

Inter-island comparison of phytoplankton growth rates and herbivory rates

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04 March 2006

[Running title: Phytoplankton growth and grazing dynamics]

## Non-technical Summary

Phytoplankton are unicellular photosynthetic organisms that are the foundation of the marine food web that use chlorophyll *a* to conduct photosynthesis. Around the Galapagos Archipelago, the marine ecosystem is characterized by regionally high primary production and chlorophyll *a* concentrations that are uncharacteristic of the surrounding waters of the Eastern Equatorial Pacific. In general, satellite images displaying surface chlorophyll concentrations show much higher concentrations west of the island of Isabela, also referred to as the “Galapagos plume”. From 20-28 January 2006, on the R/V Thomas G. Thompson, growth and cell mortality rates were measured using differing dilutions of seawater to filtered seawater and 24 hour incubations at one station within and two outside the plume. Within each sample for each of the bottles, the chlorophyll *a* was separated into 3 size fractions such that growth and grazing rates could be measured within different size phytoplankton. Additionally, a different nutrient mixture was added to the majority of the bottles and iron was added separately to half of the bottles to measure the effects of nutrients on phytoplankton growth and grazing on the total population as well as within the size fractions. Significant growth and grazing rates were determined only for the largest size fractions of phytoplankton (>20  $\mu\text{m}$ ) if iron was added at two of the stations. These results strongly imply that iron is limiting in phytoplankton growth both in and out of the plume.

## Acknowledgments

Thanks to all my fellow peers and shipmates who joined me on this cruise for all of the help and support. This project greatly benefited from excellent guidance by Gabrielle Rocap, Llyd Wells and the rest of the OCEAN 444 teaching team. Additionally, I am indebted to AJ LeFevre, Tasha Snow, Joni Werdeman and Hilary Hall for their help with data collection. Thank you to Benjamin Gilmore, Jonathan Herzog and Wes Thompson for your beneficial reviews.

## Abstract

Phytoplankton growth and microzooplankton grazing rates were investigated during a cruise from 20-28 January 2006 aboard the R/V Thomas G. Thompson at three stations around the Galapagos Archipelago. Using a seawater dilution technique and size-fractionated chlorophyll measurements, intrinsic growth and grazing rates were estimated. Additionally, half of the dilution bottles in each experiment were enriched with a final concentration of  $2.0 \text{ nmol L}^{-1} \text{ FeCl}_3$ . Overall, intrinsic phytoplankton growth and grazing rates were insignificant except for  $>20\text{-}\mu\text{m}$  size fraction in the presence of added iron at stations Bio-2 and Bio-4 (west and east of the island of Isabela, respectively). The growth and grazing rates were respectively  $0.07 \text{ h}^{-1}$  and  $0.02 \text{ h}^{-1}$  (Bio-2) and  $0.05 \text{ h}^{-1}$  and  $0.03 \text{ h}^{-1}$  (Bio-4), in both cases suggesting growth rates were not balanced by microzooplankton grazing in the presence of iron enrichment. These results strongly imply that iron is a limiting factor in phytoplankton growth both east and west of Isabela Island.

## Introduction

The Equatorial Pacific is a known High-Nitrate Low-Chlorophyll (HNLC) oceanic regime. HNLC regions are characterized by high macronutrient concentrations but low chlorophyll concentrations year round (Banse and English 1994). Mesoscale, open-ocean experiments in HNLC areas have shown increased phytoplankton standing stock following iron enrichment (Martin et al. 1994, Landry et al. 2000), especially in phytoplankton with diameters greater than 10  $\mu\text{m}$  (Tsuda et al. 2003, Cavendar-Bares et al. 1999). In addition to observed increases in larger phytoplankton due to iron enrichments, microzooplankton grazing rates observed in the Equatorial Pacific within an iron enriched patch ( $\sim 0.05\text{-}0.06\text{ h}^{-1}$ ) were approximately twice those measured outside the patch ( $\sim 0.02\text{ h}^{-1}$ ) (Landry et al. 2000). Landry et al. (2000) also observed a lag of 6 days in the increase in grazing rates suggesting that phytoplankton may briefly escape grazing pressure but will eventually be controlled by increased grazer abundances (as argued by Banse 1995). This is consistent with the so-called “Ecumenical iron hypothesis” stated by Price et al. (1994) as, “grazer controlled phytoplankton populations in an iron limited system”. Therefore, grazing rates on phytoplankton within the plume will be relatively unaffected by iron enrichment compared to stations with low standing stocks of phytoplankton in short incubations.

Although the Galapagos archipelago resides within a conventional HNLC zone, a patch of high chlorophyll from the western side of Isabela and sometimes extending a hundred kilometers westward is found there (Feldman 1986). This high chlorophyll patch is seldom seen on the eastern isles of the Galapagos, nor on the eastern side of Isabela (Feldman 1986). This phenomenon can be attributed to the comparatively iron rich Equatorial Undercurrent waters (Mackey et al. 2002) upwelling against the western archipelago platform. If a cause for the

plume is larger concentrations of iron, then grazing rates should be adapted to higher phytoplankton standing stocks due to higher concentrations of iron there. Then the lag in mesozooplankton response to iron enrichment may therefore not be observed (Banse 1995, Landry et al. 2000). Additionally, in accordance to the “ecumenical iron hypothesis”, in an iron-limited environment, growth rates should increase in the iron-enriched treatments and grazing rates for the small phytoplankton. Since smaller phytoplankton have a favorable surface area to volume ratio compared to larger phytoplankton, they are limited by iron at smaller concentrations (Morel et al. 1991). Thus, the growth rates and standing stocks of small phytoplankton (<20  $\mu\text{m}$ ) remain relatively constant (Tsuda et al. 2003) due to a lack of nutrient limitation in addition to higher grazing pressure.

The study described here compared the effects of iron on phytoplankton growth rates, microzooplankton grazing rates and the phytoplankton size distribution at stations both east and west of Isabela Island. Based on responses observed in previous dilution experiments in the HNLC equatorial waters (Latasa et al. 1997, Landry et al. 2000), I expected that iron might induce enhanced growth in all sized phytoplankton but that microzooplankton grazing would maintain control on smaller phytoplankton in the iron enriched bottles. The experiments conducted were meant to test this in addition to the hypothesis that the waters east of Isabela were iron limited compared to the western side. From the results, an understanding of the intrinsic growth and grazing rates were obtained in addition to the effects of iron on those rates within different size fractions.

