CULTURE BOTTLE EXPERIMENTS ON THE GROWTH OF PHYTOPLANKTON IN WATER SAMPLES TAKEN FROM SULU SEA AND ENRICHED WITH NUTRIENTS

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INTRODUCTION

Increasing the carrying capacity of a body of water by adding the limiting nutrients has been demonstrated in several works, particularly in freshwater environments. This was demonstrated in very early studies (Swingle and Smith, 1938; Ball, 1948; Hall et al., 1970). More recent studies where phosphorus fertilization was carried out in the Kuparuk River, Alaska, during 1985–90 resulted in a 1.4- to 1.9-fold increase in the size of age 0+ fish and a 1.5- to 2.4-fold increase in the weight gain of adult grayling in some years (Deegan and Peterson, 1992). In another study in the same river, it was shown that fifty-six percent of the variance in adult grayling growth rate in said river was associated with nutrient level and mean summer discharge which suggested that river discharge and water temperature may influence long-term survival and population dynamics of grayling in Arctic tundra streams (Deegan et al., 1999). A long-term (16 years) of stream (Kuparuk River) fertilization (phosphorus, H_3PO_4) experiment was performed to evaluate the potential eutrophication of an arctic stream ecosystem. A positive response to fertilization was observed at all trophic levels with increases in epilithic algal stocks, some insect densities, and fish growth rates (Slavik et al., 2004)

In Toolik Lake, Alaska, it was demonstrated that the addition of inorganic nitrogen and phosphorus dramatically increased phytoplankton productivity. The phytoplankton biomass however was not sufficient to draw down all the nitrogen and phosphorus that was added and these nutrients reached high levels in the last half of the summer (O'Brien et al., 1992). In artificial lakes and fishponds, nutrient enrichments has

been undertaken and has been shown to increase biological productivity and fish production. This has been practiced for several years in China, Indonesia, Philippines and other parts of Asia.

In the sea, purposeful enrichment with nutrients is less common. Kyle Scotnish, a loch in Scotland, was fertilised during the Second World War with the aim of increasing the fish biomass. According to Gross (1950) the nourishment brought about an increase of four to five times the weight of first year plaice and an increases also in second year plaice. This experiment was not deemed to be an economic success but the experimenters noted they did not have the data to allow an assessment of the economic factors in optimised enrichment demonstrations. While this experiment can be criticised for the lack of a control and other problems like poor fertiliser distribution, it showed that nourishment led to "fatter fish". The feed stock limitation to fish production had been removed. Heartened by these results Jones and Young (1997) proposed the concept of Ocean Nourishment to inject nutrients into the upper ocean both to store carbon to slow climate change and to increase the base of the marine food web to provide low cost protein for the world's poor. Jones (2001) reported that the more fortunate have an ethical need to

contribute to feeding these additional people. The extra food production will inevitably require further change of the environment but if done efficiently it might increase the standard of living to allow more education.

The above experiences indicate the positive effect of fertilization in general, on biological productivity and fish production in several types of water bodies. Several trials have been done by the Ocean Nourishment Corporation (macronutrients fertilization) and LOHAFEX (e.g. iron fertilization), among others. However, large–scale oceanic iron fertilization appears not a feasible strategy to sequester anthropogenic CO_2 (Zeebe and Archer, 2005; Allsopp et al., 2007). It is along this vein that this experiment was embarked on with the aspiration of enriching the barren areas of the sea thus increasing biological productivity and fish production in the selected area and enhancing the protein source needed by the inhabitants of that locale. With proper protocols this can be repeated in other parts of the world.

With this as the concept, culture bottle experiments were carried out wherein samples of ocean water were collected from an area in the Sulu Sea. As earlier indicated, the proposed sampling site of this investigation (third in a series) was in a location of Sulu Sea as close as possible to where the first and second investigations (Latitude $10^0$19'58.54"N; Longitude $122^0$5'28.83"E) were carried out.

Objectives

The following objectives were pursued:

- To carry out an *in situ* collection of sea water at a location in the Sulu Sea (similar to those done earlier) then compare them with those taken in July and October 2007);
- To investigate the effects of the added nutrients on the growth of phytoplankton taken with the seawater samples;
- To attempt in determining limiting nutrients to the standing stock of phytoplankton at the location of sampling and to possibly ascertain if there are sufficient micro nutrients to support additional new primary production.

