U.S. Environmental Protection Agency
Region 4, Science and Ecosystem Support Division
Athens, Georgia

OPERATING PROCEDURE

Title: **Bottom Water Sampling for Sulfide**	
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Revision History

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1 General Information

1.1 Purpose

The purpose of this operating procedure is to describe the procedures, methods and considerations to be used when obtaining a bottom water sample for sulfide. It was developed as a rapid surrogate for pore water sampling in the Everglades. The rationale for the procedure is discussed in Appendix A of this document. Appendix A is a proposal from 2015 to conduct a pilot study to demonstrate the utility of the method. The study was conducted and the findings are presented in Appendix B. This method is potentially applicable to any shallow lentic water body and to other gases and volatile organic compounds.

1.2 Scope/Application

This document describes procedures generic to all bottom water sampling methods to be used by field personnel when collecting and handling sulfide samples in the field. On the occasion that Science and Ecosystem Support Division (SESD) personnel determine that any of the procedures described in this section are inappropriate, inadequate or impractical and that another procedure must be used to obtain a bottom water sample, the variant procedure will be documented in the field logbook, along with a description of the circumstances requiring its use. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

1.3 Documentation/Verification

This procedure was prepared by persons deemed technically competent by SESD management, based on their knowledge, skills and abilities, and has been tested in practice and reviewed in print by a subject matter expert. The official copy of this procedure resides on the SESD local area network (LAN). The Document Control Coordinator is responsible for ensuring the most recent version of the procedure is placed on the LAN and for maintaining records of review conducted prior to its issuance.

1.4 References

SESD Operating Procedure for Control of Records, SESDPROC-002, Most Recent Version.

SESD Operating Procedure for Sample and Evidence Management, SESDPROC-005, Most Recent Version.

SESD Operating Procedure for Logbooks, SESDPROC-010, Most Recent Version.

SESD Operating Procedure for Field Equipment Cleaning and Decontamination,

SESDPROC-205, Most Recent Version.

SESD Operating Procedure for Field Equipment Cleaning and Decontamination at the FEC, SESDPROC-206, Most Recent Version.

USEPA. ASBLOQAM. Analytical Support Branch Laboratory Operations and Quality Assurance Manual. Region 4, Science and Ecosystem Support Division, Athens, GA. Most Recent Version.

USEPA. SHEMP. Safety, Health and Environmental Management Program Procedures and Policy Manual. Science and Ecosystem Support Division, Region 4, Athens, GA. Most Recent Version.

SESD Operating Procedure for Field Sampling Quality Control, SESDPROC-011, Most Recent Version.

1.5 General Precautions

1.5.1 Safety

Proper safety precautions must be observed when collecting bottom water samples. Refer to the SESD Safety, Health and Environmental Management Program Procedures and Policy Manual (most recent version) and any pertinent site-specific Health and Safety Plans (HASP) for guidelines on safety precautions. These guidelines, however, should only be used to complement the judgment of an experienced professional. When using this procedure, minimize exposure to potential health hazards through the use of protective clothing, eye wear, and gloves. Address chemicals that pose specific toxicity or safety concerns and follow any other relevant requirements, as appropriate.

1.5.2 Procedural Precautions

The following precautions should be considered when collecting bottom water samples:

- Special care must be taken not to contaminate samples. This includes storing samples in a secure location to preclude conditions which could alter the properties of the sample. Samples shall be custody sealed during long-term storage or shipment.
- Collected samples are in the custody of the sampler or sample custodian until the samples are relinquished to another party.
- If samples are transported by the sampler, they will remain under his/her custody or be secured until they are relinquished.
- Shipped samples shall conform to all U.S. Department of Transportation (DOT) rules of shipment found in Title 49 of the Code of Federal Regulations (49 CFR parts 171 to 179), and/or International Air Transportation Association (IATA)

hazardous materials shipping requirements found in the current edition of IATA's Dangerous Goods Regulations.