## Methods

To determine the phytoplankton (my work) and bacterial (LeFevre 2006) response with respect to grazing and growth rates, dilution bottle incubation experiments modeled after Landry

et al. (2000) were conducted from 20-28 January 2006 aboard the R/V Thomas G. Thompson. At each of our sampling sites (Table 1, Fig. 1), seawater was collected using a CTD rosette at the chlorophyll maximum which was approximately 20 m below the surface.

At each sampling, all water collected from Niskin bottles was filtered with a 209- $\mu\text{m}$  mesh to remove macrofauna and most mesozooplankton. Filtered seawater for the dilutions was obtained using the same collected water and a Pall 0.2- $\mu\text{m}$  pore size filter. Filtration was gravity directed to minimize cell lysis. Four sets of five 2.0 L transparent bottles were prepared in each set; four of the bottles were used as dilution treatments consisting of macro-nutrient enriched bottles with 10%, 20%, 50% or 100% unfiltered seawater brought to 2-L total volume with 0.2- $\mu\text{m}$  filtered seawater (Fig. 2). The nutrient addition was based on a mixture after Landry et al. (2000) of 0.5  $\mu\text{mol L}^{-1}$  N-ammonium( $(\text{NH}_4)_2\text{SO}_4$ ), 0.03  $\mu\text{mol L}^{-1}$  phosphate ( $\text{NaH}_2\text{PO}_4$ ) and 0.1  $\text{nmol L}^{-1}$  manganese ( $\text{MnCl}_2$ ) to promote constant phytoplankton growth. The fifth bottle was an undiluted seawater control receiving no nutrients. Two sets of bottles, 10 in total, providing duplication of each treatment, were enriched with  $\text{FeCl}_3$  to 2.0  $\text{nmol L}^{-1}$  final concentration of iron, a concentration chosen to accord with Tsuda et al. (2003) and Landry et al. (2000). The remaining 10 bottles received no additional iron.

After each bottle was prepared, 510 ml was extracted out of each bottle for chlorophyll *a* and bacteria abundance measurements. 500 ml was allotted to initial size fractionated chlorophyll *a* measurements and 10 ml was devoted to bacterial count slides (LeFevre 2006). Each bottle was then placed in deck incubators with shading sheets and running seawater to simulate the ambient temperature and 50% of on deck light conditions. After a 24 hour incubation period, 500 mL from each bottle were sampled. For size fractionated chlorophyll *a* measurements, the water was passed successively through 20-  $\mu\text{m}$  and 0.7-  $\mu\text{m}$  filters. Additional

size fractions of 2-  $\mu\text{m}$  or 3-  $\mu\text{m}$  were included at stations Bio-6 and Bio-4, respectively. 2 -  $\mu\text{m}$  filters were also used for the initial timepoint at station Bio-2. These size fractions were chosen to accord with other projects. Chlorophyll was extracted from the filters by the sonification and centrifugation techniques described in Arar and Collins (1997) and quantified using a Turner fluorometer 10-AU. The initial and final time points ( $T_0$  and  $T_f$ , respectively) used to determine net growth rates were based on when each sample finished filtering. Growth rate was calculated assuming exponential growth. Linear regressions between empirically measured dilutions and net growth rates were then used to estimate intrinsic growth and grazing rates.

## Results

### *Phytoplankton community compositions*

Comparison with Snow's (2006) fractionated chlorophyll measurements of unamended seawater revealed differences in the  $>20 \mu\text{m}$  size fraction of the initial seawater (Fig. 3). This suggests that the use of the 209- $\mu\text{m}$  mesh to prefilter the seawater removed a significant portion of phytoplankton  $>20 \mu\text{m}$  in size. Bio-2 had the greatest standing stock of chlorophyll ( $\sim 0.51 \mu\text{g L}^{-1}$ ) (Snow 2006) and also the greatest loss of  $>20 \mu\text{m}$  size fraction of chlorophyll after the 209- $\mu\text{m}$  mesh filtration ( $\sim 0.12 \mu\text{g L}^{-1}$ ). Bio-4 and Bio-6 samples shared similar initial chlorophyll concentrations after the 209- $\mu\text{m}$  mesh filtration ( $0.23 \mu\text{g L}^{-1}$  and  $0.24 \mu\text{g L}^{-1}$ , respectively). But according to Snow (2006), Bio-4 had a greater standing stock of chlorophyll of  $0.30 \mu\text{g L}^{-1}$ . At Bio-6, Snow (2006) measured a similar value of  $0.23 \mu\text{g L}^{-1}$ . The percent fraction of the smallest size fraction varies between Bio-4 and Bio-6. This difference may be partially attributed to the use of 3- $\mu\text{m}$  filter at Bio-4 and a 2-  $\mu\text{m}$  filter at Bio-6. Additionally, Bio-6 was also the first station sampled.

### *Growth and Grazing rates*

Intrinsic growth and grazing rates were determined using least squares linear regressions between all of the nutrient amended bottles, using the dilution factor as the independent variable and net (apparent) growth rate as the dependent variable. The y-intercept for the regression equation determined the intrinsic growth rate and the slope of the line determined the intrinsic grazing (phytoplankton mortality) rates (Table 2). An  $r^2$  value of greater than 0.49 was needed to satisfy a statistical significance level set at  $p < 0.05$ .

Most of the regressions determined by the data within the size fractions and total chlorophyll concentrations were not statistically significant ( $p > 0.05$ ). Thus growth and grazing rates significantly greater than zero could not be determined (Figs. 4-5). However at two stations (Bio-2 and Bio-4), the linear regressions were significant for the  $>20\text{-}\mu\text{m}$  fraction in the presence of added iron. The growth rates were  $0.07\text{ h}^{-1}$  and  $0.05\text{ h}^{-1}$ , respectively, and grazing rates were  $0.02\text{ h}^{-1}$  and  $0.03\text{ h}^{-1}$  (Figs. 6-7).