It has been reported several times that in many areas in Sulu and China Seas, fishing effort has been increasing but the catch per unit effort has been decreasing. The Ocean Nourishment project, if successful, could increase fish population in these areas similar to the experiences, cited earlier, in lakes and rivers.

MATERIALS AND METHODS

Sampling site. The site where samples were collected was in the village or barangay Lawigan, San Joaquin, Iloilo, Philippines. The coordinates of the sampling site was Latitude 10^o36'53.98"N; Longitude 122^o18'12.29E (See **Figure 1**) which is a little different from the site where Harrison (2007) collected their samples (Latitude $10^0$19'58.54 N"; Longitude 122^0 15'28.83"E).

Collection of samples. Ocean water **s**amples were collected on site by means of buckets at around 12:00 noon of December 9, 2008. From these buckets, two 20-liter transparent plastic bottles were filled up. These were then transported by boat to Barangay Baybay

Sur, Miagao, Iloilo which took about 3 hours then transferred to a waiting van by which were brought to the experimental site at the Brackishwater Aquaculture Center, Institute of Aquaculture, College of Fisheries and Ocean Sciences of the University of the Philippines Visayas located at Barangay Gui-gui, Leganes, Iloilo, Philippines. It took around 90 minutes by car to reach the experimental site.

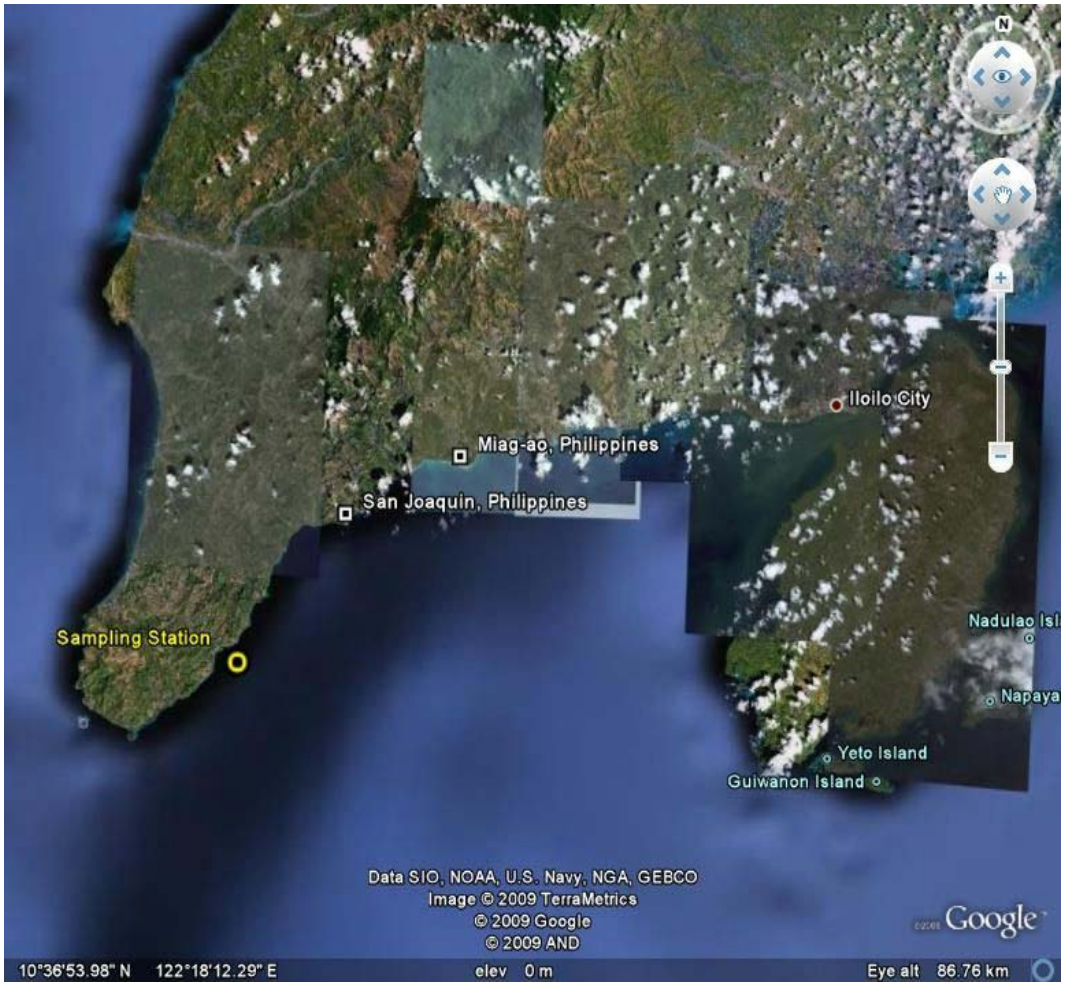

Figure 1. A- Map showing the location of the sampling station in Barangay Lawigan, San Joaquin, Iloilo, Philippines.

