4-27-1993

# Nutrient enrichment studies of Tampa Bay phytoplankton general information and laboratory procedures

City of Tampa Department of Sanitary Sewers

# NUTRIENT ENRICHMENT STUDIES OF TAMPA BAY PHYTOPLANKTON
# GENERAL INFORMATION AND LABORATORY PROCEDURES

APRIL 27, 1993

THE CITY OF TAMPA
DEPARTMENT OF SANITARY SEWERS
BAY STUDY GROUP

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## INTRODUCTION

The Florida Department of Environmental Regulation (DER) is requiring the City of Tampa to conduct algal assays on waters of Tampa Bay prior to issuing Permit Number D029-184532B for the Hookers Point AWWTP (Specific Condition No. 12). The Hillsborough Environmental Protection Commission concurs with this condition. In response to the requirement by DER, the City of Tampa, Department of Sanitary Sewers, has contracted with Dr. Vargo at USF-MSI to conduct nutrient enrichment experiments using the U.S. EPA approved "Marine Algal Assay Procedure: Bottle Test" (U.S. EPA 1974) with the green alga Dunaliella tertiolecta as the assay organism. Refer to the USF study plan (Vargo 1993) for details of this bioassay test. All field sampling for the USF bioassay will be performed by City of Tampa Bay Study Group (COT-BSG) personnel. Laboratory analyses of ambient nutrient concentrations will be done by the EPC laboratory and possible by the COT chemical laboratory.

In addition to the USF study, the COT-BSG will conduct in-house phytoplankton nutrient bioassay using natural Tampa Bay phytoplankton populations. These experiments will be conducted simultaneously with the USF bioassays. The response in growth rate to changes in nutrient additions will be indicated through changes in algal biomass measured as chlorophyll-a. The COT-BSG method is similar to methods currently used by the states of Maryland and Virginia for determination of nutrient limitation in Chesapeake Bay waters (Fisher et al. 1992a and b). The proposed study will, therefore, not only yield valuable information about nutrient limitation in Tampa Bay, and a comparison to the USF study results, but will also allow for comparison of information between Tampa Bay and Chesapeake Bay.

## METHODS

The following information details field sampling procedures for both the USF and the COT-BSG bioassays, and laboratory procedures for the COT-BSG bioassay.

### Prior to Field Sampling:

1. Coordinate all sampling plans with Dr. Vargo (893-9167 or 893-9130). Dr. Vargo may want to send a student on sampling trips. The student will then be responsible for bringing sampling equipment and transporting samples to USF-MSI.

2. Notify the Tampa Port Authority Security section (248-1924) to let them know that we will be at Berth 205 the day of sampling and the following day.

3. Notify the EPC laboratory about the sampling date and the number of samples to be delivered.

4. Notify the COT laboratory about the sampling date and the number of SiO4-Si samples to be delivered.

Sampling Locations:

The following stations will sampled starting in May, 1993 and continue on a monthly or quarterly basis, as indicated below, through April 1993 (see Figure 1 for locations):
1. Hillsborough Bay (COT4)-quarterly (May, Aug., Nov. and Feb.)
2. Old Tampa Bay (EPC40)-quarterly as above
3. Middle Tampa Bay (COT13)-quarterly as above
4. Lower Tampa Bay (EPC95)-monthly (12 samplings)

Field Sampling of In Situ Measurements:

1. Light attenuation (3 depths), sea-state, wind speed and direction, wave-height and cloud cover.

2. Temperature, salinity, dissolved oxygen, and pH (Hydrolab) readings at each meter interval from surface to near bottom.

3. Secchi disk depth, water color and station depth.

4. COT-BSG ammonia concentrations at surface and near bottom.

5. Chlorophyll-a concentrations and turbidity units at surface and near bottom.

6. Nutrient concentrations. Nitrogen series, PO4-P, TP, and SiO4-Si at surface and near bottom (PO4-P and SiO4-Si require separate bottles). Follow proper QA\QC procedures for sample collection, acidification and identification.

7. Phytoplankton composition at surface.

Field Sampling of Bioassay Water:

The USF bioassay will need <10 liters for each station. Dr. Vargo will supply bottles, screen and coolers. The COT-BSG bioassay will need 30 liters (2x5 gallon jugs not completely

filled) for each station.