#### *Nutrients effects on net growth rates*

At all three stations, there was close agreement between the net growth rates in the undiluted bottles without added iron with and without the added nutrients. This indicates that the intended nutrient additions had little demonstrable effect on growth rate estimates for the 24-hour experimental incubations. The nutrient additions between the size fractions at all three stations also demonstrated little effect on the net growth rates in the incubation bottles (Fig. 8).

However, different effects of the nutrient additions were observed on net growth rates depending on whether iron was present. LeFevre (2006) showed that our nutrient addition of  $0.5\text{ }\mu\text{mol L}^{-1}$  N-ammonium ( $(\text{NH}_4)_2\text{SO}_4$ ),  $0.03\text{ }\mu\text{mol L}^{-1}$  phosphate ( $\text{NaH}_2\text{PO}_4$ ) and  $0.1\text{ nmol L}^{-1}$  manganese ( $\text{MnCl}_2$ ) increased bacteria net growth rates in the undiluted bottles without the addition of iron at Bio-2 and Bio-6. This trend is observed with phytoplankton to a smaller

degree at stations Bio-2 and Bio-4 (Fig.9). At Bio-6, the addition of nutrients demonstrated a smaller growth rate for nutrients added and no nutrients added. However, the net growth rate of the nutrient amended without added iron is still higher than the net growth rate of the nutrient amended treatment with the added iron.

## Discussion

In the experimental bottles, a significant fraction of larger than  $>20 \mu\text{m}$  chlorophyll was missing from my samples than were present at the sampling sites (Fig. 3) in comparison to the size fractionated data provided by Snow (2006). Since we prefiltered our sampled water with a  $209\text{-}\mu\text{m}$  mesh, it is possible that chain forming phytoplankton were not able to pass through, or that the mesh became clogged and blocked larger phytoplankton from entering our bottles. However, the  $>20 \mu\text{m}$  phytoplankton are still a small fraction of the total community chlorophyll composition, and so significant insight into the total standing stock growth and grazing cannot be gathered through my experiments. The majority of the intrinsic growth and grazing rates (Table 2) found were not statistically different from zero, suggesting that overall, there was little activity in the 24 dilution incubations conducted in my experiment.

As expected, Bio-2 had the highest measured standing stock of chlorophyll out of the three stations ( $\sim 0.51 \mu\text{g L}^{-1}$ ) (Snow 2006). Bio-4 had a greater standing stock of chlorophyll of  $0.30 \mu\text{g L}^{-1}$  and at Bio-6, Snow (2006) measured a chlorophyll concentration of  $0.23 \mu\text{g L}^{-1}$ . All of these values were higher or equal to an average  $0.2 \mu\text{g L}^{-1}$  found in the equatorial Pacific by Chavez et al. (1990). The highest portion of  $>20 \mu\text{m}$ -size fraction phytoplankton was found at Bio-2, suggesting that Bio-2, at the time of sampling, was within the Galapagos plume, and that the plume is dominated by large phytoplankton.

Although the majority of the measured growth and grazing rates were not significant from zero, significant growth and grazing rates were measured in the  $>20\ \mu\text{m}$ -size fraction in the presence of  $2.0\ \text{nmol L}^{-1}$  of added iron at Bio-2 and Bio-4. Growth exceeded grazing at both Bio-2 and Bio-4 in the  $>20\ \mu\text{m}$ -size fraction in the presence of iron. This suggests that the growth rates and grazing with the presence iron and the nutrient mixture of N-ammonium, phosphate and manganese were greater than all of the other size fractions and at Bio-6. This also implies that with the additional iron at Bio-2 and Bio-4, the  $>20\ \mu\text{m}$  phytoplankton are able to escape microzooplankton grazing. However, similar subsurface primary productivity rates were found at Bio-2 and Bio-4 (Gilmore 2006) which suggests that there must be less grazing pressure on the phytoplankton at Bio-2 to be able to maintain a higher standing stock of phytoplankton and yet the same primary production, in accordance with the slower grazing rate on the phytoplankton at Bio-2.

The  $>20\ \mu\text{m}$  phytoplankton responded to the addition of iron after an incubation period of 24 hours. The lack of temporal lag as observed in Coale et al. (2004) and Landry et al. (2000) suggest that the larger phytoplankton at Bio-2 and Bio-4 are adapted to environmental increases in iron concentrations. However, the larger phytoplankton growth at those two stations is still iron limited. The growth rates observed with the addition of iron also may have just been intrinsic growth rates of the area excluding the phytoplankton lost in the  $209\text{-}\mu\text{m}$  mesh prefiltration step.

The trend of a greater growth and grazing rate with the presence of iron in the greatest size fraction is not observed in Bio-6. Bio-6 also had the smallest measured chlorophyll concentrations of the three stations and the smallest measured chlorophyll in the  $>20\mu\text{m}$ -size fraction. Snow (2006) also observed this station in addition to others east of Isabela was

dominated by smaller phytoplankton (2-20  $\mu\text{m}$ ). This suggests that Bio-6 may have not displayed the similar trend due to the lack of larger phytoplankton. But, if the addition of iron is due the Equatorial Undercurrent, then Bio-6, being the furthest station away from the upwelling, would display a similar temporal response found in Landry et al. (2000) and Coale et al. (2004). Thus, the 24-hour incubations of our experiments would not capture a phytoplankton growth and grazing response to iron in the  $>20$   $\mu\text{m}$ -size fraction. However, Bio-6 was the first station sampled and the first dilution experiment conducted by LeFevre and myself, which may have affected initial chlorophyll measurements.

According to silicate data collected by Dickson (2006), the silica measured at the same depth as my sampling depths was comparable at both Bio-2 and Bio-4 ( $\sim 7$   $\mu\text{M}$ ). According to Coale et al. (2004), this concentration is classified as low. However, since my nutrient additions did not include silica, the significant growth and grazing rate in the  $>20$   $\mu\text{m}$  size fraction cannot be attributed to additional silica added for diatom growth. Therefore, the difference between the growth and grazing rates between Bio-2 and Bio-4 in the greatest size fraction was probably not due to silica as a limiting nutrient for diatoms which generally compose the bulk of larger phytoplankton. These results suggest that the presence iron is a strong factor in 20- 209  $\mu\text{m}$  phytoplankton growth and grazing both east and west of the Galapagos Islands.