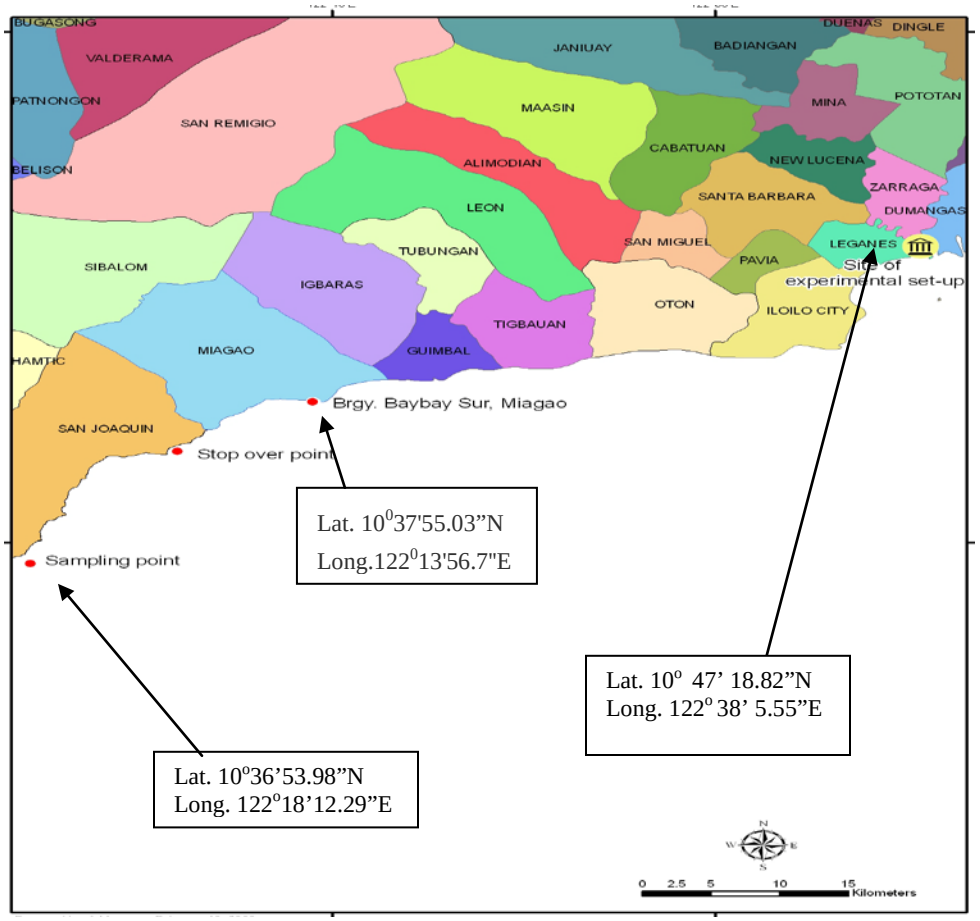

Figure 2. Map showing the location of the sampling point (Lawigan) relative to the town of Miagao (near the UPV Main Campus) and the Experimental Site at the Brackishwater Aquaculture Station, Institute of Aquaculture, College of Fisheries and Ocean Sciences, U.P. Visayas, Leganes, Iloilo, Philippines.

Culture bottles. Ocean water from the two 20-liter container were mixed and 27 units of 6-liter transparent plastic bottles were filled. These 6-liter plastic bottles served as the culture bottles.

Treatments. There were 7 treatments including 2 controls with 3 replicates each, these are as follows:

TREATMENT	REPLICATES	INPUT	CONCENTRATION
I. CO (Cap open)	**3**	None	---
II. CC (Cap closed)	**3**	None	---
III. N (Nitrogen)	**3**	Urea	16µM
IV. P (Phosphorus)	**3**	Disodium hydrogen phosphate dodecahydrate	16µM
V. NP (N + P)	**3**	Ammonium phosphate	16µM
VI. Fe - 5	**3**	$FeCl_3.6H_2O$	5mg/L
VII. Fe - 10	**3**	$FeCl_3.6H_2O$	10mg/L

Experimental design. The experiment utilized a Completely Randomized Design (CRD). The different replicates of each treatment were randomly distributed in the different compartments of the concrete tank as shown in **Figure 3**.