1. Take Niskin (5 liter) hydrocasts just below surface and partially fill the all jugs with water from each hydrocast to ensure a good mix of water in each jug. Use a 153um screen, supplied by Dr. Vargo, when filling the jugs to remove large zooplankton from the sample.

2. Place the jugs in coolers (each 5 gallon jug needs a 48 quart Igloo cooler) and add a very small amount of ice. The samples should not be chilled, but maintained at near ambient temperature.

3. If needed, deliver the USF samples to the USF-MSI laboratory (Dr. Vargo's laboratory is upstairs in the north section of Building A) and if possible pick up the eqiupment needed for the next sampling. Proper sample transfer procedures should be followed.

4. Deliver nutrient samples to the EPC laboratory. Proper sample transfer procedures should be followed.

5. Deliver SiO4-Si nutrient samples to the COT laboratory. Proper sample transfer procedures should be followed.

COT-BSG Laboratory Procedures:

1. Prepare bioassay incubation bottles (clean, label, etc.)

2. Organize incubation bottle array as follows:
   a. 2 Controls (sample id. example: C-1-95 and C-2-95)
   b. 2 N-additions (sample id. example: N-1-95 and N-2-95)
   c. 2 P-additions (sample id. example: P-1-95 and P-2-95)
   d. 2 N+P-additions (sample id. example: N+P-1-95 and N+P-2-95)

3. Vigorously shake the collection jugs and rinse each incubation bottle with sample water.

4. Add 1.5 liter of sample water from each of the two jugs into a 4 liter graduated cylinder to get a good mix of sample water from each jug. Add the 3 liter subsamples to the incubation bottles.

5. Add the following nutrient treatments to the incubation bottles:
   a. Control-add nothing
   b. N-add 5 ml of the N stock solution. The final NH3-N concentration in the sample should be close to 50uM.

c. P-add 5 ml of the N stock solution. The final PO4-P concentration in the sample should be close to 10uM.

d. N+P-add 5 ml of each of the stock solutions. The final NH3-N and PO4 concentrations in the sample should be as above.

6. Shake all samples, place them in coolers (no ice) and transport them and the incubator as soon as possible to Berth 205 in East Bay.

7. Launch the incubator and place sample bottles randomly inside. Attach the incubator screen and secure the incubator between the dock and shore on the south end of the pier. Record the starting time of incubation. No shaking is needed during incubation. The gate at Berth 205 must be locked every time we leave the premises.

8. After approximately 24 hours of incubation retrieve the samples, place them in coolers (no ice) and transport them to the COT-BSG laboratory. Don't forget to lock the gate.

9. Filter incubation samples for chlorophyll-a in a random order.

10. Perform the needed laboratory analyses, such as spectrophotometer work, NTU readings etc., and prepare for the next bioassay sampling.

Interpretation of Bioassay Results:

The responses of the bioassay experiments to the different nutrient additions will be categorized into 7 classes. The following definitions of categories are modified from (Fisher et al. [1992a and b]):

Class 1: 'Exclusive' N limitation of phytoplankton growth rates is defined as: (1) the addition of P induced no response relative the control, and (2) the addition of N alone had virtually the same effect as the addition of N+P.

Class 2: 'Exclusive' P limitation of phytoplankton growth rates is defined as: (1) the addition of N induced no response relative the control, and (2) the addition of P alone had virtually the same effect as the addition of N+P.

Class 3: 'Primary' N limitation of phytoplankton growth rates is defined as: (1) the addition of P alone induced little response relative the control, (2) the addition of N alone induced a significant response, and (3) the addition of N+P induced the largest response.

Class 4: 'Primary' P limitation of phytoplankton growth rates is defined as: (1) the addition of N alone induced little response relative the control, (2) the addition of P alone induced a significant response, and (3) the addition of N+P induced the largest response.

Class 5: 'Balanced' NP limitation of phytoplankton growth rates is defined as: (1) the addition of N and P alone induced no response relative the control, (2) the addition of N+P induced a large response.

Class 6: No response to any nutrient addition. Indicating nutrient saturation or light limitation.

Class 7: Inconsistent results

Results from the COT-BSG bioassay experiments will be evaluated on a seasonal basis using appropriate non-parametric or parametric statistics. Two main seasons, the wet season (June through September) and the dry season (remaining months) will be discussed and compared in relation to nutrient limitations measured during these experiments. Only limited conclusions will be drawn from individual monthly results.