## Conclusions

Significant growth and grazing rates were found in the  $>20$   $\mu\text{m}$ -size fraction with the addition of  $2.0 \text{ nmol L}^{-1} \text{ FeCl}_3$  at two stations. Higher standing stocks of total chlorophyll were measured at Bio-2, which was west of Isabela than at Bio-4 and Bio-6 which were east of Isabela. The phytoplankton growth and grazing rates found in the  $>20$   $\mu\text{m}$ -size fraction at Bio-2 was  $0.03 \text{ h}^{-1}$  faster than the growth rate found in the same size fraction at Bio-4 at the same

depth. Even though the  $>20\ \mu\text{m}$ -size fraction represented the smallest size fraction, it displayed the most activity throughout the experiment and both east and west of Isabela. The results of the conducted study strongly imply iron-limitation for the greatest size fraction east and west of Isabela Island.

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Table 1. Sampling stations with their respective sampling times, location and depth at which the experimental water was collected.

Station	Date	Time on Station (GMT)	Latitude	Longitude	Depth Sampled(m)
Bio-2	23/01/06	8:02	S 00 36.9759	W 091 18.9998	23.4
Bio-4	25/01/06	8:49	S 00 00.9827	W 091.07.9866	22.1
Bio-6	21/01/06	7:04	S 00 55.0189	W 089 59.9560	21.7

Table 2. The intrinsic growth and grazing numbers determined by linear regression in each of the size fractions, treatments and station and the corresponding  $r^2$  values.

Station	Treatment	Growth( $h^{-1}$ )	Grazing( $h^{-1}$ )	$r^2$
Bio-2	Total Chl	0.03	-0.01	0.08
	+Fe	0.03	0.01	0.19
	GF/F	0.06	-0.01	0.40
	+Fe	0.01	0.00	0.15
	>20um	0.07	-0.01	0.11
	+Fe	0.07	0.02	0.56
Bio-4	Total Chl	0.03	0.00	0.07
	+Fe	0.02	-0.01	0.14
	GF/F	0.03	0.03	0.08
	+Fe	0.02	0.00	0.05
	2-20um	0.01	0.01	0.04
	+Fe	-0.01	-0.01	0.11
	>20um	0.03	0.02	0.20
	+Fe	0.05	0.03	0.58
Bio-6	Total Chl	0.02	0.03	0.17
	+Fe	0.00	0.00	0.00
	GF/F	0.01	0.04	0.21
	+Fe	0.00	0.01	0.03
	2-20um	0.00	0.01	0.02
	+Fe	-0.02	-0.01	0.05
	>20um	0.07	0.03	0.22
	+Fe	0.02	0.00	0.00

## Figure Captions

Figure 1. Map of the Galapagos Islands and the station sites and names. White circles outlined in black denote station locations.

Figure 2. Schematic of the conducted dilution experiments. Half of the bottles received 2 nM iron additions, and each treatment was uplicated such that there will be 20 bottles total. SW stands for seawater prefiltered with a 209- $\mu\text{m}$  mesh. FSW stands for gravity driven filtration with a 0.2- $\mu\text{m}$  pore size filter.

Figure 3. Size fractionated chlorophyll concentrations in the unamended bottles at the initial and final timepoints at each of the stations. Bars labeled Snow was from size fractionated chlorophyll measurements made by Snow (2006). Bars labeled Guo were size fractionated chlorophyll measurements made by me after filtering the water with a 209- $\mu\text{m}$  mesh from the CTD. Error bars denote the first standard deviation.

Figure 4. Results of the dilution experiments from Bio-6 in total chlorophyll, the smallest size fraction (0.7  $\mu\text{m}$ -2  $\mu\text{m}$ ), and the intermediate size fraction of 2 $\mu\text{m}$ -20  $\mu\text{m}$ . Regression lines are fit to the data are for incubations with added nutrients. The  $r^2$  value for the linear regression lines for the iron/no iron treatments are displayed on the figure.

Figure 5. Results of dilution experiments from Bio-6 in the largest size fraction (>20 $\mu\text{m}$ ). Regression lines are for incubations with added nutrients. The dotted line is the regression line for nutrients but no added iron. The  $r^2$  value for the linear regression lines for the iron/no iron treatments are displayed on the figure.

Figure 6. Results of dilution experiments from Bio-4 in the largest size fraction (>20 $\mu\text{m}$ ). Regression lines are for incubations with added nutrients. The dotted line is the regression line

for nutrients but no added iron. The  $r^2$  value and equation for the linear regression line for the iron added treatment are displayed on the figure.

