



Ocean Nourishment® in the Philippines - Proof of Concept Report for the Sulu Sea

DATE: 1ST AUGUST 2007

PROJECT: EOS 07-008

DOC NO.: EOS-REP-07-008 REV E PR.DOC



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DOCUMENT REVISION STATUS					
REV	DESCRIPTION	AUTHOR	REVIEW	APPROVAL	DATE
A	1 st Draft	D Harrison			13/8/07
B	2 nd Draft	D Harrison	I. S. F. Jones		4/11/07
C	3 rd Draft	D Harrison	Peter Wheen		4/1/08
D	Final Draft	D Harrison	John Ridley		10/1/08
E	Public Release	D Harrison	John Ridley		13/2/08



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1 INTRODUCTION

1.1 Background

For thousands of years the Sulu Sea has provided a rich source of available protein to the populations of the Philippines and Malaysia. With an increase in commercial fishing and the pressures of an ever increasing population this valuable resource may have reached the limit of its sustainable fish catch (Jones, 2002). Current catches have levelled off since 1991 (at a level near estimated maximum sustainable yield) and existing fishing effort is clearly too high. (Barut, 1997)

As the fishing effort increases, the catch per unit effort is decreasing. In discussions with the author artisan fisherman in the Philippines village of Aniniy-i in the east of the Sulu Sea reported that their catch for a days fishing had dropped from an average of 20 kgs of small pelagic fish to 3-5 kgs during their career. Increasing population both in the Philippines and Malaysia will continue to provide enlarged demand for protein from the Sulu Sea. Increasing population is also affecting the local environment, which may also have a further detrimental effect on fisheries resources. Could the carrying capacity of the Sulu Sea be increased to supply protein to the additional population by increasing the primary production?

Ocean Nourishment is the purposeful introduction of nutrients into the upper ocean to capture and store atmospheric carbon and to increase the supply of marine protein. Ocean Nourishment® Corporation (ONC) is considering the feasibility of undertaking an Ocean Nourishment project in the Exclusive Economic Zone (EEZ) of the Philippines. The project involves the manufacture of urea from natural gas and the subsequent introduction of this urea to the upper layers of the ocean. This stimulates the marine food chain, increasing both ocean sequestration of atmospheric CO₂ and the carrying capacity of the region for fish stocks. The vision of expanded sustainable fisheries is provided by Jones and Young (1997).

Are there enough trace nutrients in offshore waters of the Sulu Sea to support some growth of the natural assemblage of algae when provided with nitrogen and phosphate? What species will prosper under an enrichment regime?



1.2 Objectives

The experimentation was carried out with the following objectives.

- To collaborate with the University of the Philippines in the Visayas.
- To demonstrate the Ocean Nourishment Process in the waters of the Sulu Sea.
- To investigate the limiting nutrients for phytoplankton growth in waters of the Sulu Sea.
- To provide verification of sea surface temperature from a publicly available satellite data assimilation model.

2 COLLABORATION

The experiment was performed in collaboration with staff and students, and associate researchers of the University of the Philippines in the Visayas (UPV), and was performed at the Miag-ao campus of the University.

Associate researchers from UPV assisted in the collection of water samples, in situ measurements, sampling during the experiment, and also with phytoplankton / zoo plankton analysis.

Many of the staff were familiar with the concepts of Ocean Nourishment having themselves practiced nourishment of lakes in the Philippines using phosphorous, and also practiced various forms of nourishment within their aquaculture industry research.

UPV researchers undertook the species analysis of the phytoplankton samples at the conclusion of the experiment.



3 THE EXPERIMENT

3.1 Background

3.1.1 Biomass

The phytoplankton biomass is dependent on the difference between the growth of phytoplankton less that lost by processes such as grazing and senescence. Phytoplankton growth in turn depends amongst other things on the availability of nutrients which are in short supply in oligotrophic ocean waters.

3.1.2 Chlorophyll-a

In order to monitor the response of a phytoplankton assemblage to nutrient enrichment and measure phytoplankton biomass in situ, a method of measuring biomass was adopted. The most useful method for determining the total quantity of phytoplankton in seawater is to estimate the amount of chlorophyll-a present. All photosynthetic algae contain chlorophyll-a which is a highly fluorescent blue-green water insoluble pigment (Falkowski & Kiefer, 1985). chlorophyll captures photons from the sun in order to synthesise cellular components from CO₂.