- Documentation of field sampling is done in a bound logbook. Chain-of-custody documents shall be filled out and remain with the samples until custody is relinquished.
- All shipping documents, such as bills of lading, will be retained by the project leader and stored in a secure place.

1.5.3 Records

Information generated or obtained by SESD personnel will be organized and accounted for in accordance with SESD records management procedures found in SESD Operating Procedure for Control of Records, SESDPROC-002 (most recent version). Field notes, recorded in a bound field logbook, will be generated, as well as chain-of-custody documentation, in accordance with SESD Operating Procedure for Logbooks, SESDPROC-010 (most recent version), and SESD Operating Procedure for Sample and Evidence Management, SESDPROC-005 (most recent version).

2 Sampling Methodology

2.1 General

The bottom water sampling techniques and equipment described in this procedure are designed to minimize effects on the chemical and physical integrity of the sample. If the procedures in this section are followed, a representative sample of sulfide in bottom water should be obtained.

2.2 Collection Considerations

Wading may disrupt bottom sediments causing biased results; therefore, the sampler should enter the area by boat, or by other platform such as a helicopter. If sampling in water deeper than the length of the sampler, extensions may be utilized. The sampling device is suitable for use only over fine-grained material (no gravel or cobble).

2.3 Summary of Procedure

Bottom water is the water at the bottom of the water column, immediately above the soil-water interface. It is collected using a bottom water sampling device (Figure 1). The device is constructed of readily available PVC plumbing parts, a magnetic filter holder, a particulate filter, Tygon® tubing, and a sampling syringe with a three-way valve. The sampling end of the device is placed on the bottom at the soil-water interface, and bottom water is extracted through the tubing and into the sampling syringe. Other similar devices may be used provided that the integrity of the sample is maintained and no ambient air or surface water is allowed in contact with the sample.

2.4 Sampling Equipment

The bottom of the sampler consists of a PVC toilet flange, a reducing adapter, a filter holder, an inside adapter, tubing, and a riser. The tubing extends about a meter beyond the end of the riser. Coffee filters, cut or purchased to size, work well as filters for particulate matter. The particulate filter is placed in the magnetic filter holder. The tubing is sealed to the inside adapter with silicone caulk, forming a water tight seal, so that only bottom water suctioned through the particulate filter can enter the tubing and sampling syringe. A three-way valve is attached to the top end of the tubing. A 60-ml syringe is attached to the in-line port of the valve for purging the sampler of entrained surface water and air before drawing the sample. Once the tubing is purged, the valve is switched and the sample is collected into a pre-preserved 60-ml syringe attached to the side port.

There are many modifications that can be incorporated into the procedure to satisfy data quality objectives for a specific application. For example, Teflon® or other tubing may be used instead of Tygon®. The procedures discussed in the following sections provide guidance on the basic operation of bottom water sampling devices and issues to consider when collecting bottom water.

2.5 Bottom Water Sampler Deployment Considerations

When deploying the bottom water sampling device, care must be taken to not disturb the sampling area. This method was developed specifically to collect a small sample of water at the soil-water interface from a floating platform for the dissolved gas hydrogen sulfide. Any modifications for other purposes must be carefully evaluated to minimize the risk of drawing unintended waters into the sample.

2.6 Bottom Water Collection Procedure

With gloved hands, attach a purging syringe to the valve and sample syringe assembly. Set aside. If floating periphyton covers the water, gently move it aside by hand or with the sampler. Rinse the bottom of the sampler in ambient surface water. Install a new filter in the filter holder. Select a location on the bottom where the device will be placed. Ideally, the spot will be level and clear of plant stems or other obstacles that could prevent complete contact with the bottom. Gradually lower the sampler through the water, slowly tilting and waving it to avoid vegetation as much as possible. Do not trap floating periphyton or plant stems under the sampler. Pause before contacting the bottom, then gently touch the device down.

