**PHYSIOLOGICAL ECOLOGY OF SRS CAROLINA BAY PHYTOPLANKTON
COMMUNITIES: EFFECTS OF NUTRIENT CHANGES AND CO₂ SOURCES**

Renewal Year One Report

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Date Published - November 1992

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**PREPARED FOR THE
U.S. DEPARTMENT OF ENERGY
UNDER CONTRACT DE-FG09-88SR18049**

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GENERAL PROJECT BACKGROUND:

Among the abundant wetland systems within the United States Department of Energy (DOE) Savannah River Site (SRS) are unique aquatic environments known as Carolina bays. Carolina bays represent especially critical habitat areas because they are the only naturally occurring aquatic systems above the floodplain in otherwise dry upland areas. This first renewal year project effort had as its primary objective to determine the effects of nutrient enrichment on phytoplankton communities in SRS Carolina Bays. An additional objective included comparing phytoplankton community changes during the present extended hydroperiod conditions with sampling results from previous drier years. One of our primary study sites is Lost Lake, a Carolina Bay formerly impacted by SRS operations, but now undergoing restoration. These restoration activities have included adding fertilizers to encourage macrophyte growth and our study efforts will help to quantify how potential nutrient increases in bay waters might cause shifts in algal communities.

The high ecological value of wetlands to the nation has been emphasized by the Federal policy of "no net loss of wetlands". With greater than 150 larger Carolina bays within its borders (Shields, *et al.*, 1982) the SRS National Environmental Research Park (NERP) potentially provides protection to one of the greatest concentrations of relatively undisturbed Carolina bays in the nation. SRS bays take on special significance considering the description of Carolina bays within South Carolina as "relatively endangered systems" with only 4,000 bays existing in the State (Bennett and Nelson, 1991).

Progress on the ongoing two renewal years of Carolina Bay phytoplankton research has accomplished most of the field and laboratory nutrient enrichment studies and the population sampling under extended hydroperiod conditions. Additionally, numerous recounts and reanalyses were conducted on phytoplankton and zooplankton samples collected during this project's first three years. These recounts of samples were conducted as a quality control effort to validate outlier values or unusually high sample variances that had been detected during manuscript preparation of the first three project year results. These reanalyses have been completed and final manuscript submissions should be out by the next two months.

SUMMARY STATUS OF RENEWAL YEAR ONE OBJECTIVES:

All experimental work on Year One project objectives is nearly complete. The final microcosm nutrient enrichment experiment is underway and will be completed in two weeks.

Laboratory processing and counting of almost all field samples for the extended hydroperiod is also nearly completed. Computer processing and statistical analyses of these experimental results must still be completed, however. Due to the reanalysis and recreation of all SAS data bases for the three previous project years, statistical and computer work on this present year's data has been delayed.

PROGRESS ON SPECIFIC OBJECTIVES:

Objective: Determine the effects of nutrient enrichment (fertilizing during wetlands restoration) on phytoplankton communities.

A total of four experiments on phytoplankton community responses to nutrient enrichment were conducted. Two field experiments were held using microcosms semi-submerged in both Lost Lake and in Flamingo Bay. The other two experiments were conducted using microcosms under controlled conditions in the laboratory. The field microcosms consisted of 27 four-liter transparent pyrex bottles each filled with 3 liters of ambient water from the bay surface. The different treatment combinations consisted of 3 replicate bottles of nine different combinations of inorganic phosphate and nitrate additions. The different nitrate additions were classified as: high (13.3 ppm), medium (4.4 ppm), and none (0 ppm). The corresponding phosphate additions were: high (6.7 ppm), medium (2.2 ppm), and none (0 ppm). The treatment array was such that each nitrate level occurred with each different level of added phosphate. The three bottles with no nitrate or phosphate addition served as controls. Following nutrient additions, the bottles were capped with a rubber stopper and each replicate bottle was randomly arranged in one of three rows of bottles anchored in the bay at a depth of < 1.0 m.

The Lost Lake experiment was begun on February 11, 1992 and each bottle was sampled for phytoplankton and nutrient concentrations again on February 27, March 3, and March 12, 1992. Phytoplankton samples (500 ml each) were immediately preserved with an acid-Lugol's solution. In the laboratory each sample was successively concentrated by fluid volume reductions following gravity settling of the plankton. The final settled sample was collected on the coverslip bottom of a plankton counting chamber using the Utermohl technique (Utermohl, 1931, in Wood, 1971) and counted on an inverted microscope at 1000x total magnification under oil immersion. Phytoplankton cells in successive fields of view were counted until a maximum number of cells for the most common species was reached or 100 fields of view had been counted, whichever came first. If the average number of cells per field for the most common species were 20 or more, the maximum number of cells counted was 200. If the average number of cells of the most common species were less than 20, then a maximum of 100 cells of that species were counted. The total area of chamber bottom observed in the counts was next determined and the counts per mm^2 will be expanded for the entire chamber bottom area and transformed into numbers per liter.