Ancillary information collected during this study, as well as information from other sources, such as the EPC and City of Tampa monitoring programs, may be used in addition to the bioassay results to evaluate nutrient limitation of the Tampa Bay phytoplankton population. Further, nutrient limitation measured during these experiments may be discussed in relationship to the current status and trend of Tampa Bay water quality and biological indicators, such as chlorophyll-a, macroalgae and seagrass.

Quality Assurance/Quality Control:

All water collections, field determinations, and laboratory analyses conducted by the City of Tampa Bay Study Group will follow the City of Tampa Department of Sanitary Sewers Quality Assurance Plan.

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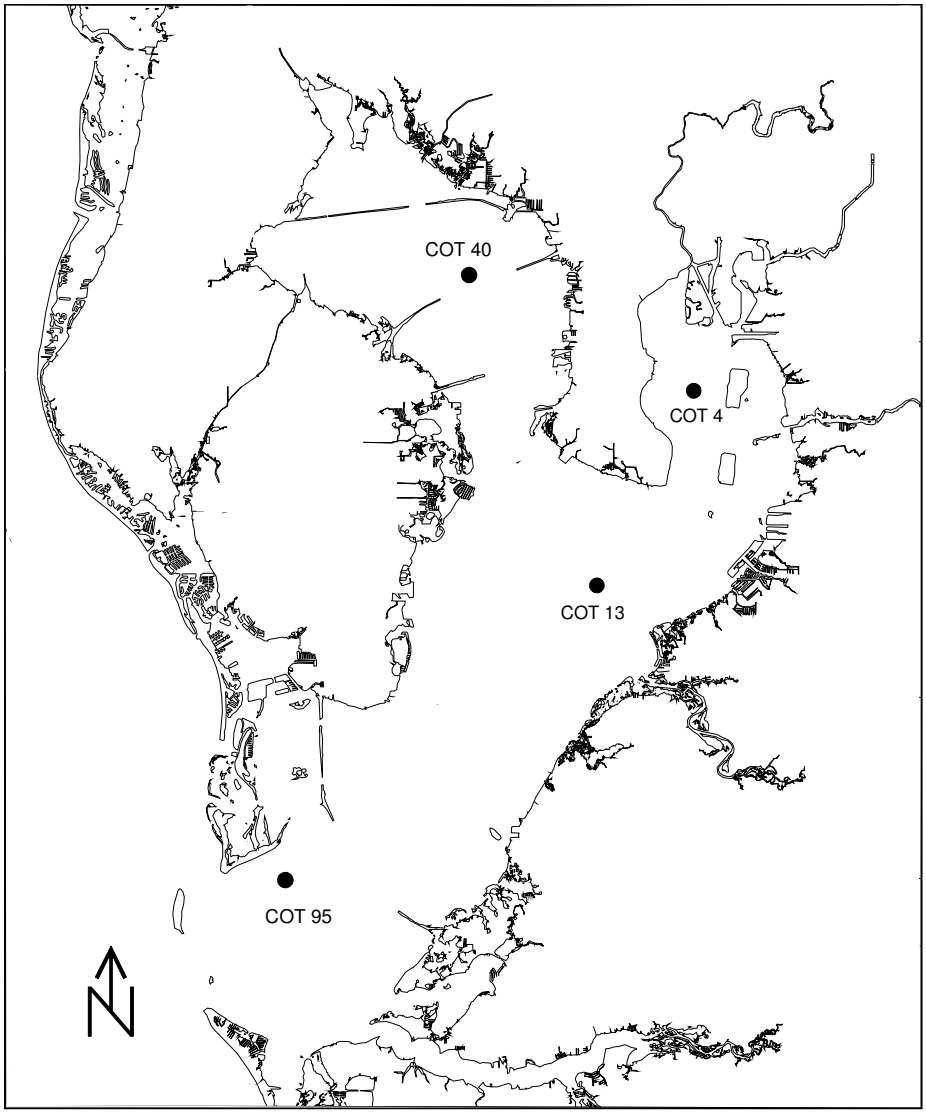


Phytoplankton nutrient bioassay monitoring stations in Tampa Bay sampled by the COT-BSG.

Figure 1. Locations of stations to be used for nutrient enrichment experiments.