After the bottom water device has been successfully deployed, attach the valve and syringes assembly to the end of the tubing (Figure 2). Before collecting the sample, purge all air and surface water from the device, including the tubing, with the appropriate amount of bottom water. The volume to be purged is determined by the length and diameter of the tubing and by the volume of the inside adapter. After the sample syringe is filled, close the valve, detach the tubing, remove the purging syringe, and check the sample syringe for air bubbles. Purge as necessary and re-close the valve. Discard the filter and drain the tubing. Empty the purging syringe and save for re-use.

2.7 Quality Control

For any one sample collected, control or background samples are constituted of all other samples collected in the same survey. Equipment blanks must be collected since equipment is cleaned in the field and reused on-site.

2.8 Specific Sampling Equipment Quality Assurance Techniques

All equipment used to collect bottom water samples shall be cleaned as outlined in the SESD Operating Procedure for Field Equipment Cleaning and Decontamination, SESDPROC-205 (most recent version) or SESD Operating Procedure for Field Equipment Cleaning and Decontamination at the FEC, SESDPROC-206 (most recent version) and repaired, if necessary, before being stored at the conclusion of field studies. Cleaning procedures utilized in the field or field repairs shall be thoroughly documented in field records.

3 Special Sampling Considerations

3.1 Special Precautions for Bottom Water Sampling

- A clean pair of new, non-powdered, disposable latex gloves will be worn each time a different location is sampled and the gloves should be donned prior to handling sampling equipment and sampling. The gloves should not come in contact with the media being sampled and should be changed any time during sample collection when their cleanliness is compromised.
- Samplers must use new, verified, certified clean disposable equipment, or pre-cleaned non-disposable equipment. Non-disposable equipment should be pre-cleaned according to procedures contained in SESD Operating Procedure for Field Equipment Cleaning and Decontamination (SESDPROC-205).

3.2 Sample Handling and Preservation Requirements

1. The pre-preserved 60-ml sample syringe becomes the sample container after it is filled, purged, closed, and labeled. Sulfide syringes will be stored in the field in a separate case and kept out of the sun. Since sulfide is volatile and easily oxidized into sulfate, the syringe must not be opened until analysis.
2. The preservative for sulfide is zinc acetate. Since the holding time for sulfide is only 12 hours, samples are analyzed at the field base laboratory in the evening. Other requirements for analysis of water samples are found in the USEPA Analytical Support Branch *Laboratory Operations and Quality Assurance Manual (*USEPA ASBLOQAM).

