Dialysis Tubing 2018 Equilibration Study

Becky Norris
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Three Lakes Association
P.O. Box 689
Bellaire, MI 49615

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Introduction

In 2017 as part of our ongoing study of golden-brown algae (GBA) on the benthic surfaces of lakes in northern Michigan, we undertook a study to explore the hypothesis that declining availability of phosphorus over the growing season tended to favor the growth of diatoms that were both low-nutrient tolerant and highly pigmented. To obtain the necessary chemical analysis of benthic phosphorus, we used "peepers" consisting of baggies made of dialysis tubing housed in protective rigid PVC pipes with drilled holes to allow contact of the membrane with the environment. For reasons largely unknown and unknowable, the 2017 study was inconclusive. The pattern of diatom populations found over the growing season was markedly different from that observed the preceding year. The phosphorus levels in replicate samples showed large differences, making interpretation unreliable.

Three particular concerns emerged from the outcome of the 2017 study. One was that the amount of contact between the dialysis membrane and the environment may have been too limited because of the size and number of holes in the PVC protective housing. Another was that the length of deployment of the peepers in the environment (approximately 4 weeks for each study point) may have allowed the formation of a biofilm with colonization of the membrane by bacteria and other organisms that could have altered the character of the membrane and variably affected phosphorus concentrations inside the dialysis tubing baggies. And a third was that contamination of the samples might have occurred during their collection, storage, or handling in the laboratory. I was told by the chemist at the University of Michigan Biological Station (where the samples were analyzed) that once the dialysis tubing baggies were thawed, they began to weep into the zip-lock bags used in the field to store them; any extraneous phosphorus from clinging lake floor sediment, other organic material, or human handling, could have affected the analytical results.

Because of the value of reliable measurements of benthic nutrient levels (phosphorus and nitrogen) to our understanding of the GBA phenomenon, we have sought to modify our peeper structure and test it in a controlled, in vitro, environment.

Materials

Laboratory grade gloves (Kimberly-Clark professional blue nitrile gloves, item 38520) were worn for all activities in this study.

Dialysis tubing (Sigma-Aldrich cellulose membrane, item D9527) was used to make the dialysis tubing baggies.

Rigid plastic mesh tubes (Industrial Netting RN4430) were used as protective housing for the dialysis tubing baggies.

Polystyrene (Sterlite) clear storage bins with locking lids were used for water baths.

Deionized water obtained from the University of Michigan Biological Station Laboratory, Pellston, Michigan was used to fill the dialysis tubing baggies and the water baths. Vials of concentrated laboratory reagent nutrients (NO_3-N, NH_4-N, and PO_4-P) were obtained from the same laboratory. Six

molar hydrochloric acid, also from UMBS, was used to clean the rigid mesh tubes and the water baths prior to their use in the study.

Commercial sandbox sand (Ace Hardware) was used to provide an approximation to the lake floor environment in one of the water baths.

Methods

Dialysis tubing was cut into approximately 30 inch lengths, wetted and softened in deionized water, knotted at one end, filled with deionized water, and knotted at the other end, creating baggies with a sample volume of approximately 450 cc. The baggies were placed inside the rigid mesh tubes to mimic the anticipated use in the natural environment. (Figure 1.)

Three water baths were prepared. One contained only deionized water (DW). One contained deionized water spiked with an aliquot of the concentrated laboratory reagent nutrients (DN). One contained sand in a depth adequate to cover the rigid mesh tubing plus deionized water spiked with an aliquot of the concentrated laboratory reagent nutrients (SN). (Figure 2.)

Two dialysis tubing baggies in rigid mesh tubes were placed in bath DW. Four dialysis tubing baggies in rigid mesh tubes were placed in bath DN and in bath SN. After set-up, all baths were sealed with locking covers at all times except when samples were being harvested.

Grab samples of the water of each bath were harvested at hours 0, 24, 48, 72, and 96. Dialysis tubing baggie samples were harvested from the DW bath at hours 24 and 48 and from the DN and SN baths at hours 24, 48, 72, and 96. Selected triplicate samples to evaluate within-laboratory reproducibility were collected: grab samples from baths DN and SN at hour 0 and dialysis tubing baggie samples from baths DN and SN at hour 96. The laboratories were blinded during analysis to the identity of the samples.

