Nutrients enrichment experiment on seawater samples at Pulau Perhentian, Terengganu

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Abstract

The northeast monsoon wind over about maximum of 30 knots to the east coast of Peninsular Malaysia, literally drive the vertical and upwelling activities which lift nutrient-rich water to the ocean surface water. The availability of these nutrients was essential for the phytoplankton growth and its compositions play an important role in ocean primary production, nutrient cycle and food web. Hence, the mean elemental ratio of marine organic particle 106C/16N/1P must be highly conserved, to ensure the phytoplankton productivity, associated with the availability of iron that critically depends on the speciation. To clarify the nutritional status at Pulau Perhentian, Terengganu during the post northeast monsoon, we conducted nutrients enrichment incubation experiment. The growth of phytoplankton in different combination of nutrients was observed during incubation period. Our initial data could suggest a limited condition of phytoplankton growth during pre-northeast monsoon event at study area.

Keywords: North-East Monsoon; nutrient; incubation experiment; phytoplankton growth; productivity

1. Introduction

The northeast monsoon season usually establishes in early November until the ends of March. Regular easterly or north-easterly winds of 10 to 20 knots prevail during these periods. The winds over the east coast states of Peninsular Malaysia may reach 30 knots or more during periods of strong surges of cold air from the north (cold surges). The winds are generally light and variable during the two inter-monsoon seasons.

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Based on the previous synergy of three recent satellite data sets (SeaWiFS, TRMM and QuikScat) [1], showed a 30-fold increase in surface chlorophyll-a concentrations (3.2 ± 4.4 mg/m$^3$) due to strong nutrient pumping by vertical mixing and upwelling. The monsoonal seasons brings up winds that will drive the vertical and upwelling activities at the ocean. The relative motion between air and the seawater surface currents induces a positive (negative) wind stress curl, which upwelling and the vertical mixing. The pumping lifts the nutrients-rich water into the euphotic zone (seawater surface) thereby enhance the primary production. Phytoplankton was known to have an essential role in primary production, nutrient cycling, and food webs related to primary production in aquatic systems. Widely applied as an indicator and guideline of ecosystem productivity and trophic status, phytoplankton mostly gave impact on the ecosystem body. However, the primary production was usually limited by mineral nutrients (e.g. N, P or Fe) [2]. Sufficient intensities of light, nutrient availability and composition among other factors, indirectly related to the phytoplankton bloom and stock standing [3].

The blooms enable one to identify useful microalgae biomass potential as feedstock for biofuels and co-products that may have applications as food and health supplements [4]. Beside, through photosynthesis, phytoplankton consumes carbon dioxide CO$_2$ on a scale equivalent to forests and other land plants. Some of this carbon is carried to the deep ocean when phytoplankton die, and some is transferred to different layers of the ocean as phytoplankton are eaten by other creatures, which themselves reproduce, generate waste, and die (biological carbon pump) [5]. Even small changes in the growth of phytoplankton may affect atmospheric carbon dioxide concentrations, which would feedback to surface temperatures especially at Pulau Perhentian, Malaysia. Also, as they convert inorganic nutrients and sunlight into vegetative matter, most marine food chains depend on their presence as a primary food source [4]. Phytoplankton is important for the functioning of marine system. They affect on how the ocean absorbs atmospheric carbon dioxide and also the biological cycling of organic compound though the food-web [5]. Recent studies have shown that the accessible source of nutrient (especially nitrogen) affect the size and growth of phytoplankton [6]. It has been proposed that netplankton are mostly nitrate user [6], while nanoplancktons are ammonium user [7]. From the previous studies, which relating to the size and composition of phytoplankton to nutrient, we pursue to study this relationship using incubation method [8].

This study aims to evaluate the influence of different types of nutrient (Fe and nitrogen) on the growth of phytoplankton’s community during the pre-monsoon season at Pulau Perhentian, Terengganu.

2. Methodology

The seawater sampling was done on 22 to 24 October 2014 at Pulau Perhentian, Terengganu (Fig. 1). That was prior to the pre-monsoon before the monsoon seasonal from November until the end of March. Surface seawater samples (>3m depth) were collected from at a few selected stations at Pulau Perhentian, Terengganu by using Van dom water sampler. 10L seawater samples were collected for each station for nutrients enrichment experiment. The in-situ parameters for each station were measured by using water quality parameters probes, SCT YSI Model 30, YSI Model 54 conductivity meter, Thermo Orion AQ 4500 turbidity meter, Thermo Orion 230A Plus pH meter as shown at Table 1.

