

Final report on the installation and operation of PraxSoft nutrient sensors and the comparison of real time N and P concentrations from these nutrient sensors to manual samples; at Immokalee and Hastings

Deliverables 5b and 5d
(FDACS – Contract # 019693)
(UF – Contract # 0088308)
(UF-Project # 00106368)

By

Kelly T. Morgan¹

Faculty Investigator

Lincoln Zotarelli²

Faculty Investigator

Patrick Moran²

Biological Scientist

Soil and Water Science Department¹

Horticultural Sciences Department²

Institute of Food and Agricultural Sciences

University of Florida

June, 2013

Executive Summary

The description and details of the installation and methodology for comparison of N and P concentrations in periodic water samples and PraxSoft nutrient sensors at Immokalee and Hastings were presented in deliverables 5a and 5c, respectively. The PraxSoft nutrient sensors were tested at the Southwest Florida Research and Education Center in Immokalee (deliverable 5b) and the Florida Partnership for Water, Agriculture and Community in Hastings (deliverable 5d).

- a) In Immokalee, the nutrient sensors were installed adjacent to a row segment and water samples were taken from shallow wells in a tomato field. Manual water samples (grab samples) were taken twice a day between 8:00 am and 3:00 pm from April 22 to April 30, 2013. Irrigation was provided by seepage instead of drip, in order to develop a water table to take the samples from. Water samples taken at the Immokalee site were placed on ice and stored at 4°C until analysis at the soils laboratory at the SWFREC within 24 hours of collection.
- b) In Hastings, three nutrient sensors were installed at water control structures along a perimeter ditch in a seepage irrigated potato field. Grab samples were taken manually three times a week from April 10 to June 10, 2013. The collected samples were stored at 4 °C and sent to NELAC accredited University of Florida Analytical Research Laboratories (ARL) for analysis within 48 hours after sampling.

Laboratory analysis of nitrate and ortho-phosphate were conducted for both sites. The nitrate and ortho-phosphate concentrations from grab samples were compared with the results from the PraxSoft nutrient sensors.

The ortho-phosphate results had a better correlation to the 1:1 ratio line than the nitrate results. Correlation coefficients for ortho-phosphate were 0.75, 0.69, and 0.69 for Immokalee, Hastings, and combined data, respectively. The higher correlation for ortho-phosphate is high enough to infer that the results from the laboratory were in reasonable agreement with the PraxSoft nutrient sensor measurements for both sites. The correlation of nitrate concentrations among laboratory and PraxSoft sensor analyses were 0.50, for nitrate at Hastings indicating low

correlation between laboratory and sensor results. The numbers of samples taken at Immokalee were low due to the limited number of times in which the water table was high enough for sampling. Also, due to the limited number of samples above the detection limit, no statistical comparison could be performed for the PraxSoft nitrate sensor and grab samples in Immokalee. Potential methods for correcting disagreement in analysis by the PraxSoft nutrient sensors and IFAS laboratories are: 1) to install a bottom capped screened well to prevent coarse particles from clogging the filters; 2) to use a black tubing to prevent algae formation inside the tubing, or installation of the ceramic filter inside the enclosure; and 3) a complementary study of the variation of the internal temperature in the nutrient sensor enclosures under open field conditions. Drastic variation of internal temperature could be negatively impacting the quality of the reagents.

Deliverable description:

Deliverable 5b. Provide a final report on the installation and operation of the nutrient sensors; and the comparison of real time N and P concentrations from the nutrient sensors, to the ones taken from grab samples at a drip irrigated crop from a field in the Immokalee research center. (Morgan)

Deliverable 5d. Provide a final report on the installation and operation of the nutrient sensors; and the comparison of real time N and P concentrations from the nutrient sensors, to the ones taken from grab samples at a seepage irrigated crop from a field in the Hastings research center. (Zotarelli)

Introduction

The main objective of this project was to evaluate the PraxSoft instruments for analysis of nitrate and ortho-phosphate from soil and ditch water samples from tomato and potato crops grown at UF/IFAS research sites in Immokalee and Hastings under farm-like settings throughout Florida. Use of nutrient sensor systems with validated data quality could be invaluable in testing

water quality impacts of BMPs under field conditions. Excess applications of nitrogen and phosphorus from fertilizer can become dissolved contaminants and they can move through the soil profile and into drainage ditches and the water table. Analysis of soil and ditch water provides information on contaminant movement and fate. This analysis can also provide early warnings for contaminants that may reach major water bodies, and help with alternative strategies for irrigation and nutrient management.