Although chlorophyll-a only makes up a very small percentage of the total biomass, it can be related to total plant carbon by applying a factor. This factor has been found to vary between 25 – 100 and can be estimated by taking into account such things as temperature, species composition, and state of nutrition (Flemer, 1969, Cloern et al, 1995). By analysing the chlorophyll-a concentration in seawater, it enables us to determine the concentration of living phytoplankton in the presence of zooplankton, detritus and dead phytoplankton.

3.1.3 Fluorescence

Fluorescence is the property of a material to absorb specific wavelengths of light and almost instantaneously emit longer wavelengths of light. The fluorescence technique is a common means used to calculate the concentrations of chlorophyll-a present in seawater.

chlorophyll-a naturally absorbs blue light and emits, or fluoresces, red light. The basic assumption of the fluorescence technique is that there is a constant ratio between the observed fluorescence intensity of an *in-vivo* (in living algal cells) sample and the extractable chlorophyll-a concentration (Cloern et al 1995).

Measurements of chlorophyll-a were taken with an ECO-FLRT (serial number:100) fluorometer (Western Environmental Technology Laboratories Inc.). This fluorometer uses a light emitting diode (LED) to provide the excitation source. An interference filter is used to reject the small amount of out-of-band light emitted by the LED. The light from the source enters the water at an angle of



approximately 55°– 60° with respect to the end face of the unit. The fluoresced light is received by a detector positioned where the receiving angle forms a 140° intersection with the source beam. An interference filter is used to reject the scattered excitation light.

3.2 In Situ Measurements

3.2.1 Aim

To investigate the chlorophyll and temperature conditions at locations in the Panay Gulf in the Sulu Sea during July. This data will then be compared with satellite data for verification of the systems.

3.2.2 Method

A southerly heading was sailed from a location near Miag-ao (N10°34.980' E122°06.486') 32nm out into the Sulu sea to a location of (N10°03.316' E122°00.904'). Measurements were taken at 5 nautical miles from the coast and approximately every 10 nm thereafter. Each measurement consisted of sea surface temperature, and a depth profile of chlorophyll with readings at 2m, 4m, 6m, & 7m of depth. 4 locations were sampled in total. Chlorophyll measurements were taken by lowering the fluorometer described in section 3.1.2 by cable from the side of a small fishing vessel into the water column. The method of profile sampling is shown in Figure 3.1.



Figure 3.1 : Ramon Cruz of the University of the Philippines in the Visayas, In-situ sampling with the Wet Labs ECO-FLRT Fluorometer.

3.2.3 Results

The locations of interest are shown in Figure 3.2 location 001 was the departure point of the voyage. Sampling was performed at locations 002, 003, 004, and 005. Chlorophyll concentrations were calculated using the manufacturers published scale factor of 0.0078 and the Clean Water Offset (CWO) that was obtained by taking a calibration in clear chlorinated pool water, Appendix 2. The results for the four sampled locations are presented in Table 3.1.

Location	002		003		004		005	
Lat Long								
Temp °C	30		30		30		31	
Depth (m)	Counts	Chlorophyll µg/L	Counts	Chlorophyll µg/L	Counts	Chlorophyll µg/L	Counts	Chlorophyll µg/L
2	109	0.1035	111	0.1166	108	0.0944	112	0.1211
4	110	0.1059	111	0.1162	109	0.0981	112	0.1211
6	108	0.0953	110	0.1088	109	0.0981	114	0.1396
7	109	0.1043	110	0.1084	109	0.1014	115	0.1449

