.4 times higher in SERIES.

In the SEEDS experiment, a 20 times increase in Chl-*a* (>15  $\mu\text{g l}^{-1}$ ) was observed in a week after the Fe fertilization (Tsuda et al. 2003), while it took more than two weeks to attain the maximum Chl-*a* (6.3  $\mu\text{g l}^{-1}$ ) in the SERIES experiment (Boyd et al 2004). The maximum rate of apparent Chl-*a* increase at 5 m in the Fe-patch was 1.15 d<sup>-1</sup> (from D7 to D8) in SEEDS and 0.73 d<sup>-1</sup> (from D14 to D15) in the SERIES experiment. This increase in *in situ* rates of the Fe patch was consistent with the  $\mu_{\max}$  value for the micro-sized fraction in this study and that in the SEEDS experiment (Noiri et al., 2005).

The high  $\mu_{\max}$  value for the micro-sized fraction at the SEEDS site may explain the rapid formation of diatom bloom in a week after the Fe fertilization in the SEEDS experiment. As is mentioned previously, the specific growth rate obtained in this study is a net rate. It is reported that growth rate of nano and pico-sized phytoplankton was well balanced with the grazing loss in summer of the subarctic Pacific Ocean (Liu et al., 2002). However, the apparent growth rate of the nano-sized fraction increased as a function of added Fe. It suggested that the gross (potential) growth rate of nano-sized fraction may be higher than the observed rate and the growth of this size seemed to be limited by Fe.

A half-saturation constant ( $K_{\text{Fe}}$ ) from the Monod equation indicates an affinity for substrate (Fe); a lower  $K_{\text{Fe}}$  value indicates higher affinity for Fe and vice versa. The surface mixed layer temperature during SEEDS and SERIES experiments was 9 and 12°C, respectively. The comparison of  $K_{\text{Fe}}$  at the *in situ* temperature between SEEDS and SERIES (Fig. 6) elucidates that  $K_{\text{Fe}}$  for the micro-sized fraction in the SERIES site was 0.1 nM at 12°C, significantly lower ( $p < 0.05$ ) than that of 0.58 nM at 9°C in the SEEDS site (Noiri et al., 2005). This large difference in  $K_{\text{Fe}}$  for the micro-sized fraction

suggests that the phytoplankton assemblage composing of this size fraction at the SERIES experimental site had a higher affinity for Fe uptake than at the SEEDS site. In other words, phytoplankton at the SERIES site can attain their maximal growth rate at a lower ambient Fe concentration while the maximal growth rate was much lower that at the SEEDS site.

Most of dissolved Fe in the ocean is present as organically-complexed forms (Boye et al, 2001, Nolting et al, 1998, Rue and Bruland, 1997, Witter and Luther III, 1998). However, if an artificially enriched Fe concentration exceeds the organic ligand concentration *in situ*, excess dissolved Fe may change to the particulate or colloidal form. The organic ligand concentration was not measured in the incubation bottles, but this concentration was a few nM during the SEEDS and SERIES experiments (Takeda pers. Comm.). This suggests that the amended Fe may form organic complexes and no obvious difference seems present in the biologically available Fe pool in the incubation experiments at both sites. However, it is not clear whether there is some difference in stability of the Fe complex at both sites, which may affect the availability of the iron pool for phytoplankton and  $K_{Fe}$  estimation in this study.

Nishioka et al. (2003) reported dissolved iron and labile particulate iron concentrations in the subarctic Pacific Ocean covering Alaskan gyre and western subarctic gyre and Oyashio region. No difference was found in the dissolved iron concentration in the surface mixed layer between the western and eastern subarctic Pacific Ocean. However, a significant increasing trend from the eastern to western subarctic Pacific was found in the labile particulate iron concentration. Dissolved Fe is defined as the iron concentration in the 0.22  $\mu\text{m}$  filtrate, which may represent a truly dissolved ionic or organically-complexed form and colloidal Fe. The labile particulate iron is defined as the particulate form of Fe that is liberated under a weak acidic condition (pH 3.2). A high concentration of the labile particulate iron was also observed during the SEEDS experiment, while the dissolved Fe concentration decreased rapidly after the Fe infusion (Nishioka et al., 2003). However, the mechanism of Fe acquisition by phytoplankton is not clear, especially how this labile particulate iron fraction relates to iron availability for phytoplankton. This trend of a higher concentration of the labile particulate iron in the western subarctic Pacific Ocean may be a reflection of higher atmospheric iron depositional flux than in the eastern subarctic Pacific Ocean (Duce and

Tindale, 1991). Fujishima et al. (2001) reported that the dissolved iron concentration in the deep water (>200 m) in an area southwest of the WSG, was ~1 nM, which was about two times higher than that of the AG. This result suggests that a higher flux of iron from the deeper layer to the surface could be expected in the western subarctic Pacific Ocean than in the eastern subarctic Pacific Ocean (Harrison et al., 2004).

