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Monitoring of Spunky Bottoms Restored Wetland in Southern Illinois for Biotic and Abiotic Pollution Indicators

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ABSTRACT

A study was conducted to determine and compare biotic (bacterial) and abiotic (physicochemical) pollution indicator levels generated from water samples collected from eleven sites within an aquatic wetland under restoration in Brown County in Southern Illinois. The approximately 700-acre "Spunky Bottoms" wetland, purchased by The Nature Conservancy, is currently being restored by The Wetlands Initiative to conditions prior to levying of the Illinois River and draining of adjacent floodplain for intensive agriculture (circa 1900). Water samples of approximately 200-ml were collected aseptically and analyzed for indicator bacteria (total coliform and *Escherichia coli*) concentrations using a membrane filtration technique and culturing methods. Predominant bacterial genera were also isolated from selected water samples and identified using standard culturing, microscopic, and biochemical techniques. Temperature, pH, dissolved oxygen and conductivity were also monitored concurrently in the field at water sampling sites. Levels of bacterial and physicochemical pollution indicators in water samples taken from the Illinois River and wetland sites adjacent to agricultural land use were substantially higher than levels found at other sampling sites, possibly due to agricultural runoff. Predominant bacterial genera recovered from all sampling sites were Pseudomonas and Bacillus, which may contribute to biogeochemical cycles. Results suggest that restored wetlands may contribute to pollution indicator reduction, and that wetland microbial populations may contribute to biogeochemical (N, P, C) element cycling. Further research is necessary to determine more specific contributions of aquatic wetlands to indicator bacteria concentration reduction and biogeochemical cycles.

INTRODUCTION

Preservation of aquatic wetland ecosystems is vital to protect wildlife habitats, protect water quality, and provide for aesthetically pleasing environmental sanctuaries for recreational purposes. Aquatic wetland ecosystems are being lost or degraded at a dramatic rate throughout the world. National wetland area loss since European settlement is roughly estimated near 50% (Whigham, et al., 1993). Installation of drain tiles and river levying to facilitate agricultural land use practices has been a primary contributor to wetland loss in many U.S. states, including Illinois since circa 1900 (Hey and Philippi, 1999). Illinois has shown a significant loss of aquatic wetlands to agricultural and residential land use since circa 1800. Approximately 3-4% (500,000 acres) of the state of Illinois was designated as wetland in 1990.

Surface and ground water quality is important both nationally (U.S. Environmental Protection Agency - USEPA, 1996) and in Illinois (Illinois Environmental Protection Agency - IEPA, 1994; 1995; 1996). Aquatic wetlands protect water quality by serving as a buffer system to slow water runoff from storm events and allow infiltration into soils, percolation into soil-groundwater systems, and allow time for water purification through natural physical, chemical, and biological processes (Hey and Philippi, 1999; Hammer, 1997; Kadlec and Knight, 1996; National Research Council, 1995; Whigham, et al. 1993). However, there has been some criticism of the ability of restored wetlands to mimic natural wetlands, including failure to attract targeted endangered waterfowl species (Malakoff, 1998). Residential, municipal and industrial wastes are discharged into the Illinois River (IEPA, 1994; 1995; 1996). Aquatic contaminants include pathogenic microorganisms, toxic chemicals, and plant nutrients such as nitrate and phosphate that can lead to eutrophication and depletion of aquatic dissolved oxygen (Salvato, 1992). Microbial populations in aquatic systems may also contribute to biogeochemical cycling of elements, including N and P (Atlas and Bartha, 1987; Whigham, et al., 1993; Hurst, et. al., 1997). These processes may reduce nutrient loading of rivers and ultimately, degradation of estuarine ecosystems (e.g., the hypoxic “dead zone” in the Gulf of Mexico). Wetland restoration has been proposed as a means of reducing N transport to coastal waters (Fleischer and Stibe, 1991). However, limited research has been conducted on the potential for aquatic wetland microbial populations to contribute to biogeochemical cycling of elements.

