Biological Analysis Report

NW-DIST-2022-10-14-01

Florida DEP Laboratory
2600 Blair Stone Road
Tallahassee, FL 32399-2400
DOH Accreditation E31780

Overflow Analyses Performed By:
University of West Florida
11000 University Parkway
Pensacola, FL 32514
DOH Accreditation E71969

Event Description:	**Walton Washinton Run**
Request ID:	**RQ-2022-10-10-37**
Customer:	**NW-DIST**
Project ID:	**SMP**

Send Reports to:
FL Dept. of Environmental Protection
160 W Government Street
Suite 208
Pensacola, FL 32502-5794
Attn: Myranda Butler

For additional information please contact
Cheryl Swanson - Administrator
Sarah Menz, Ph.D. - Bench Biology & Aquatic Toxicity
Puja Jasrotia, Ph.D. - Molecular Biology & Taxonomy
Thekkekalathil Chandrasekhar, Ph.D, QA Officer
Phone (850) 245-8177

Certified by: Cheryl A. Swanson, Program Administrator

Date Certified: 04-NOV-2022 16:01

Case Narrative

Unless otherwise noted, all samples included in this report were received in accordance with protocols referenced in Chapter 62-160, Florida Administrative Code (F.A.C.). Results published in this report pertain only to the samples as submitted to, and received by the laboratory. All times in this report are adjusted to the applicable Eastern Time Zone (EST or EDT).

Results for the following analytical groups are included in this report: Chlorophyll/Grain Size/BOD, Molecular and Overflow.

Scientific notation may be used in reporting very large or small values. Values reported using scientific notation will take the form of the following example: 1.3E+03, which is equivalent to 1.3×10^3 or 1300.

Unless otherwise noted, analytical values for soil and sediment samples are reported on a dry weight basis, and analytical values for waste and tissue samples are reported on a wet weight basis.

Results for TNI accredited tests met requirements established by The NELAC Institute. A double asterisk (**) is used to indicate an analyte/matrix/method for which the laboratory is not TNI accredited by the Florida Department of Health Environmental Laboratory Certification Program or where accreditation for that field of testing is not applicable.

Any significant anomalies or deviations from established protocols are documented in Non-Conformance Reports, which, where appropriate, are included within this analytical report. Additional comments related to specific analytical tests may be included as remarks following the analytical results for each sample. Such comments and remarks are for informational purposes only and are not intended to convey judgement about the usability of the reported data.

A quality control report on the performance of the test method for the submitted samples is included. Uncertainty associated with the analytical results contained in this report can be estimated from the reported quality assurance results and from published quality control acceptance limits for each analytical test. Matrix quality control results (matrix spike recoveries and matrix sample precision) pertain only to the matrix sample tested and do not necessarily reflect test method performance for other samples.

Typical matrix quality control (QC) measurements may include matrix spike recovery, matrix spike duplicate recovery, matrix spike precision and matrix sample precision. Not all matrix QC results may be available or reportable; where they are not an explanation is provided. Typical reasons for unavailable QC results include, but are not limited to, a) insufficient matrix sample to perform some or all QC measurements; b) analyte concentration in the sample replicated was too low for a meaningful measurement of precision and c) analyte concentration in the matrix sample spiked was too high (relative to the amount of analyte spiked) for a meaningful measurement of recovery. Where matrix QC results are unavailable, other method performance metrics (e.g., LCS recovery, LCS precision, surrogate recovery) may be used to assess performance of the method. Comments explaining any missing QC measurements are not intended to convey any adverse conclusions about the quality of the reported data.

Precision is reported as relative percent difference unless otherwise noted.

Quality Control codes as defined below may be used in this report to indicate results that are associated with one or more quality control elements which did not fall within established test method criteria. Such results may be qualified as estimates using a J qualifier as required by 62-160 F.A.C. Explanations are included in the report for any results that were reported as estimates for other reasons.

QC Codes used in this report may include:

LCS – Recovery for the batch Laboratory Control Sample (LCS) was outside existing control limits;
MS – Recovery for the batch matrix spike (MS) was outside existing control limits;
CCV – Recovery for a continuing calibration verification (CCV) standard was outside existing control limits;
SUR – Recovery of a surrogate (SUR) for associated analytes was outside existing control limits;
RPD – The precision, measured as relative percent difference (RPD), of batch replicate measurements was outside existing control limits;
RSD – The precision, measured as relative standard deviation (RSD), of batch replicate measurements was outside existing control limits;
SMP – Sample - used precision derived from replicate analyses of a sample;

The following data qualifiers are used, where applicable, in this report as specified in 62-160 F.A.C.

