

Biological Analysis Report

NW-DIST-2021-01-15-01

Florida Department of Environmental Protection
Central 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: **Pensacola Run**
Request ID: **RQ-2021-01-11-37**
Customer: **NW-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
160 Government Center
Suite 208
Pensacola, FL 32502-5794
Attn: Cheryl Bunch

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

Certified by: Jacqueline Savage, Environmental Administrator

Date Certified: 19-FEB-2021 08:48

A handwritten signature in black ink that reads "Jackie Savage". The signature is written in a cursive, flowing style.

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: Muscogee Creek 300 downstream of Beulah Road

Collection Date/Time: 01/14/2021 11:30

Field ID: G5NW0162

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2219510	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	153		MPN/100 mL		

Sample Location: Jones Creek Upstream Of N Navy Blvd

Collection Date/Time: 01/14/2021 09:26

Field ID: G4NW0429

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2219513	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	160		MPN/100 mL		
2219514	SOP-PCR-1.0	HF183-qPCR**	140	U	GEU/100 mL	P392217	
	SOP-PCR-1.2	GULL2-qPCR**	1.8E+03	U	TSC/100 mL	P392217	
	SOP-PCR-1.3	GFD-qPCR**	2.4E+03	J	TSC/100 mL	P392217	LCS, MS
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	U	TSC/100 mL	P392217	
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	U	TSC/100 mL	P392217	

Ref. Method and Comment:

SOP-PCR-1.2: Precision data unavailable for Analysis Batch A103217

SOP-PCR-1.3: Precision data unavailable for Analysis Batch A103246. Refer to the Lab Analysis Report for an explanation of QC Codes.

Sample Location: Carpenter Creek Upstream Of 9th Avenue

Collection Date/Time: 01/14/2021 14:10

Field ID: G4NW0415

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2219491	SM 10200 H (mod.)	Chlorophyll-a, Corrected	0.82	U	ug/L	P392526	
		Phaeophytin-a	0.90	U	ug/L	P392526	
		Chlorophyll-a, Uncorrected	0.60	U	ug/L	P392526	

Sample Location: Bayou Marcus upstream of Mobile Hwy

Collection Date/Time: 01/14/2021 09:56

Field ID: G5NW0161

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2219490	SM 10200 H (mod.)	Chlorophyll-a, Corrected	1.4	I	ug/L	P392526	
		Phaeophytin-a	0.90	U	ug/L	P392526	
		Chlorophyll-a, Uncorrected	1.8		ug/L	P392526	
2219509	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	31		MPN/100 mL		

Sample Location: Ten Mile Creek Above Powerlines

Collection Date/Time: 01/14/2021 10:55

Field ID: G5NW0045

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2219511	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	121		MPN/100 mL		

Sample Location: Ten Mile Creek Upstream Of Pine Forest Road

Collection Date/Time: 01/14/2021 10:37

Field ID: G5NW0051

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2219512	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	86		MPN/100 mL		

Sample Location: Rest Area Run Below Beaver Pond Creek

Collection Date/Time: 01/14/2021 12:15

Field ID: G5NW0068

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2219489	SM 10200 H (mod.)	Chlorophyll-a, Corrected	0.99	I	ug/L	P392526	
		Phaeophytin-a	0.90	U	ug/L	P392526	
		Chlorophyll-a, Uncorrected	1.2	I	ug/L	P392526	
2219508	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	10	U	MPN/100 mL		

Quality Assurance Report
Method Blank Results

Reference Method: SM 10200 H (mod.)
Batch ID: P392526

Component	Result	Code	Units
Chlorophyll-a, Corrected	0.82	U	ug/L
Chlorophyll-a, Uncorrected	0.60	U	ug/L
Phaeophytin-a	0.90	U	ug/L

Reference Method: SOP-PCR-1.0
Batch ID: P392217

Component	Result	Code	Units
HF183-qPCR	280	U	GEU/100 mL

Reference Method: SOP-PCR-1.2
Batch ID: P392217

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

Reference Method: SOP-PCR-1.3
Batch ID: P392217

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

Reference Method: SOP-PCR-1.4
Batch ID: P392217

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

Reference Method: SOP-PCR-1.5
Batch ID: P392217

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: P392526

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	108		P	84 - 116

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P392217

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	130	108	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P392217

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	87.3	94.0	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P392217

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	178	123	F/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P392217

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	98.1	58.8	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P392217

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	79.4	56.8	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P392217

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2218027	HF183-qPCR	108		P	50 - 175
2218028	HF183-qPCR	116	116	P/P	50 - 175
2218340	HF183-qPCR	130		P	50 - 175
2218923	HF183-qPCR	118		P	50 - 175
2219367	HF183-qPCR	124		P	50 - 175
2219368	HF183-qPCR	110		P	50 - 175
2219369	HF183-qPCR	109		P	50 - 175
2219514	HF183-qPCR	106		P	50 - 175
2220199	HF183-qPCR	101		P	50 - 175
2220200	HF183-qPCR	109		P	50 - 175
2220201	HF183-qPCR	110		P	50 - 175
2220202	HF183-qPCR	105		P	50 - 175
2220203	HF183-qPCR	99.6		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P392217

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
---------------	-----------	---------	---------	-----------	----------------

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.2

Batch ID: P392217

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2219367	GULL2-qPCR	79.0		P	50 - 300
2219368	GULL2-qPCR	78.3		P	50 - 300
2219369	GULL2-qPCR	69.1		P	50 - 300
2219514	GULL2-qPCR	61.6		P	50 - 300
2220199	GULL2-qPCR	98.4		P	50 - 300
2220200	GULL2-qPCR	68.0		P	50 - 300
2220202	GULL2-qPCR	65.2		P	50 - 300
2220203	GULL2-qPCR	61.4		P	50 - 300