Much of the flood plain adjacent to the Illinois River was levied and drained for intensive agricultural use by pumping water collected in the wetland into the Illinois River (circa 1900). The rich alluvial soils allowed highly productive agriculture due to the accumulation of nutrients during thousands of years of natural flood cycles and resulting silt deposition. Restoration of arable land to wetland conditions has been extensively studied (Manchester, et al., 1999; Mitsch, et al., 1999; Young, 1996). Spunky Bottoms wetland in Southern Illinois was purchased by The Nature Conservancy in 1998 with a goal of restoration to more natural conditions prior to intensive agricultural use. This approximately 1,500-acre site (containing approximately 700-acres of wetland) in Southern Illinois is being restored in cooperation with The Wetlands Initiative, funded by a grant from The National Fish and Wildlife Foundation. Restoration goals include increasing the level of water in the area and planting native grasses and other wetland flora and provide habitats for aquatic organisms. Water flow through Spunky Bottoms wetland is

primarily from northwest to southeast, entering the wetland through runoff from the upland topography to the west. Water then flows through drainage ditches (North Market, Main Road, and South Cox) to the Pumphouse. Excess water collected was then pumped over the levy into the Illinois River to facilitate agricultural land use (Fig. 1). This technique may also be used to control water levels within the wetland during further restoration efforts. Sources of potential water contaminants include both point and non-point due to adjacent agricultural land use.

Transfer of potentially pathogenic microorganisms, nutrients, and toxic chemicals from agricultural, residential, and industrial wastes through soil to surface water or groundwater is a recognized environmental health concern (Pancorbo, et al., 1991; Kelley, et al., 1994, 1995). Sampling and characterization of local surface water systems is also used to educate students and the general public concerning important environmental health concepts, such as aquatic ecosystem's response to various contaminants, and reduction of pollution (Kelley, et al., 1994, 1995; National Science Foundation - NSF, 1993). This study determined and compared the distribution of physicochemical and bacterial indicators of pollution, as well as predominant bacterial populations in water samples collected from the Spunky Bottoms wetland from June-September 1999. Quantitative data was generated concerning coliform concentrations while qualitative data only (identification of predominant genera) was generated concerning predominant bacterial genera. Appropriate physical, chemical, and bacterial parameters monitored were chosen from those recommended and described by the Clean Water Act National Pollution Discharge Elimination System (NPDES), Environmental Engineering and Sanitation, 4th Ed. (Salvato, 1992), and Standard Methods for the Examination of Water and Wastewater, 20th Ed. (Eaton, 1998).

Monitoring of water samples collected from Spunky Bottoms wetland for biotic and abiotic pollution indicators is being conducted to provide information concerning the current state of water quality, and changes in water quality as restoration proceeds. Data gathered on predominant bacterial populations generated from this and future studies may be used to determine the microbe's potential role in biogeochemical cycling of N, P, and C elements in the wetland.

MATERIALS AND METHODS

Seven sites within the wetland were initially identified by The Wetlands Initiative as indicating a representative geographical distribution of surface water flow throughout the area. Four test wells were later sampled for a total of eleven sampling sites (Table 1). All seven sites initially identified were sampled five times each during June-September 1999. Test wells spb-5 and 13 were sampled twice each for physicochemical pollution indicators. Test well spb-13 was sampled three times for bacterial indicators. Test wells spb-12 and 19 were sampled only once each. Samples taken from four sites (South Cox, Main road, Illinois River, and Snyder's Landing) were analyzed for predominant bacterial populations using techniques described below.

Physicochemical and Bacterial Analyses

Temperature, conductivity, total dissolved solids, and dissolved oxygen levels were measured in the field from June-September, 1999 using a Corning® Multimeter (Corning

Inc., Science Products Division, Corning, NY). Methods used for physicochemical water analyses followed directions recommended by the instrument manufacturer. For bacterial analyses, water samples of approximately 500-ml were taken from eleven selected sites (Table 1) using aseptic technique and stored in sterile Whirlpak®- type sealed plastic bags according to collection, shipment, and storage procedures outlined in Standard Methods for the Examination of Water and Wastewater, 20th Ed. (Eaton, 1998). Bacterial analyses were completed within 48-72 hours of sampling. Appropriate volumes of undiluted water samples or appropriate dilutions of water samples were filtered through 0.45-µm pore size 47-mm diameter gridded filters (Micron Separations Inc., Westborough, MA) using a Nalgene® (Nalgene Co., Rochester, NY) filtration apparatus attached to a vacuum pump. Filters were transferred to the surface of Methyl-Ulbelliferyl β-D-Glucaronide (MUG)-based m-TMM (Tergitol Monensin MUG) culture media contained in 50-mm petri dishes and incubated at 35^0 C for 24 hrs (Dry-type Bacteriological Incubators, Blue M Electric Company, Blue Island, IL) for culturing of bacterial groups. MUG-based media allowed for concurrent culturing and identification of total coliform and *Escherichia coli* (Freier and Hartman, 1987). Aseptic technique was applied during all microbiological analyses. Characteristic colonies were counted and bacterial group concentrations reported on a colony forming unit per milliliter basis (cfu ml^{-1}).