Laboratory support for the chemical analyses was provided by Great Lakes Environmental Center (GLEC, Traverse City, Michigan) for PO_4-P and by UMBS for PO_4-P, NO_3-N and NH_4-N. All samples were harvested into sample containers supplied by the laboratories performing the analyses, stored according to the laboratories' requirements (on ice or refrigerated for GLEC, frozen for UMBS), and delivered within the laboratories' specified holding times (24 hours for GLEC, 7 days – actual, less than required – for UMBS).

Results

The analytical results are shown in Appendix B. Laboratory to laboratory comparability was assessed with the PO_4-P analyte. There was good replication of results by the two different laboratories.

Equilibration between the dialysis tubing baggies and the water baths was shown to occur by 24 hours. Some variation began to emerge after 24 hours. The baths were not prepared as sterile and possible colonization or coating of the membrane by unknown substances that could have been present in the baths may have influenced the results.

Discussion

This in vitro study successfully demonstrated that a 24 hour deployment time with the equipment used is adequate for equilibration between the water bath and the dialysis tubing baggies. It also provided an

indication that greater deployment time may affect the analytical results and influence the conclusions that can be drawn.

The study also provided assurance that the PO_4-P results obtained by the two laboratories used in this study are similar enough that we can rely on results from either.

Given the positive results of this study, we are planning to revisit the pattern of phosphorus levels flux over the growing season in 2018. We will use the peeper design tested in this study. We will also be collecting benthic algae samples to track the seasonal growth patterns of the diatoms.