Materials and Methods

The field test evaluation of PraxSoft wireless N and P sensors were carried out at two sites operated by the University of Florida. The first site ([site for deliverable 5b](#)) was the University of Florida's Southwest Florida Research and Education Center (SWFREC), near Immokalee, FL. The second site ([site for deliverable 5d](#)) was the University of Florida, Florida Partnership for Water, Agriculture and Community (PWAC) in Hastings, FL in the Tri-County Agricultural Area (TCAA). The soil at the SWFREC site is a sandy Spodosol with a confining spodic layer at less than one meter, classified as Immokalee fine sand (Sandy, siliceous, hyperthermic Arenic Alaquods). The soils in the TCAA production areas are sandy and loamy soils with an underlying impermeable layer within approximately 0.3 to 0.5 meters of the soil surface (Zhiwei, 2010). The soil at the experimental site is classified as sandy, siliceous, hyperthermic Arenic Ochraqualf belonging to Ellzey series (USDA, 1983). Tomatoes (*Solanum lycopersicum*) in Immokalee and potatoes (*Solanum tuberosum*) in Hastings and, were planted on March 13, 2013 and February 8, 2013; respectively.

Evaluations of N and P concentration in water samples

PraxSoft supplied the three nutrient sensors for each location. In Immokalee the nutrient sensors were installed in monitoring wells in the field and in Hastings each nutrient sensor was installed at each water control structure in a seepage irrigation area. The nutrient sensors were installed in Immokalee on April 3, 2013 and in Hastings on March 27, 2013. The nutrient sensors utilize a colorimetric method to measure concentrations of analytes in water samples.

Measurements were based on the proven spectrophotometric technique wherein the analyte of interest reacts with a reagent to form a colored product. The intensity of light that passes through a sample before and after addition of the reagent is measured and the absorbance is calculated using:

$$A = \text{Log} (I^0/I)$$

where ' I^0 ' is the intensity of light that passes through a sample before reagent is added, and ' I ' is the intensity of light after the reagent is added.

The absorbance can then be used to calculate the concentration of the analyte using Beer's Law.

$$A = \epsilon lc$$

where ' A ' is the measured absorbance, ' ϵ ' is the wavelength-dependent molar absorptivity, and ' c ' is the concentration. The equation can be rearranged to solve for concentration. In the case of the phosphate measurement, the intensity of the blue colored product that forms after addition of the reagent is measured at 700 nm, where it has a molar absorptivity of $17,000 \text{ cm}^{-1} \text{ M}^{-1}$.

The fluid flow path of the sensor/instrument is comprised of three pumps, each capable of driving two fluid lines; user-selected connective tubing; and an optical cell. The sample enters the instrument through the sample line and is pumped into an optical cell, where a reference measurement (I^0) is taken. Using a second pump, the reagent is then added to the flowing fluid line through a Y connector and permitted to thoroughly mix with the sample and fill the optical cell. Because the color formation requires time to develop, the pumps are then turned off. After sufficient time has elapsed for color development, sample measurements (I) are taken and the sample concentration is calculated. Once the measurement is complete, the optical cell and fluid lines are flushed in preparation for the next sample.

Water sample collection and analysis

Water sample collection and analysis protocols were discussed and agreed to by IFAS and PraxSoft during a joint meeting at Immokalee on January 29, 2013 and at Hastings on

January 30th, 2013, as required under each of their contracts with FDACS. IFAS followed such protocols, every time they took, handled, and analyzed a sample as described in Appendix 2.

PraxSoft nutrient sensors were set to take measurements of PO₄-P and NO₃-N every 12 hours if water was present in the ditch at Hastings or if there was enough water available in the sample wells at Immokalee. The analyzed sample data for N and P were sent wirelessly to a computer connected to the internet. The data were available on line at the following web site www.agnetlive.com.