Table 3.1 : In-situ sampling results on 20/7/07



Figure 3.2 : In-situ sampling locations



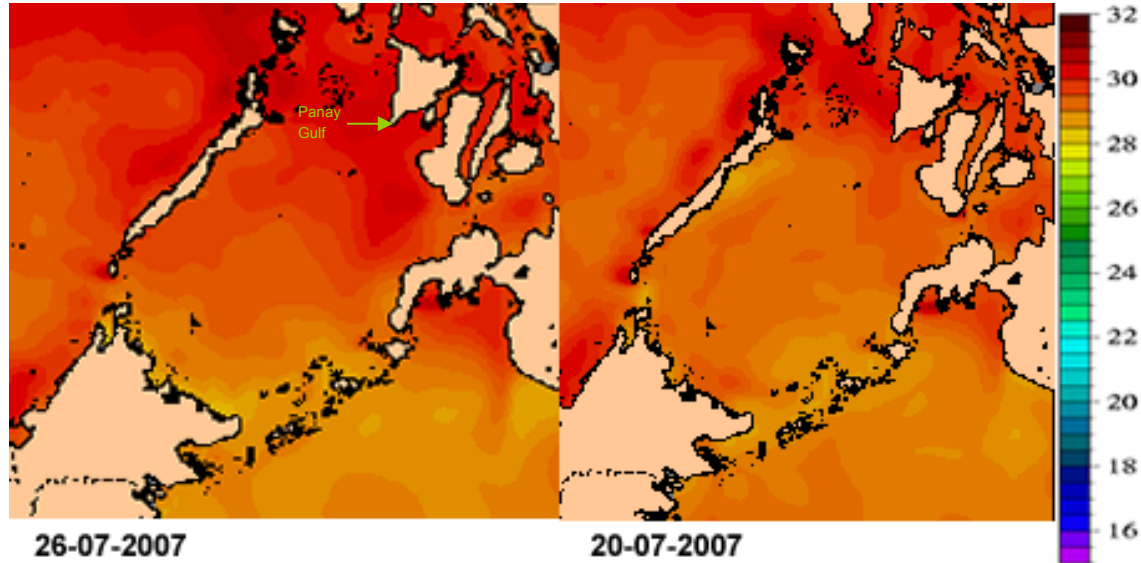


Figure 3.3 : NCOM Sea Surface Temperature °C.

Figure 3.3 Shows the Sea Surface Temperature (SST) for the days measurements were taken from the United States of America Naval Research Laboratory, Navy Coastal Ocean Model (NCOM) available at http://www7320.nrlssc.navy.mil/global_ncom/.

It can be seen (by comparison against table 3.3) that the NCOM ocean model correlates with the measured sea surface temperatures to within 1°C accuracy.

3.2.4 Discussion

It can be seen that the chlorophyll concentrations in the locations sampled are very low values typical of areas classed as ocean deserts. The level of chlorophyll was approximately the same throughout the area sampled and across the depth range sampled.

The expected result of decreasing chlorophyll with distance from the coast was not observed. The first measurement taken 5 nautical miles from the coast was possibly outside the coastal area influenced by nutrient runoff from streams and rivers which could be expected to produce higher levels of chlorophyll.

The results show low background levels of phytoplankton suggesting growth is limited, possibly due to lack of one or more nutrients. Samples were collected from location 5 and cultured in simulated natural conditions to test this theory.

3.3 Culture Experiments

3.3.1 Aim

To investigate the effects on growth of adding various nutrients in a number of concentrations to seawater samples from the Sulu Sea in the Visayas region. Such a study will assist identification of limiting nutrients to the standing stock of phytoplankton at the location of sampling and ascertain if there are sufficient micro nutrients to support additional new primary production.

3.3.2 Method

In July 2007 seawater samples were collected from location 005 (Refer Figure 3.2) in the Sulu Sea at co-ordinates 10°03.316'N 122°00.904'E. Samples were cultured in simulated natural conditions to observe the growth of phytoplankton.

The experiment included controls, and samples nourished with various concentrations of nutrients including, nitrogen alone, nitrogen and phosphorous in the Redfield ratio, and a commercial fertiliser called Grow Giant. Sample 0 was frozen soon after collection to provide a control for the species analysis. Sample 1 and 2 were used as controls. Samples 3-6 were created by adding prepared solutions of Urea and Sodium Phosphate. Samples 7 & 8 were nourished from a solution made by dissolving approximately 1 gram of Grow Giant in 1 litre of distilled water. 1ml of the resulting solution was added to sample 7 and 5ml to sample 8. The Sample identification and nutrients introduced are provided in Table 3.2 the amount of nutrients for samples 7 & 8 are approximate only. The constituents of Grow Giant are provided in Appendix 4.