Suzuki et al. (2002) compared photosynthetic competence of phytoplankton such as the photochemical quantum yield ( $F_v/F_m$ ) and the functional absorption cross-section of Photo System II ( $\sigma_{\text{PSII}}$ ) between the NW and NE subarctic Pacific Ocean. They found a decreasing trend in  $F_v/F_m$  and an increase in  $\sigma_{\text{PSII}}$  from west to east, suggesting that iron limitation becomes more severe in the NE Pacific. This difference may be a reflection of an adaptation to lower Fe concentrations because the eastern subarctic Pacific is characterized as a lower atmospheric dust (Fe) deposition region than the western subarctic Pacific (Duce and Tindale, 1991).

Coale et al. (2003) conducted ship-board Fe enrichment experiments in the Southern Ocean and reported a  $\mu_{\text{max}}$  and  $K_{\text{Fe}}$  for bulk Chl-*a* and phytoplankton taxa. In summer, in the Antarctic Circumpolar Current (ACC) region, Chl-*a* based  $\mu_{\text{max}}$  values

were 0.13-0.3 d<sup>-1</sup> and K<sub>Fe</sub> values were 0.15-0.18 nM. They suggested that the phytoplankton community in the ACC experiences severe iron stress in summer. The  $\mu_{\max}$  values in the Southern Ocean were much lower than those in the subarctic Pacific Ocean (Noiri et al., 2005; the present study). The K<sub>Fe</sub> values were almost the same as that for the micro-sized fraction in SERIES, but much lower than that for the same fraction in SEEDS. Thus, a lower K<sub>Fe</sub> value in SERIES also indicates a more severe iron stress than in the SEEDS region. However, Coale et al. (2003) also reported that the K<sub>Fe</sub> values changed seasonally (i.e., high K<sub>Fe</sub> value in spring for the ACC) region. Therefore,  $\mu_{\max}$  and K<sub>Fe</sub> values may change seasonally responding to the ambient concentration of Fe. Phytoplankton reduce their cell size under Fe-limited condition (Sunda and Huntsman, 1995, 1997). Smaller cell size may have an advantage since they can utilize lower concentrations of substrate due to the increase in surface to cell volume ratio. Thus, the lower K<sub>Fe</sub> might partly reflect a change in cell size and result of adaptation to lower Fe concentration.

In summary, ship-board iron enrichment bottle experiments during the SERIES experiment elucidated the growth response of different size fractions of phytoplankton

to iron concentration and temperature gradients. The  $\mu_{\max}$  values were 0.72 and 0.48 d<sup>-1</sup> and  $K_{\text{Fe}}$  values were 0.10 and 0.08 nM for the micro and nano-sized fraction, respectively. The  $\mu_{\max}$  agreed with the rate of increase in Chl-*a* observed *in situ* for the mesoscale iron fertilization experiment. The  $\mu_{\max}$  value for micro-sized fraction at 12°C was half of that in the western subarctic Pacific Ocean (SEEDS experiment in 2001), indicating the Chl-*a* increase rate (apparent growth rate) after iron enrichment was much higher in SEEDS than in SERIES. The  $K_{\text{Fe}}$  values were much lower than that in SEEDS, suggesting that the phytoplankton community in the NE subarctic Pacific Ocean is adapted to a lower ambient Fe concentration. This difference in  $K_{\text{Fe}}$  between SERIES (NE) and SEEDS (NW) may reflect the previously suggested gradient in Fe flux to the subarctic Pacific Ocean.

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Table 1 Regression lines obtained from silicate vs nitrate plots at 12 and 16°C. “a”, “b” and “R” denote the slope, y-intercept and the regression coefficient from the linear regression analysis, respectively. The average slope was calculated from the slope of the data for each Fe-amended bottle and the number in parenthesis indicates one standard deviation.

Fe addition (nM)	12°C			16°C		
	a	b	R	a	b	R
control	1.39	-0.69	0.97	1.45	-1.13	0.97
0.3	1.12	2.11	0.99	1.17	1.62	0.98
0.5	1.19	1.77	0.98	1.12	2.30	0.99
1.0	1.12	2.51	0.98	1.12	2.30	0.99
1.5	1.02	3.10	0.99	0.92	3.94	0.98
2.0	1.10	2.32	0.99	1.12	2.13	0.98
average	1.11 (0.06)			1.07 (0.09)		

Table 2 Phytoplankton species composition, abundance (cells ml<sup>-1</sup>), and size (length in μm of the apical axis) the control bottle (Control. with no Fe added) and Fe-added bottles (+Fe, 2.0 nM Fe added) on Day 4. Only those species exceeding 100 cells ml<sup>-1</sup> are listed.