Manual water samples (grab samples) were taken three times a week between April 10 and June 10, 2013 at Hastings. In Immokalee, manual samples were taken twice a day between 8:00 am and 3:00 pm from April 22 to April 30, 2013. At both locations, grab samples were taken by pressing the manual pump override switch located on the bottom of the boxes. Water samples were collected in 20 ml scintillation vials, acidified with H₂SO₄ and placed in a cooler. The collected samples from Hastings site were immediately stored at 4°C and sent to NELAC accredited University of Florida Analytical Research Laboratories (ARL). Samples taken at the Immokalee site were treated the same as described above and analyzed in the soils laboratory at the SWFREC. Samples analyzed at the SWFREC lab were conducted within 24 hours of collection (Immokalee) and 48 hours for ARL (Hastings). Both laboratories conducted analysis of nitrate and phosphate. The results from PraxSoft N and P sensor were compared to laboratory results. Sample duplicates were taken once a week as a quality control to verify the accuracy of the results from laboratory analysis, if the standard variation between the sample and duplicate were higher than 0.05 mg/L, the sample was discarded. The method of analysis at the SWFREC lab was flow analysis with a Lachat flow injection analysis with ion chromatography for nitrate nitrogen and ortho-phosphate. The method of analysis at the ARL in Gainesville for nitrate was EPA Method 353.2 and EPA Method 365.1 for ortho-phosphate.

Results and Discussion

Immokalee:

The pump supplying irrigation water for the drip system in Field 8 at SWFREC had to be removed for replacement. During the time that the well was out of service water was supplied

from a well in Field 10. The well and pump in Field 10 are larger than the well and pump in Field 8 and supplies more water than could be used by the drip system. Therefore, the rows of tomato in this study were irrigated by furrow supplied seepage irrigation from April 13, 2013 to May 23, 2013. Analysis of water samples collected twice daily from April 22, 2013 to April 30, 2013, data for nitrate and ortho-phosphorus are available on Tables 1 and 2, respectively. Because the dates with elevated water tables were restricted to dates during furrow irrigation, the number of samples collected were limited. Nutrient sensor 7111 only provided one water sample for analysis; however, the nitrate value was below the detection limit of 0.165 mg/L, therefore the data was not included in the analysis.

Table 1. Nitrate concentrations (mg/L) results from PraxSoft nutrient sensors and grab samples for Immokalee, FL, Spring 2013. Note: only results above the detection limit of 0.165 g/L were reported.

Sensor		7110		7111		7112	
Date	Time	UF-Immokalee	Nutrient Sensors	UF-Immokalee	Nutrient Sensors	UF-Immokalee	Nutrient Sensors
4/22/2013	AM	1.01	1.25			0.68	0.22
4/22/2013	PM	1.80	0.96				
4/24/2013	AM					1.86	0.29
4/24/2013	PM					0.17	0.0
4/25/2013	AM	0.65	1.55				
4/25/2013	PM						
4/26/2013	AM	1.34	1.29			0.38	0.0
4/29/2013	AM					0.85	0.12
4/29/2013	PM					0.57	0.39
4/30/2013	PM	1.83	0.77			0.62	1.04
5/22/2013	AM					0.30	2.33

In Immokalee, water samples were analyzed in triplicate to remove any controversy associated with sample analysis. Sample results with more than 10% variation or below the detection limit were removed prior to the regression analysis. Grab sample nitrate concentrations, when compared graphically with PraxSoft nutrient sensor measurements, indicated high variation from the 1:1 ratio line (Figure 1A). After the removal of data points with values below the detection limit, only 11 values were available for the regression analysis. Due to the small

number of available points and high variability, the regression analysis for nitrate in Immokalee was not significant (see Appendix A – Immokalee nitrate), therefore the values from Immokalee nitrate sensors could not be used. More samples are required to further evaluate the accuracy of the nitrate nutrient sensors in Immokalee conditions.

On the other hand, ortho-phosphate analytical results compared with PraxSoft nutrient sensors had much smaller variation ($R^2 = 0.75$) from the 1:1 ratio line indicating much better correlation for the two analytical methods (Figure 1B).

Table 2. Ortho-Phosphate concentrations (mg/L) results from PraxSoft nutrient sensors and grab samples for Immokalee, FL, Spring 2013.