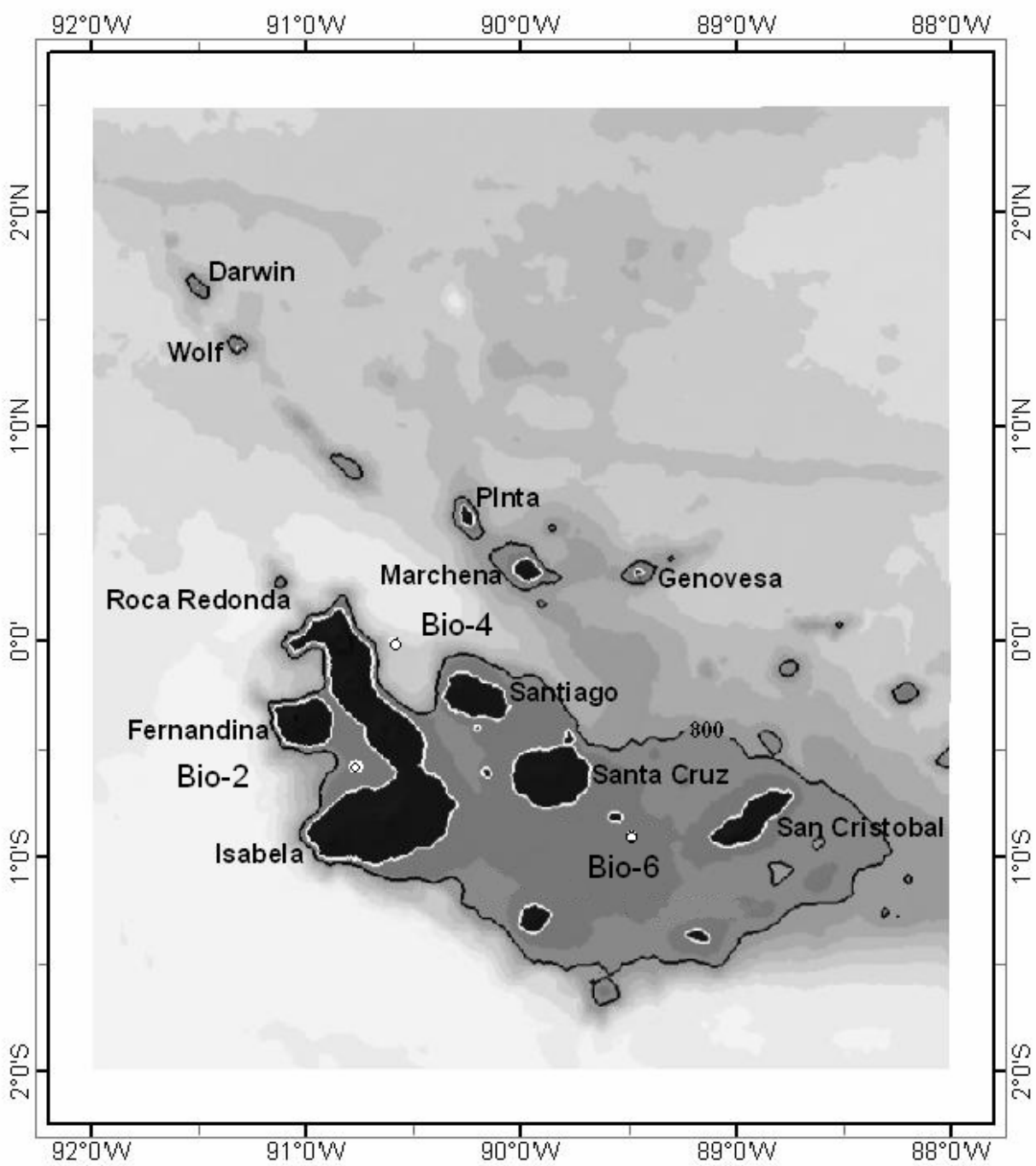
Figure 7. Results of dilution experiments from Bio-2 in the largest size fraction ( $>20\mu\text{m}$ ).

Regression lines are for incubations with added nutrients. The dotted line is the regression line for nutrients but no added iron. The  $r^2$  value and equation for the linear regression line for the iron added treatment are displayed on the figure.

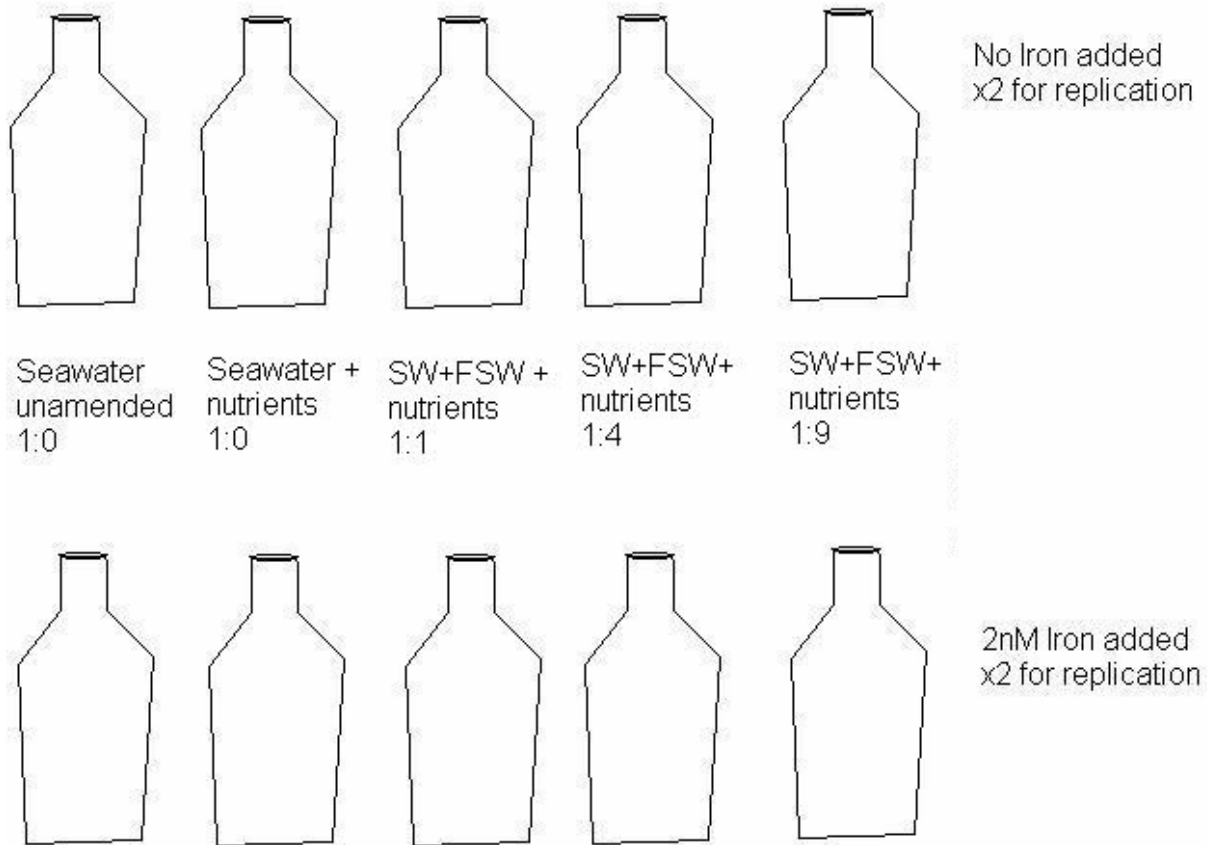
Figure 8. Comparison of averaged net growth rates in the unamended and nutrient amended undiluted bottles in the total chlorophyll reading and each of the three size fractions at Bio-4. Bio-4 was chosen because other stations displayed similar results. The error bars denote the upper range of the two replicates.