	Size	12 °C		16 °C	
		Control.	+Fe	Control.	+Fe
<b>Bacillariophyceae</b>					
<i>Pseudo-nitzschia</i> spp.	90	140	330	180	1,100
<i>Fragilariopsis</i> spp.	5-15	360	100	300	150
<b>Haptophyceae</b>					
<i>Emiliania huxleyi</i>	5-10	100	1,140	50	810
<b>Cyanobacteria</b>					
<i>Synechococcus</i> sp.	1	13,500	15,300	17,300	19,700

Table 3 Maximum growth rates ( $\mu_{\max}$ ) and half saturation constants for Fe ( $K_{\text{Fe}}$ ) for micro- and nano-sized phytoplankton grown at two incubation temperatures. Samples for the incubation were from out-patch on Day 16. These parameters were obtained from the relationship between Fe concentrations and size-fractionated Chl-*a* based specific growth rate fit to the Monod equation (see text). Number in parenthesis indicates the error from the best fit analysis.

Temperature (°C)	$\mu_{\max}$ (d <sup>-1</sup> )		$K_{\text{Fe}}$ (nM)	
	micro-sized	nano-sized	micro-sized	nano-sized
12	0.72 (0.03)	0.48 (0.08)	0.10 (0.03)	0.08 (0.03)
16	0.69 (0.07)	0.45 (0.01)	0.19 (0.08)	0.03 (0.01)

## Figure legends

Fig. 1 Time course changes in the size-fractionated Chl-*a* concentration in the Fe-amended bottle incubation at 12 and 16°C. The y-axis is a log scale and the lines indicate regression lines for specific growth rates.

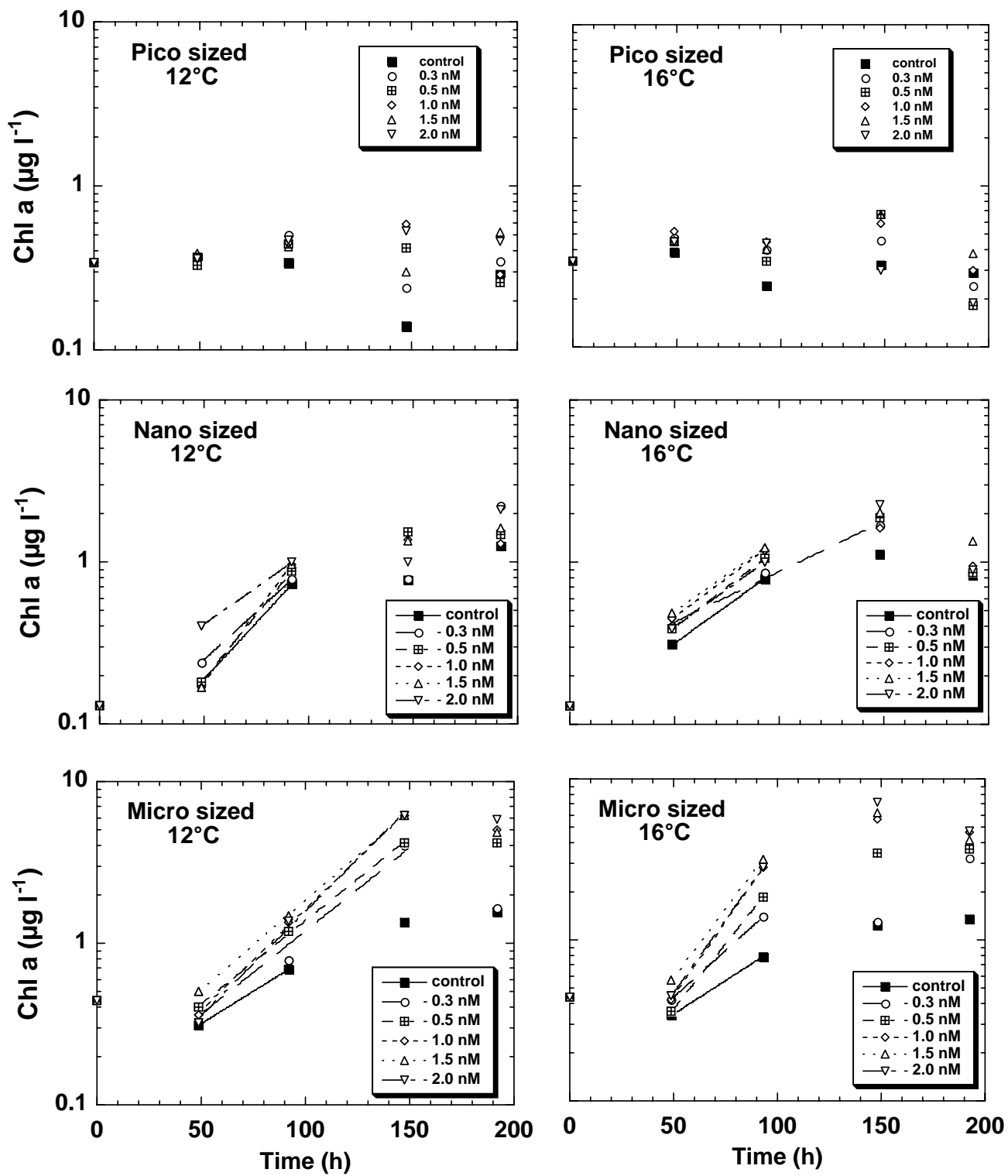
Fig. 2 Time course changes in silicate, nitrate and phosphate in the Fe-amended bottle incubation at 12 and 16°C.