Figure 3. The concrete experimental tank showing the arrangement of the 8 compartments where 2 to 3 replicates of the several treatments were randomly distributed.

Figure 4. Close-up of the concrete experimental tank showing the culture bottles at the start of the experiment when they were still filled with ocean water (A) and towards the end of the experiment when the volume of ocean water had been significantly reduced through sampling (B).

Fertilizers and Phytoplankton Growth. Samples of the different treatments from the incubated bottles in the concrete tanks were collected by siphoning out the water from the bottle with a hand pump (**Figure 5**). The samples from the various replicates were thoroughly mixed and 10 ml was taken for phytoplankton analysis. Two drops of 10 %formalin solution was added to each sample. A sample was placed on the Sedgewick Rafter counting chamber and cell and viewed under a compound microscope. The

Figure 5. Close up of the hand pump (left) and the other hand pumps used to draw sample from the different replicates of each treatment.

phytoplankton organisms present were identified and counted. Data were analyzed using the univariate analysis of variance.

Chlorophyll *a* and fertilizers. The concentration of chlorophyll *a* from the water samples were made following the methods described by Boyd (1979). The water samples were filtered through a 0.45µ membrane filter (Millipore Corp., Type HA, 47 mm Ø) in a millipore filter apparatus (Figure 6A). The filter and the plankton were macerated in 90% acetone in a homogenizer (Figure 6B) and the resulting extracts were centrifuged at 2500

Figure 6. The millipore filter apparatus (A), the centrifuge (B) and the homogenizer (C)

RPM for 10 minutes. The supernatant liquid was then transferred into the cuvettes and centrifuged for 5 minutes at 500 RPM. Light absorption was measured with Spectronic 20 spectrophotometer at 665 mµ wavelength. The chlorophyll *a* concentration was calculated from the equation given by Vollenweider (1969) as described by Boyd (1979).

Fertilizers and growth of zooplankton. The same water samples taken for phytoplankton analysis were used to determine the effects of the various fertilizers on the growth of zooplankton. Ten (10) ml seawater sample was collected from each replicate by means of a hand pump then transferred slowly from the incubated bottle to the plastic sample bottles. Two drops of 10% formalin were added into the beaker to fix the zooplankton then transferred into the sample bottles. Zooplankton count was done using Sedgwick Rafter Counting chamber and cell then recorded.

RESULTS AND CONCLUSION

To be added

ACKNOWLEDGEMENT

We wish to give due recognition to the various colleagues, friends and institutions/organizations who unselfishly extended their assistance and support that made our task a little lighter and enjoyable.

- Ocean Nourishment Foundation – provided the fund for this task;
- Brackishwater Aquaculture Station at Leganes, Iloilo, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippnes-Visayas;
- Professor Ian S. Jones, Ocean Technology Group, University of Sydney;
- Professor Valeriano L. Corre, Jr., Head, Brackishwater Aquaculture Station, for allowing the use of some staff and facilities of the station;
- Mrs. Perfecta C. Carpio, Ms. Cecilia Jaspe, Alex (laborere);
- Our students/colleagues: Cheche B. Salcepuedes, Maria Ashlee Cerdan Garcia, Dana Ermita and Renee May E. Galeos

REFERENCES

Allsopp, M., P. Johnston and D. Santillo. 2007. A scientific critique of oceanic iron fertilization as a climate change mitigation strategy. GRL-TN-07-2007. Greenpeace Research Laboratories Technical Note 07/2007 September 2007

Ball, R.C. 1948. Relationship between available fish food, feeding habits of fish and total fish production in a Michigan lake. Tech. Bull. No. 206. Micigan State College of Agr. Expt. Sta.

Boyd, P.W. and 30+ co-authors.. 2004. The decline and fate of an iron-induced subarctic phytoplankton bloom. Nature 428: 549-553.

Boyd, C.E. 1979. Water quality in warmwater fish ponds. Auburn University Agricultural

Expt. Station, Auburn, Alabama, U.S.A.