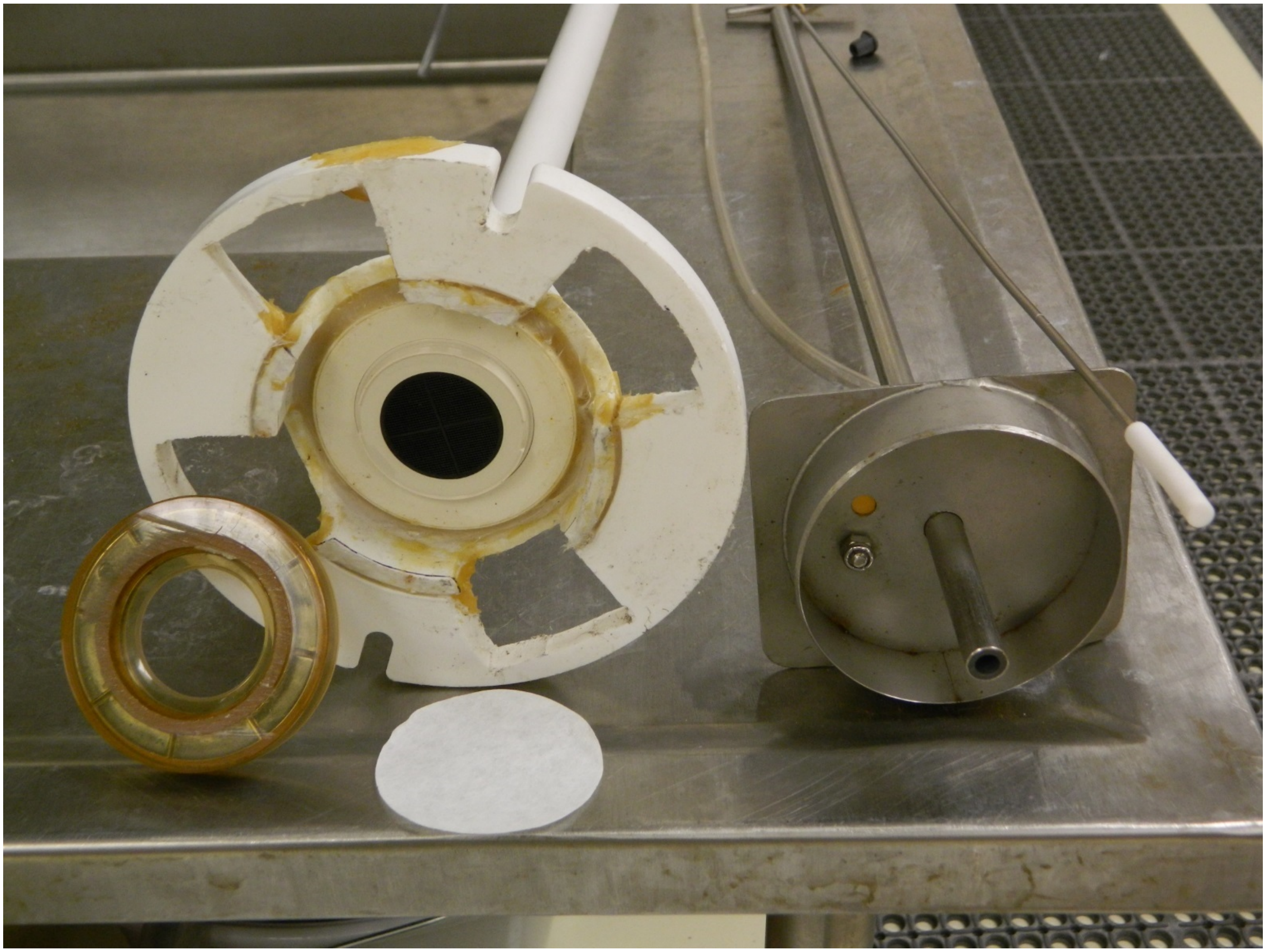

Figure 1. Bottom water sampler and pore water "sipper"
Bottom water sampling device on left; pore water sipper on right for comparison.

Figure 2. Valve and syringes assembly
Valve and syringes assembly attached to sampler in place. Purging shown.

APPENDIX A

Comparison of Sulfide in Bottom Water and Pore Water in the Everglades:
A Proposal for Collaboration with Florida International University

Peter I. Kalla, Ph.D., Leader
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Introduction

Sulfide is a constituent of interest in the Everglades for a number of reasons. It is the metabolic end-product of sulfur-reducing bacteria, which produce methylated mercury, a potent neurotoxin in birds and mammals, as a by-product (Fink and Rawlik 2000, Jeremiason et al. 2006, Mitchell et al. 2006). The source of sulfur for these bacteria is sulfate in surface water. Sulfide itself in surface water is toxic to aquatic macroinvertebrates (USEPA 2004), and in the root zone to emergent macrophytes that are characteristic of the native Everglades marsh (Li et al. 2009).

Its biogeochemical importance notwithstanding, sulfide is difficult to sample in the environment. In previous studies of the Everglades marsh (Scheidt and Kalla 2007), investigators obtained soil pore water to assess sulfide, since it is formed under the reducing conditions found in saturated soil (Barton and Fauque 2009). Pore-water “sipper” devices have been developed by the U.S. Environmental Protection Agency (EPA) (Parsons 2013), and modified for the Everglades Ecosystem Assessment Program (aka REMAP) by Florida International University (FIU). These devices are effective, but their proper deployment and use can be time-consuming.

