# The Effects of the Sumter Wastewater Treatment Plant on the Survival and Reproduction of *Ceriodaphnia dubia* in Chronic Toxicity Tests

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The Pocotaligo River is a freshwater wetland that originates in Sumter, South Carolina. The wetland has endured severe anthropogenic and natural alterations to its ecology over the last few decades. Wetlands filter excess nutrients and are crucial for biodiversity. The Sumter Wastewater Treatment Plant (WTP) discharges its treated waste into the Pocotaligo swamp and is an important source of point pollution. The objective of the present study was to determine the possible effects of the WTP on water quality of the swamp using physiochemical measurements and a biological indicator *Ceriodaphnia dubia* (*C. dubia*), a freshwater zooplankton. Surface water samples were collected every two months from an upstream (control) site, a discharge (effluent) site, and a downstream site for a period of one year. Our results indicate that there were significant differences between the effluent site, and the upstream and downstream sites for seasonally averaged temperature, pH and dissolved oxygen, ($p<0.05$); however, no significant differences ($p<0.05$) were noted between upstream and downstream sites. There was a significant difference ($p<0.05$) between the upstream and downstream sites for total dissolved solids and phosphorous, but no significant differences in concentration of ammonia, nitrite and nitrate. There was no significant difference in mortality, but some reduction in reproductive success among *C. dubia* was noted between the three sites. Presently, the WTP is adequately treating its sewage before discharge. However, a long term study is necessary for more definitive conclusions.

## Introduction

The Pocotaligo River is a freshwater wetland that originates in Sumter, South Carolina, and joins the Black River at the Clarendon-Williamsburg County line. The Pocotaligo Swamp, which encompasses 272,000 acres with over 30,000 acres as wetlands, has endured severe anthropogenic and natural alterations to its ecology over the last eight or nine decades. The Environmental Protection Agency (EPA) defines wetlands as "lands where saturation with water is the dominant factor determining the nature of the soil development and the types of plant and animal communities living in the soil and on its surface." Wetlands include swamps, marshes, bogs, mangroves, coastal beaches and flood plains. Variations in wetlands can be attributed to regional and local differences in soils, topography, climate, hydrology, water chemistry, vegetation, and other factors, including human disturbance (EPA 2009). Wetlands such as the Pocotaligo Swamp are important and often referred to as the “kidneys of the environment,” as they filter harmful pollutants (Mitsch and Gosselink 2000). Besides filtering pollutants, they also serve as an important habitat for birds, fish, mammals and insects. Wetlands also filter excess nutrients from the water. If an abundance of limiting nutrients reach rivers and lakes, they stimulate excessive algal growth, a process called "eutrophication" (Smith et al. 1999). The most frequently limiting nutrient for freshwater lakes and wetlands is usually phosphorus (Schindler 1977; Schlesinger 1997; Bedford et al. 1999).

The Pocotaligo watershed faced a major challenge during the 1950’s and early 1960’s as a result of heavy logging of cypress trees and removal of large sections of hard wood trees and their canopies (U.S. Army Corps of Engineers 1982). In 1989, Hurricane Hugo added to the destruction of the tree canopy. The removal of the canopy permitted growth of weeds and floating plants that further impeded water flow. The introduction of point sources of nutrients such as nitrogen and phosphorus began with urbanization and the construction of Sumter's Pocotaligo Wastewater Treatment Plant (WTP) in the late 1970's, and its expansion in 1987. The treatment plant at the time of our study discharged an average of over 1 billion liters per month (8.8 million gallons per day) into the Pocotaligo swamp. The discharge from the plant accounts for 5-21% of the flow in the swamp (Morris et al. 1994). Treated sewage contains nutrient rich chemicals such as nitrogen and phosphorous which in large quantities threaten the biodiversity of the swamp (Mitsch and Gosselink 2000). Morris et al. (1994) found high concentrations of phosphorus at several sites in the Pocotaligo swamp. Continued growth of the Sumter community has increased the need to raise the discharge from the WTP, and the plant is yet again being expanded, which has the potential to further increase nutrient loading into the Pocotaligo.

Evaluating water quality is critical to understanding the health of a wetland (Yi et al. 2009).