Fig. 3 Relationship between phosphate and nitrate during the incubation at 12 and 16°C. The upper-right point indicates the initial concentrations.

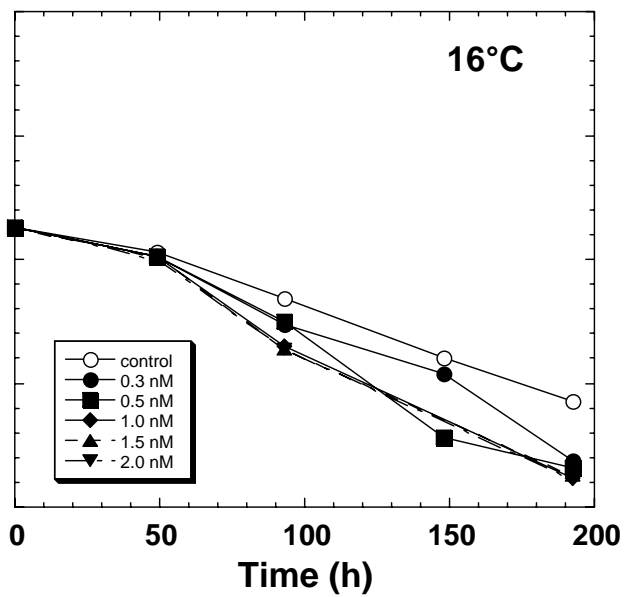
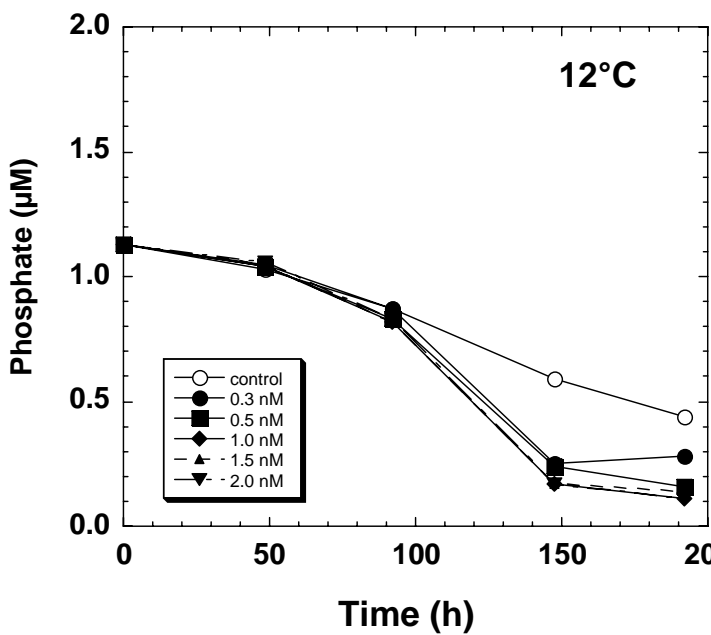
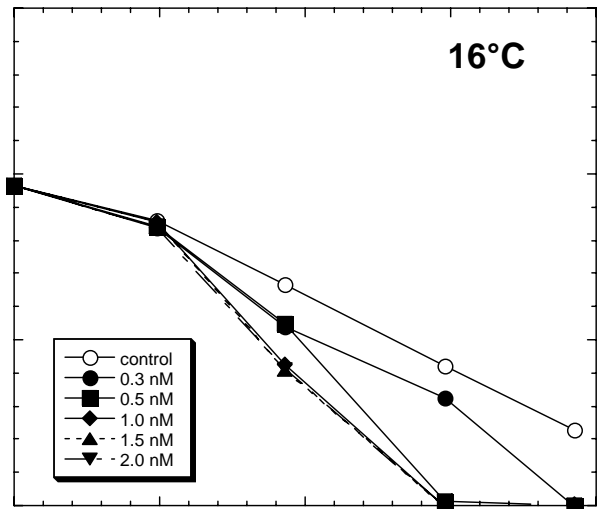
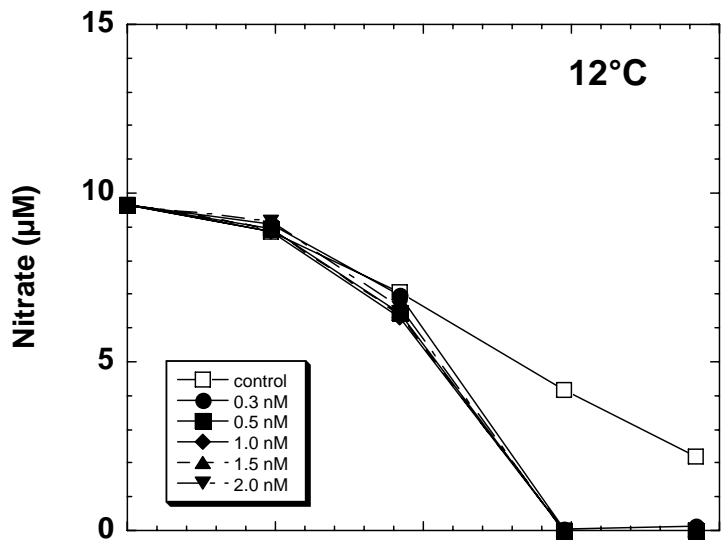
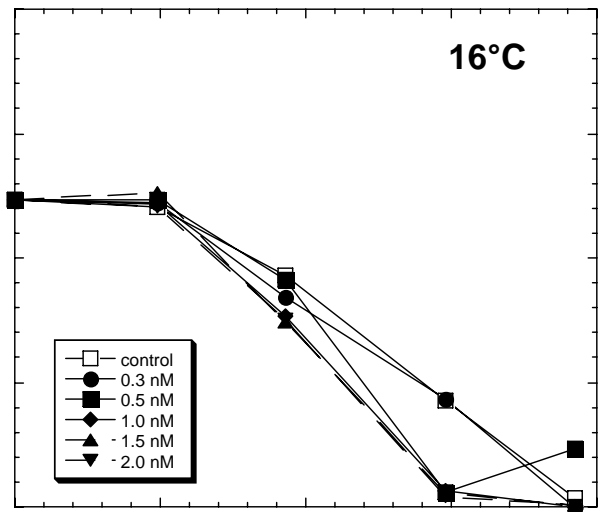
