12.2 Nutrient addition bioassay experiments

D285 **Mark Moore, Mike Lucas, Sophie Seeyave**

Nutrient addition bioassay experiments were performed using a highly replicated design to investigate the inter-dependence of iron and light availability on phytoplankton physiology, growth and nutrient drawdown. Original plans to incorporate silicate additions as a further factor were abandoned due to the increased logistical problems and time constraints involved in performing experiments of twice the size. Given the successful completion of a number of experiments during Crozex Leg 1 (D285) it is hoped that some work on silicate limitation may be possible during Leg 2 (D286).

As with all work involving manipulation of iron availability for phytoplankton populations, strict controls were required to avoid contamination of incubation containers and sampled water. Incubations were performed in 2 l polycarbonate bottles which had passed through a rigorous cleaning process involving a Decon wash and soaking in 50% HCl for 1 week, followed by rinsing then storage with acidified Milli-Q prior to sailing.

The original intention was to collect incubation water from the underway Fe fish, however this strategy presented a number of problems. Firstly it was thought that collection whilst on station might result in contamination from the ship, conversely underway collection can potentially result in variability within the sample bottles due to patchiness along track. Finally a broken hose within the fish body during sampling for the second experiment resulted in serious contamination and hence a failure to collect any usable data. This contamination may have also resulted in a noisy fourth and final experiment where replication was poor. Due to constraints on sampling time before leaving the study area, this experiment was performed in the bottles contaminated during Expt. 2, the other bottles being used in Expt. 3. It was subsequently concluded that these bottles were probably not cleaned adequately between experiments. A more rigorous cleaning procedure between experiments will thus be adopted on the second leg. Additionally, sampling from the titanium CTD (Ti) rig is considered to be the only reliable method of collecting uncontaminated water and is recommended during Leg 2.

The experimental design involved the incubation of 20 bottles in 4 sets of 5 replicates, one set each for high light (control and +Fe) and low light (control and +Fe). Two of the five replicate bottles were sub-sampled approximately every 2 days. The remaining three replicates remained sealed until the 5-6th day as a check that sub-sampling had not contaminated the time-series measurements. Such a strategy also provides more robust statistics and a large volume of water for an additional suite of final measurements.

Sampling of the time-series was routinely performed for chlorophyll, ambient macronutrients (N, P and Si) and PSII characteristics as measured by FRRf. Additional sampling at the beginning and end time points consisted of ^{14}C P vs E determinations, POC/PON and preservation of samples in lugols iodine for phytoplankton counts. In order to assess contamination, samples were also collected for analysis of total dissolvable iron (TDFe) at the end of the experiments.

Table 12.6 Sampling methods, locations, times and initial conditions for bioassay experiments

	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Sampling location	**M1**	**M6**	**M3**	**M8E**
Sampling method	Fe Fish	Fe Fish	Ti CTD, Station 15516, Depth, 20m	Ti CTD, Station 15531 Depth, 25m
Bottle set	1	2	1	2
Start point	1435 GMT, JD 316	2100 GMT, JD 326	0230 GMT, JD 330	0220 GMT, JD335
End point	1630 GMT, JD 321	1700 GMT, JD 331	1630 GMT, JD 335	1645 GMT, JD 339
Initial chlorophyll concentration	1.83 ± 0.05	0.59 ± 0.05	0.63 ± 0.02	0.80 ± 0.04
Initial Nitrate concentration	18.49 ± 0.17	22.95 ± 0.15	23.46 ± 0.13	23.10 ± 0.42
Initial Silicate concentration	1.23 ± 0.23	17.64 ± 0.52	8.84 ± 0.21	2.52 ± 0.09
Comments	Replication good, clear +Fe response	Experiment contaminated due to problems with Fe Fish	Replication good, clear +Fe response	Poor replication, some indication of +Fe response, suspect contamination of bottle remains after Expt. 2!

A total of four experiments lasting 5-6 days each were carried out during Leg 1. Of these experiments, 2 produced good quality data. A complete list of experiments along with sampling locations and initial conditions is provided in Table 12.6.

Despite the contamination problems that resulted in only half the experiments providing robust repeatable data, overall results were satisfactory, with some potentially novel outcomes. Relatively few experiments on the combined effects of iron and light availability have been performed in the field (Boyd et al. 1999, Maldonado et al. 1999). Additionally it is not known of any previous work including extensive measurements of PSII characteristics within such a framework. Preliminary results from experiment 1 are presented in Fig. 12.3. This experiment was of further interest as the incubation water was sampled within a relatively large bloom of a colonial *Pheocystis* spp.