Figure 9. Comparison of iron and nutrient amended bottles and nutrient only amended bottles in net growth rates undiluted seawater treatments at each of the stations. The error bars denote the upper range of the two replicates averaged.

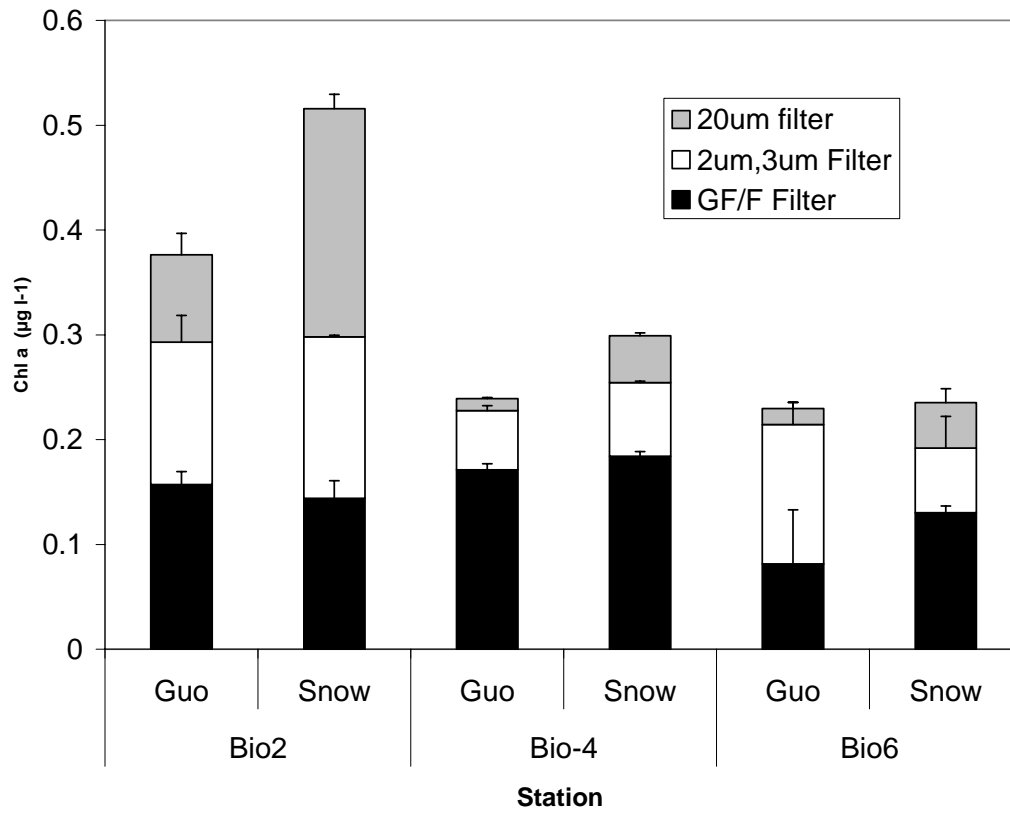
Guo Fig. 1



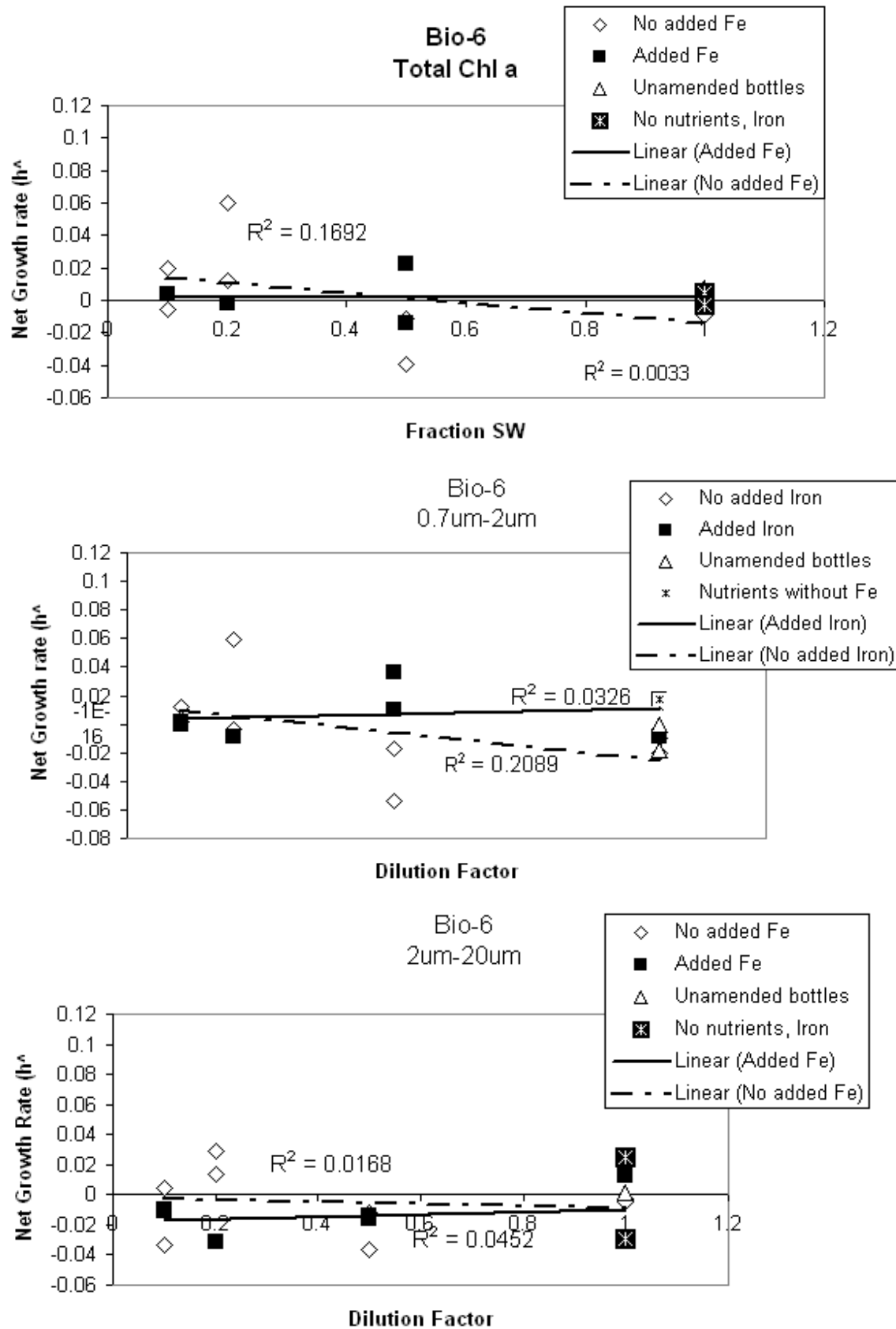
Guo Fig 2.