Coale, K. H., K. S. Johnson, S. E. Fitzwater, S. P. G. Blain, T. P. Stanton, T. L. Coley. 1998. IronEx- I, an in situe iron-enrichment experiment: Experimental design, implementation and results. Deep-Sea Research Part II, 45, 919-945.

Davey, M.,et al., 2008. Nutrient limitation of picophytoplankton photosynthesis and growth in the tropical North Atlantic. Limnology and Oceanography, 53(5):1722–1733

Deegan, L. A., H. E. Golden, C. J. Harvey and B. J. Peterson, 1999. Influence of Environmental Variability on the Growth of Age-0 and Adult Arctic Grayling. Trans. Amer. Fisheries Society, 128 (6). Nov. 1999.) pp. 1163–1175

Deegan, L. A. and B. J. Peterson, 1992. Whole-River Fertilization Stimulates Fish Production in an Arctic Tundra River. Can. J. Fish. Aquat. Sci. 49(9): 1890–1901. Canada.

Eppley, R. W., E. H. Renger, and W. G. Harrison,1979. Nitrate and phytoplankton production in southern California coastal waters. Limnology and Oceanography, 24(3)

Gross, F. ,1950. A fish cultivation experiment in an arm of a sea-loch I Introduction. *Proc. Roy. Soc. of Edinburgh*, B, **64**, 1-4.

Green, H. W., Young, T. E., Walker, D. & Scholz, C. H. Anticrack-associated faulting at very high pressure in natural olivine. Nature 348, 720–722 (1990).

Hall, D.J., W.E. Cooper and E.E. Werner. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol Oceanogr., 15 (pp. 839-928.

Harrison, D P (2007) "Ocean Nourishment™ in the Philippines - Proof of Concept Report", Earth Ocean & Space Pty Ltd, EOS-REP-07-008 Rev E. www.earthoceanspace.com

Jones, I.S.F. 2001. The global impact of ocean nourishment. Lamont Doherty Earth Observatory Columbia University, New York, U.S.A.

Jones, I.S.F. and H.E. Young, (1997) Engineering a large sustainable world fishery. *Environmental Conservation*, **24**, 99-104

Lindley, S.T., Barber, R.T., 1998. Phytoplankton response to natural and experimental iron addition. Deep-Sea Res. II 45, 1135– 1150.

Metzger E J and Hurlburt H E 1996 Coupled dynamics of the South China Sea, the Sulu Sea and Pacific Ocean; J. Geophysics Res. 101 12,331(12,352)

Mitra, A. and K. J. Flynn, 2006. Promotion of harmful algal blooms by zooplankton activity. Biology letters 2, 194-197.

O'Brien W. J., A. E. Hershey, J. E. Hobbie, M. A. Hullar, G. W. Kipphut, M. C. Miller, B. Moller and J. R. Vestal, 1992. Control mechanisms of arctic lake ecosystems: a limnocorral experiment. Hydrobiologia. 240 (1-3) September, 1992 pp.143-188

Realy, D.S., et al., 1999. Temperature Dependence of Inorganic Nitrogen Uptake: Reduced Affinity for Nitrate at Suboptimal Temperatures in Both Algae and Bacteria. Applied Environmental Microbiology, Vol 65(6): 2577–2584

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. Ecology: Vol. 85, No. 4, pp. 939-954. doi: 10.1890/02-4039. Regular Article

Swingle, H. and E.V. Smith 1938. Fertilizers for increasing the natural food for fish in ponds. Trans. Amer. Fish. Soc., 68: pp. 126-135.

Thessens, A. E., et al, 2005. Effects of Salinity on Pseudo-Nitzschia species (Bacilliariopphyceae) Growth and Distribution. Journal on Phycology, Vol 41, 21–29

Vollenweider , R.A. 1969. Chapter 1, pp. 1-3. In R.A. Vollenweider (ed.), A Manual on Methods for Measuring Primary Production in Aquatic Environments. IBP Handbook N. 12, Blackwell Scientific Publ., Oxford and Edinburgh.

Zeebe, R.E. and D. Archer, 2005. Feasibility of ocean fertilization and its impact on future atmospheric CO_2 levels. GEOPHYSICAL RESEARCH LETTERS, VOL. 32, L09703 School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, Hawaii and Geophysical Sciences, University of Chicago, Illinois, USA, doi:10.1029/2005GL022449, 2005