Preliminary testing of water samples for predominant bacterial populations was also conducted using the following protocol (United Analytical Services, Inc., Downer's Grove, IL): Fifteen-ml of each water sample were centrifuged for 15-min. at approximately 3000-rpm. Two-ml of supernatant and the remaining sediment were used as a concentrated sample to inoculate BHI (brain heart infusion) plates which were incubated for a total 10- days. Plates were observed on days five, seven and ten. Gram stains, wet mounts and biochemical analyses were performed on the growing colonies. The two predominant genera by concentration were then identified.

Statistical data analysis

Data generated were subjected to one-way analysis of variance (ANOVA) and pairwise comparison using Sheffe's S test with SPSS software. Significance was determined and probability (p) levels reported for ANOVA results. Location and sampling time were identified as independent variables. Physicochemical and bacterial pollution indicator (coliform and *E. coli*) levels were identified as dependent variables.

RESULTS

Physicochemical analyses

Mean data for physicochemical and bacterial analyses of water samples are reported in Table 1. Temperature of water at sampling sites ranged from 18.7^0 C (Middle Creek, September 27) to 31.8^0 C (Pumphouse, July 19). Levels of pH of water at sampling sites ranged from 7.04 (well spb-5) to 7.90 (Snyder's Landing). Conductivity of water at sampling sites ranged from 404 micro-Siemens (µS) to 796 µS (Pumphouse and well spb-12, respectively). Total dissolved solids (TDS) concentrations ranged from 201 to 365 mg L^{-1} measured by the instrument as correlated to conductivity. Dissolved oxygen concentrations were off scale at several sites tested, possibly due to instrument or operator error. After elimination of off-scale DO values dissolved oxygen levels in water samples ranged from 3.1 mg/L (Snyder's Landing) to 12.8 mg/L (South Cox).

Bacterial analyses

Total coliform concentrations ranged from 18 cfu 100 ml^{-1} (Pumphouse) to 85,000 cfu 100 ml^{-1} (well spb-5). *Escherichia coli* concentrations ranged from 2.0 cfu 100 ml^{-1} (Pumphouse) to 20,000 cfu 100 ml^{-1} (Middle Creek). A ranking of mean total coliform and *E. coli* concentrations (from lowest to highest) are as follows: Illinois River, Pumphouse, Middle Creek, Main Road, Snyder's Landing, well spb-5, well spb-13, South Cox, and North Market (Table 1). High variability of some microbial concentration data was primarily due to temporal variation between or among different samples (e.g., taken at different times), rather than variability of microbial concentrations within samples taken at the same time. Pseudomonas and Bacillus were the two predominant bacterial genera isolated from water samples collected and submitted for analysis.

Statistical analyses

One-way analysis of variance (ANOVA using SPSS software) of physicochemical and bacterial data (dependent variables) generated for all time periods indicated significant surface site (independent variables) differences among conductivity, $F(10, 21) = 4.001$, $p = 0.004$; total dissolved solids $F(10, 21) = 4.248$, $p = 0.003$; total coliform concentrations, $F(10, 71) = 4.083$, $p < .0001$; and *E. coli* concentrations, $F(10, 71) = 2.222$, $p = 0.026$. Pairwise comparisons using Sheffe's S test indicate no significant differences among paired sites, but this apparent inconsistency many have been due to the limited number of observations ($n \leq 5$). ANOVA and Sheffe's S test results reported were performed on data from all eleven sites. Subsequent analysis excluding well data did not substantively change results or conclusions.