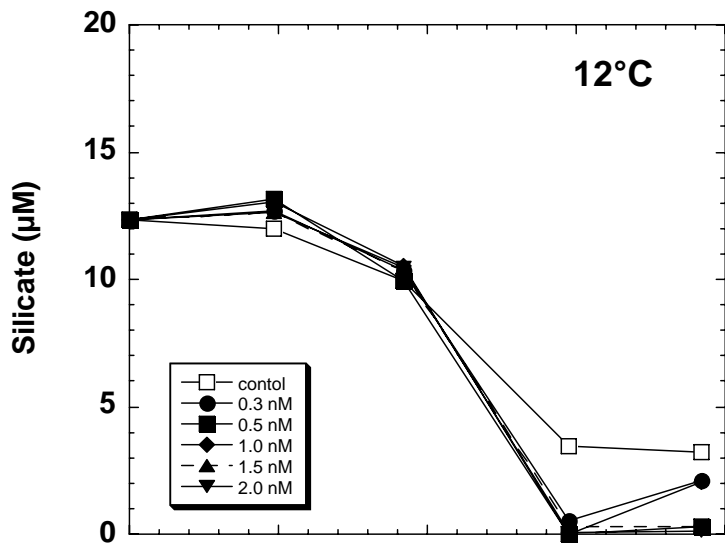
Fig. 4 Relationship between nitrate and silicate during the incubation at 12 and 16°C. The upper-right point indicates the initial concentrations. The solid line indicates the regression line for the control bottles and the dashed line indicates the regression line for the Fe-amended bottles (see Table 1).