Water quality can be evaluated through physiochemical measurements and use of biological indicators. Physical parameters may include temperature, pH, turbidity, total dissolved solids, total suspended solids, dissolved oxygen, air saturation, and flow rate. Chemical parameters may include ammonia, nitrogen, nitrate, nitrite, iron, chlorine, bisphthalate, and phosphorous. Biological indicators of water quality include fecal coliforms, macroinvertebrates, microinvertebrates, and toxicity tests using biological organisms (Hall 2004). One commonly used biological indicator of water quality is *Ceriodaphnia dubia* (*C. dubia*), a freshwater microinvertebrate. *C. dubia* are freshwater crustaceans most commonly known as “water fleas” that live in the littoral areas of lakes, ponds, marshes, and swamps throughout the world (USEPA 2002). *C. dubia* are highly sensitive organisms that respond to many of the parameters that define water quality such as pH, chemical levels, temperature, and oxygen concentrations. They also represent the lower trophic levels on which the food chain is based (Daphnia Genomics Consortium 2000). *C. dubia* have a short generation time so it is possible to complete an experiment on a whole generation of this organism in a matter of weeks or months. Since they occur naturally throughout the world, the EPA recommends them as a universal test organism (USEPA 2002). Presently, there is no other monitoring of water quality besides tests conducted by the WTP, and no published study looking at physiochemical as well as biological indicators of water quality in the Pocotaligo swamp. We therefore conducted the study with the objective of determining the possible effects of the WTP on water quality by conducting acute and chronic toxicity tests using *C. dubia* as a test organism. We hypothesized that the Sumter WTP would have an effect on downstream water quality.

## Methods

Surface water samples were collected from three locations (Fig. 1 map): one upstream from the WTP, one at the plant's discharge site, and one downstream of the plant. The first sampling location was 0.3 km upstream of the discharge site and is the "control" or "upstream" site. The second sampling location was the point of discharge of the effluent, and the third sampling location was 5.6 km downstream from the effluent discharge site. The upstream site retained canopy cover. The effluent site was an open drainage channel leading to the main river channel. The downstream site had an open canopy and a dense aquatic plant growth (Fig. 2A, 2B and 2C). The three sites were those accessible and/or open to the public. Triplicate surface samples were collected every two months from July 2008 until July 2009 in plastic bottles for the toxicity tests. Additionally, single replicates were collected in glass bottles for chemical analysis. Samples were placed in ice

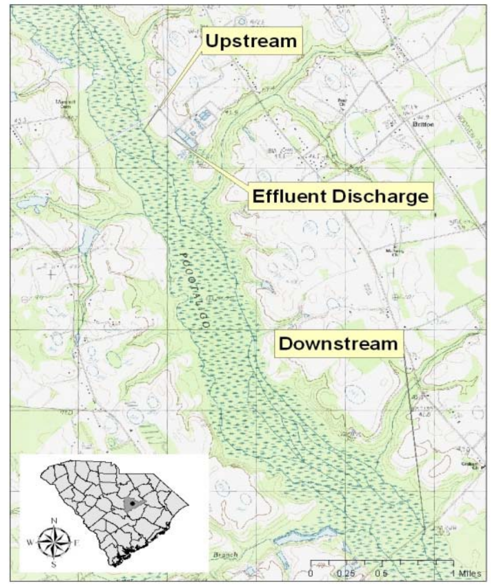


Fig. 1 Map shows the sampling locations in the Pocotaligo River Swamp. The first sampling location is 0.3 km upstream of effluent entry site and is the control or upstream site. The second sampling location is the point of effluent entry and the third sampling location is 5.6 km downstream from the effluent discharge.

and brought to the laboratory for analysis. Physical parameters such as temperature, pH, dissolved oxygen, and total dissolved solids were measured on site for accuracy using an YSI 85 instrument and an EX-stick. Averages were computed from three readings taken for each measurement. In addition, chemical analyses of nitrites, nitrates, ammonia, and phosphates were conducted using the LaMotte Smart Colorimeter kit. Distilled deionised water was used as a blank. Data were summarized and analyzed using the nonparametric Tukey Test for statistical significance to isolate the site or sites that differed from the others. A p-value $<0.05$ meant that differences in the median values among the treatment groups were greater than would be expected by chance, and that there was less