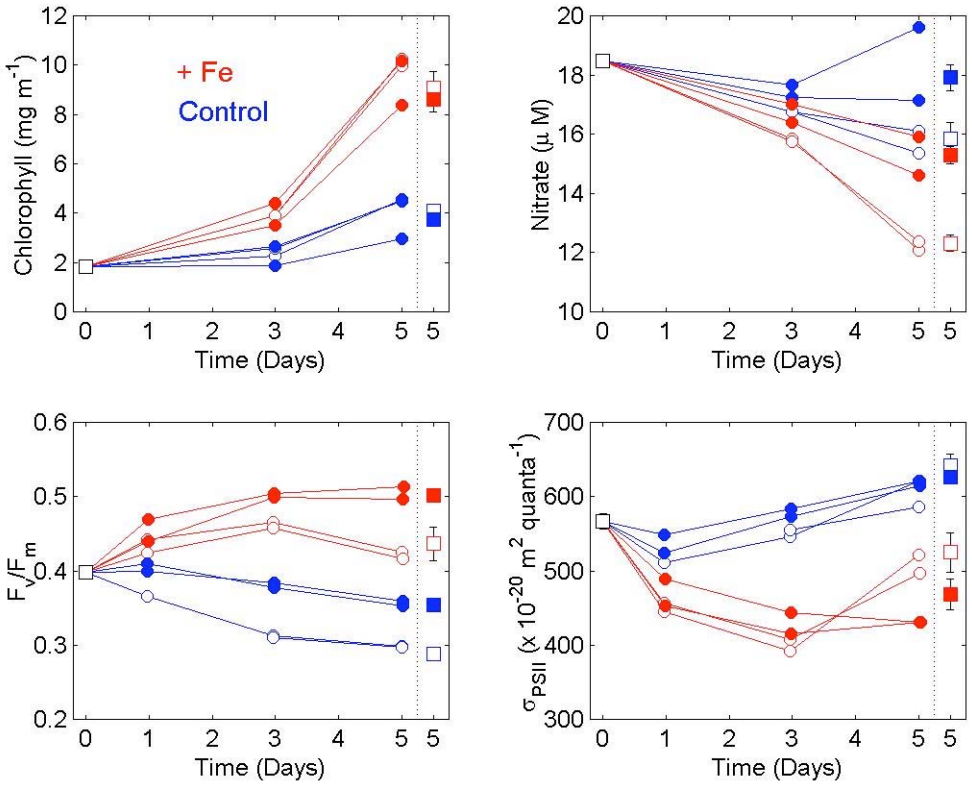

Fig. 12.3 Results from nutrient addition bioassay experiment '1'. A clear response to iron addition is observed. Distinct responses in nutrient drawdown and PSII characteristics (F_v/F_m and σ_{PSII}) to both iron availability and light level were also observed.

D286 **Anna Hickman, Mike Lucas, Sophie Seeyave**

Nutrient addition bioassay experiments were performed following the protocols designed and conducted by Mark Moore on Leg 1.

A highly replicated design was used to investigate the inter-dependence of iron and light availability on phytoplankton physiology, growth and nutrient drawdown. Original plans to incorporate silicate additions as a further factor were abandoned on Leg 1 due to the increased logistical problems and time constraints involved in performing experiments of twice the size.

As with all work involving manipulation of iron availability for phytoplankton populations, strict controls were required to avoid contamination of incubation containers and sampled water. Incubations were performed in 2 l polycarbonate bottles which had passed through a rigorous cleaning process prior to Leg 1 (involving a Decon wash and soaking in 50% HCl for 1 week, followed by rinsing then storage with acidified Milli-Q prior to sailing). On both the return and outward passages between Leg 1 and Leg 2 bottles were rinsed with 10% HCl, rinsed and subsequently stored with acidified Milli-Q. Between experiments all bottles were cleaned with 10% HCl and rinsed with milli-Q. All samples were collected from the titanium CTD (ti) rig.