DISCUSSION

Physicochemical analyses

Results of physicochemical analyses of water samples, including temperature, pH, conductivity, turbidity, and dissolved oxygen are within ranges of 50-1,500 μMhos and 6.0-9.0, respectively for natural waters as indicated in the literature (Eaton, 1998; Salvato, 1992). Mean dissolved oxygen levels were 5.75 mg L^{-1}, above levels of 5.0 mg L^{-1} recommended to support fish survival and reproduction (Salvato, 1992). Based on results generated and analyzed in this study, there was evidence to suggest that concentrations of selected physicochemical indicators of pollution (conductivity and total dissolved solids) were significantly reduced as water flowed through the wetland.

Bacterial analyses

Salvato (1992) citing the Federal Water Pollution Control Administration (FWPCA, 1968) indicated that acceptable levels of fecal coliform concentrations for general recreational use waters for which ingestion is not a significant concern should not exceed an average of 4,000 cfu 100 ml^{-1}. Fecal coliform are a sub-group of total coliform and are therefore a more specific indicator of fecal pollution (Salvato, 1992). Mean total coliform concentrations exceeding recommended levels of 4,000 cfu 100-ml^{-1} were recovered from South Cox, North Market, Snyder's Landing, and wells spb-5 and 13 (Table 1). Assuming that a majority of fecal coliform are *E. coli*, fecal coliform concentrations recovered did not appear to exceed recommended maximum levels. Potential sources of

fecal pollution include runoff of animal waste (e.g., cattle, pigs, goats, etc.) from adjacent agricultural practices, as well wild animal wastes.

Based on results generated and analyzed in this study, there was evidence to suggest that as water flowed through the wetland, concentrations of bacterial indicators of fecal pollution (total coliform and *E. coli*) were significantly reduced.

Conclusions

As described in the introduction, water flow through Spunky Bottoms wetland is primarily from northwest to southeast, entering the wetland through runoff from the upland topography to the northwest. Water then flows through drainage ditches (North Market, Main Road, and South Cox) to the Pumphouse site. Excess water collected was then pumped over the levy into the Illinois River to facilitate agricultural land use (Fig. 1). Considering this flow pattern, it is interesting to note that lowest concentrations of *E. coli*, total dissolved solids and conductivity levels were found in samples collected from the Pumphouse site. The second lowest concentration of total coliform was also recovered from the Pumphouse site. These observations support the evidence that concentration reduction of pollution indicators occurred as water passed through the wetland. It should be noted that many factors could have contributed to concentration reduction of pollution indicators, including physical processes of dilution and settling.

Several species of Pseudomonas and Bacillus function in the nitrogen cycle including *Pseudomonas nitrificans* and *P. denitrificans, Bacillus polymyxa* and *B. macerans*. *B. megatherium* also functions in the phosphorous cycle in mineralization of phosphate. *Pseudomonas* and *Bacillus* species also function in the carbon cycle to degrade recalcitrant compounds such as chitin, lignin, and xylan (Atlas and Bartha, 1987). Therefore, there is limited evidence from preliminary results of this study that bacterial populations in the wetland may contribute to biogeochemical cycling of N, P, and C elements in the environment. Further study is necessary to support or reject this contention.

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REFERENCES

Atlas, R.M. and R. Bartha. 1987. Microbial Ecology, 2nd Ed. Benjamin/Cummings Pub. Co., Menlo Park, CA.

Eaton, A. (Ed.) 1998. Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF).

Fleischer, S. and L. Stibe. 1991. Restoration of wetlands as a means of reducing nitrogen transport to coastal waters. Ambio 20(6)271-272.

Freier, T.A., and P. Hartman. 1987. Improved membrane filtration media for enumeration of total coliforms and *Escherichia coli* from sewage and surface waters. Appl. & Environ. Micro. 53, (6): 5-9.

Hammer, D.A. 1997. Creating Freshwater Wetlands, 2nd Ed. CRC Lewis Publishers, Boca Raton, FL.

Hey, D. and N. Philippi. 1999. A Case for Wetland Restoration. John Wiley and Sons, Pub. , New York NY.

Hurst, C.J., G. Knudson, M. McInerney, L. Stetzenbach, M. Walter. 1997. Manual of Environmental Microbiology. American Society for Microbiology Press, Washington, D.C.