Phase IV of Everglades REMAP was conducted in 2013 and 2014 with a budget far less than that of previous phases. In REMAP, helicopters are used to access over 100 stations scattered throughout the marsh. Since helicopter time is one of the most expensive items in the budget, it was imperative to reduce time on-station in order to reach all the stations before running out of funds.

As a rapid alternative to sampling sulfide in pore water, in Phase IV EPA developed a device to sample sulfide in bottom water. The device consists of tubing enclosed in a pipe and attached to a filter holder that is supported by a flange. The target medium of this device is the centimeter of water in immediate contact with the soil surface. This soil-water, biogeochemically active, interface is sampled by divers in EPA nutrient flux studies in estuaries (Parsons 2015, McNeal et al. 2014), and by many other investigators for various purposes (Gardner et al. 2009).

Bottom-water sulfide in Phase IV did not correlate with surface-water sulfate as well as pore-water sulfide did in previous phases (Kalla 2014). It is therefore advisable to further evaluate bottom water as a medium for assessing sulfide by doing side-by-side comparisons with pore water.

Methods

The two media would be sampled together by FIU airboat at locations covering a range of expected sulfide concentrations (Table 1). These locations were selected on the basis of historical pore-water concentrations (Scheidt and Kalla 2007) and relative ease of access by airboat. Triplicate samples of both media would be collected at a minimum of three places, with additional stations included if time allows. Selection of all places would be at the discretion of the airboat captain and other collaborating personnel at FIU with local knowledge of current conditions in the Everglades.

The samples would be collected in accordance with established protocols (Parsons 2013, Kalla et al. 2014) and analyzed by EPA Method 8131-modified [Hach]. Analyses would be performed nightly upon return to FIU by one of EPA's in-house contract field chemists. Bivariate statistics (correlation and regression) would be performed in the Statistica package (StatSoft 2014) to explore and evaluate the relationship between the two media.

Benefits and Implications of the Proposed Work

If sulfide in bottom water is closely correlated to sulfide in pore water, the former could be used as a rapid and therefore inexpensive surrogate for the latter in future REMAP surveys. Applications in other environments are also possible. By positing that concentrations in water near the bottom of the water column are driven by those in soil near the top of the soil surface, one could estimate pore-water sulfide from bottom-water data.

If there is no relationship between the two media, the Phase IV sulfide data would be presented in a more cautious light. Of greater consequence, in future phases EPA would have to return to pore water sampling to assess sulfide.

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Table 1. Potential places for comparative sampling of sulfide in bottom water and pore water in the Everglades. This table lists nine potential places to get triplicate samples of both media.

Relative Bottom-water Sulfide Concentration	Location	Access point	2014 REMAP Stations	Coordinates
High	Western Water Conservation Area (WCA) 2A	Boat Ramp at Twenty-six Mile Bend on east side of US-27	159 303 301	-80.406352 26.246430 -80.395323 26.281749 -80.478601 26.345474
Medium	Eastern WCA3A	Boat Ramp west of US-27 near C-11 Extension	107	-80.501221 26.061269
Medium	Northeastern WCA3A	Boat Ramp at Twenty-six Mile Bend on west side of US-27	115 255	-80.488833 26.183911 -80.512131 26.187276
Low	Southwestern WCA3A	Boat Ramp on US-41 at S-12C	116	-80.741667 25.786713
Low	Southwestern WCA3A	Boat Ramp on US-41 at S-12A	244 249	-80.831421 25.789500 -80.815010 25.786471

[ADDENDUM: Most of the stations in the table above were sampled in 2015. In 2016 station 107 was sampled, as were three additional stations in the Eastern Marl Prairie in the Chekika District of Everglades National Park. Peter I. Kalla, 23 February 2017]

APPENDIX B

Results of Demonstration Study

See Appendix A for background material, including introduction, study area, and methods.