Fig. 5 Chl-*a* based specific growth rates ( $\mu$ ) vs Fe concentration in the bottle for the micro-sized (>10  $\mu\text{m}$ ) and nano-sized (2-10  $\mu\text{m}$  size) fraction at 12 and 16°C. See text for the curve fitting protocol.

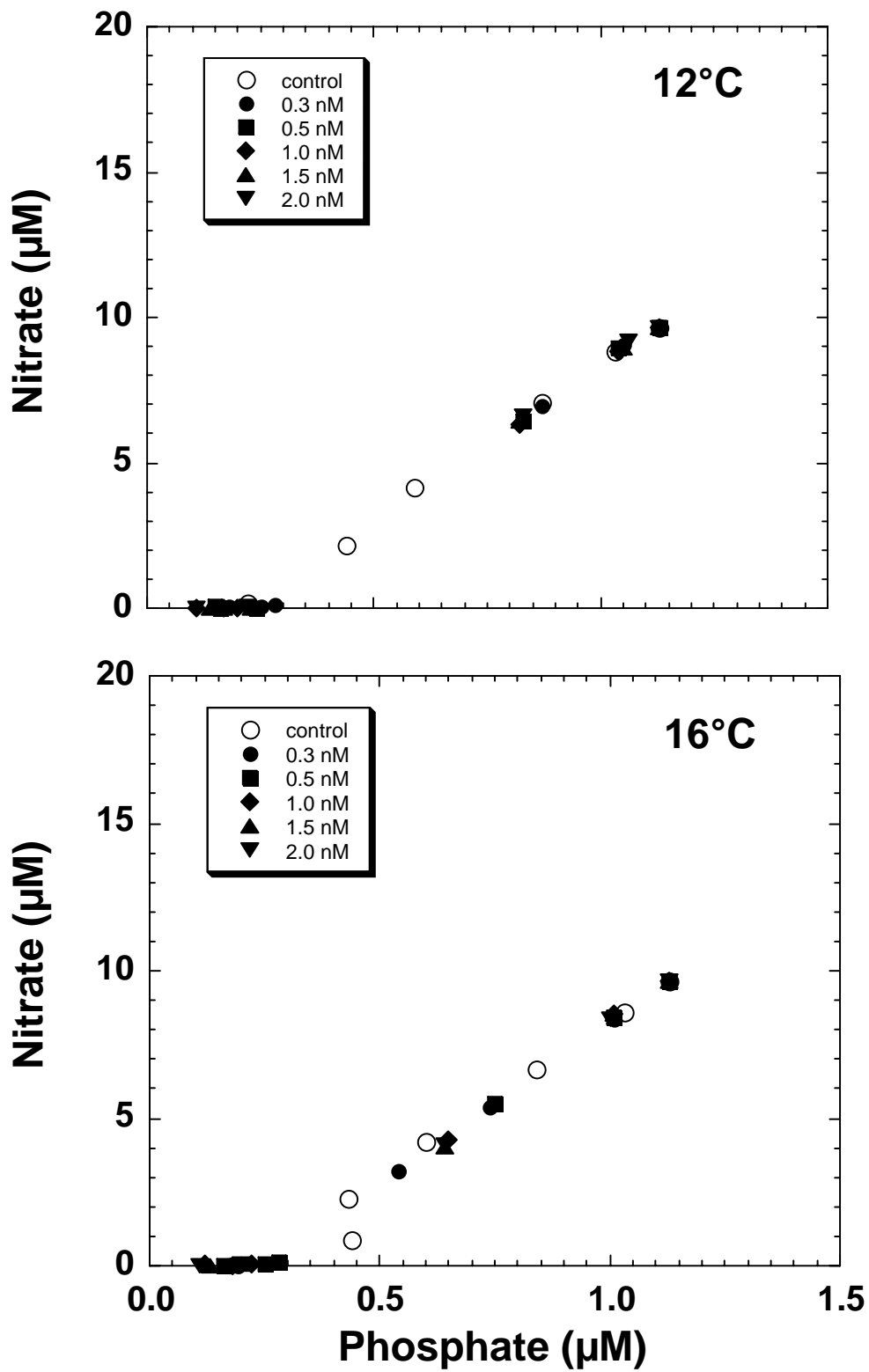
Fig. 6 The maximum growth rate ( $\mu_{\text{max}}$ ) and the half-saturation constant for Fe ( $K_{\text{Fe}}$ ) vs temperature relationship for the micro-sized (>10  $\mu\text{m}$ ) and nano-sized (2-10  $\mu\text{m}$  size) fractions in SERIES (this study) and SEEDS (Noiri et al., 2005). Error bars represent errors from the best fit analysis (Table 3) and are not visible when smaller than the symbol.