Appendix A, Figures

Figure 1. Dialysis tubing baggie and rigid mesh tube housing

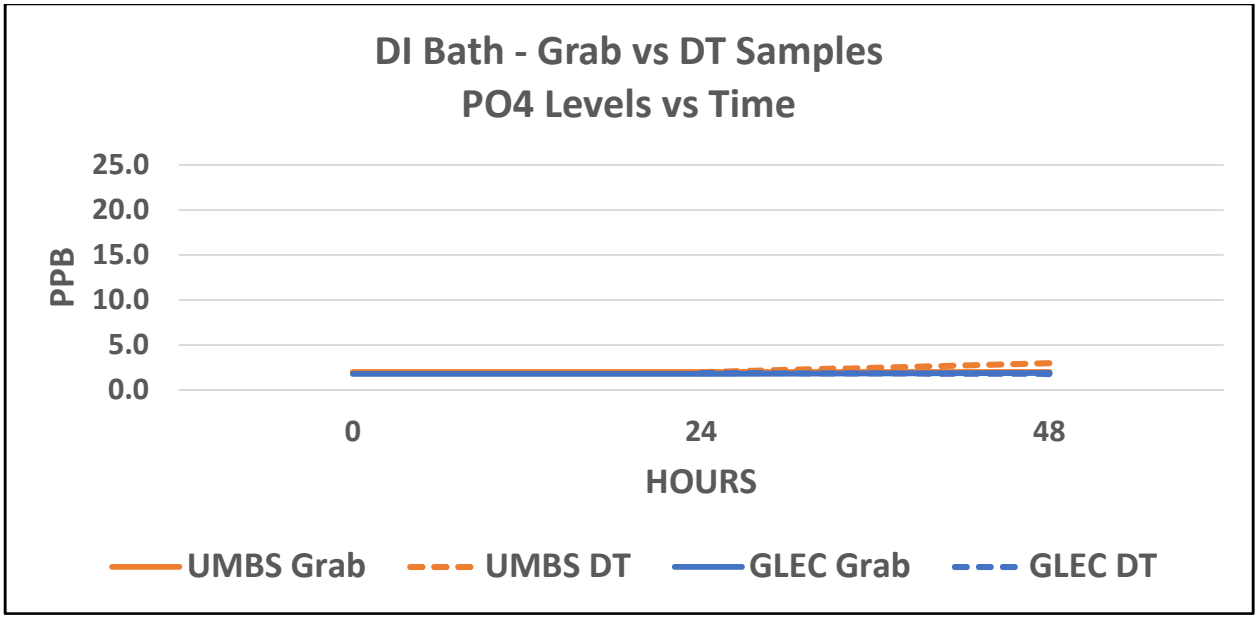
Figure 2. Water bath DW with peepers

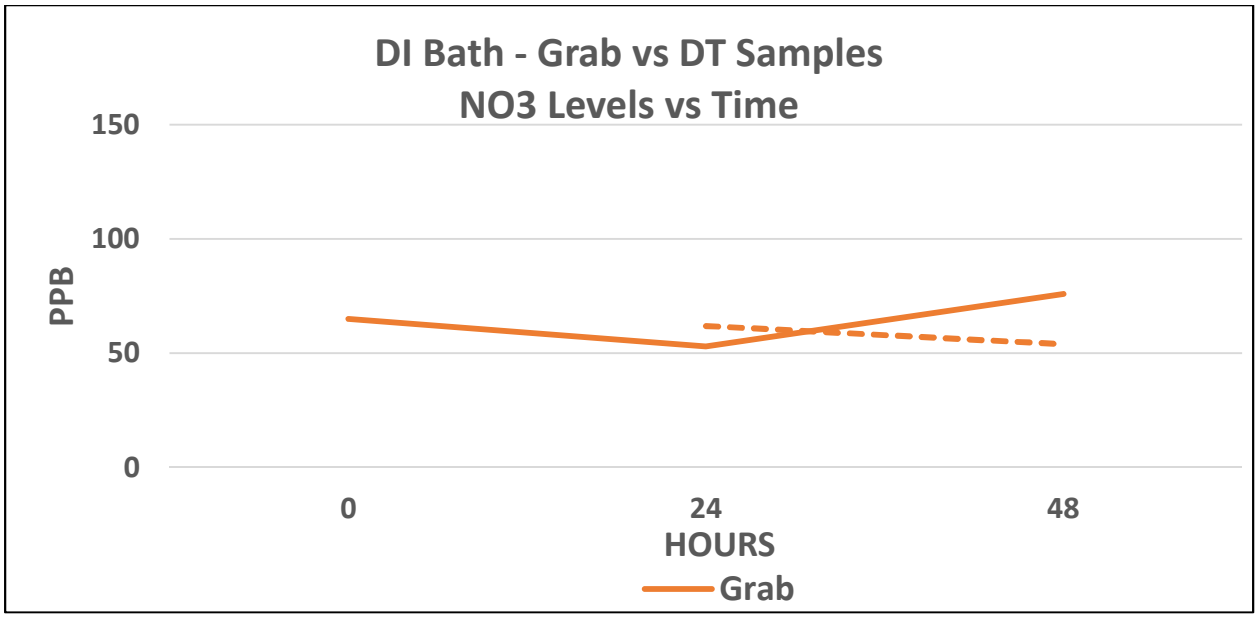
Appendix B, Analytical Results

Nutrient Levels by Bath, Sample Type and Study Hour

Bath	Sample Type	Hour	UMBS NO3-N (mg L-1)	UMBS NH4-N (ug L-1)	UMBS PO4-P (mg L-1)	GLEC PO4-P (mgL-1)
DW	Grab	0	0.065	8.8	0.002	0.0018
DW	Grab	24	0.053	<3.0	0.002	0.0018
DW	DT	24	0.062	<3.0	0.002	0.0019
DW	Grab	48	0.076	<3.0	0.002	0.0019
DW	DT	48	0.054	10.5	0.003	0.0018
DN	Grab	0	0.137	<3.0	0.010	0.0024
DN	Grab	0	0.139	<3.0	0.011	0.0032
DN	Grab	0	0.151	4.1	0.012	0.005
DN	Grab	24	0.138	<3.0	0.011	0.0273
DN	DT	24	0.138	4.3	0.011	0.0103
DN	Grab	48	0.159	30.8	0.011	0.0097
DN	DT	48	0.154	12.2	0.011	0.0091
DN	Grab	72	0.122	<3.0	0.012	0.0086
DN	DT	72	0.223	<3.0	0.012	0.0114
DN	Grab	96	0.120	<3.0	0.012	0.0064
DN	DT	96	0.130	<3.0	0.013	0.0074
DN	DT	96	0.121	<3.0	0.013	0.0056
DN	DT	96	0.122	<3.0	0.014	0.0079
SN	Grab	0	0.352	23.7	0.059	0.069
SN	Grab	0	0.344	13.2	0.056	0.0669
SN	Grab	0	0.478	18.3	0.057	0.0692
SN	Grab	24	0.346	9.7	0.056	0.0894
SN	DT	24	0.321	4.4	0.096	0.1064
SN	Grab	48	0.325	10.0	0.103	0.0931
SN	DT	48	0.384	16.6	0.120	0.1362
SN	Grab	72	0.340	14.7	0.093	0.0814
SN	DT	72	0.356	15.3	0.091	0.086
SN	Grab	96	0.342	23.6	0.087	0.0456
SN	DT	96	0.329	6.5	0.074	0.054
SN	DT	96	0.319	6.7	0.074	0.0557
SN	DT	96	0.315	10.7	0.085	0.0664