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| Station 1 | Station 2 | Station 3 | Station 4 | Station 5 |
|-----------|-----------|-----------|-----------|-----------|
| Depth (m) | 38.40     | 37.50     | 36.74     | 34.23     | 28.76     |
| pH        | 6.19      | 7.86      | 7.68      | 7.91      | 7.82      |
| Temperature (°C) | 29.90 | 29.60 | 29.40 | 29.30 | 29.10 |
| Conductivity (mS/cm) | 52.30 | 52.50 | 52.50 | 52.80 | 52.70 |
| Salinity (ppt) | 31.50 | 31.50 | 31.50 | 31.70 | 31.70 |
| Dissolved Oxygen (mg/L) | 7.53 | 8.68 | 6.89 | 6.43 | 5.90 |
| Turbidity (NTU) | 0.10 | 0.05 | 0.04 | 0.00 | 0.06 |
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The homogenized seawater sample from 10L carboy was distributed into acid-cleaned 1L polycarbonate bottle. Each seawater sample from each station will be replicated into 7 bottles (1 control and 6 for nutrient-enrichment experiment). The seawater sample (1L) was enriched with NO$_3^-$, NH$_4^+$, Fe, C and PO$_4$, addition of concentration of 200mM, 9mM, 90mM, 100mM and 20mM, respectively (Table 2). The bottles for incubation were placed into the incubator machine with temperature set up to ±1 °C of the sampling site temperature to mimic the natural condition in the seawater at Pulau Perhentian. The sample was incubated for 6 days. The
The growth of phytoplankton on each incubation sample was monitored everyday by spectrophotometer at 500-550 µm wavelengths.

Table 2 The nutrient-enrichment experiment was added by nutrients and Fe at different concentration

| Nutrients                  | Replicates |
|----------------------------|------------|
| Carbon, C (100mM)          | A B C D E F G |
| Nitrate, NO₃ (200mM)       |            |
| Ammonia, NH₄ (200mM)       |            |
| Phosphorus, PO₄ (20mM)     |            |
| Iron, Fe (9mM)             |            |
| Iron, Fe (90mM)            |            |

Fig. 1. Sampling location at the Pulau Perhentian, Terengganu. Nutrient enrichment experimental was done on surface seawater samples collected from point X₁ until X₅ as plotted

3. Results and discussion

3.1 Growth pattern

The absorption (A) graph indicated the growth of phytoplankton during the incubation experiment in each nutrient enrichment bottles (Fig. 2). On Day 1, there were no growths of the phytoplankton abundance in seawater enrichment for Station 1 to Station 5 (Fig. 2). The lag phase in the beginning of the growth, due to the adaption of phytoplankton to new source of nutrient, light exposure intensity and temperature changed shock. Previous study reported that those factors were taken into consideration as the chlorophyll a level in phytoplankton is adequately delicate and sensitive to any environmental changes. The growth of phytoplankton started to increase on Day 2 in all incubation bottles (Fig. 2). It started to drastically increase as the phytoplankton community had successfully adapted to the new changes (during experiment) and begin to growth and expend their community and size [9]. The initial phytoplankton was believed to compose of diatoms and picocyanobacteria (Mohamed et al, unpublished data, 2014). However, the diatoms composition size increased more rapidly than picocyanobacteria and dominated in all incubation bottles [9]. Our suggestion was supported by previous study [6], which present the diatoms domination over other phytoplankton species when in the high concentration volume of nutrient.

The highest growth of phytoplankton was observed at Day 3 at all stations in all incubation bottles (Fig. 2).
Station 1 shows a maximum growth of phytoplankton with 0.082A reading (nutrient enrichment G). In addition, Station 2, 3, and 4 has a maximum growth of phytoplankton in nutrient enrichment D with 0.111A, 0.047A and 0.121A respectively. This might indicated that, the phytoplankton possess to grow after the addition of nutrient (Fe, N, P, C), express that the seawater sample in study area was might be insufficient of nutrients for its growth. It could indicate that the seawater at Pulau Perhentian was insufficient amount of nutrient during pre-monsoon event that limited the growth of phytoplankton.

However, the growth of phytoplankton was decreased after Day 3 until the end of the incubation period (Fig. 2). This suggested that the maximum growth of the phytoplankton was induced by the maximum used of nutrients provided and the colony growth aggregate to decrease as some of the phytoplankton could be die as inadequate of nutrient. Studies by [1] results on the decline of Chl a, Thalassiosirana sp. correlating with depletes of nutrient. The nutrients added were also suggested to be totally used and depleted. The addition nutrients were believed to be exhausted and reduced level of phytoplankton growth. It is believe that after Day 3, the nutrients added were depleted as grazing by phytoplankton was effective in our incubation experiment. The addition of nutrient enhanced phytoplankton growth in term of cell abundances and composition [10], but as the day passed, the nutrients added seem to be insufficient for phytoplankton. Many studies on the effect of nutrient on the phytoplankton size and composition, shows decreasing in the Chl a abundances as the nutrient added depletes [11].