Sample	Description	N	P	K	Fe	Mn	B	Zn	Cu	Mo
0	Collection									
1	Control									
2	Control									
3	8uM N	8.00								
4	16uM N	16.00								
5	8uM N + P	8.00	0.50							
6	16uM N + P	16.00	1.00							
7	1ml Grow Giant	10.71	2.11	6.37	0.02	0.01	0.02	<0.01	<0.01	<0.01
8	5ml Grow Giant	53.55	10.57	31.84	0.09	0.05	0.09	0.01	0.01	<0.01

Table 3.2 : Nutrients Added in μM

The samples were cultured on the rooftop of a UPV laboratory in Miag-ao, they were exposed to natural sunlight for the full day. Sunlight reaching the samples was reduced by covering the sample containers with black nylon stockings. Temperature variance from in situ conditions was minimised by immersion of the samples in a running water bath, fed by constantly flowing tap water.

3.3.3 Apparatus

The samples were cultured in 2.2 litre polycarbonate sample bottles chosen to support ultra clean techniques. In particular, care was taken to ensure the bottle walls and measuring equipment were free of iron and to avoid introducing any soluble iron to the samples. The bottles and equipment were cleaned and sterilized in accordance with the method adopted by Fitzwater et al, 1982, designed specifically to minimise risk of contamination in phytoplankton culture experiments.

Measurements of chlorophyll-a were taken daily with the ECO-FLRT100 fluorometer described in section 3.1

A plastic fitting was used to accurately position the fluorometer within the sample bottles as the background fluorescence is dependent on the location of the sensor. The sample bottles were placed in the same location each day before taking the reading for the same reason. The setup used to take fluorescence readings can be seen in Figure 3.4.

The sample jars were covered by stockings while in direct sunlight to reduce ultraviolet radiation. The sample jars were tested using a light meter with and without the stockings, the stockings were found to reduce light intensity by around 60%. The experimental setup is shown in Figure 3.5.



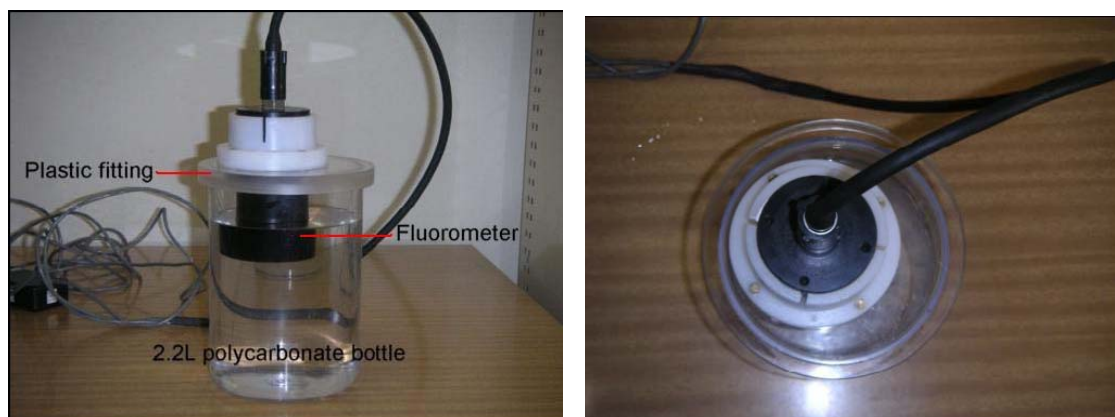


Figure 3.4 : Plan and Elevation of Fluorometer setup



Figure 3.5 : Samples in water bath during experiment

3.3.4 Phytoplankton Species

The culture bottles after 7 days contained mainly diatoms from the families Chaetoceros and Skeletonema costatum. There were very few dinoflagellates which is reassuring with regards to harmful algal blooms, HAB. The concentration of cells in each culture bottle is shown at Appendix 5. It is the biomass of phytoplankton that is of interest and it can be estimated from the chlorophyll concentration but not the cell count, as different phytoplankton families have different mass per cell.

3.3.5 Concentration Results

For each measurement, two sets each of 20 fluorescence readings (raw counts) were taken using the ECOView software that is distributed with the fluorometer. The data was then exported to Microsoft Excel to calculate the average chlorophyll-a concentration.