Raw and Natural Log-Transformed Data, by Station

Station	PW sulfide (mg/l)	BW sulfide (mg/l)	nlog PW	nlog BW
159A	10.2	4.4	2.322388	1.481605
159B	7.2	5.2	1.974081	1.648659
159C	7.6	4.8	2.028148	1.568616
303A	6	4.4	1.791759	1.481605
303B	6.4	3.8	1.856298	1.335001
303C	9	3.2	2.197225	1.163151
115A	2.72	2.58	1.000632	0.947789
115B	1.8	2.88	0.587787	1.05779
115C	2.72	2.22	1.000632	0.797507
107A	1.008	1.496	0.007968	0.402795
107B	0.964	1.812	-0.03666	0.594431
107C	1.728	3.008	0.546965	1.101275
244A	0.03	0.024	-3.50656	-3.7297
244B	0.035	0.016	-3.35241	-4.13517
244C	0.024	0.034	-3.7297	-3.38139
249A	0.012	0.011	-4.42285	-4.50986
249B	0.009	0.024	-4.71053	-3.7297
249C	0.005	0.032	-5.29832	-3.44202
255A	0.073	0.072	-2.6173	-2.63109
255B	0.127	0.108	-2.06357	-2.22562
255C	0.077	0.066	-2.56395	-2.7181
ENP1A	0.023	0.01	-3.77226	-4.60517
ENP1B	0.038	0.004	-3.27017	-5.52146
ENP1C	0.038	0.003	-3.27017	-5.80914
ENP2A	0.026	0.004	-3.64966	-5.52146
ENP2B	0.037	0.005	-3.29684	-5.29832
ENP2C	0.056	0.006	-2.8824	-5.116
ENP3A	0.051	0.003	-2.97593	-5.80914
ENP3B	0.093	0.003	-2.37516	-5.80914

Linear Estimation of Pore Water Sulfide (PW) on Bottom Water Sulfide (BW), by Least Squares:

(natural log-transformed data)

PW = 0.775863*BW + 0.151417

Linear Regression Statistics:

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.90787646
R Square	0.82423967
Adjusted R Square	0.81773002
Standard Error	1.0608193
Observations	29

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	142.4883089	142.4883089	126.6183	1.0654E-11
Residual	27	30.38411492	1.12533759		
Total	28	172.8724238			

Linear Regression Statistics, Continued:

RESIDUAL OUTPUT

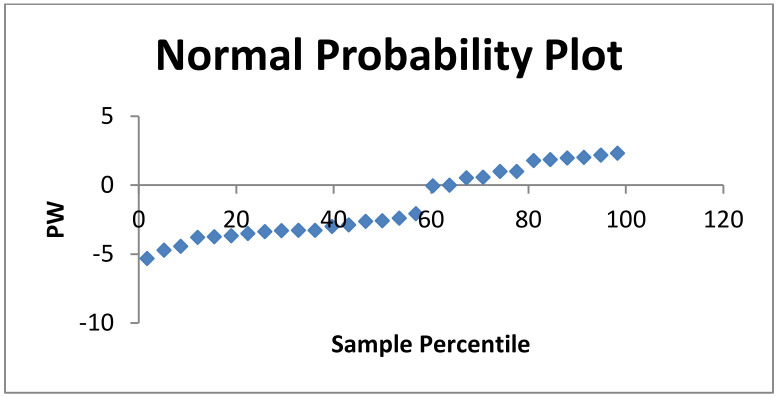
Observation	*Predicted PW*	*Residuals*	*Standard Residuals*
1	1.30093974	1.021447983	0.980555039
2	1.43055084	0.543530189	0.521770344
3	1.36844865	0.659699594	0.633288989
4	1.30093974	0.490819732	0.471170113
5	1.18719551	0.669102479	0.642315437
6	1.05386324	1.143361339	1.097587681
7	0.88677227	0.113859614	0.109301325
8	0.9721179	-0.38433124	-0.368944809
9	0.77017385	0.23045803	0.221231806
10	0.46393113	-0.45596296	-0.437708807
11	0.61261468	-0.64927867	-0.623285259
12	1.0058564	-0.45889173	-0.440520321
13	-2.7423203	-0.76423762	-0.733641908
14	-3.0569057	-0.29550152	-0.283671329
15	-2.472082	-1.25761948	-1.207271578
16	-3.3476165	-1.07523211	-1.03218596
17	-2.7423203	-1.96821042	-1.889414517
18	-2.5191184	-2.77919899	-2.667935735
19	-1.8899475	-0.72734829	-0.698229422
20	-1.5753621	-0.48820606	-0.468661081
21	-1.9574565	-0.6064934	-0.582212864
22	-3.4215642	-0.3506969	-0.336656995
23	-4.1324803	0.862311215	0.827789199
24	-4.3556822	1.08551312	1.042055373
25	-4.1324803	0.482821594	0.463492174
26	-3.9593515	0.662514121	0.635990839
27	-3.8178949	0.935491331	0.8980396
28	-4.3556822	1.379752593	1.324515178
29	-4.3556822	1.980526453	1.901237484