Data Graphs


DI Bath - Grab vs DT Samples
PO4 Levels vs Time
25.0
20.0
15.0
10.0
5.0
0.0
PPB
0
24
48
HOURS
UMBS Grab
UMBS DT
GLEC Grab
GLEC DT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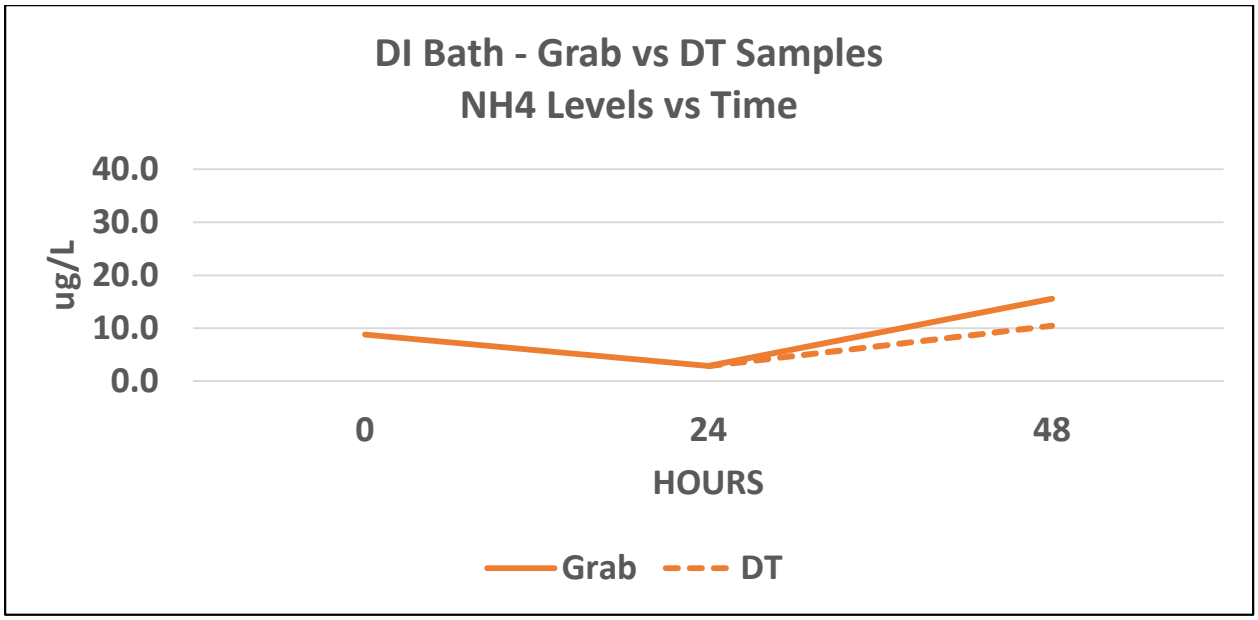
NO3 Levels vs Time
150
100
50
0
PPB
0
24
48
HOURS
Grab