The chlorophyll-a concentration is proportional to the fluorescence output and can be calculated using the following equation:

$$\text{Chlorophyll-a} = \text{SF} \times (\text{Output} - \text{CWO})$$

Where:

Chlorophyll-a = chlorophyll-a concentration ($\mu\text{g/l}$ or mg/m^3)

Output = Fluorescence output from fluorometer (counts)

CWO = The Clean Water Offset was determined by measuring the average fluorescence output (from 20 measurements) using a sample of deionised water. When the fluorometer is used in a bottle the CWO is higher than for in-situ measurements. The CWO was measured for one culture bottle. Tests performed on the culture bottles in the Ocean Technology Laboratory at the University of Sydney showed that there is a negligible difference in CWO between the 8 culture bottles used in this experiment, see Appendix 3. CWO of 101 counts was used.

Relative Fluorescence = (Output – CWO)

SF = Scale Factor, which is used to derive the chlorophyll-a concentration from the output of the fluorometer. The scale factor is given by the manufacturer to be 0.0078 but the validity of this is not universal and it can be determined independently using the spectrophotometric technique.

In producing the results for this report, the more conservative manufacturers scale factor was used, this allows direct comparison with previous results. The scale factor determined by the manufacturer was determined using a mono-culture of phytoplankton (*Thalassiosira weissflogii*). The population was assumed to be reasonably healthy and the concentration was determined using the absorption method. The most recent calibration of the fluorometer used in this experiment, carried out in the Tasman Sea in May 2005 gave a SF of 0.0094 which indicates that when examining natural phytoplankton assemblages a variability of 20% is possible from the manufacturers published scale factor. For more accurate results of actual chlorophyll concentrations, the fluorometer needs to be calibrated against a sample from the experiment being considered. In this experiment calibration was planned using the spectrophotometric technique but due to the unavailability of suitable equipment at the local location, was not performed.

The raw results and calculated chlorophyll concentrations are presented in Table 3.3

Figure 3.6 is a plot of chlorophyll concentration against time for the culture bottles.



Days	CWO	0	1	2	3	4	5	6	7
Date		20/07/07	21/07/07	22/07/07	23/07/07	24/07/07	25/07/07	26/07/07	27/07/07
Time (PST)		14:55	12:18	13:00	10:30	13:00	14:00	15:20	11:00
Temp °C		31	32	32	33	32	31	31	31
Cloud		0/8	1/8	1/8	1/8	3/8	3/8	5/8	OVCT
Counts									
1 Control	101	169	162	175	193	369	783	635	664
2 Control	101	169	158	160	169	365	552	475	779
3 8uM N	101	169	161	157	175	296	570	538	650
4 16uM N	101	169	145	167	192	363	635	553	712
5 8uM N +P	101	169	121	165	201	649	1846	871	755
6 16uM N +P	101	169	137	155	165	271	800	2043	2568
7 1ml Crop Giant	101	169	132	166	209	932	2520	1303	1068
8 5ml Crop Giant	101	169	134	169	202	1383	1504	1104	1277
Chlorophyll (ug/L)									
1 Control		0.5311	0.4721	0.5762	0.7190	2.0883	5.3165	4.1629	4.3906
2 Control		0.5311	0.4456	0.4635	0.5320	2.0596	3.5209	2.9160	5.2911
3 8uM N		0.5311	0.4674	0.4399	0.5768	1.5226	3.6555	3.4053	4.2814
4 16uM N		0.5311	0.3407	0.5140	0.7080	2.0420	4.1675	3.5268	4.7664
5 8uM N +P		0.5311	0.1576	0.5002	0.7816	4.2713	13.6077	6.0087	5.1000
6 16uM N +P		0.5311	0.2777	0.4198	0.5013	1.3233	5.4528	15.1470	19.2393
7 1ml Crop Giant		0.5311	0.2379	0.5076	0.8453	6.4834	18.8664	9.3760	7.5451
8 5ml Crop Giant		0.5311	0.2578	0.5322	0.7894	9.9980	10.9401	7.8203	9.1689