(A) (B) (C)

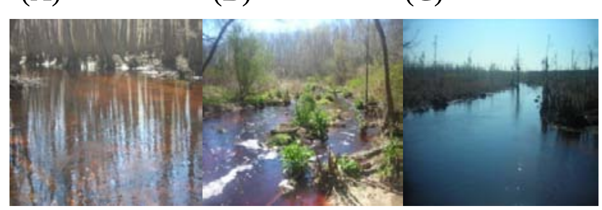

Fig. 2. Upstream Site (Panel A), Discharge site (Panel B) Downstream Site (Panel C)

than a 5% chance that the difference was random.

Chronic (seven day) toxicity tests were conducted as per the USEPA (2002) protocol. Neonates were grown in the USC Aiken laboratory. Twenty replicates, each containing one *C. dubia* neonate, were incubated in 25 ml of each type of water sample at $25^0$C under a 16:8

light:dark photoperiod. Neonates were fed daily a diet of *Selenastrum capricomutum* and YCT (yeast, cerophyll and trout chow). Reconstituted moderately hard water was used as a control. After three days, *C. dubia* adults were moved into fresh sample cups, and the number of offspring was recorded. This procedure was repeated after six days. The data were summarized in MS Excel for analysis and significant differences between the results in the reconstituted control hard water and the water samples were analyzed by the application of Mann–Whitney test ANOVA to determine if the WTP was affecting reproducibility of *C. dubia*.

**Results**

Physical tests showed significant differences between the effluent site, and the upstream and downstream sites for seasonally averaged temperature, pH and dissolved oxygen, ($p<0.05$); however, no significant differences ($p<0.05$) were noted between upstream and downstream sites (Fig. 3). There was a significant difference ($p<0.05$) between upstream and downstream sites for total dissolved solids (Fig. 4). The average in mg/L was 89.75 at the upstream site, 907.50 at the effluent site and 57.4 at the downstream site.

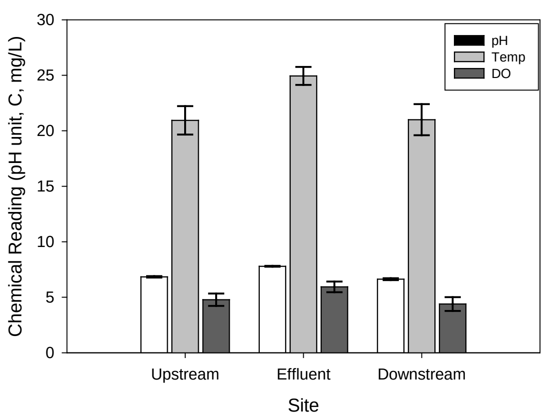


Fig. 3. Variation in pH, temperature $^0$C, and dissolved oxygen (DO) mg/L among the three sampling sites.

Chemical tests showed significant differences between the effluent site, and upstream and downstream sites for ammonia, nitrite and nitrate ($p<0.05$); however, no significant differences ($p<0.05$) were noted between upstream and downstream sites (Fig.5 and 6). There was a significant difference between upstream and downstream sites for phosphorus (($p<0.05$). The average (mg/L) was 0.860 at the upstream site, was 0.480 at the effluent site, and 0.450 at the downstream site.

For the toxicity testing, mortality was less than 10% in all exposures for all tests, and therefore, not significant. *C. dubia* reproduction was widely variable over the study

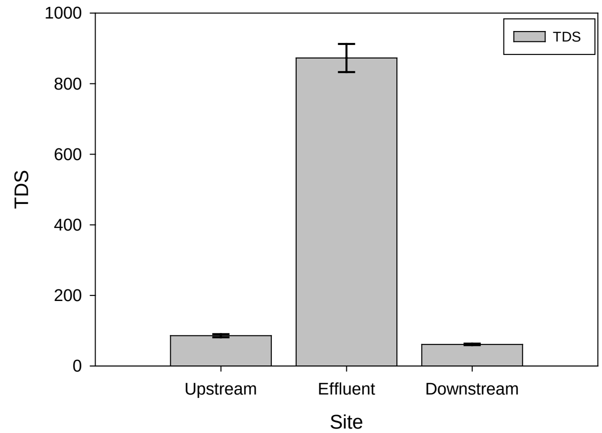


Fig. 4. Variation in Total Dissolved Solids among the three sampling sites.