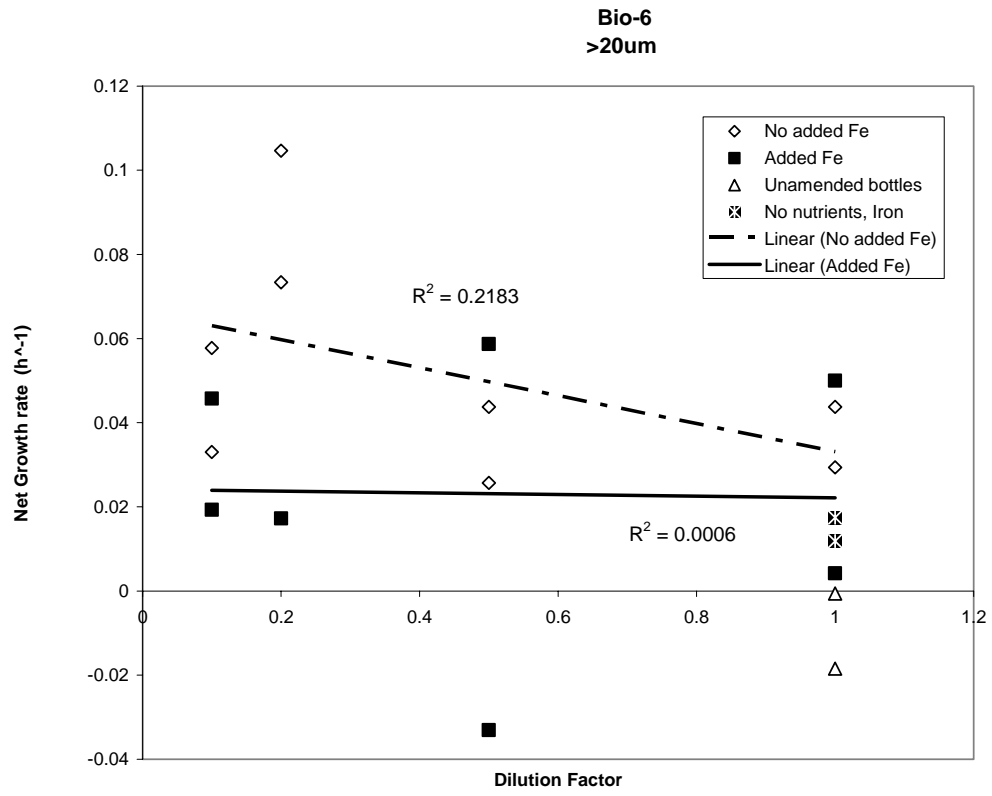
Guo Fig. 3



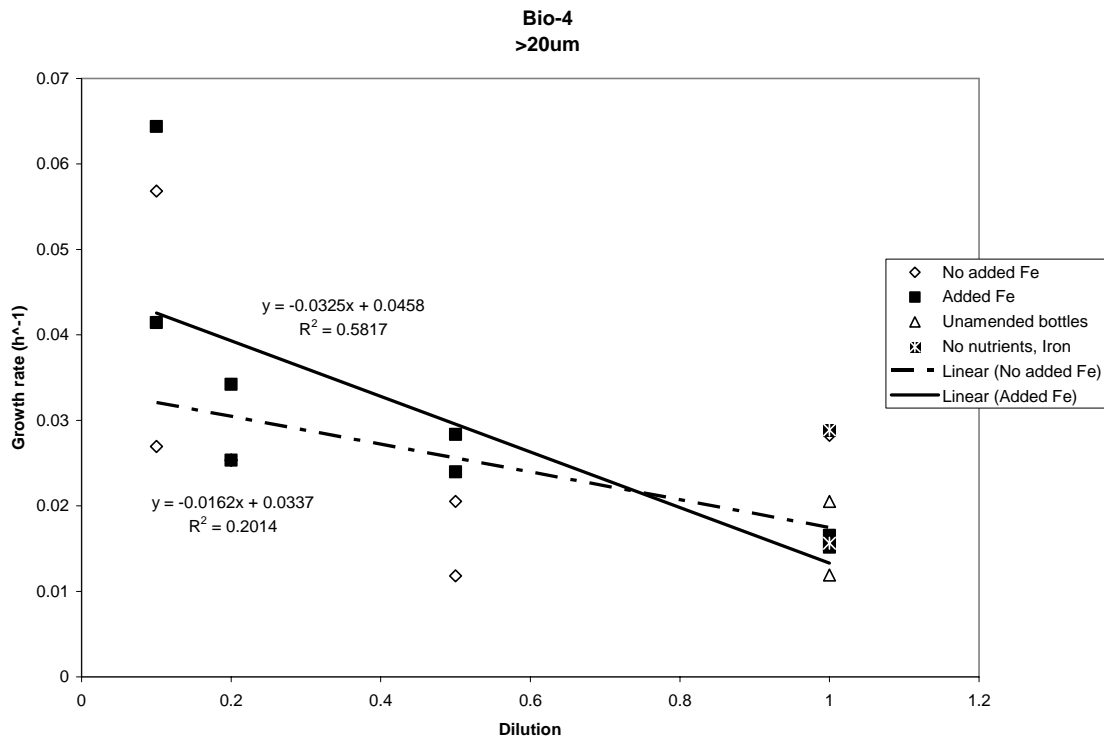
Guo Fig. 4