Table 3.3 : Results of Culture Bottle Experiments

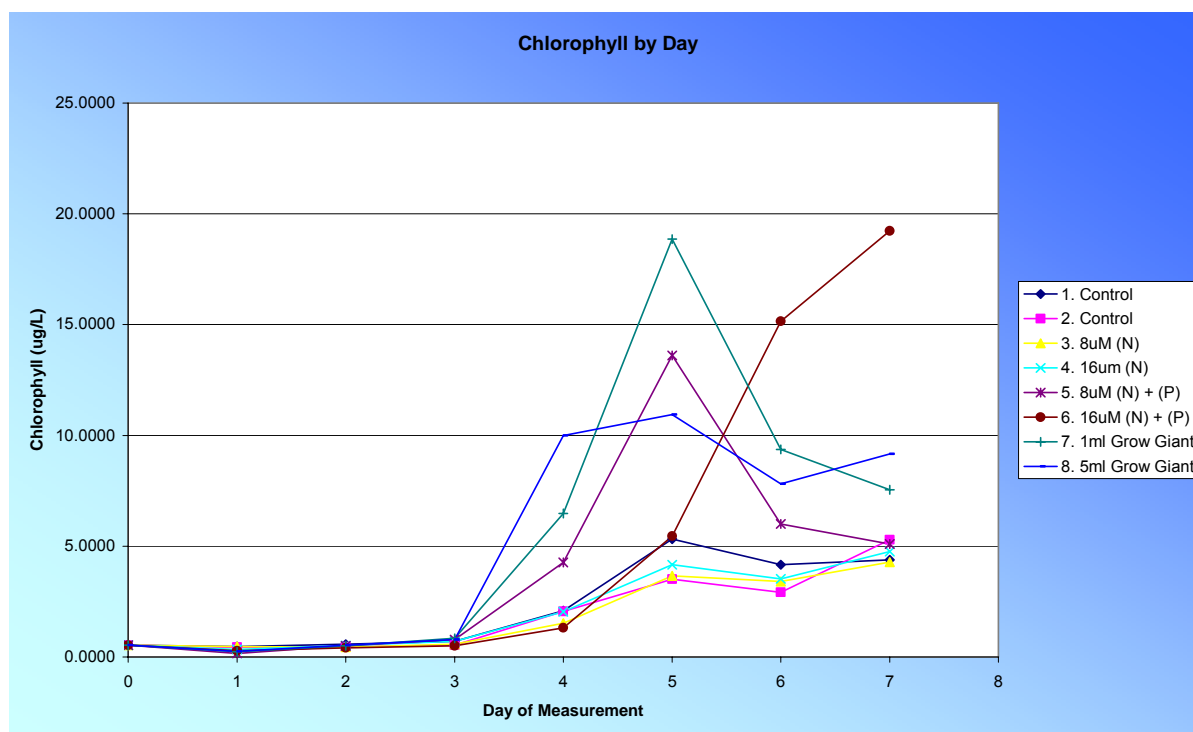


Figure 3.6 : Culture bottle results. Note that initial bottle results do not match insitu observations



3.3.6 DISCUSSION

Some growth was experienced in the two control bottles indicating the conditions in bottles are not fully representative of the in situ conditions. To confirm that it was not natural factors increasing the concentration of phytoplankton in the controls, site 002 was revisited and the chlorophyll again measured in situ. If it were a natural factor that had increased the growth such as an increase in sunlight then the natural in situ assemblages could also have been expected to increase in concentration. It was found that the natural assemblages were the same concentration as at the time of sample collection. Others, for example, DiTullio (1993) have also observed growth of chlorophyll concentration in control bottles possibly due to the wall effect, and altering of the predication regime.

It can be seen that Samples 5 and 6 nourished with nitrogen and phosphate in the Redfield ratio showed significant growth over and above the controls. Sample 5 reached a maximum concentration of 13.6 µg/L chlorophyll and Sample 6 reached 19.2 µg/L. It is not known whether Sample 6 had yet reached its maximum growth as the experiment was completed while this sample was still in a growth phase. Samples 7 & 8 treated with commercial fertiliser also showed significant growth when compared with the controls.

The samples nourished with nitrogen alone showed growth comparable with that of the controls. A conservative view is to only consider the growth in excess of what was experienced by the controls as that resulting from the addition of nutrients. From this approach it could be concluded that phosphorous limitation or co limitation by nitrogen and phosphorous is occurring at the site sampled.

The failure of the samples nourished with Grow Giant to out perform those samples nourished only with nitrogen and phosphate suggests it is the macronutrients that are limiting and not micronutrients such as iron which has previously been shown to limit primary production in some areas of the worlds oceans eg Coale et al (1996).