Sensor		7110		7111		7112	
Date	Time	UF-Immokalee	Nutrient Sensors	UF-Immokalee	Nutrient Sensors	UF-Immokalee	Nutrient Sensors
4/22/2013	AM	2.17	3.31			0.72	0.82
4/22/2013	PM	2.53	2.50			0.89	1.00
4/23/2013	AM	2.78	2.75			0.97	1.20
4/24/2013	AM					0.81	1.68
4/24/2013	PM					1.40	1.62
4/25/2013	AM	1.85	0.30			1.43	1.90
4/25/2013	PM					2.11	2.27
4/26/2013	AM	1.85	0.21			1.79	1.83
4/26/2013	PM					1.53	1.72
4/29/2013	AM					1.04	1.17
4/29/2013	PM					1.06	1.44
4/30/2013	AM					0.75	3.64
4/30/2013	PM	0.92	0.06	0.01	6.02	0.02	1.17
5/22/2013	AM					1.29	1.04

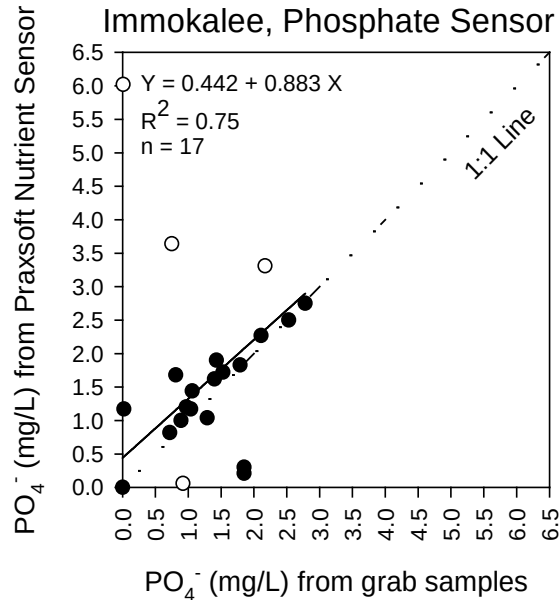


Figure 1. Correlation between ortho-phosphate (mg/L) concentrations from grab samples vs. vs. ortho-phosphate concentration from PraxSoft nutrient sensors in Immokalee. Observation: outliers were represented by white circles were not included in the regression analysis.

Hastings

The measurement of nitrate and ortho-phosphate by the Praxsoft nutrient sensors and the correspondent grab samples started on April 10, 2013, immediately after the equipment installation. Grab samples from each nutrient sensor were taken manually three times a week. Enough water for sampling was available in the ditch for most of the days during the evaluation period, except for the days reported in the Table 3. A total of 127, 123 and 147 samples were taken by nutrient sensors 7210, 7211 and 7212, respectively, during the evaluation period. A few days after the installation, the ceramic filter (outside the enclosure) clogged. The clogging problem was reduced after the installation of pre-filter before the ceramic filter, which required a weekly manual flushing of both filters (pre-filter and ceramic filter).

Table 3 - Nutrient sensor log describing manual sampling problems with nutrient sensors in Hastings, Spring 2013.

Nutrient Sensor	Date	Issue
7210	4/24	Clicking noise every 10-15 sec no water to sample
	5/1	Clogged
	5/6	Clogged
	5/9	Water stopped flowing after first sample
	5/14	No sample, low water in ditch. See Fig. 2a, 2b
	5/15	No sample, low water in ditch
	5/16	No sample, low water in ditch
	5/21	No sample, low water in ditch
	5/22	Two inch rain event filled ditches. See Fig. 3, sensor clogged
	5/30	Clogged
7211	4/29	Clogged
	5/6	Clogged
	5/15	No sample, low water in ditch. See Fig. 2a
	5/16	No sample, low water in ditch
	5/21	No sample, low water in ditch
	5/22	Two inch rain event filled ditches. See Fig. 3b, sensor clogged
	5/30	Clogged
7212	5/6	Clogged
	5/7	Down for repairs
	5/22	Clogged
	6/5	Pump down
	6/6	Pump down
	6/10	Pump down



Figure 2. Low water in ditch at Nutrient Sensor 7210 (left) and low water in ditch at nutrient sensor 7211 (right), Hastings, FL, Spring 2013.



Figure 3. Nutrient sensor 7210 after 2" rain event (left) and nutrient sensor 7211 (right) on May 22, 2013.

Analysis of water sample results collected from April 10, 2013 to June 10, 2013 for nitrate and ortho-phosphorus from nutrient sensors 7210, 7211, and 7212 are presented in Table 4) and Table 5, respectively.

Table 4. Nitrate concentrations (mg/L) results from PraxSoft nutrient sensors and grab samples for Hastings, FL, Spring 2013.

