

Biological Analysis Report

SO-DIST-2023-10-25-01

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

Overflow Analyses Performed By:
Benchmark EnviroAnalytical, Inc. - North Port
1001 Corporate Avenue, Suite 102
North Port, FL 34289
DOH Accreditation E85086

Event Description: **SMP : Hatchett Creek SCI**
Request ID: **RQ-2023-10-23-22**
Customer: **SO-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
FLDEP South District Office
2295 Victoria Ave. Suite 314
Fort Myers, FL 33901-3381
Attn: Nicole Reistad

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Certified by: Puja Jasrotia, Environmental Administrator

Date Certified: 17-JAN-2024 09:08



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: AlgalBio, Chlorophyll/Grain Size/BOD, InvertZool, 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: HATCHETT CREEK AT PINEBROOK RD

Collection Date/Time: 10/24/2023 10:25

Field ID: G3SD0103

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2446989	SOP-AB18	Dominant sample taxon**	Other				
2446990	SOP-IZ06	Stream Condition Index (SCI)**	21		Points	P439837	
		Macroinvert-FW-Qual-DipnetX20-#Taxa-Rep2**	23		# Taxa	P439837	
2447007	SM 10150 B	Chlorophyll-a, Corrected	7.2		ug/L	P437246	
		Phaeophytin-a	1.6	I	ug/L	P437246	
		Chlorophyll-a, Uncorrected	8.3		ug/L	P437246	
2447008	SOP-PCR-1.0	HF183-qPCR**	1.7E+03	U	TSC/100 mL	P436911	
	SOP-PCR-1.2	GULL2-qPCR**	1.8E+03	U	TSC/100 mL	P436911	
	SOP-PCR-1.3	GFD-qPCR**	910	I	TSC/100 mL	P436911	
	SOP-PCR-1.4	BacR-qPCR**	1.5E+03	U	TSC/100 mL	P436911	
	SOP-PCR-1.5	DG3-qPCR**	1.3E+03	U	TSC/100 mL	P436911	
2447009	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	228		MPN/100 mL		

Ref. Method and Comment:
 SOP-AB18: The co-dominant taxa were Plectonema wollei (Cyanophyceae) and Vaucheria sp. (Xanthophyceae).
 SM 10150 B: 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 10150 B
 Batch ID: P437246

Component	Result	Code	Units
Chlorophyll-a, Corrected	0.96	U	ug/L
Chlorophyll-a, Uncorrected	0.70	U	ug/L
Phaeophytin-a	1.0	U	ug/L

Reference Method: SOP-PCR-1.0
 Batch ID: P436911

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

Reference Method: SOP-PCR-1.2
 Batch ID: P436911

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

Reference Method: SOP-PCR-1.3
 Batch ID: P436911

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

Reference Method: SOP-PCR-1.4
 Batch ID: P436911

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

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.5
Batch ID: P436911

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

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SM 10150 B
Batch ID: P437246

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
Chlorophyll-a, Corrected	103		P	84 - 116

Reference Method: SOP-PCR-1.0
Batch ID: P436911

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	85.7	77.5	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P436911

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	97.1	65.5	P/P	50 - 175

Reference Method: SOP-PCR-1.3
Batch ID: P436911

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-qPCR	102	75.2	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P436911

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	93.4	91.9	P/P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P436911

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
DG3-qPCR	92.8	70.2	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P436911

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2446832	HF183-qPCR	87.7	83.5	P/P	50 - 175
2446833	HF183-qPCR	86.0		P	50 - 175
2446949	HF183-qPCR	82.2		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P436911

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2447008	HF183-qPCR	91.2		P	50 - 175
2447186	HF183-qPCR	94.3		P	50 - 175
2447322	HF183-qPCR	89.0		P	50 - 175
2447323	HF183-qPCR	84.7		P	50 - 175
2447983	HF183-qPCR	87.3		P	50 - 175
2448285	HF183-qPCR	86.2		P	50 - 175
2448286	HF183-qPCR	64.2		P	50 - 175
2448287	HF183-qPCR	98.1		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P436911

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2447008	GULL2-qPCR	86.2		P	50 - 175
2447186	GULL2-qPCR	94.9		P	50 - 175
2447323	GULL2-qPCR	82.5		P	50 - 175
2447983	GULL2-qPCR	70.0		P	50 - 175
2448287	GULL2-qPCR	68.7		P	50 - 175

Reference Method: SOP-PCR-1.3
Batch ID: P436911

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2447008	GFD-qPCR	94.3		P	50 - 175
2447186	GFD-qPCR	102		P	50 - 175
2447323	GFD-qPCR	95.1		P	50 - 175
2447983	GFD-qPCR	96.4		P	50 - 175
2448287	GFD-qPCR	84.7		P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P436911

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2446832	BacR-qPCR	80.4	85.0	P/P	50 - 175
2446949	BacR-qPCR	54.4		P	50 - 175
2447008	BacR-qPCR	95.4		P	50 - 175
2447186	BacR-qPCR	77.6		P	50 - 175
2447322	BacR-qPCR	82.2		P	50 - 175
2447323	BacR-qPCR	92.2		P	50 - 175
2447983	BacR-qPCR	92.0		P	50 - 175
2448285	BacR-qPCR	97.2		P	50 - 175
2448286	BacR-qPCR	93.1		P	50 - 175