Improvements that could be made to the experiment are;

1. Keep one control sealed until other samples are at maximum growth before testing.
2. Include sampling with phosphorous as the only added nutrient.



4 SUMMARY

The collaboration with UPV allowed enriched water samples to be cultured and identification of the resulting phytoplankton species distribution. This proof-of-concept experiment in the waters of the Sulu Sea showed that phytoplankton concentration is limited by a combination of nitrogen and phosphorous. The experiment provides a positive indication that phytoplankton growth in the location sampled could be increased by macronutrients and that there were sufficient micronutrients present to significantly increase the standing stock of phytoplankton. In particular the trace metal clean techniques suggest iron is not a limiting nutrient. No harmful phytoplankton were discovered in the enriched cultures.

Acknowledgements

The author would like to acknowledge the contribution of Dr Romeo Fortes of UPV.

The collaboration was very successful and relied on the skill, equipment, and facilities at UPV. The UPV researchers offered valuable help with the experiment and offered their facilities at Miag-ao as a location for the culture of samples for the duration of the experiment.



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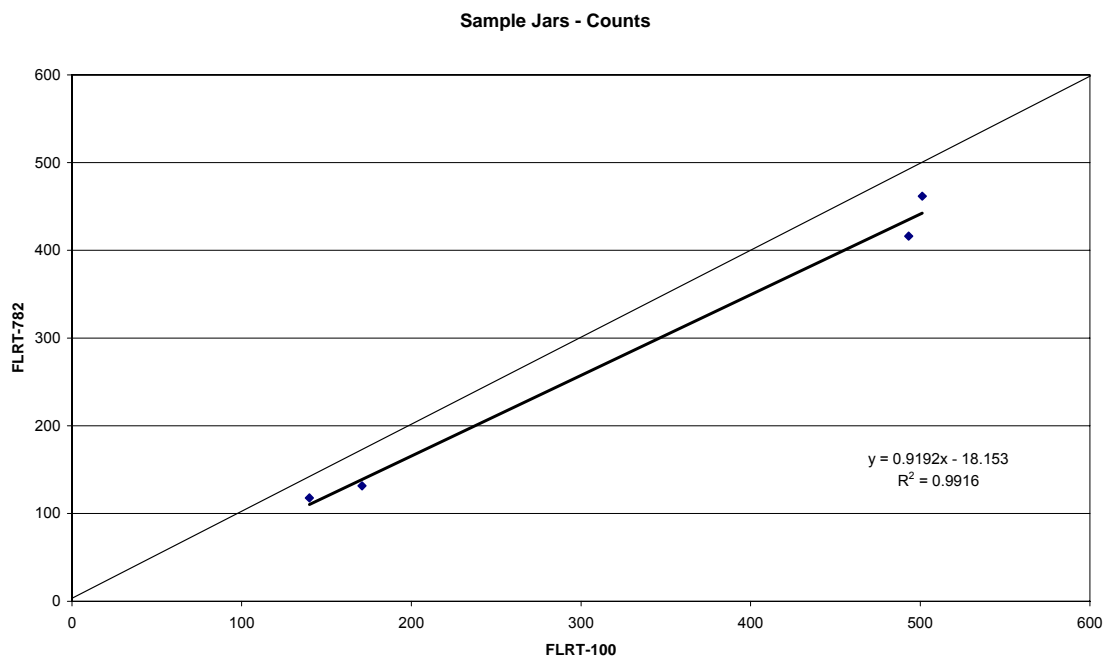
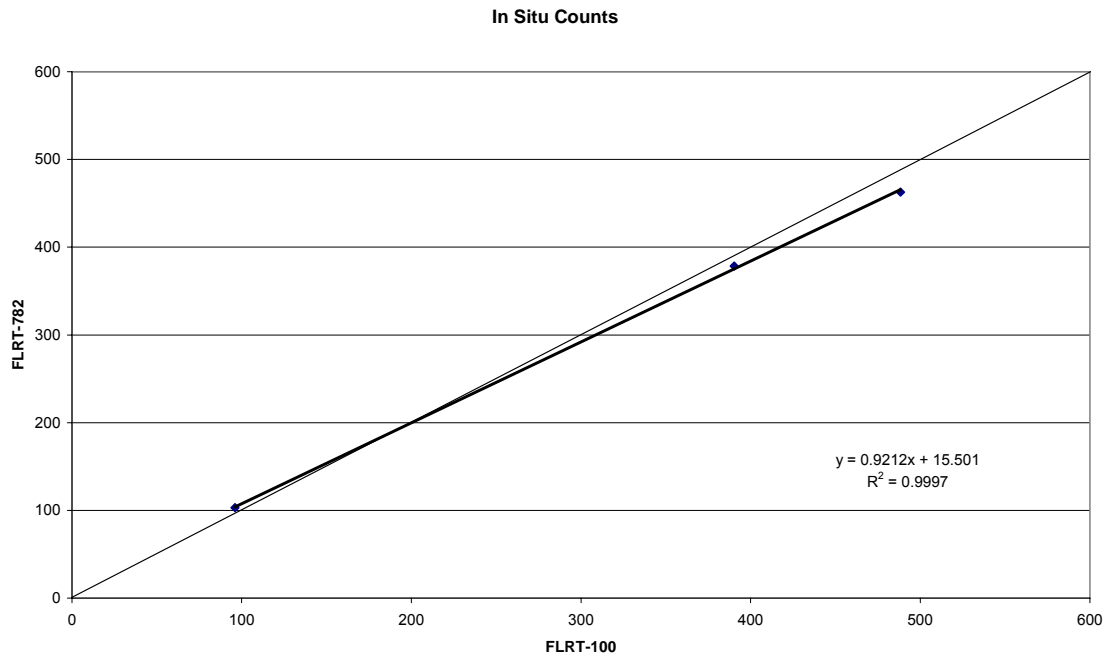
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Appendix 1– Inter-calibration of Fluorometers, Serial Number 100 & 782