Illinois Water Quality Report, 1994-1995. 1996. Illinois Environmental Protection Agency, Bureau of Water.

Kadlec, R.H., and R. L. Knight. 1996. Treatment wetlands. CRC Lewis Publishers, Boca Raton, FL.

Kelley, T.R. 1994. Geographic and seasonal distribution of microbial indicators in the Withlacoochee River and adjacent groundwater. Final Report, Faculty Research Grant, Valdosta State University, Valdosta, GA.

Kelley, T.R., O. Pancorbo, W. Merka, S. Thompson, M. Cabrera, and H. Barnhart. 1994. Fate of microbial indicators and pathogens in fractionated poultry litter during storage. Journal of Applied Poultry Research, 3:279-288.

Kelley, T. R., O. Pancorbo, W. Merka, S. Thompson, M. Cabrera, and H. Barnhart. 1995. Fate of selected bacterial pathogens and indicators in fractionated poultry litter during reutilization. Journal of Applied Poultry Research, 4:366-373.

Malakoff, D. 1998. Restored wetlands flunk real-world test. Science 280(5362), 371-372.

Manchester, S.J., S. McNally, J. Treweek, T. Sparks, and J. Mountford. 1999. The cost and practicality of techniques for the reversion of arable land to lowland wet grassland - an experimental study and review. Journal of Environmental Management, 55, 91-109.

Mitsch, W.J., X. Wu, R. Nairn, P. Weihe, N. Wang, R. Deal, and C. Boucher. 1998. Creating and restoring wetlands: a whole-ecosystem experiment in self-design. Bioscience 48(12), 1019-1030.

National Research Council. 1995. Wetlands: Characteristics and Boundaries. National Academy Press, Washington, D.C.

National Water Quality Inventory, 1995. 1996. United States Environmental Protection Agency Office of Water, Washington DC.

Pancorbo, O. C., T. Kelley, and T. Cai. 1991. Toxicity assessment of groundwater, pond water, and pond sediment samples taken adjacent to an industrial landfill in McDuffie County, Georgia. Final report of independent testing by Environmental Health Science Program Water Quality Laboratories, University of Georgia, Athens, GA.

Report of the Committee on Water Quality Criteria. 1968. Federal Water Pollution Control Administration, U.S. Department of the Interior, Washington, D.C., April 1, 1968, pp. 8-14.

Salvato, J. 1992. Environmental Engineering and Sanitation. John Wiley & Sons, New York, NY.

Stressed Stream Analysis: New Approaches and Techniques for Undergraduate Faculty. 1993. Center for Applied Aquatic Science and Aquaculture, National Science Foundation Summer Program, State University of New York, Brockport, NY.

The Condition of Illinois Water Resources 1972 - 1994. 1994. Illinois Environmental Protection Agency, Springfield, IL.

Water Pollution Control Program Plan, Fiscal Year 1996. 1995. Illinois Environmental Protection Agency, Division of Water Pollution Control, Springfield, IL.

Whigham, D.F., D. Dykyjova, and S. Hejny, Eds. 1993. Wetlands of the world I: Inventory, ecology, and management, Vol I, from Handbook of vegetation science Vol. 15/2, H. Lieth, Ed. In Chief. Kluwer Academic Pub., Dordrecht, The Netherlands.

Young, P. 1996. The “new science” of wetland restoration. Environmental Science and Technology, 30(7) 292-296A.

Figure 1. Map of wetlands area and sampling site locations.