Microcosm water samples were stored on ice for transportation back to the lab. pH was measured immediately upon returning to the lab while the remainder of the sample volume was then filtered to remove animals, silt, and detritus. Following EPA guidelines, water samples were stored at 5°C and analyzed for nitrate and inorganic phosphate. Water samples from nearby stations in Lost Lake were analyzed for additional chemical parameters. Analytical methods used in these analyses included:

Compound	Method
NH ₃	Nessler Reagent
PO ₄	Amino-naphthol reduction
NO ₂	Diazotization
NO ₃	Cadmium reduction & Diazotization
SiO ₂	Sodium Molybdate -Citric Acid, heteropoly blue
Alkalinity	Standard H ₂ SO ₄ titration

Concentrations of the above reacted compounds were analyzed on a Bausch and Lomb Spectronic 20 spectrophotometer at visible and near infrared wavelengths. Standard curves were developed to convert wavelength absorption into concentrations.

The Flamingo Bay experiment was begun on April 2, 1992 and each bottle was sampled for phytoplankton and nutrient concentrations again on April 7, and April 16, 1992. Phytoplankton and water chemistry samples were processed as described above for Lost Lake. Sampling data are currently being computerized, however in both field experiments concentrations of phytoplankton in the high nitrate and high inorganic phosphate treatments apparently reached bloom proportions with water appearing light green in color. In contrast the control treatments with no added nutrients did not indicate algal blooming with their water remaining relatively clear.

Laboratory microcosm experiments were begun on June 10, 1992. Eighteen aquaria (ca 57 l in volume) were filled with 42 l of tap water and treated with chlorox to kill bacteria, algae, or other organisms. Residual chlorine was removed by treating the water with sodium thiosulfate. Each microcosm was inoculated with 1.0 l of water from Lost Lake. The different treatment combinations consisted of 3 replicate microcosms of six different combinations of inorganic phosphate and nitrate additions. The different nitrate additions were classified as: high (13.3 ppm), medium (4.4 ppm), and none (0 ppm). The corresponding phosphate additions were medium (2.2 ppm) and none (0 ppm). Treatments were randomly assigned to the microcosms which were artificially illuminated using fluorescent lighting on 12 hour photoperiod. Each microcosm was sealed with a clear glass top with two access holes for sampling, closed by rubber stoppers. Phytoplankton and water chemistry samples were collected similarly to the methods described

above for the field microcosms on June 10, June 17, June 24, June 30, and July 8, 1992. This experiment is presently being repeated and was begun October 20, 1992.

Although statistical analyses of these nutrient enrichment data are still ongoing, qualitative observations of Lost Lake phytoplankton did not reveal algal blooming near the study area in the southeastern quadrat of Lost Lake. Supplemental observations of aquatic macrophyte growth indicated good success of cattails (*Typha* sp.) along the shoreline and islands. Submerged aquatic species were also becoming established, however water lily (*Nymphaea odorata*) plantings observed during winter months were not observed on summer and fall sampling trips, presumably due to dessication / heat stress during exposure to drying in shallow water during early summer. The cleared upland areas immediately adjacent to the Lost Lake shoreline had become covered with dense early stage growths of annual weeds typical of early stage old field succession.

Objective: Compare phytoplankton community dynamics during the current extended hydroperiod for Carolina Bays with patterns in previous drier years.

The hydroperiod of Carolina Bays is characterized by submergence from winter to late summer with extensive drying during fall. Since 1991, Flamingo Bay and Ellenton Bay have remained more completely full, even in summer, than in our previous years of sampling. These conditions have provided a unique opportunity to compare bay phytoplankton community changes under continuously wet conditions with sampling during years of more typically dry periods. Samples have been collected as part of the efforts described for the nutrient enrichment experiments above along with some additional sampling trips. In addition to determining phytoplankton changes, the abundance of zooplankton taxonomic groups (calanoid copepods, cyclopoid copepods, and cladocera) is also being analyzed. Following computerization, these phytoplankton changes for the 1991-1992 extended hydroperiod will be compared with our results from 1989 - 1991 when bay hydroperiods included more typically dry periods.

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