Earth Ocean & Space maintains fluorometer # 782 as a reference, the lowest reading is the value in clear chlorinated water.



Appendix 2 – Clean Water Offsets

The clean water offset for the Wet Labs ECO FLRT Fluorometer depends on the objects in the field of view. It has been shown previously to be different in open water than when used in the 2 litre polycarbonate culture bottles. To determine the CWO for open ocean measurements the fluorometer was calibrated using values taken in a fresh water chlorinated swimming pool.

The results for the two fluorometers owned by Earth Oceans and Space are shown below.

	FLRT-100	FLRT-782
	counts	counts
Pool Water 1	95.47	103.14
Pool Water 2	96.74	103.12
Pool Water 3	95.95	
Average	96.05	103.13
Published Offset	110	84
Difference	-13.95	19.13
Black Tape CWO	101.00	93.25

The difference in Clean Water Offset for the two fluorometers from the published specifications was 13 and 19 counts.

The measured difference between the two fluorometers of black tape CWO was 7 counts.

Note there is also a difference between the SF value for insitu situations and culture bottles.

Appendix 3 – Results Calibration of reading in 2 litre Polycarbonate Culture Bottles

Sample	Av Counts	
Bottle	From 20 readings	This test was performed using deionised water in the 8 culture jars used in the experiment PoC 3. The polycarbonate bottles were all of the same type and tested under the same conditions. It can be seen that the individual bottle has a negligible effect on the value of CWO.
1	145.10	
2	144.65	
3	144.85	
4	146.50	
5	145.45	
6	147.50	
7	144.50	
8	145.45	
Average	145.5	
Range	3.00	

Appendix 4 – Calculations and Specifications of Adiz Crop Giant**Aldiz Crop Giant**

Manufacturer Specifications					Solution	Sample 7	Sample 8
Element	ppm	%wt	wt added (ug)	Molec Wt	uM/L	1ml uM	5ml uM
N		15.00%	150000	14.0067	10709.16	10.71	53.55
P (as P₂O₅)		6.55%	65500	30.9738	2114.69	2.11	10.57
K (as K₂O)		24.90%	249000	39.0983	6368.563	6.37	31.84
Fe	1000		1000	55.8470	17.90606	0.02	0.09
Mn	500		500	54.9381	9.101152	0.01	0.05
B	200		200	10.8110	18.49968	0.02	0.09
Zn	150		150	65.3900	2.293929	0.00	0.01
Cu	110		110	63.5460	1.731029	0.00	0.01
Mo	70		70	95.9400	0.729623	0.00	0.00



Appendix 5 – Phytoplankton Cell Counts