Reference Method: SOP-PCR-1.5
Batch ID: P436911

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2446832	DG3-qPCR	89.2	87.6	P/P	50 - 175
2446833	DG3-qPCR	88.5		P	50 - 175
2446949	DG3-qPCR	80.5		P	50 - 175
2447008	DG3-qPCR	95.3		P	50 - 175
2447186	DG3-qPCR	97.5		P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.5
Batch ID: P436911

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2447322	DG3-qPCR	80.3		P	50 - 175
2447323	DG3-qPCR	92.9		P	50 - 175
2447983	DG3-qPCR	84.9		P	50 - 175
2448285	DG3-qPCR	93.7		P	50 - 175
2448286	DG3-qPCR	82.5		P	50 - 175
2448287	DG3-qPCR	89.3		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P436911

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2446832	HF183-qPCR	4.95	Spike	P	0 - 25

Reference Method: SOP-PCR-1.2
Batch ID: P436911

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2446832	GULL2-qPCR	3.89	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P436911

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2446832	GFD-qPCR	3.53	Spike	P	0 - 25

Reference Method: SOP-PCR-1.4
Batch ID: P436911

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2446832	BacR-qPCR	5.63	Spike	P	0 - 25

Reference Method: SOP-PCR-1.5
Batch ID: P436911

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2446832	DG3-qPCR	1.82	Spike	P	0 - 25

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

Quality Assurance Report Surrogates

Quality Assurance Report Surrogates

Lab Sample ID: 2447008
 Field Sample ID: G3SD0103

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	108	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	103	P	50 - 300
SOP-PCR-1.3	SKETA-qPCR	96.2	P	50 - 300
SOP-PCR-1.4	SKETA-qPCR	98.3	P	50 - 300
SOP-PCR-1.5	SKETA-qPCR	101	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery		MS % Recovery			LCS	Precision SMP	MS
SM 10150 B	Chlorophyll-a, Corrected	103							
SOP-PCR-1.0	HF183-qPCR	77.5	85.7	87.7	83.5	86.0			4.95
SOP-PCR-1.2	GULL2-qPCR	65.5	97.1	86.2	94.9	82.5			3.89
SOP-PCR-1.3	GFD-qPCR	75.2	102	94.3	102	95.1			3.53
SOP-PCR-1.4	BacR-qPCR	91.9	93.4	92.2	92.0	97.2			5.63
SOP-PCR-1.5	DG3-qPCR	70.2	92.8	89.2	87.6	88.5			1.82

Reference Method Descriptions

Method	Description	Associated Samples
SM 10150 B	Phytoplankton chlorophyll-a corrected, uncorrected, and phaeophytin by spectrophotometry	2447007
SM 9223 Quanti-Tray	E. coli by Colilert/QT by BEA South in North Port	2447009
SOP-AB18	Qualitative assessment of algal taxa present without counts	2446989
SOP-IZ06	No. Taxa of FW macroinverts, qual samp, 20 dipnets.	2446990
SOP-PCR-1.0	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2447008
SOP-PCR-1.2	Detection and quantification of coastal bird specific Catellicoccus marimammalum Gull2 genetic marker with quantitative polymerase chain reaction	2447008
SOP-PCR-1.3	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2447008
SOP-PCR-1.4	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2447008
SOP-PCR-1.5	Detection and quantification of canine-specific DG3 Bacteroides genetic marker with quantitative polymerase chain reaction	2447008

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 10150 B	10/25/2023	10/25/2023 15:15	Tynan A Branch	10/31/2023 14:42	Keanu Thijm	2447007
SM 9223 Quanti-Tray	10/24/2023	10/24/2023 15:32		10/25/2023 15:49	Benchmark EnviroAnalytical in North Port	2447009
SOP-AB18	10/25/2023			10/25/2023 13:20	Isaura Lorenzo-Perez	2446989
SOP-IZ06	10/25/2023	01/08/2024 08:54	Connor Lee	01/12/2024 13:25	Todd R. Risk	2446990
SOP-PCR-1.0	10/25/2023	10/25/2023 13:38	Madeline Gruver	11/16/2023 08:29	Roberta Tatti	2447008
SOP-PCR-1.2	10/25/2023	10/25/2023 13:38	Madeline Gruver	11/20/2023 12:31	Roberta Tatti	2447008
SOP-PCR-1.3	10/25/2023	10/25/2023 13:38	Madeline Gruver	11/20/2023 14:54	Jennifer Mihalic	2447008
SOP-PCR-1.4	10/25/2023	10/25/2023 13:38	Madeline Gruver	11/17/2023 16:08	Jennifer Mihalic	2447008
SOP-PCR-1.5	10/25/2023	10/25/2023 13:38	Madeline Gruver	11/20/2023 08:21	Roberta Tatti	2447008