

Biological Analysis Report

NW-DIST-2022-03-10-01

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: **Holmes Run**
Request ID: **RQ-2022-02-14-58**
Customer: **NW-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
160 Government Center
Suite 208
Pensacola, FL 32502-5794
Attn: Myranda K 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: Sarah Menz, Environmental Administrator

Date Certified: 19-APR-2022 10:26

A handwritten signature in black ink, appearing to be 'Sarah Menz', written in a cursive 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: Wrights Creek upstream of HWY 2

Collection Date/Time: 03/09/2022 11:00

Field ID: G3NW0236

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2311724	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	226		MPN/100 mL		

Sample Location: Limestone Branch upstream of Lake Victor Rd

Collection Date/Time: 03/09/2022 11:40

Field ID: G3NW0232

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2311727	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	10	U	MPN/100 mL		
2311729	SOP-PCR-1.0	HF183-qPCR**	1.7E+03	UJ	TSC/100 mL	P410798	SUR
	SOP-PCR-1.2	GULL2-qPCR**	3.6E+03	U	TSC/100 mL	P410798	
	SOP-PCR-1.3	GFD-qPCR**	720	U	TSC/100 mL	P410798	
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	U	TSC/100 mL	P410798	LCS
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	U	TSC/100 mL	P410798	LCS

Ref. Method and Comment:
 SOP-PCR-1.0: Refer to the Lab Analysis Report for an explanation of QC Codes.

Sample Location: Limestone Branch upstream of CR179A

Collection Date/Time: 03/09/2022 11:50

Field ID: G3NW0229

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2311725	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	1246		MPN/100 mL		

Sample Location: Sikes Creek upstream of Harris Stevenson Rd

Collection Date/Time: 03/09/2022 12:30

Field ID: G3NW0450

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2311726	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	17329		MPN/100 mL		

Sample Location: Sikes Creek upstream of Bonifay Gritney Rd

Collection Date/Time: 03/09/2022 12:50

Field ID: G3NW0159

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2311723	SM 10200 H (mod.)	Chlorophyll-a, Corrected	4.4	I	ug/L	P411289	
		Phaeophytin-a	2.3	I	ug/L	P411289	
		Chlorophyll-a, Uncorrected	5.9		ug/L	P411289	
2312995	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	4611		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.

Sample Location: Wrights Creek upstream of CR 179

Collection Date/Time: 03/09/2022 13:30

Field ID: G3NW0228

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2311728	SM 9223 B Quanti-Tray	Escherichia Coli-Quanti-Tray	281		MPN/100 mL		
2311730	SOP-PCR-1.0	HF183-qPCR**	1.1E+03	U	TSC/100 mL	P410798	
	SOP-PCR-1.2	GULL2-qPCR**	2.4E+03	U	TSC/100 mL	P410798	
	SOP-PCR-1.3	GFD-qPCR**	480	U	TSC/100 mL	P410798	

Field ID: G3NW0228

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2311730	SOP-PCR-1.4	BacR-qPCR**	1.0E+03	U	TSC/100 mL	P410798	LCS
	SOP-PCR-1.5	DG3-qPCR**	860	U	TSC/100 mL	P410798	LCS

Quality Assurance Report
Method Blank Results

Reference Method: SM 10200 H (mod.)
Batch ID: P411289

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: P410798

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

Reference Method: SOP-PCR-1.2
Batch ID: P410798

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

Reference Method: SOP-PCR-1.3
Batch ID: P410798

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

Reference Method: SOP-PCR-1.4
Batch ID: P410798

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

Reference Method: SOP-PCR-1.5
Batch ID: P410798

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: P411289

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	94.1		P	84 - 116

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
 Batch ID: P410798

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	75.0	82.8	P/P	50 - 175

Reference Method: SOP-PCR-1.2
 Batch ID: P410798

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	97.5	82.3	P/P	50 - 300

Reference Method: SOP-PCR-1.3
 Batch ID: P410798

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	66.8	75.4	P/P	50 - 175

Reference Method: SOP-PCR-1.4
 Batch ID: P410798

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	79.6	20.1	P/F	50 - 175

Reference Method: SOP-PCR-1.5
 Batch ID: P410798

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	75.2	21.7	P/F	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
 Batch ID: P410798

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2310518	HF183-qPCR	74.5	73.1	P/P	50 - 175
2311119	HF183-qPCR	74.1		P	50 - 175
2311120	HF183-qPCR	82.1		P	50 - 175
2311451	HF183-qPCR	69.8		P	50 - 175
2311453	HF183-qPCR	82.8		P	50 - 175
2311510	HF183-qPCR	81.9		P	50 - 175
2311511	HF183-qPCR	89.6		P	50 - 175
2311512	HF183-qPCR	83.2		P	50 - 175
2311691	HF183-qPCR	77.0		P	50 - 175
2311729	HF183-qPCR	76.2		P	50 - 175
2311730	HF183-qPCR	87.9		P	50 - 175
2311753	HF183-qPCR	93.4		P	50 - 175

Reference Method: SOP-PCR-1.2
 Batch ID: P410798

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2310518	GULL2-qPCR	78.8	75.2	P/P	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.2

Batch ID: P410798

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2311451	GULL2-qPCR	50.4		P	50 - 300
2311510	GULL2-qPCR	55.0		P	50 - 300
2311511	GULL2-qPCR	84.2		P	50 - 300
2311512	GULL2-qPCR	60.4		P	50 - 300
2311729	GULL2-qPCR	95.4		P	50 - 300
2311730	GULL2-qPCR	84.3		P	50 - 300
2311753	GULL2-qPCR	93.9		P	50 - 300