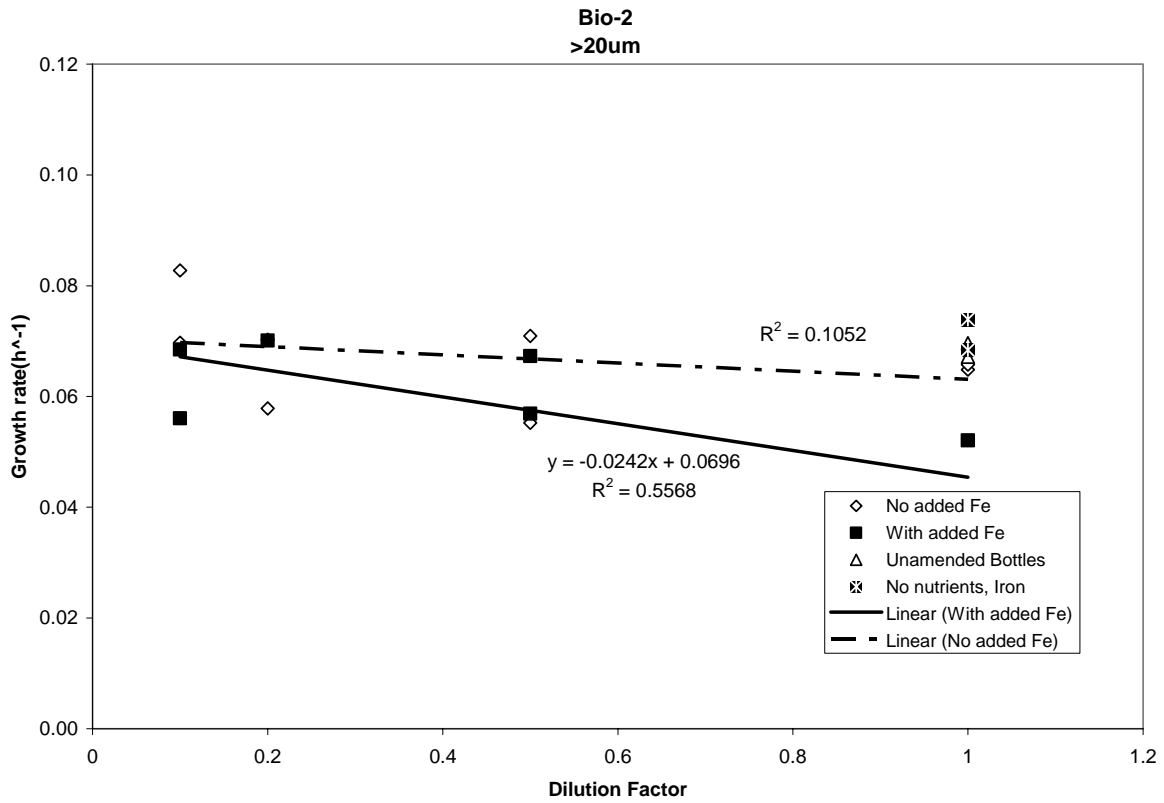
Guo Fig. 5



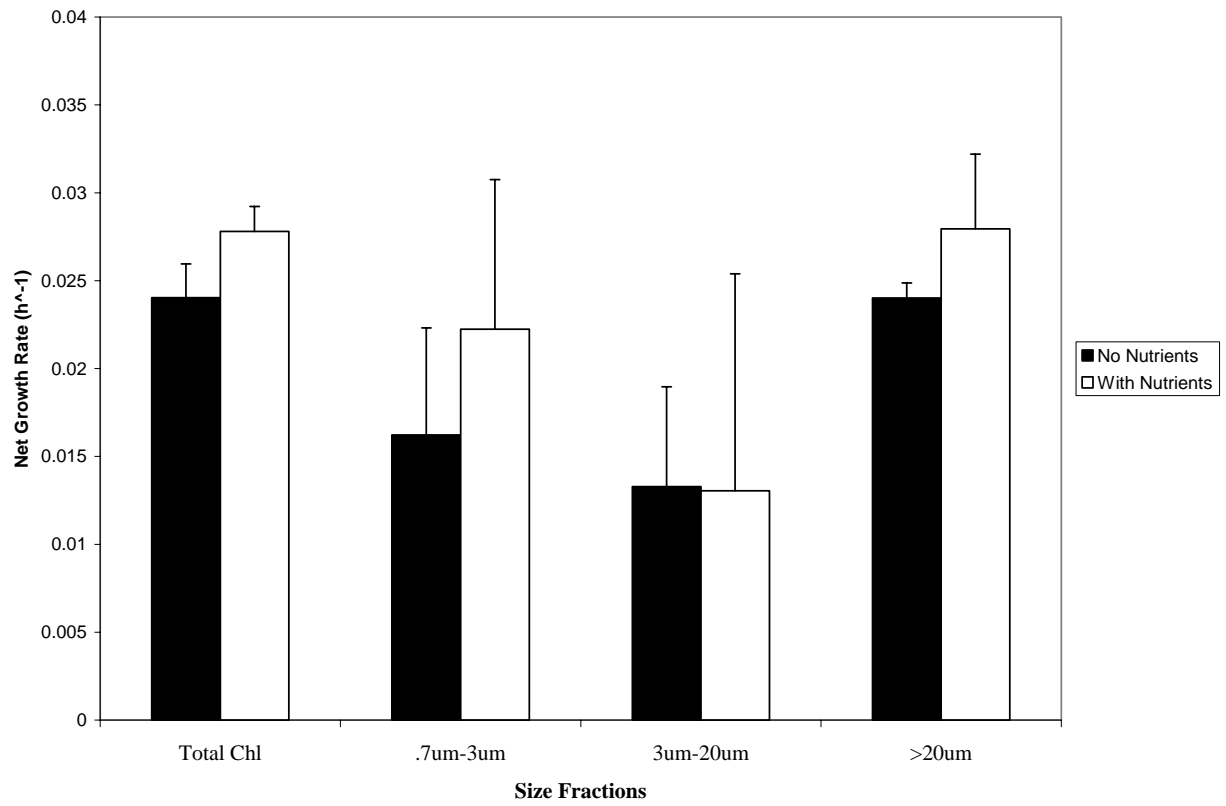
Guo Fig. 6



Guo Fig. 7



Guo Fig. 8



Guo Fig. 9