DI Bath - Grab vs DT Samples
NH4 Levels vs Time
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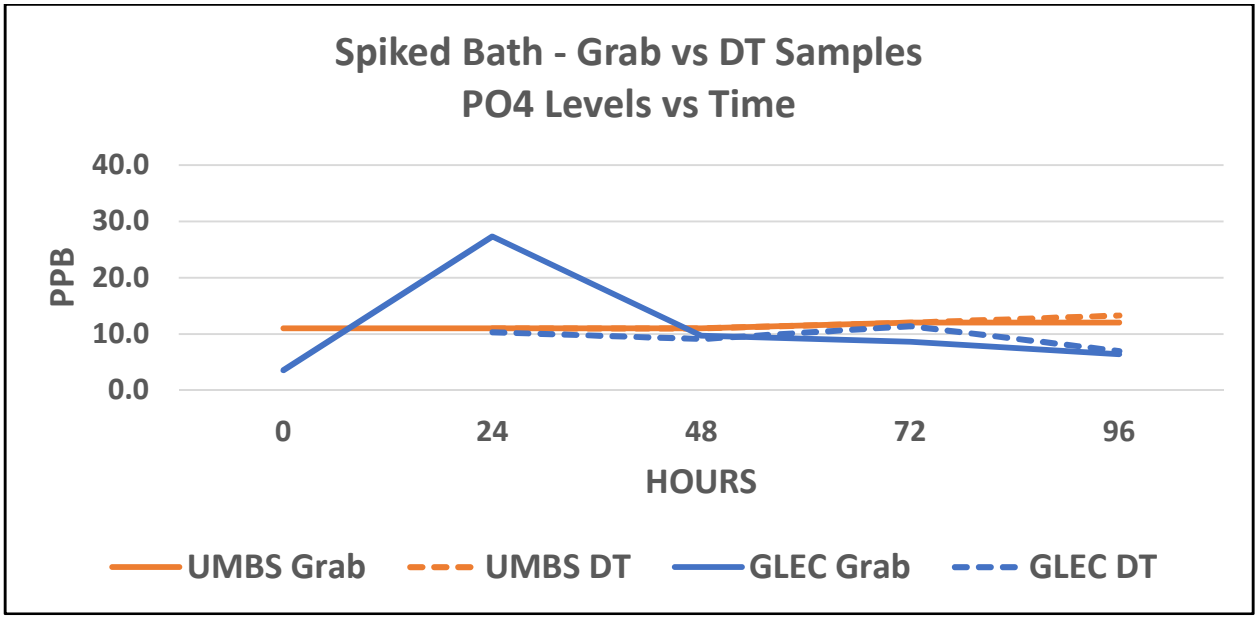
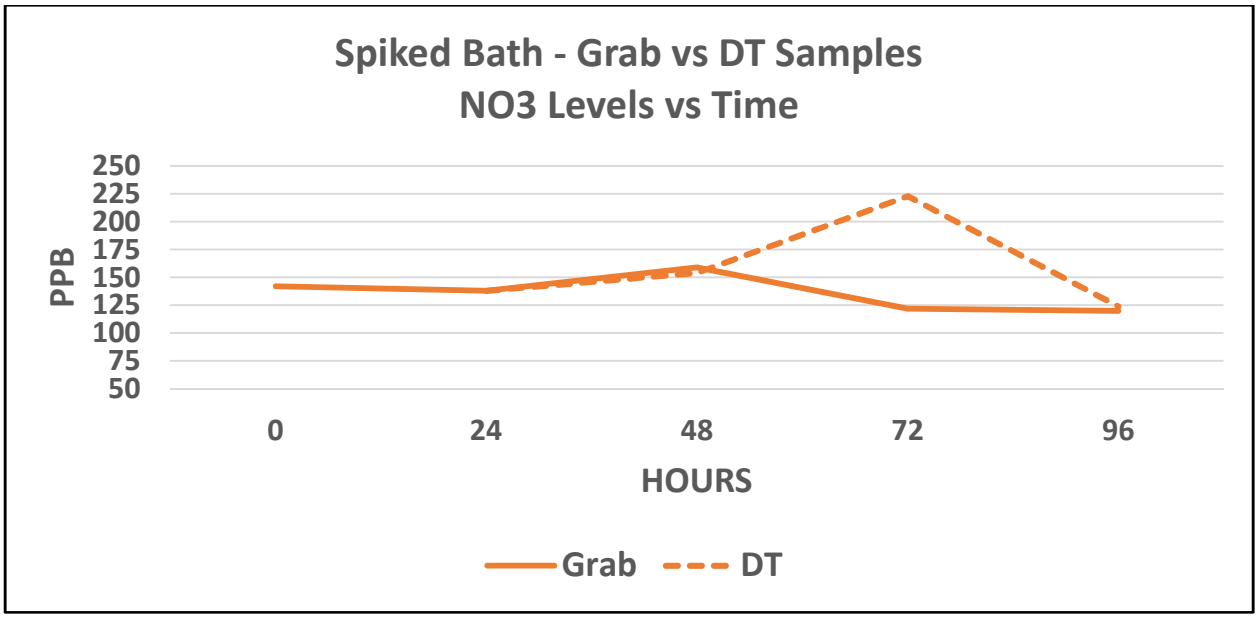
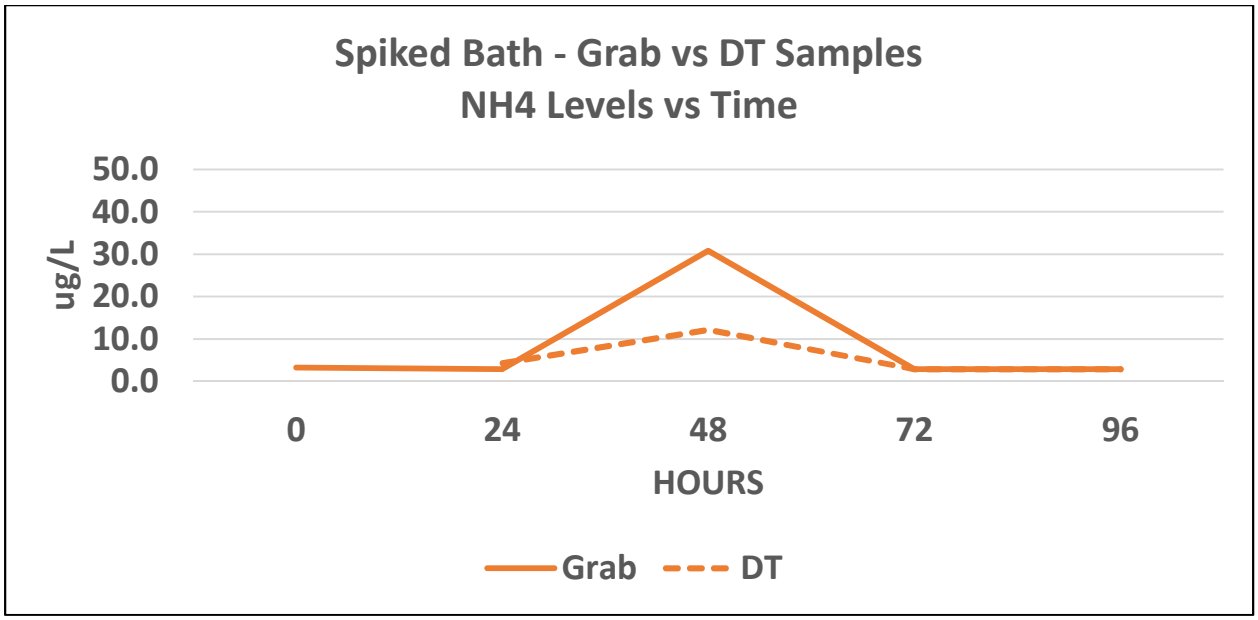