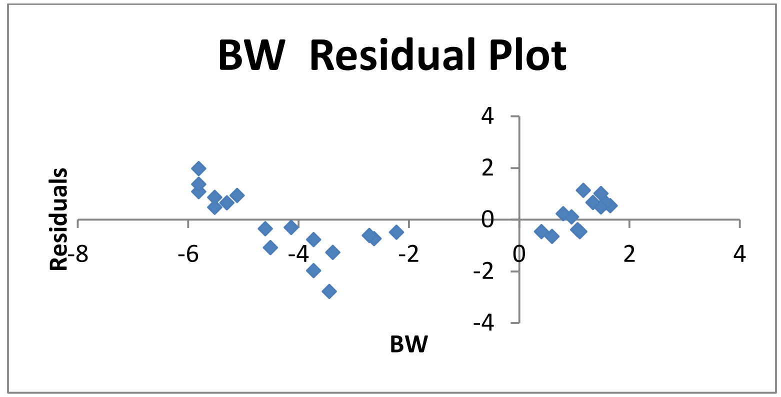
PROBABILITY OUTPUT

Percentile	*PW*
1.72413793	-5.298317
5.17241379	-4.710531
8.62068966	-4.422849
12.0689655	-3.772261
15.5172414	-3.729701
18.9655172	-3.649659
22.4137931	-3.506558
25.862069	-3.352407
29.3103448	-3.296837
32.7586207	-3.270169
36.2068966	-3.270169
39.6551724	-2.97593
43.1034483	-2.882404
46.5517241	-2.617296
50	-2.56395
53.4482759	-2.375156
56.8965517	-2.063568
60.3448276	-0.036664
63.7931034	0.007968
67.2413793	0.546965
70.6896552	0.587787
74.137931	1.000632
77.5862069	1.000632
81.0344828	1.791759
84.4827586	1.856298
87.9310345	1.974081
91.3793103	2.028148
94.8275862	2.197225
98.2758621	2.322388