Reference Method: SOP-PCR-1.3

Batch ID: P392217

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2219367	GFD-qPCR	172		P	50 - 175
2219368	GFD-qPCR	175		P	50 - 175
2219369	GFD-qPCR	142		P	50 - 175
2219514	GFD-qPCR	193		F	50 - 175
2220199	GFD-qPCR	180		F	50 - 175
2220200	GFD-qPCR	167		P	50 - 175
2220202	GFD-qPCR	167		P	50 - 175
2220203	GFD-qPCR	168		P	50 - 175

Reference Method: SOP-PCR-1.4

Batch ID: P392217

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2218027	BacR-qPCR	74.4		P	50 - 175
2218028	BacR-qPCR	87.5	82.7	P/P	50 - 175
2218340	BacR-qPCR	80.4		P	50 - 175
2218923	BacR-qPCR	88.5		P	50 - 175
2219367	BacR-qPCR	81.8		P	50 - 175
2219368	BacR-qPCR	79.8		P	50 - 175
2219369	BacR-qPCR	74.1		P	50 - 175
2219514	BacR-qPCR	66.6		P	50 - 175
2220199	BacR-qPCR	79.4		P	50 - 175
2220200	BacR-qPCR	76.9		P	50 - 175
2220201	BacR-qPCR	83.3		P	50 - 175
2220202	BacR-qPCR	72.3		P	50 - 175
2220203	BacR-qPCR	71.5		P	50 - 175

Reference Method: SOP-PCR-1.5

Batch ID: P392217

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2218027	DG3-qPCR	85.5		P	50 - 175
2218028	DG3-qPCR	92.4	81.7	P/P	50 - 175
2218340	DG3-qPCR	95.2		P	50 - 175
2218923	DG3-qPCR	87.9		P	50 - 175
2219367	DG3-qPCR	85.2		P	50 - 175
2219368	DG3-qPCR	82.4		P	50 - 175
2219369	DG3-qPCR	80.2		P	50 - 175
2219514	DG3-qPCR	87.1		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
Batch ID: P392217

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2220199	DG3-qPCR	84.1		P	50 - 175
2220200	DG3-qPCR	82.2		P	50 - 175
2220201	DG3-qPCR	91.3		P	50 - 175
2220202	DG3-qPCR	71.4		P	50 - 175
2220203	DG3-qPCR	80.4		P	50 - 175

Quality Assurance Report Precision

Reference Method: SM 10200 H (mod.)
Batch ID: P392526

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2219684	Chlorophyll-a, Corrected	1.62	Sample	P	0 - 25
2219684	Chlorophyll-a, Uncorrected	0.168	Sample	P	0 - 25
2219684	Phaeophytin-a	2.52	Sample	P	0 - 25

Reference Method: SOP-PCR-1.0
Batch ID: P392217

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2218028	HF183-qPCR	0.194	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P392217

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2218028	BacR-qPCR	5.52	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P392217

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2218028	DG3-qPCR	12.3	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2219514
Field Sample ID: G4NW0429

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	98.1	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	105	P	50 - 300

Quality Assurance Report Surrogates

Lab Sample ID: 2219514
 Field Sample ID: G4NW0429

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.3	SKETA-qPCR	96.3	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	108	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	96.4	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SM 10200 H (mod.)	Chlorophyll-a, Corrected	108						1.62	
	Chlorophyll-a, Uncorrected							0.168	
	Phaeophytin-a							2.52	
SOP-PCR-1.0	HF183-qPCR	108	130	101	109	110			0.194
SOP-PCR-1.2	GULL2-qPCR	94.0	87.3	79.0	78.3	69.1			
SOP-PCR-1.3	GFD-qPCR	123	178	172	175	142			
SOP-PCR-1.4	BacR-qPCR	98.1	58.8	87.5	82.7	74.4			5.52
SOP-PCR-1.5	DG3-qPCR	56.8	79.4	92.4	81.7	85.5			12.3
				95.2					

Reference Method Descriptions

Method	Description	Associated Samples
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2219489, 2219490, 2219491
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2219508, 2219509, 2219510, 2219511, 2219512, 2219513
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2219514
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2219514
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2219514
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2219514
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2219514

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10200 H (mod.)	01/15/2021	01/15/2021 13:51	Jessica Sherman	01/18/2021 11:48	Yuliya Danyuk	2219489, 2219490, 2219491
SM 9223 B Quanti-Tray	01/14/2021	01/14/2021 14:40		01/15/2021 12:12	UWF Lab	2219508, 2219509, 2219510, 2219511, 2219512, 2219513
SOP-PCR-1.0	01/15/2021	01/15/2021 12:15	Lauren Campbell	01/21/2021 14:33	Lauren Campbell	2219514
SOP-PCR-1.2	01/15/2021	01/15/2021 12:15	Lauren Campbell	01/25/2021 14:16	Lauren Campbell	2219514
SOP-PCR-1.3	01/15/2021	01/15/2021 12:15	Lauren Campbell	01/26/2021 11:58	Lauren Campbell	2219514
SOP-PCR-1.4	01/15/2021	01/15/2021 12:15	Lauren Campbell	01/22/2021 09:00	Mailin Lopez	2219514
SOP-PCR-1.5	01/15/2021	01/15/2021 12:15	Lauren Campbell	01/22/2021 11:27	Mailin Lopez	2219514