RIOS 2004 The Effects of Containment on Oceanic Bacteria

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I. Abstract

Water from the Hudson River plume, benthic upplume and surface downplume, are kept at two different temperatures (4°C and 20°C) in the dark. Various measurements are thought to show an increase in bacteria that is greater for the 4°C water than the samples kept at 20°C. Another experiment using benthic water at an open ocean site (latitude 39.45 longitude 74.23) was preformed to measure the bacterial numbers over a period of 24 hours. These measurements showed an approximate double in numbers between the times of 3 hours and 18 hours. The increase in the bacteria is thought to be due to a decrease in predation pressure brought on by the colder temperature.

Finally the leftover microbes from the upplume and downplume sites were used in an experiment where half the flasks had dissolved nutrients and the other half did not. The results did not come out very well as there must have been some contamination of some sort. The possible source of this contamination is then discussed.

II. Introduction

In the study of oceanic microbes the storage and culturing are often necessary to study many phenomena associated with them. These treatments present complications for the interpretation and analysis of data.

The most forthcoming problem has to do with the effects of storage on these samples. The question of whether or not the microbes grow, stay the same, or decline has obvious effects on how samples are collected and treated. By various measurements it can be determined if microbes from benthic upplume water and surface downplume water of the Hudson River kept in different storage conditions (4°C and 20°C) vary as a function of time.

Another problem is the consequences of the media nutrient concentrations on growth and size. Kirchman (2000) points out that the growth medium ratio (C:N:P) has consequences on the growth rates of bacteria. He goes on further to relate that bacteria grown on richer media (laboratory cultures) are faster growing and larger that bacteria grown in natural environments (Kirchman 2000). This causes a large surface area to volume ratio difference severely affecting internal solute concentrations that in turn influence the bacteria's metabolism (Kirchman 2000). Another experiment was carried out with the hope of eventually finding out what concentrations would best suit the bacteria by determining the minimum amount of added dissolved nutrients needed through a sequence of experiments hoping to correlate growth with different C:N:P ratios. Unfortunately due to lack of time only the first phase of this project was completed where the two extremes were compared, half the flasks were saturated with dissolved nutrients and the other half had none.

III. Methods

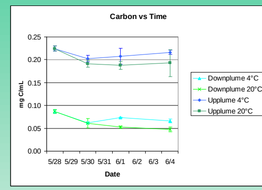
Samples were collected from benthic upplume waters (latitude 40.41.142, longitude 74.01.713) and surface downplume waters (latitude 40.20.54, longitude 73.56.74) of the Hudson River plume with a surface pump. Before half of each sample was stored at 4°C and 20°C in a total of 36 flasks in the dark, 6 time (t)=0 samples were taken as a baseline. Three repetitions were then filtered through Whatman GF/F filters in order to separate the dissolved and particulate matter over time intervals of t=0, t=2, t=4, and t=7 days. These samples were then analyzed for particulate C, N, P, chlorophyll, phaeopigments, and bacterial activities. Samples were also later taken from latitude 39.45 longitude 74.23 with niskins and kept at room temperature. Bacterial counts were preformed on these with time intervals of t=0, t=1.5, t=3, t=18, and t=24 hours.

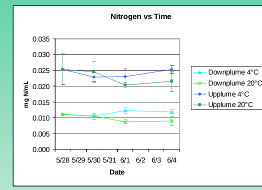
With the microbes leftover from the benthic upplume and surface downplume Hudson plume waters, cultures were made with artificial seawater that included dissolved nutrients. Then 12 flasks of 50mL artificial seawater were made up with half containing no dissolved N or P and the other half containing 15mM NH_4 and 6 mM PO_4. One each of the different flasks containing dissolved nutrients and containing no nutrients were then inoculated with 1mL of a 1:100 solution of Chaetocerous (chae), Isochrysis (iso), and Pavlova (pav) algeas. Measurements were then taken for the initial day and day 4.

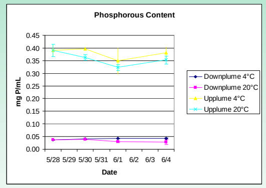
IV. Results

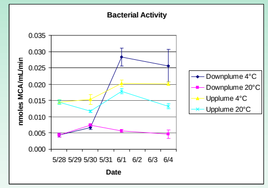
The upper middle graphs represent the data from the experiment run on the Hudson River plume water for particulate C, N, and P content. From this data the C:N:P ratios were calculated. A graph of the bacterial activity is also shown.

Obviously the upplume benthic water contains more of all the nutrients (C,N,P) which isn't surprising considering the amount of anthropogenic input in the Hudson (Hetling 1999). What is more interesting is the apparent increase in particulate nutrients over time for the water kept at 4°C, more so at least than the 20°C water.