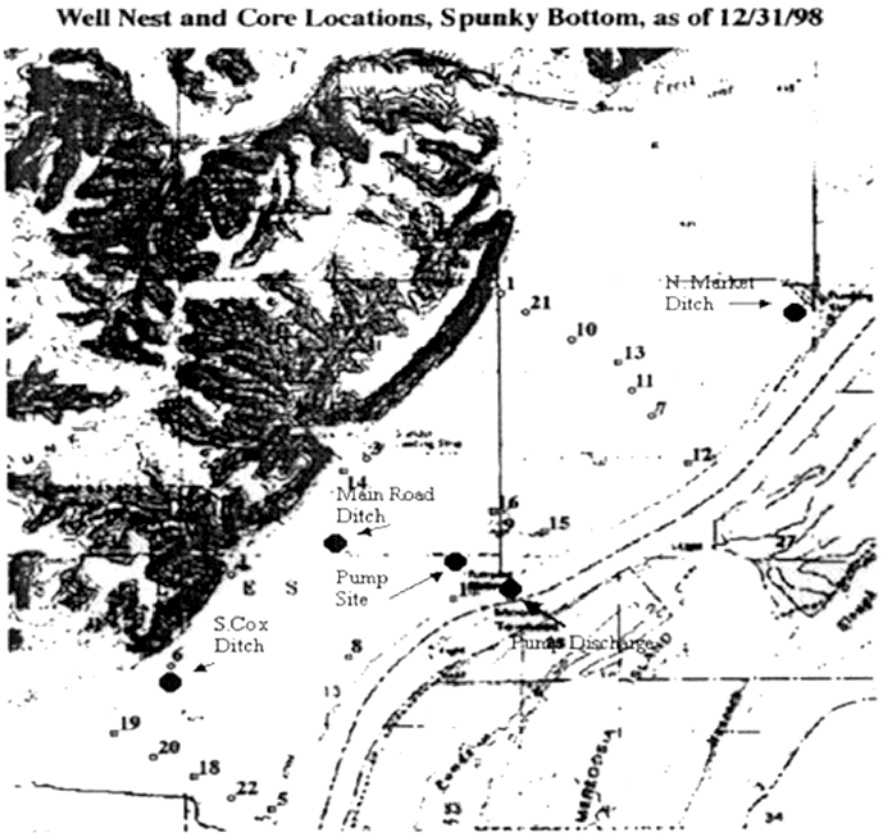

Table 1: Results of Spunky bottoms wetland water sample analyses for bacterial and physicochemical pollution indicators (Mean ± 1 Standard Deviation).

	South Cox[1]	Middle Creek[1]	North Market[1]	Main Road[1]	Snyder's Landing[1]	Pump-house[1]	Illinois River[1]	Well spb-5[2]	Well spb-12[3]	Well spb-13[2]	Well spb-19[3]
Total coliform (cfu ml^{-1})	74.9 ± 32.8	34.0 ± 20.6	79.1 ± 77.2	36.4 ± 23.5	55.8 ± 47.8	28.9 ± 15.5	22.2 ± 14.8	57.0 ± 26.0	ND*	65.3 ± 44.1	ND
E. coli (cfu ml^{-1})	6.7 ± 4.9	5.75 ± 6.36	7.08 ± 5.98	5.33 ± 4.10	6.90 ± 5.63	3.00 ± 1.10	3.40 ± 2.42	5.0 ± 0.7	ND	6.17 ± 3.98	ND
Temp. (^{0}C)	25.80 ± 1.89	22.47 ± 2.68	27.50 ± 5.05	26.05 ± 3.46	27.00 ± 4.93	26.90 ± 4.04	28.30 ± 0.30	21.15 ± 1.65	23.90	23.15 ± 2.55	24.10
D O (mg/L)	5.8 ± 1.1	7.4 ± 3.9	4.0 ± 0.35	7.0 ± 3.4	7.9 ± 2.6	5.3 ± 2.2	6.7 ± 2.6	4.8 ± 1.9	3.1	5.2 ± 2.0	3.4
pH (0-14)	7.41 ± 0.22	7.26 ± 0.21	7.30 ± 0.19	7.38 ± 0.16	7.40 ± 0.30	7.35 ± 0.19	7.28 ± 0.17	7.14 ± 0.10	7.22	7.17 ± 0.10	7.44
Conductivity (µS)	490.25 ± 35.75	648.33 ± 60.54	591.75 ± 55.60	535.00 ± 131.87	532.25 ± 22.75	469.00 ± 43.97	696 ± 28.99	605.50 ± 28.50	796	655 ± 11	612
TDS (mg/L)	248.75 ± 19.64	331.00 ± 29.20	301.00 ± 29.28	266.25 ± 67.40	267.00 ± 11.11	235.75 ± 23.56	352 ± 10.61	304.50 ± 14.50	401	344.00 ± 0	313

[1]Sampled five times, [2]Sampled twice (spb-13 three times for bacterial indicators), [3]Sampled once only, *ND = No data generated