Reference Method: SOP-PCR-1.3

Batch ID: P410798

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2310518	GFD-qPCR	86.6	83.8	P/P	50 - 175
2311451	GFD-qPCR	66.5		P	50 - 175
2311510	GFD-qPCR	89.3		P	50 - 175
2311511	GFD-qPCR	86.7		P	50 - 175
2311512	GFD-qPCR	88.9		P	50 - 175
2311729	GFD-qPCR	78.8		P	50 - 175
2311730	GFD-qPCR	87.8		P	50 - 175
2311753	GFD-qPCR	82.2		P	50 - 175

Reference Method: SOP-PCR-1.4

Batch ID: P410798

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2310518	BacR-qPCR	57.9	56.3	P/P	50 - 175
2311119	BacR-qPCR	71.5		P	50 - 175
2311120	BacR-qPCR	64.4		P	50 - 175
2311451	BacR-qPCR	45.9		F	50 - 175
2311453	BacR-qPCR	57.8		P	50 - 175
2311510	BacR-qPCR	48.1		F	50 - 175
2311511	BacR-qPCR	75.6		P	50 - 175
2311512	BacR-qPCR	52.6		P	50 - 175
2311691	BacR-qPCR	53.6		P	50 - 175
2311729	BacR-qPCR	66.4		P	50 - 175
2311730	BacR-qPCR	54.3		P	50 - 175
2311753	BacR-qPCR	66.3		P	50 - 175

Reference Method: SOP-PCR-1.5

Batch ID: P410798

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2310518	DG3-qPCR	92.0	84.6	P/P	50 - 175
2311119	DG3-qPCR	88.6		P	50 - 175
2311120	DG3-qPCR	89.6		P	50 - 175
2311451	DG3-qPCR	70.9		P	50 - 175
2311453	DG3-qPCR	94.1		P	50 - 175
2311510	DG3-qPCR	73.7		P	50 - 175
2311511	DG3-qPCR	91.6		P	50 - 175
2311512	DG3-qPCR	74.6		P	50 - 175
2311691	DG3-qPCR	80.6		P	50 - 175
2311729	DG3-qPCR	81.4		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
Batch ID: P410798

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2311730	DG3-qPCR	88.4		P	50 - 175
2311753	DG3-qPCR	88.4		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P410798

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2310518	HF183-qPCR	1.86	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P410798

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2310518	GULL2-qPCR	4.65	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P410798

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2310518	GFD-qPCR	3.23	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P410798

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2310518	BacR-qPCR	2.73	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P410798

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2310518	DG3-qPCR	8.33	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Lab Sample ID: 2311729
Field Sample ID: G3NW0232

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
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Quality Assurance Report Surrogates

Lab Sample ID: 2311729
Field Sample ID: G3NW0232

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	0.430	F	50 - 300
SOP-PCR-1.2	SKETA-qPCR	105	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	117	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	92.2	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	78.5	P	50 - 300

Lab Sample ID: 2311730
Field Sample ID: G3NW0228

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	171	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	102	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	115	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	101	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	82.5	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	94.1							
SOP-PCR-1.0	HF183-qPCR	82.8	75.0	74.5	73.1	74.1			1.86
				82.1					
SOP-PCR-1.2	GULL2-qPCR	82.3	97.5	78.8	75.2	50.4			4.65
				55.0					
SOP-PCR-1.3	GFD-qPCR	75.4	66.8	86.6	83.8	66.5			3.23
				89.3					
SOP-PCR-1.4	BacR-qPCR	20.1	79.6	57.9	56.3	71.5			2.73
				64.4					
SOP-PCR-1.5	DG3-qPCR	21.7	75.2	92.0	84.6	88.6			8.33
				89.6					

Reference Method Descriptions

Method	Description	Associated Samples
SM 10200 H (mod.)	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2311723
SM 9223 B Quanti-Tray	E. coli by Colilert-18/QT by UWF in Pensacola	2311724, 2311725, 2311726, 2311727, 2311728, 2312995
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2311729, 2311730
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2311729, 2311730
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2311729, 2311730
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2311729, 2311730
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2311729, 2311730

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10200 H (mod.)	03/10/2022	03/10/2022 15:25	Joel Wharton	03/16/2022 10:40	Ethan Wickline	2311723
SM 9223 B Quanti-Tray	03/09/2022	03/09/2022 16:40		03/10/2022 12:57	UWF Lab	2311724, 2311725, 2311726, 2311727, 2311728, 2312995
SOP-PCR-1.0	03/10/2022	03/10/2022 13:50	Jennifer Mihalic	04/11/2022 14:21	Roberta Tatti	2311729, 2311730
SOP-PCR-1.2	03/10/2022	03/10/2022 13:50	Jennifer Mihalic	04/14/2022 09:52	DeAsia Armster	2311729, 2311730
SOP-PCR-1.3	03/10/2022	03/10/2022 13:50	Jennifer Mihalic	04/14/2022 11:31	DeAsia Armster	2311729, 2311730
SOP-PCR-1.4	03/10/2022	03/10/2022 13:50	Jennifer Mihalic	04/11/2022 11:46	DeAsia Armster	2311729, 2311730
SOP-PCR-1.5	03/10/2022	03/10/2022 13:50	Jennifer Mihalic	04/12/2022 10:12	Roberta Tatti	2311729, 2311730