A - Value reported is the mean of two or more determinations.
B - Results based on colony counts outside the acceptable range.
I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
J - Estimated value and/or the analysis did not meet established quality control criteria.
K - Actual value is known to be less than value given.
L - Actual value is known to be greater than value given.
N - Presumptive evidence of presence of material.
O - Sampled, but analysis lost or not performed.
Q - Sample held beyond normal holding time.
T - Value reported is less than the criterion of detection.
U - Material was analyzed for but not detected. The reported value is the method detection limit for the sample analyzed.
V - Analyte was detected in both sample and method blank.
X - Too few individuals to calculate SCI value.
Y - The laboratory analysis was from an unpreserved or improperly preserved sample. The data may not be accurate.
Z - Colonies were too numerous to count (TNTC).

Quality control information from overflow laboratories may not be included in this report. Please refer to the associated report from the overflow laboratory for additional information.

Sample Location: Bay Branch below Coy Burgess Loop **Collection Date/Time: 10/13/2022 11:03**

Field ID: G3NW0231 Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2362502	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	4569		MPN/100 mL		
2362503	SOP-PCR-1.0	HF183-qPCR**	6.6E+03	U	TSC/100 mL	P420720	
	SOP-PCR-1.2	GULL2-qPCR**	7.2E+03	U	TSC/100 mL	P420720	
	SOP-PCR-1.3	GFD-qPCR**	1.4E+03	U	TSC/100 mL	P420720	
	SOP-PCR-1.4	BacR-qPCR**	6.1E+03	U	TSC/100 mL	P420720	
	SOP-PCR-1.5	DG3-qPCR**	5.1E+03	U	TSC/100 mL	P420720	

Sample Location: Panther Creek **Collection Date/Time: 10/13/2022 10:34**

Field ID: G3NW0061 Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2362500	SM 10200 H (mod.)	Chlorophyll-a, Corrected	2.1	U	ug/L	P421293	
		Phaeophytin-a	2.2	U	ug/L	P421293	
		Chlorophyll-a, Uncorrected	1.5	U	ug/L	P421293	
2362501	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	3873		MPN/100 mL		

Ref. Method and Comment:
SM 10200 H (mod.): Precision data is not available for at least one component due to the small amount of analyte in the QC sample. Refer to QA report for available precision data.

Quality Assurance Report
Method Blank Results

Reference Method: SM 10200 H (mod.)
Batch ID: P421293

Component	Result	Code	Units
Chlorophyll-a, Corrected	0.82	U	ug/L
Chlorophyll-a, Uncorrected	0.60	U	ug/L
Phaeophytin-a	2.5	I	ug/L

Reference Method: SOP-PCR-1.0
Batch ID: P420720

Component	Result	Code	Units
HF183-qPCR	3.3E+03	U	TSC/100 mL

Reference Method: SOP-PCR-1.2
Batch ID: P420720

Component	Result	Code	Units
GULL2-qPCR	3.6E+03	U	TSC/100 mL

Reference Method: SOP-PCR-1.3
Batch ID: P420720

Component	Result	Code	Units
GFD-qPCR	720	U	TSC/100 mL

Quality Assurance Report
Method Blank Results

Reference Method: SOP-PCR-1.4
Batch ID: P420720

Component	Result	Code	Units
BacR-qPCR	3.0E+03	U	TSC/100 mL

Reference Method: SOP-PCR-1.5
Batch ID: P420720

Component	Result	Code	Units
DG3-qPCR	2.6E+03	U	TSC/100 mL

Quality Assurance Report
Laboratory Control Sample Accuracy

Reference Method: SM 10200 H (mod.)
Batch ID: P421293

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	100		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P420720

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	88.5	92.2	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P420720

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	97.8	84.7	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P420720

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	89.9	94.6	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P420720

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	93.1	75.2	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P420720

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	57.8	74.5	P/P	50 - 175

Quality Assurance Report
Matrix Spike Accuracy

Quality Assurance Report
Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P420720

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2361364	HF183-qPCR	74.8	81.1	P/P	50 - 175
2361365	HF183-qPCR	51.5		P	50 - 175
2361698	HF183-qPCR	94.5		P	50 - 175
2361699	HF183-qPCR	88.1		P	50 - 175
2361700	HF183-qPCR	90.3		P	50 - 175
2362002	HF183-qPCR	90.1		P	50 - 175
2362125	HF183-qPCR	83.0		P	50 - 175
2362503	HF183-qPCR	64.1		P	50 - 175
2363299	HF183-qPCR	89.9		P	50 - 175
2364066	HF183-qPCR	99.1		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P420720

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2361364	GULL2-qPCR	89.8	85.7	P/P	50 - 300
2361698	GULL2-qPCR	89.6		P	50 - 300
2361699	GULL2-qPCR	94.3		P	50 - 300
2361700	GULL2-qPCR	83.2		P	50 - 300
2362002	GULL2-qPCR	70.4		P	50 - 300
2362125	GULL2-qPCR	90.5		P	50 - 300
2362503	GULL2-qPCR	64.0		P	50 - 300
2364066	GULL2-qPCR	70.5		P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P420720