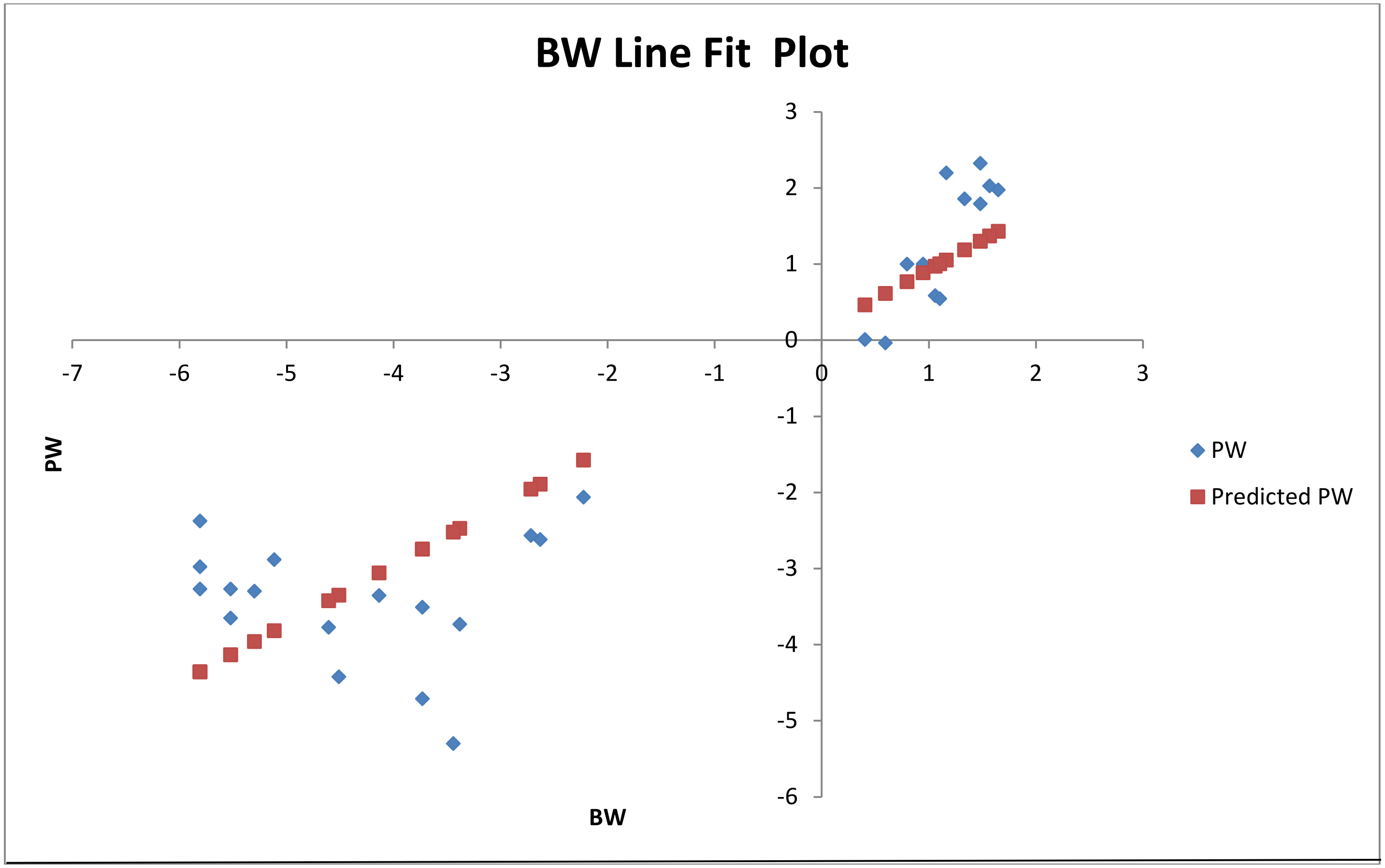
Linear Regression Statistics, Continued:

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.15141745	0.243795231	0.621084529	0.539752	-0.34880905	0.651644	-0.34880905	0.65164394
BW	0.7758631	0.068950416	11.25247879	1.07E-11	0.63438853	0.917338	0.63438853	0.91733767





Linear Regression Statistics, Continued:



Conclusion

A method validation was completed to compare bottom water to pore water for sulfide, utilizing the Science and Ecosystem Support Division of EPA Region 4 Operating Procedure for Field Sampling and Measurement Procedures and Procedure Validation (Lewis 2012). With a large coefficient of determination and a highly significant F statistic, the procedure indicated that bottom water was comparable to pore water. Therefore, bottom water is an acceptable surrogate for pore water for assessing sulfide in the Everglades.