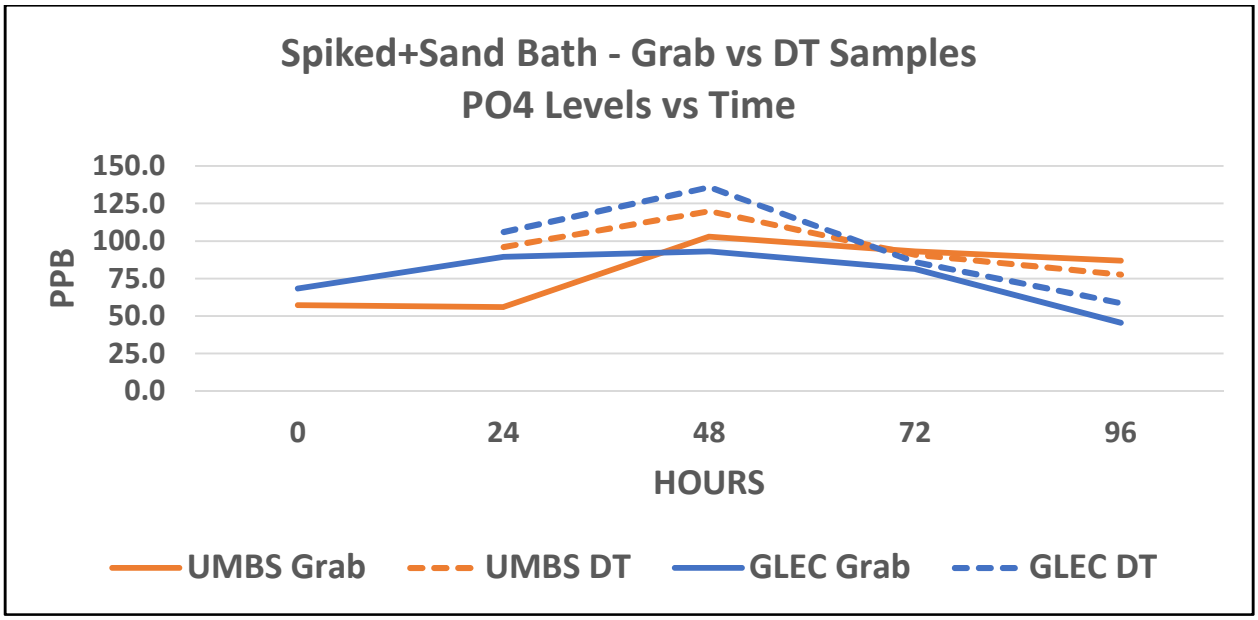
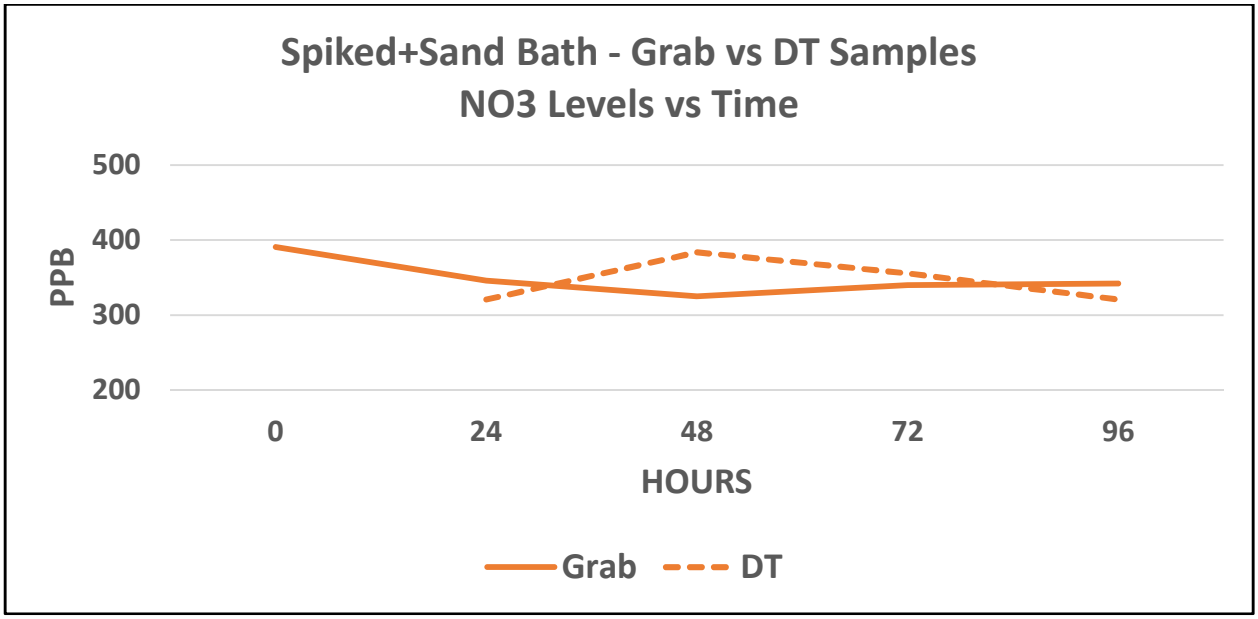
30.0
20.0
10.0
0.0
ug/L
0
24
48
HOURS
Grab
DT