3.2 Effect on different N source (nutrient enrichment B and E)

An addition of 200mM for both NO$_3^-$ and NH$_4^+$ along with other nutrients (Glucose, Phosphate, Fe) into the
samples was commensurable to derive a significant increase in the phytoplankton growth by different source of nitrogen. During the incubation period, a higher growth of phytoplankton was observed in the addition of NH$_4^+$ compared to NO$_3^-$ (Fig. 3) at all stations. The higher uptake of NH$_4^+$ as the nitrogen source for the phytoplankton growth correlated to NO$_3^-$ although its concentration are was low (>4 µmol L$^{-1}$) [12]. Detailed field experiments results shows that the low NO$_3^-$ utilization was cause by the NH$_4^+$ inhibition process most of the year. Phytoplankton was thought to be prefer NH$_4^+$ as a N source since the energetic costs of assimilating that species of nitrogen are less than that for NO$_3^-$ [12]. That was proven by [13] on the ability of phytoplankton to separate out the use of NO$_3^-$ and NH$_4^+$ using stable isotope $^{15}$N. Parts from that, growth of phytoplankton during Day 1 until Day 3, were constantly increased (Fig. 3). However, it begins to decline as results of the total deplete of NH$_4^+$ corresponding to the absolute use by phytoplankton.

3.3 Effect on different Fe concentration (nutrient enrichment F and G)

Fig. 4. Comparing phytoplankton growth in the presence of different concentration of Fe represented by F (9mM) and G (90mM) during 5-days of incubation experiment at all stations. All five stations except station 2, show 90mM Fe addition gives higher phytoplankton growth compared to 9mM Fe addition

The growth of phytoplankton at Station 1, 3, 4 and 5 show an increasing with addition of 90mM Fe (nutrient enrichment G) (Fig. 4) which likely enhance the phytoplankton growth. In the incubation experiment from Station 1 sample, the 90mM Fe addition (nutrient enrichment G) gave about 2-fold higher phytoplankton growth (0.082A) compared to 9mM Fe addition (nutrient enrichment F) (0.040A) at Day 3 (Fig. 4). These results suggested that the phytoplankton critically needs more of Fe for its growth. As higher amount of Fe was added (nutrient enrichment G), its boost the phytoplankton growth and activated the drawdown of macronutrient [14]. The less amount of Fe concentration attends to have slow phytoplankton growing rates [15]. After Day 3, all incubation samples shows decreasing in phytoplankton growth (Fig. 4). It might be resulting of the insufficient Fe, in consequences of complete used of Fe addition. Station 1, 2, 3, and 4 in Figure 1 appeared to have higher growth of phytoplankton in the addition of more Fe concentration (90mM) in comparison with 9mM Fe. Fe demand is varies between taxa and their growth condition [16].

Marine phytoplankton utilized Fe bound in vary stable chelates by reductive process and some species via specialized transport mechanisms [17]. It can be consider that Fe uptake system was divide into 3 categories, (1) Fe uptake, (2) Fe(II) uptake, and (3) FeL uptake [18]. Discharge of macronutrient input to the ocean demand on a newly accessible source of Fe. Consequently, such production of Fe cannot support the initial collection of biomass, although recycling of Fe at the ocean surface may be necessary for prolonging bloom period [19]. Fe limitation, thus, occurs in both ocean and coastal regions when massive available macronutrients are not correlated by sufficient Fe to fulfill the demands for optimal phytoplankton growth [20]. The phytoplankton growths are significantly effect by the present of nutrients and Fe. The insufficient amount of nutrient of Fe might resulting to the declination of phytoplankton colony, altogether will impact the primary production and global carbon cycle. These study, suggested that the phytoplankton composition were basically increase with the addition of nutrient and Fe.

4. Conclusion
This study could suggest that the seawater sample at Pulau Perhentian was lacking of nutrients and Fe during pre-monsoon event as the phytoplankton growth was increased when the nutrient and Fe added. However, the composition and type of phytoplankton species is needed to be analysed for further understanding on their dynamics of nutrient uptakes, along with Fe bioavailability. Series of studies will be conducted as a comparison between pre-monsoon and post-monsoon season.

**Acknowledgements**
This study was funded by the Ministry of Higher Education, Vote no. 5524617FRGS-2014. Allowance and scholarship from Universiti Putra Malaysia is gratefully acknowledged. Special thanks to Environment Science Department, Universiti Putra Malaysia, for assistance given during the sampling expeditions and during the analyses.

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