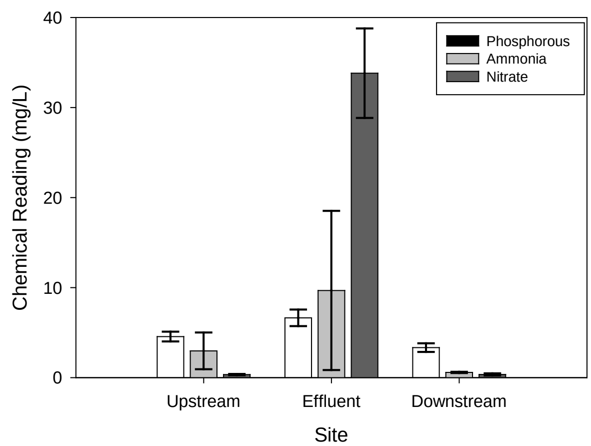


Fig. 5. Variation in phosphorus, ammonia, and nitrate mg/L among the three sampling sites.

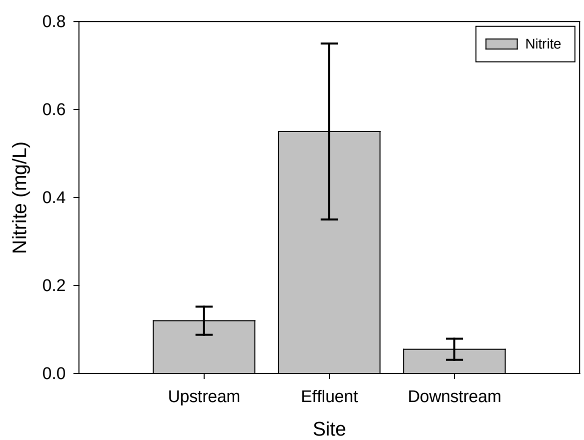


Fig. 6. Variation in nitrite mg/L among the three sampling sites.

period, with the average number of young per female ranging from 15.7 to 35.3 in the upstream samples, and with a low average of 2 neonates per female in the July 2008 effluent (Fig.7). There was a statistically significant ($p<0.05$) difference in reproduction rates at the effluent site in the months of July, October, and March as compared to the upstream site. There was a significant ($p<0.05$) drop in reproduction rates at the downstream site in the months of July (2008), February, and March 2009 as compared to the upstream site.

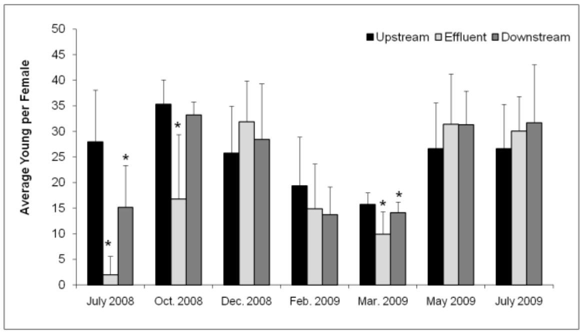


Fig. 7. Results of *Ceriodaphnia dubia* chronic toxicity testing (average young per female). Asterisk indicates those values that are significantly lower when compared to the Upstream sample ($p<0.05$).

## Discussion

The primary objective was to study the effects of the WTP on water quality using physiochemical and biological indicators. Routine chemical monitoring gives insight regarding some, but not all contaminants in surface waters. The benefit of using bioassays such as *C. dubia* for testing of surface waters is that the biological effects of known as well as unknown compounds can be assessed. Toxicity tests play a viable role as a water quality screening tool, because they provide a means to characterize the bioavailability of pollutants as they relate to actual aquatic communities (Cooney 1995).