Sensor	7210		7211		7212	
Date	UF-ARL	Nutrient Sensors	UF-ARL	Nutrient Sensors	UF-ARL	Nutrient Sensors
4/10/2013						
4/16/2013	0.47	2.66				
4/18/2013	0.28	1.95				
4/24/2013	NS					
4/25/2013	0.18	0.52				
4/29/2013			NS			
5/1/2013	NS		NS			
5/7/2013						
5/8/2013			0.17	0.05		
5/9/2013					0.42	0.10
5/14/2013	NS				0.42	0.79
5/15/2013	NS		NS		0.48	1.05
5/16/2013	NS		NS		0.69	1.14
5/18/2013	NS		NS		NS	
6/5/2013			0.20	0.04	NS	
6/6/2013					NS	
6/10/2013	2.60	3.21	0.20	0.06	NS	

NS – no sample available

Table 5. Ortho-Phosphate concentrations (mg/L) results from PraxSoft nutrient sensors and grab samples for Hastings, FL, Spring 2013.

Sensor	7210		7211		7212	
Date	UF-ARL	Nutrient Sensors	UF-ARL	Nutrient Sensors	UF-ARL	Nutrient Sensors
4/16/2013	0.10	0.38	0.08	0.18	0.04	0.18
4/18/2013	0.18	0.67	0.02	0.09	0.02	0.11
4/24/2013	NS		0.01	2.87	0.01	0.11
4/25/2013	0.03	0.14	0.01	0.07	0.01	0.08
4/29/2013	NS		NS		0.01	0.13
5/1/2013	NS		NS		0.01	0.09
5/7/2013	0.77	0.77	0.09	0.25	NS	
5/8/2013	0.71	0.44	0.08	0.13	0.05	0.14
5/9/2013	0.88	0.15	0.08	0.15	0.06	0.14
5/14/2013	NS		0.05	0.60	0.05	0.16
5/15/2013	NS		NS		0.05	0.13
5/16/2013	NS		NS		0.05	0.15
5/18/2013	NS		NS		NS	
6/5/2013	1.32	1.75	0.04	0.98	NS	
6/6/2013	1.46	1.30	0.06	0.09	NS	
6/10/2013	3.21	6.50	0.06	0.19	NS	

NS – no sample available

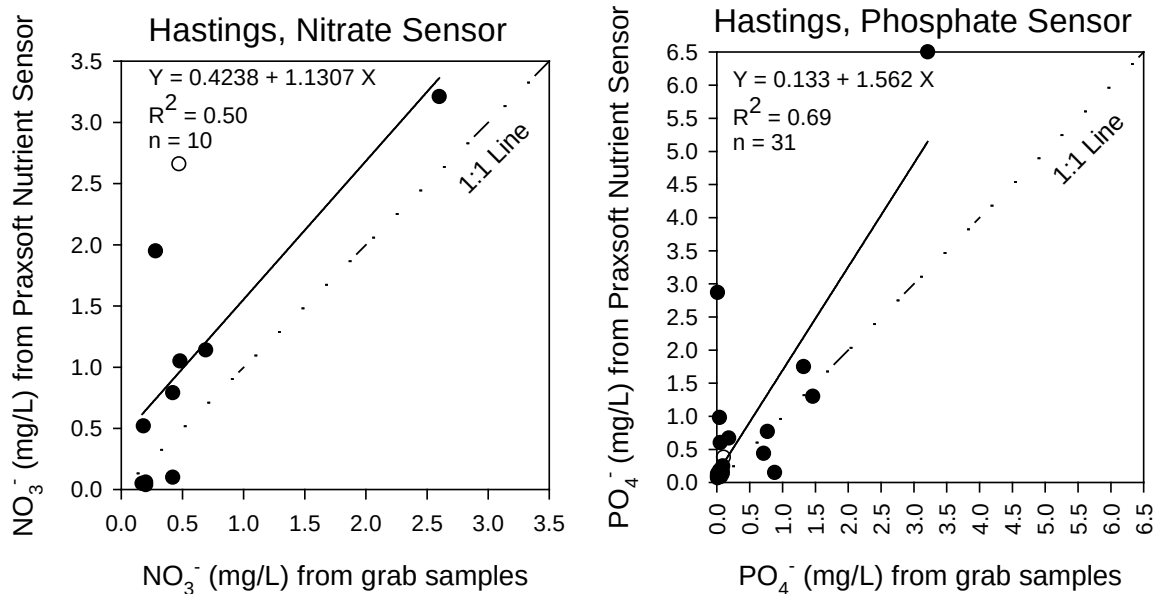


Figure 4. Correlation between nitrate concentrations (mg/L) and ortho-phosphate from grab samples vs. nitrate (A, left graph) and vs. ortho-phosphate (B, right graph) concentrations from PraxSoft nutrient sensors in Hastings.