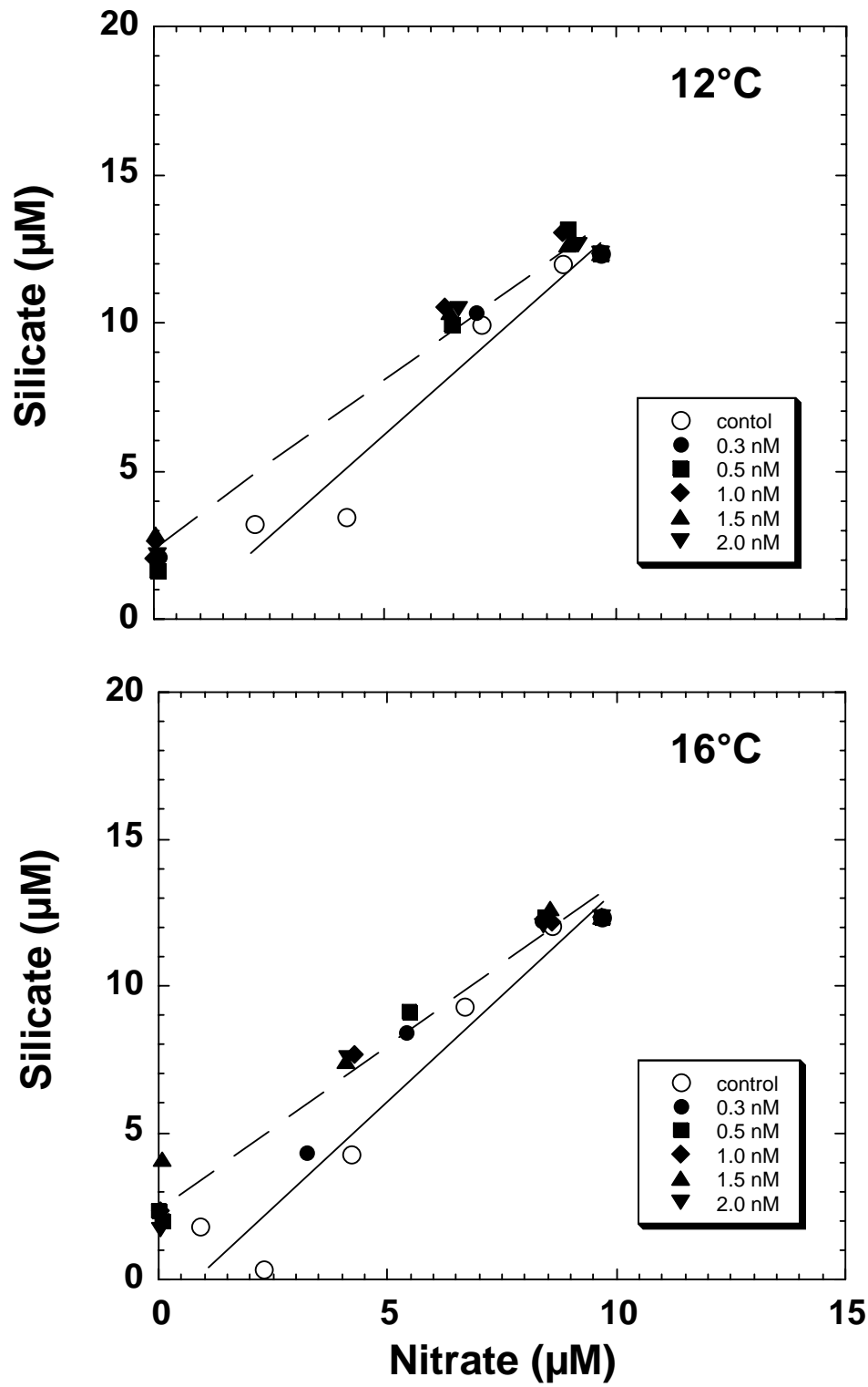
Kudo et al., Fig 1



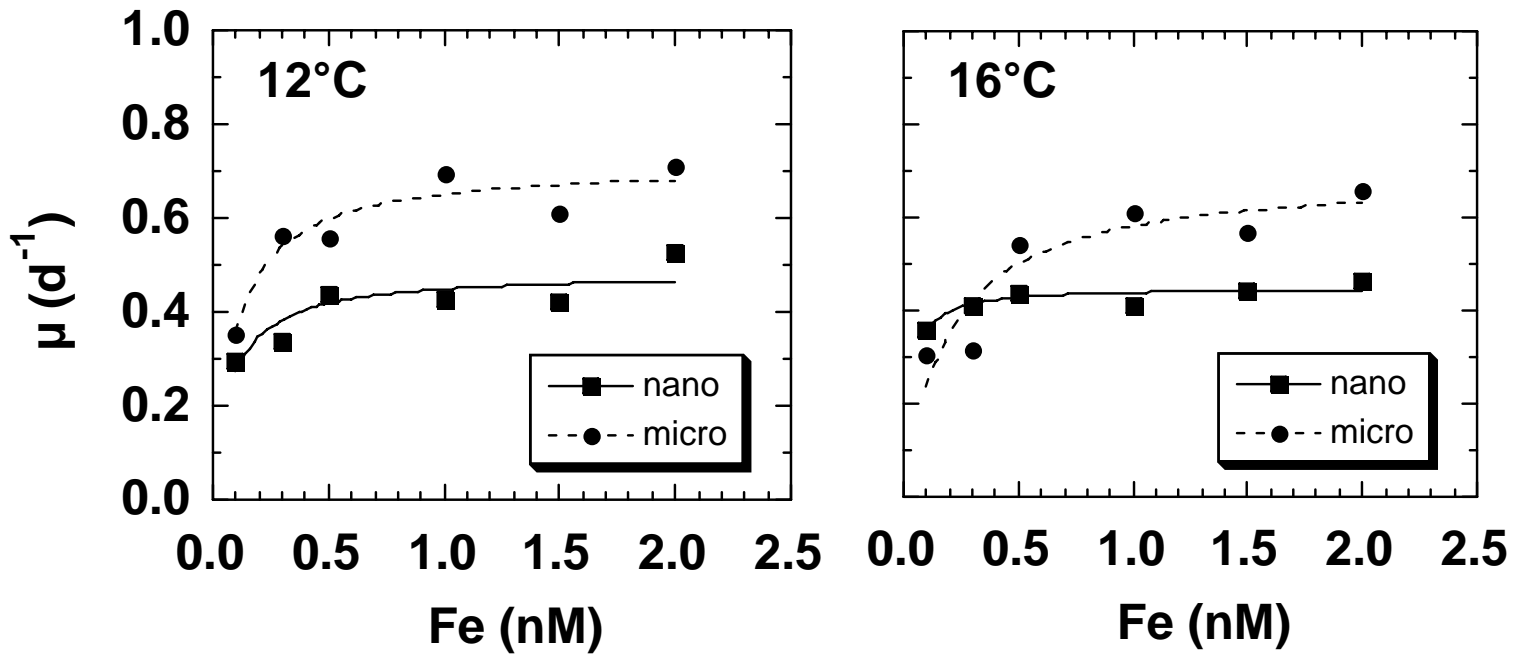
Kudo et al., Fig.2