The experimental design involved the incubation of 20 bottles in 4 sets of 5 replicates, one set each for high light (control and +Fe) and low light (control and +Fe). Two of the five replicate bottles were sub-sampled approximately every 2 days. The remaining three replicates remained sealed until the 5-6th day as a check that sub-sampling had not contaminated the time-series measurements. Such a strategy also provides more robust statistics and a large volume of water for an additional suite of final measurements.

Table 12.7 Sampling for bioassay experiments on D286

	Expt. 5	**Expt. 6**	**Expt. 7**	**Expt. 8**
Sampling location	**M10**	**M3**	**M2**	**M3**
Sampling method	Ti CTD Station 15561 Depth, 25m	Ti CTD Station 15592 Depth, 25m	Ti CTD, Station 15602 Depth, 40m	Ti CTD, Station 15621 Depth, 20m
Bottle set	1	1	1	2
Start point	1845 GMT, JD 355	1400 GMT, JD 366	1600 GMT, JD 006	1800 GMT, JD 010
End point	1700 GMT, JD 361	1700 GMT, JD 005	1700 GMT, JD 012	1630 GMT, JD 015
Initial chlorophyll concentration	0.79 ± 0.05	0.78 ± 0.01	0.36 ± 0.02	4.84 ± 0.07
Initial Nitrate concentration	20.78 ± 0.10	22.87 ± 0.14	21.47 ± 0.02	18.40 ± 0.07
Initial Silicate concentration	1.11 ± 0.01	2.89 ± 0.02	2.02 ± 0.01	0.12 ± 0.02
Comments	Replication good, clear but minimal +Fe response	Replication poor, minimal +Fe response	Replication good, minimal +Fe response	Replication good, clear +Fe response in early stages.

Sampling of the time-series was routinely performed for chlorophyll, ambient macronutrients (N, P and Si) and PSII characteristics as measured by FRRf. Additional sampling at the beginning and end time points consisted of ^{14}C P vs E determinations, POC/PON and preservation of samples in lugols iodine for phytoplankton counts. Samples were also filtered for dissolved iron measurements at the beginning and end of the experiments.

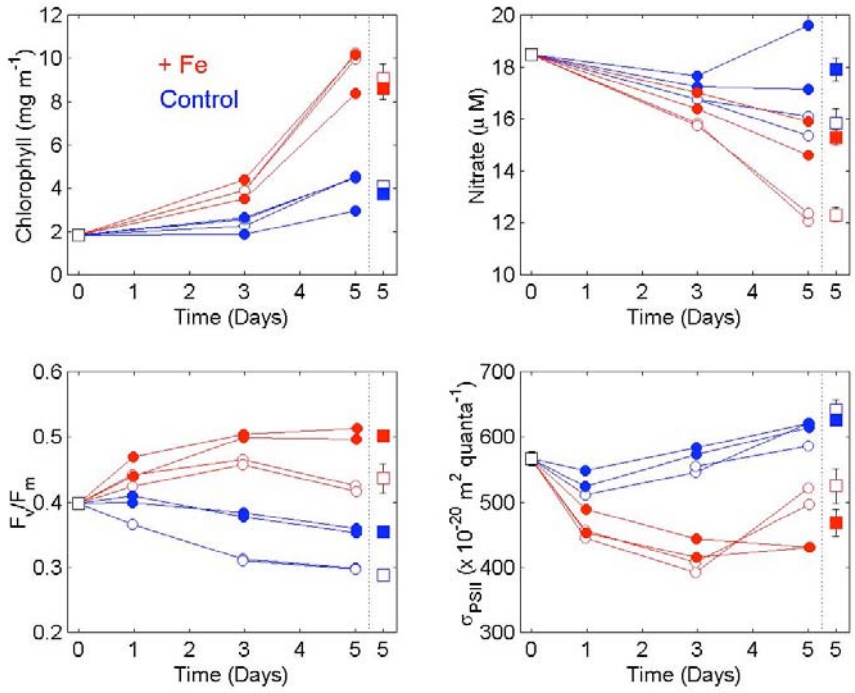

Fig. 12.4 Results from nutrient addition bioassay experiment '5'. A clear iron response is observed, although there is minimal difference between incubations of high and low light levels.

A total of four experiments lasting 5-6 days each were carried out during Leg 2. A complete list of experiments along with sampling locations and initial conditions is provided in Table 12.7. Preliminary results from all experiments are promising, showing no sign of contamination of samples. Preliminary results from Experiment 5 are shown in Fig. 12.4.

References:

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