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2361364	GFD-qPCR	90.3	89.2	P/P	50 - 175
2361698	GFD-qPCR	104		P	50 - 175
2361699	GFD-qPCR	97.7		P	50 - 175
2361700	GFD-qPCR	95.7		P	50 - 175
2362002	GFD-qPCR	100		P	50 - 175
2362125	GFD-qPCR	90.9		P	50 - 175
2362503	GFD-qPCR	89.0		P	50 - 175
2364066	GFD-qPCR	87.3		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P420720

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2361364	BacR-qPCR	72.3	77.1	P/P	50 - 175
2361365	BacR-qPCR	71.8		P	50 - 175
2361698	BacR-qPCR	91.1		P	50 - 175
2361699	BacR-qPCR	96.2		P	50 - 175
2361700	BacR-qPCR	78.4		P	50 - 175
2362002	BacR-qPCR	66.1		P	50 - 175
2362125	BacR-qPCR	79.3		P	50 - 175
2362503	BacR-qPCR	50.9		P	50 - 175
2363299	BacR-qPCR	89.1		P	50 - 175
2364066	BacR-qPCR	74.6		P	50 - 175

Quality Assurance Report
Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
Batch ID: P420720

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2361364	DG3-qPCR	86.7	89.6	P/P	50 - 175
2361365	DG3-qPCR	76.5		P	50 - 175
2361698	DG3-qPCR	85.2		P	50 - 175
2361699	DG3-qPCR	89.2		P	50 - 175
2361700	DG3-qPCR	88.9		P	50 - 175
2362002	DG3-qPCR	84.3		P	50 - 175
2362125	DG3-qPCR	82.3		P	50 - 175
2362503	DG3-qPCR	70.8		P	50 - 175
2363299	DG3-qPCR	86.7		P	50 - 175
2364066	DG3-qPCR	83.5		P	50 - 175

Quality Assurance Report
Precision

Reference Method: SM 10200 H (mod.)
Batch ID: P421293

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2362520	Chlorophyll-a, Corrected	2.77	Sample	P	0 - 25
2362520	Chlorophyll-a, Uncorrected	2.77	Sample	P	0 - 25

Reference Method: SOP-PCR-1.0
Batch ID: P420720

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2361364	HF183-qPCR	8.04	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P420720

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2361364	GULL2-qPCR	4.71	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P420720

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2361364	GFD-qPCR	1.25	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P420720

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2361364	BacR-qPCR	6.36	Spike	P	0 - 25

Quality Assurance Report
Precision

Reference Method: SOP-PCR-1.5
Batch ID: P420720

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2361364	DG3-qPCR	3.27	Spike	P	0 - 25

* Sample, spike and/or laboratory control sample precision (LCS) is reported.

Quality Assurance Report
Surrogates

Lab Sample ID: 2362503
Field Sample ID: G3NW0231

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	79.7	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	92.0	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	99.5	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	86.5	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	70.9	P	50 - 300

Quality Assurance Report
Summary

Ref. Method	Analyte	LCS % Recovery	MS % Recovery	Precision		
				LCS	SMP	MS
SM 10200 H (mod.)	Chlorophyll-a, Corrected	100			2.77	
	Chlorophyll-a, Uncorrected				2.77	
SOP-PCR-1.0	HF183-qPCR	92.2 88.5	74.8 81.1 51.5 94.5			8.04
SOP-PCR-1.2	GULL2-qPCR	84.7 97.8	94.3 83.2 70.4 90.5			4.71
SOP-PCR-1.3	GFD-qPCR	94.6 89.9	90.3 89.2 104 97.7			1.25
SOP-PCR-1.4	BacR-qPCR	75.2 93.1	72.3 77.1 71.8 91.1			6.36
SOP-PCR-1.5	DG3-qPCR	74.5 57.8	86.7 89.6 76.5 85.2			3.27

Reference Method Descriptions

Method	Description	Associated Samples
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2362500
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2362501, 2362502
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2362503
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2362503
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2362503
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2362503

Reference Method Descriptions

Method	Description	Associated Samples
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2362503

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10200 H (mod.)	10/14/2022	10/14/2022 13:41	Megan Higbee	10/20/2022 14:07	Megan Higbee	2362500
SM 9223 B Quanti-Tray	10/13/2022	10/13/2022 13:50		10/14/2022 11:30	UWF Lab	2362501, 2362502
SOP-PCR-1.0	10/14/2022	10/14/2022 13:35	Claudia Castro	10/24/2022 10:07	Roberta Tatti	2362503
SOP-PCR-1.2	10/14/2022	10/14/2022 13:35	Claudia Castro	10/25/2022 16:29	Roberta Tatti	2362503
SOP-PCR-1.3	10/14/2022	10/14/2022 13:35	Claudia Castro	10/26/2022 09:13	Roberta Tatti	2362503
SOP-PCR-1.4	10/14/2022	10/14/2022 13:35	Claudia Castro	10/24/2022 14:12	DeAsia Armster	2362503
SOP-PCR-1.5	10/14/2022	10/14/2022 13:35	Claudia Castro	10/25/2022 09:00	Roberta Tatti	2362503