The effluent showed a spike that could be attributed to temperature of water released from the WTP, which averages around 20°C. Chemical tests showed a significant increase in phosphorous between the upstream and downstream sites, with the upstream site having a significantly higher concentration of phosphorus than the downstream site. The higher levels of phosphorous at the upstream sites could be attributed to nonpoint sources such as runoff from agricultural wastes, and at the effluent site could be attributed to the discharge from the WTP. Chemicals are used to precipitate phosphorus and heavy metals at WTP's (Patoczka 2006) which could have resulted in an increase in total dissolved solids.

Toxicity tests conducted over the 12-month period of this study indicated varying degrees of chronic toxicity to *C. dubia* exposed to WTP effluent. These responses are probably indicative of in-stream conditions, where effluent composition reflects the day-to-day variability of complex influents. Interpretation of effluent toxicity results must be done with caution, as these data work best when combined with other information related to site-specific conditions and when considering native invertebrate populations (Chapman 2000). While we noted chronic toxicity during some periods, it is assumed that the Sumter WTP is operating within its NPDES permit limits and that effluents, at normal in-stream dilutions, are not chronically toxic. Our hypothesis that the WTP has an effect on downstream water quality could not be entirely supported.

Additional limitations of the study were that it was conducted only for one year, and therefore no definite conclusions can be drawn. A long term study looking at non-point sources and seasonal variations affecting water quality needs to be conducted. In addition, the effects of the WTP in sediments and downstream communities, especially after the plant expansion is complete, would be potentially beneficial.

## Acknowledgments

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## References

1. Bedford B.L., Walbridge M.R., Aldous A. Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology*, 1999, **80(7),** 2151-2169.
2. Cooney J.D. Freshwater tests. In: Rand G, ed. Fundamentals of Aquatic Toxicology. 2nd ed. Washington (DC): Taylor and Francis. 1995, p. 71-98.
3. Chapman P.M. Whole effluent toxicity testing: usefulness, level of protection, and risk assessment. *Environ Toxicol Chem*, 2000, **19(1),** 3-13.
4. Hall T. Self Evaluation Techniques: Evaluating Water Quality. In: Farm Water Quality Planning (Publication 8118). 2004, Fact Sheet 5.2.
5. Mitsch W.J., Gosselink J.G. Wetlands. 3rd ed. John Wiley & Sons, Inc., 2000, p. 469-511.
6. Morris J.T., Gross J., McAninch S. The Pocotaligo Swamp Reclamation Study, Columbia, SC, 1994, p. 1-51.
7. Patoczka J. TDS and Sludge Generation Impacts from Use of Chemicals in Wastewater Treatment. Water Environment Foundation, 2006, p. 5209-5214. http://www.environmental-expert.com/. Cited 2009 Dec. 10.
8. Schindler D.W. Evolution of phosphorus limitation in lakes. *Science*, 1977, **195,**260-262.
9. Schlesinger W.H. Biogeochemistry: An Analysis of Global Change. 2nd ed. Acad. Press, San Diego, C.A, 1997, p. 159-163.

10. Smith V.H., Tilman G.D., Nekola J.C. Eutrophication: impacts of excess nutrient inputs on freshwater, marine and terrestrial ecosystems. *Environ. Pollut.*, 1999, **100,**179-196.

11. The Daphnia Genetic Consortium Organization (Internet). (updated 2009). Compelling Reasons for Choosing Daphnia as a Model Species. Indiana University: Bloomington Campus (cited 2009 Dec 01). Available from: http://daphnia.cgb.indiana.edu/people/modelsystem/.

12. U. S. Environmental Protection Agency. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 2002, 4th ed. EPA-821-R-02-013.

13. U.S. Environmental Protection Agency. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 2002, 4th ed. EPA-821-R-02-012.

14. U.S. Army Corp of Engineers. Special Report Pocotaligo Swamp Sumter County, SC, 1982 p.1-8.

15. Wetlands. http://www.epa.gov/wetlands. Updated 2009 Nov. 16. Cited 2009 Dec. 10.

16. Yi K., Kim H., Je J., Schlenk D, Jung J. A toxicity monitoring study on identification and reduction of toxicants from a wastewater treatment plant. Ecotoxicology and Environmental Safety. 2009, 1-6.