In Hastings, grab sample analysis concentrations that were compared graphically with PraxSoft nutrient sensor measurements indicate some variation from the 1:1 ratio line (Figure 4A) with 70% of the samples above the 1:1 ratio line. A significant linear regression was fitted for the available nitrate data from grab samples and Praxsoft nutrient sensors (see Appendix – Hastings Nitrate), and the coefficient of correlation for both methods was $R^2 = 0.50$). For ortho-phosphate results, the comparison between laboratory analysis and PraxSoft nutrient sensors showed smaller variations from the 1:1 ratio line compared to nitrate results. With the exception of two outliers, there was higher correlation for the two analytical methods (Figure 4B). For phosphate, a linear regression was also fitted for the available data, which showed a better coefficient of correlation ($R^2 = 0.69$) compared to the nitrate sensor.

Hastings and Immokalee combined analysis

The ortho-phosphate concentrations from grab samples were compared to the results from the PraxSoft nutrient sensors (combined data from Hastings and Immokalee) resulted in 69% correlation ($R^2 = 0.69$) to the 1:1 ratio line (Figure 5). The ortho-phosphate results would indicate that the measurements from the laboratory were in agreement with the PraxSoft measurements for both sites. The data from Immokalee and Hastings for nitrate could not be combined because of the lack of statistical correlation between grab samples and nutrient sensor in Immokalee.

In order to improve the correlation of nitrate and phosphate measurements by the PraxSoft nutrient sensors at both Immokalee and Hastings, we suggest the following:

- a) to periodically inject a known standard (for nitrate and phosphate) in the nutrient sensor as reference to evaluate the performance of the unit, and this same known standard needs also to be sent to the laboratory for analysis.
- b) to install a bottom capped screened well to prevent coarse particles from clogging the filters;

- b) to use a black tubing to prevent algae formation inside the tubing, or installation of the ceramic filter inside the enclosure; this procedure was performed by Praxsoft during the evaluation period;
- c) to perform a complementary study of the variation of the internal temperature in the nutrient sensor enclosures under open field conditions. Drastic variation of internal temperature could be negatively impacting the quality of the reagents.

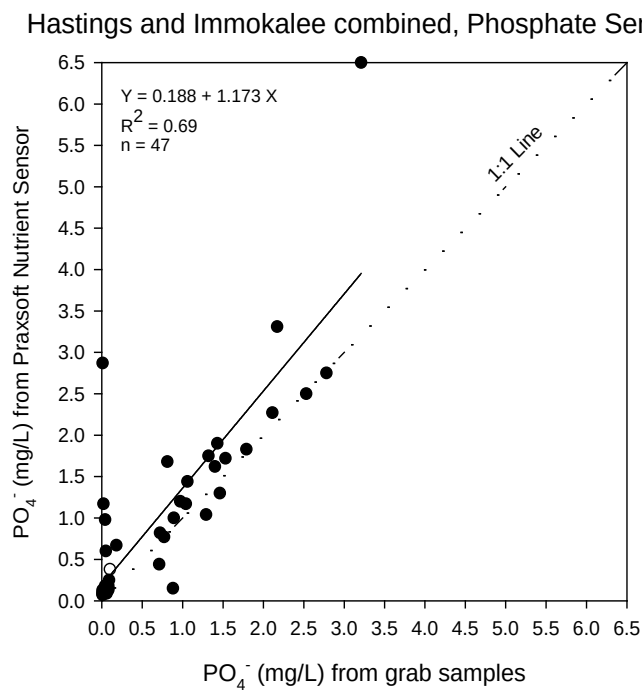


Figure 5. Correlation between ortho-phosphate concentrations in mg/L from grab samples vs. ortho-phosphate concentrations from PraxSoft nutrient sensors in combined Hastings and Immokalee data.