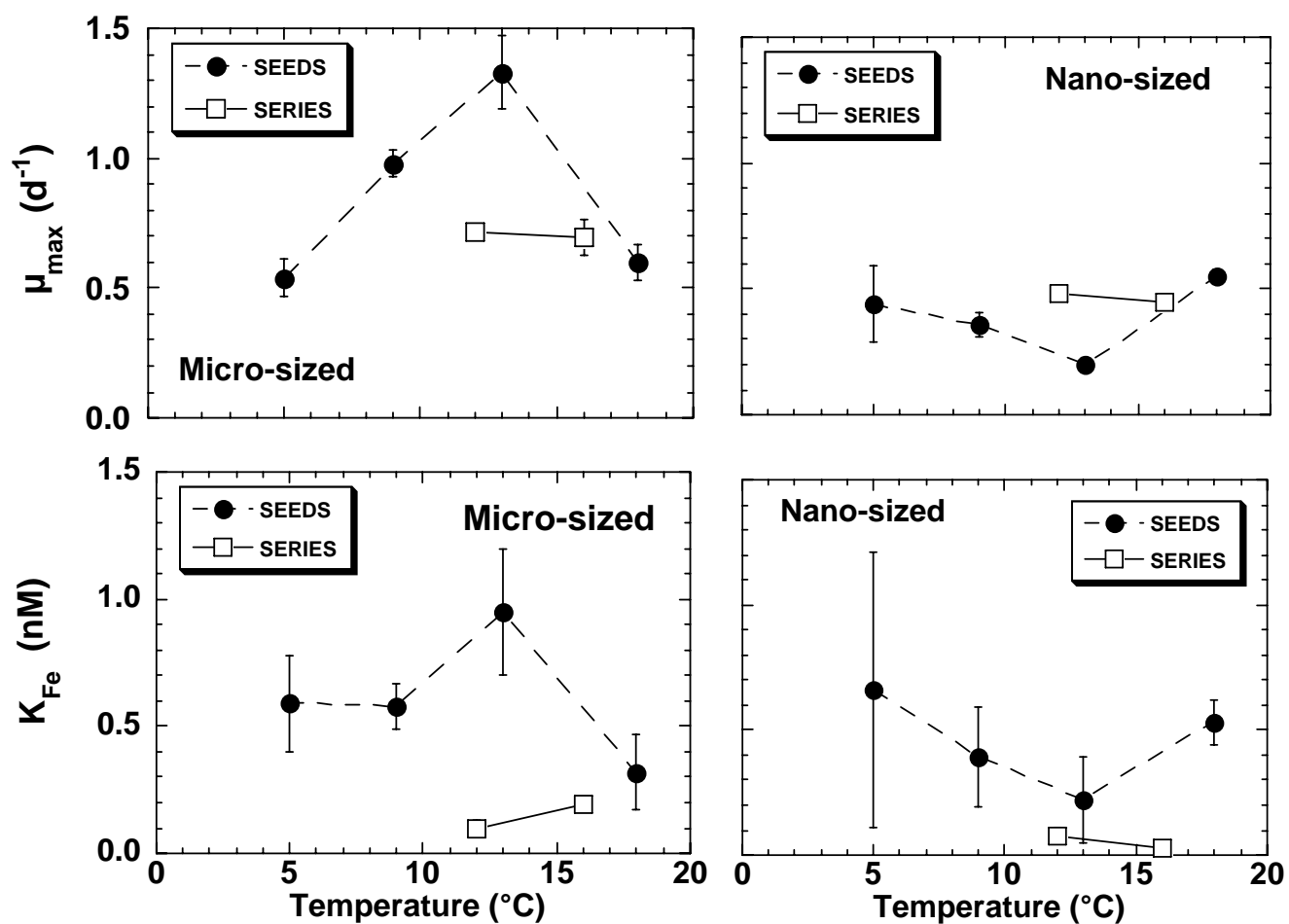
Kudo et al., Fig. 3



Kudo et al., Fig. 4



Kudo et al., Fig. 5



Kudo et al., Fig. 6