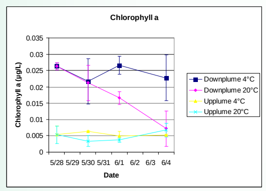


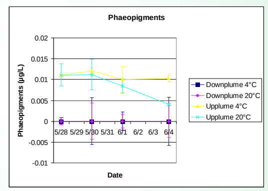


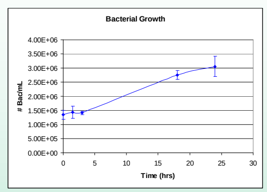




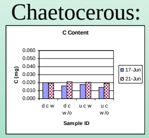
Particulate C:N:P ratio				
Date	Downplume 4°C	Upplume 4°C	Downplume 20°C	Upplume 20°C
5/28	122:13:1	74:7:1	122:13:1	74:7:1
5/30	80:12:1	66:6:1	79:12:1	68:7:1
6/1	89:13:1	77:7:1	92:13:1	75:7:1
6/4	81:13:1	73:7:1	89:14:1	71:7:1

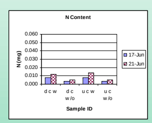


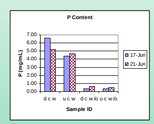




Chaetocerous:







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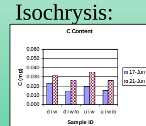
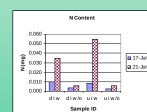
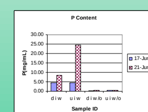
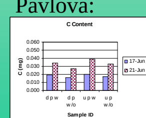
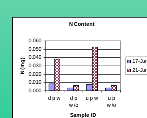


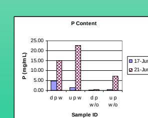




Pavlova:







The bacterial activity measurements also effectively support this explanation. Bacterial activity is measured by how much a chemical called MCA (a food source) is consumed by the bacteria. The bacterial activities for this experiment showed a large increase for downplume 4°C over downplume 20°C. The same development is shown for the upplume bacteria, though not as strongly.

The chlorophyll a and phaeopigments (decomposing algae) data, the middle graphs, also support this trend. The chlorophyll a measurements are used in this case to represent living algae where as phaeopigments represent dead algae. The phaeopigment calculations for the downplume 4°C and 20°C were originally negative numbers that were taken to mean 0 because a flask obviously can not have a negative number of phaeopigments. The strong decreases in the chlorophyll a for downplume 20°C and upplume 20°C could be interpreted as the bacteria eating the algae.

The bacterial growth measurements show that there is an increase in the bacteria over time. The graph shows that the bacteria approximately double in numbers from t=3 to t=18 at room temperature.

The bottom middle graphs show the particulate C, N, and P content of the microbes in the experiment conducted with the dissolved nutrients grouped by which type of algae was used as the C source. The samples are identified as follows: The first letter indicates d for downplume water sample microbes or u for upplume water sample microbes. The second letter indicates which type of algae was the C source: c chae, i for iso, or p for pav. The final identifying letter/s are either w for with dissolved nutrients or w/o for without dissolved nutrients. Almost all of the measurements show a marked increase in growth of the microbes in the flasks containing the dissolved nutrients.

V. Conclusions

The first experiment, which tried to relate bacterial growth to time, shows at first a decline and then an increase in particulate C, N, and P. This was not surprising and could be because of a number of explanations. The explanation that seems to be the best is that there was a loss of predation. Another possible reason is that algae grew in the flasks as well. However, these flasks were kept in the dark while incubating so that does not seem to be a reasonable interpretation.

As for the difference in growth relating to the temperature difference, it appears that, although contrary to intuition, these bacteria grow better in a colder environment. If the explanation that the bacteria grew because of a loss of predation is accepted as true, one could then also conclude that perhaps it is not that the bacteria acclimate better to the 4°C environment but that instead the predators did not acclimate to the 4°C environment and this allowed more bacteria to survive.

The results for the portion of the experiment considering the dissolved nutrients were perplexing. It seems most likely that even though the algaes used as a C source were supposed to be non-viable algae. The increase in C content in the flasks with no dissolved C content something autotrophic must have been present. There were only 3 possible sources of algae though. One from the manufacture's algae sold as non-viable. Another possible source was from the filtered seawater used to dilute the algal paste. The other possible source was the inoculate used from the upplume and downplume water, but this water had been kept in the dark for several weeks.

Another problem with this experiment was the data from the spectroflorometer for the chlorophyll a and phaeopigment measurements. These confusing results could be due to the photomultiplier tube, which was newly installed to increase sensitivity to the lower wavelengths where chlorophyll and phaeopigments are detected. When the results were calculated they seemed to indicate that there was no chlorophyll when the individual samples were considered. Yet, when one looked at the readings as a time series there was a definite increase in the numbers.

Clearly this experiment, even though it had just begun, would need to be repeated this time in the dark. This would hopefully provide more reliable results before attempting to correlate growth to C:N:P ratios.

VI. Works Cited

Kirchman, David L. (2000) Uptake and Regeneration of Inorganic Nutrients by Marine Heterotrophic Bacteria. *Microbial Ecology of the Oceans*. (ed. K.L. Kirchman), 261-288. Wiley-Liss, New York.

Hetling, Leo; Norbert A. Jaworski and David J. Garretson. (1999). Comparison of nutrient input loading and riverine export fluxes in large watersheds. *Water Science and Technology.* 39.12. 189-196.