Conclusions:

Measurement of nitrate and phosphorus from ditch water or ground water sources by an automated device that samples and analyzes these nutrients can be useful in providing continuous data on water quality for best management practices studies. Samples of nitrate and ortho-phosphate by automated sensors provided by PraxSoft were compared at two locations. Ditch water was sampled at PWACS in Hastings and ground water was sampled at SWFREC in Immokalee. Sampling procedures were agreed to by IFAS and PraxSoft prior to study initiation. Manual water samples were collected using the sampling button at the bottom of the nutrient sensor box provided by PraxSoft. The samples were acidified in the field and stored on ice until placed in a refrigerator at 4°C prior analysis. Two separate laboratories were used for analysis at each location. Samples were analyzed within 24 hours after sampling at SWFREC and within 48 hours by ARL for samples taken at Hastings. Nitrate and ortho-phosphate measurements from PraxSoft nutrient sensors from ditch and ground water samples were compared to laboratory analysis. The coefficient of correlation (R^2) between the automated sensor and laboratory analysis was 0.50 for Hastings. The number of samples taken at Immokalee was low due to a limited number of times in which water tables were elevated enough for sampling. Also, due to the limited number of samples with results above the detection limit, no statistical comparison could be performed for PraxSoft nitrate sensor and grab samples in Immokalee. The automated measurement of ortho-phosphate by the PraxSoft sensors was very similar ($R^2 = 0.69$ for Hastings and 0.75 for Immokalee) to the laboratory analysis, indicating reasonable but highly variable agreement. However, a lower coefficient of correlation for nitrate was obtained for PraxSoft sensors compared to laboratory results, which was due to the short period of evaluation and due to the need to discard all data points from samples below the detection limit of the laboratory analysis. More data points comparing results from grab samples and nutrient sensor for nitrate and ortho-phosphate are required to improve the robustness of the proposed evaluation under field conditions. It is our recommendation that further evaluation of both nitrate and ortho-phosphate nutrient sensors under field conditions is required, including the use of known standards.

Appendix – 1

Statistical Analysis:

Immokalee - Nitrate

Data Source: Data 1 in Nutrient Sensor N and P - 2013 - Immok and Hast 3 with detection limits

Equation: Polynomial, Linear

$$f = y_0 + a * x$$

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.459	0.211	0.123	0.466

	Coefficient	Std. Error	t	P
y0	0.196	0.282	0.696	0.5039
a	0.375	0.242	1.551	0.1553

Analysis of Variance:

	DF	SS	MS
Regression	2	4.166	2.083
Residual	9	1.956	0.217
Total	11	6.122	0.557

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	0.523	0.523	2.406	0.1553
Residual	9	1.956	0.217		
Total	10	2.479	0.248		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.1700)

W Statistic= 0.8970 Significance Level = 0.0500

Constant Variance Test Passed (P = 0.6932)

Immokalee – Phosphate

Nonlinear Regression

Data Source: Data 1 in Nutrient Sensor N and P - 2013 - Immok and Hast 2

Equation: Polynomial, Linear

$$f = y_0 + a * x$$

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.868	0.754	0.737	0.406

	Coefficient	Std. Error	t	P
y0	0.442	0.199	2.224	0.0419
a	0.883	0.130	6.777	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	2	51.813	25.907
Residual	15	2.478	0.165
Total	17	54.291	3.194

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	7.586	7.586	45.923	<0.0001
Residual	15	2.478	0.165		
Total	16	10.064	0.629		

Hasting - Nitrate

Data Source: Data 1 in Nutrient Sensor N and P - 2013 - Immok and Hast 3 with detection limits

Equation: Polynomial, Linear

$f = y_0 + a \cdot x$

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.7104	0.5047	0.4497	0.8237

	Coefficient	Std. Error	t	P
y0	0.4238	0.3236	1.3097	0.2227
a	1.1307	0.3734	3.0282	0.0143

Analysis of Variance:

	DF	SS	MS
Regression	2	18.3908	9.1954
Residual	9	6.1057	0.6784
Total	11	24.4965	2.2270

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	6.2212	6.2212	9.1703	0.0143
Residual	9	6.1057	0.6784		
Total	10	12.3270	1.2327		

Statistical Tests:

Normality Test (Shapiro-Wilk)

Failed (P = 0.0154)

W Statistic= 0.8163

Significance Level = 0.0500

Constant Variance Test

Passed (P = 0.4667)

Hastings – Phosphate**Nonlinear Regression****Data Source:** Data 1 in Nutrient Sensor N and P - 2013 - Immok and Hast 2**Equation:** Polynomial, Linear $f = y_0 + a \cdot x$ **R Rsqr Adj Rsqr Standard Error of Estimate**