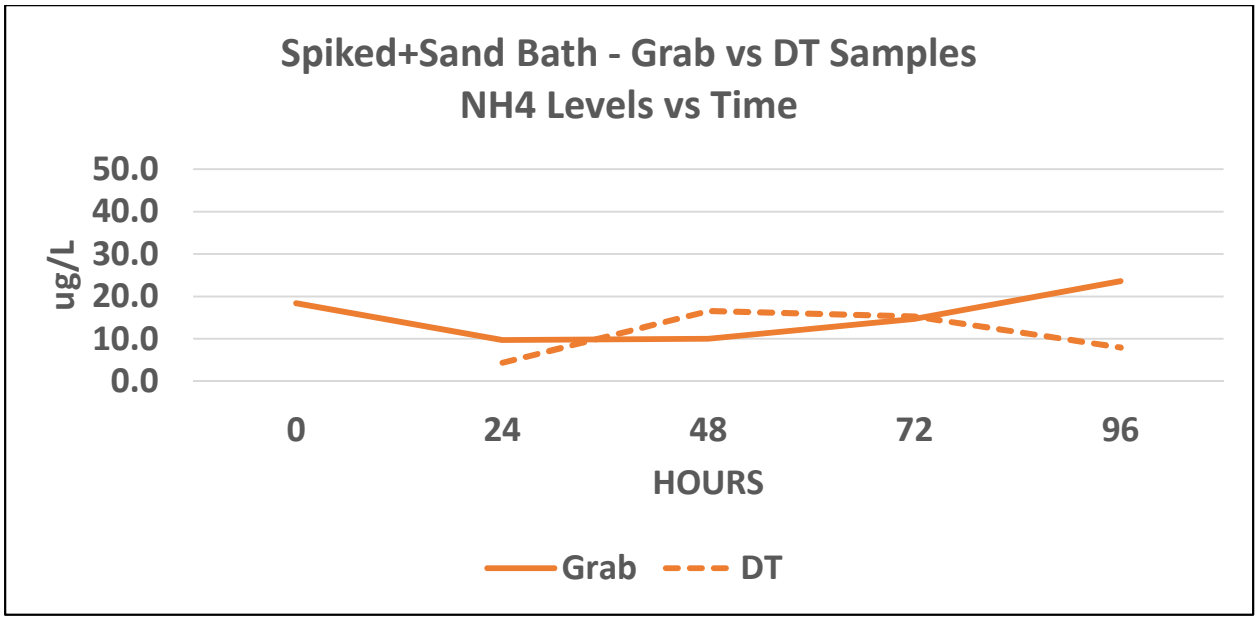

Spiked Bath - Grab vs DT Samples
PO4 Levels vs Time
40.0
30.0
20.0
10.0
0.0
PPB
0
24
48
72
96
HOURS
UMBS Grab
UMBS DT
GLEC Grab
GLEC DT


Spiked Bath - Grab vs DT Samples
NO3 Levels vs Time
PPB
250
225
200
175
150
125
100
75
50
0
24
48
72
96
HOURS
Grab
DT


Spiked Bath - Grab vs DT Samples
NH4 Levels vs Time
ug/L
50.0
40.0
30.0
20.0
10.0
0.0
0
24
48
72
96
HOURS
Grab
DT


Spiked+Sand Bath - Grab vs DT Samples
PO4 Levels vs Time
150.0
125.0
100.0
75.0
50.0
25.0
0.0
PPB
0
24
48
72
96
HOURS
UMBS Grab
UMBS DT
GLEC Grab
GLEC DT


Spiked+Sand Bath - Grab vs DT Samples
NO3 Levels vs Time
500
400
300
200
PPB
0
24
48
72
96
HOURS
Grab
DT



Acknowledgment

Thanks to Rick Doornbos for preparing the graphic representations of the chemical data.