0.831 0.690 0.680 0.705

	Coefficient	Std. Error	t	P
y0	0.133	0.140	0.949	0.3506
a	1.562	0.194	8.038	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	2	43.938	21.969
Residual	29	14.428	0.498
Total	31	58.365	1.883

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	32.145	32.145	64.613	<0.0001
Residual	29	14.428	0.498		
Total	30	46.573	1.552		

Combined Immokalee and Hastings – Nitrate**Data Source:** Data 1 in Nutrient Sensor N and P - 2013 - Immok and Hast 3 with detection limits**Equation:** Polynomial, Linear $f = y_0 + a \cdot x$ **R Rsqr Adj Rsqr Standard Error of Estimate**

0.668 0.446 0.415 0.615

	Coefficient	Std. Error	t	P
y0	0.147	0.209	0.703	0.4910
a	0.806	0.212	3.808	0.0013

Analysis of Variance:

	DF	SS	MS
Regression	2	16.655	8.328
Residual	18	6.803	0.378
Total	20	23.459	1.173

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	5.480	5.480	14.500	0.0013
Residual	18	6.803	0.378		
Total	19	12.283	0.646		

Statistical Tests:

Normality Test (Shapiro-Wilk) Passed (P = 0.1648)

W Statistic= 0.9315 Significance Level = 0.0500

Combined Immokalee and Hastings – Phosphate**Nonlinear Regression**

Data Source: Data 1 in Nutrient Sensor N and P - 2013 - Immok and Hast 2

Equation: Polynomial, Linear

$$f = y_0 + a \cdot x$$

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.835	0.698	0.691	0.668

	Coefficient	Std. Error	t	P
y0	0.188	0.125	1.503	0.1398
a	1.173	0.115	10.198	<0.0001

Analysis of Variance:

	DF	SS	MS
Regression	2	92.552	46.276
Residual	45	20.105	0.447
Total	47	112.656	2.397

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	46.467	46.467	104.007	<0.0001
Residual	45	20.105	0.447		
Total	46	66.572	1.447		

Appendix 2. Grab sample methods.

PraxSoft Nutrient Sensors: Methods

Nitrate – US EPA 353.4

Phosphate, Ortho – US EPA 365.3

Nitrate Standard: 2.5 mg/L NO_3^-

Stock Solution Information: Ricca Chemical Company

Nitrogen Standard

Cat. No. 5457-4

Lot 2201695

1 mL = 0.100 mg N (100 ppm N)

1.00 mL = 442.7 $\mu\text{g NO}_3^-$ (442.7 ppm NO_3^-)

Phosphate Standard: 0.25 mg/L PO_4^{3-}

Stock Solution Information: **Diluted from ENCO Labs' 50 mg/L PO_4^{3-} stock. **

J.T. Baker

Potassium Phosphate, Monobasic, Crystal

Baker Analyzed A.C.S. Reagent

Product No. 3246

Lot No. H21149

ENCO Labs: Methods

Nitrate – SM18 4500- NO_3^- -H

Phosphate, Ortho – US EPA 365.1 or 365.3 (depends on sample volume & equipment available)

Grab Sample Methods

1. Grab a sterile plastic 250 mL bottle that has the correct label for the specific site location and analysis. Carefully, place the bottle under the filtered sample waste line and fill container. Close the lid tightly. Do this for all sample bottles.
NOTE: The flow through valve contains a 60-micron filter. Orthophosphate does not require a preservative, but must be filtered at the site. Pre-preserved sample bottles containing concentrated Sulfuric Acid (H_2SO_4) will be used for grab samples involved in the Nitrite + Nitrate analysis to have a final pH equivalent to 2.0.
2. Place in cooler filled with ice sitting up and maintain a temperature of 4°C. Do NOT freeze the samples.
3. Head to next sampling site and repeat steps 1 and 2.
4. After all samples have been taken at each site and placed into the cooler, send samples to a laboratory that has been certified by the Florida Department of Health, Bureau of Laboratories, and the National Environmental Laboratory Accreditation Program (NELAP) (i.e. ENCO Laboratories).
NOTE: The analysis for Nitrite + Nitrate is SM18 4500- NO_3^- -H and has a hold time of **28 days**. For Orthophosphate, the analytical method is EPA 365.1 (or 365.3) with a hold time of **48 hours**. Please make sure the same standard concentrations (see above) are used when monitoring equipment performance.

Thomas

1/28/2013