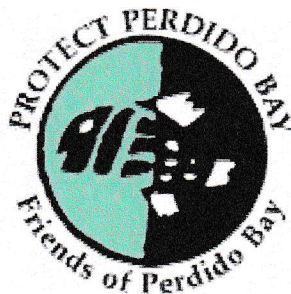




Friends of Perdido Bay
38 S. Blue Angel Parkway
PMB 350
Pensacola, FL 32506



Prepared by:
Hydrosphere Research

Test Location:
11842 Research Circle
Alachua, FL 32615

Contact Information:
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Test Number:
FPB-CH 22173

Initiated:
September 7, 2022

Test Type:
14-day Algal Growth Potential Test



Hydrosphere Research is a NELAP Certified Lab (E82295)

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Appendix A. Chain of Custody

Appendix B. Laboratory Notes & Statistical Results

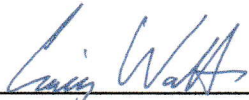
Report of Algal Growth Potential Tests Performed for Friends of Perdido Bay

Abstract

Personnel at Friends of Perdido Bay collected samples from Perdido Bay, FL. At the request of Friends of Perdido Bay, Hydrosphere Research conducted a 14-day Algal Growth Potential test with the saltwater algae *Dunaliella tertiolecta* using the samples collected.

The results are summarized in the accompanying report. This report shall not be reproduced, except in full, without the written approval of the laboratory. The results discussed in this report relate only to the sample as identified on the Chain of Custody form in Appendix A. The Laboratory Bench Sheets and Statistical Results are in Appendix B.

Testing was conducted using generally accepted lab practices and adhere to the standards set forth by the National Environmental Laboratory Accreditation Program (NELAP). The Algal Growth Potential test method is not a NELAP accredited test. Hydrosphere Research believes the results are true and accurate.

	10/05/22
Craig Watts, Lab Director	Date

Introduction

Two grab samples were provided by Friends of Perdido Bay. Using these samples, Hydrosphere Research conducted a 14-day AGP test with the algae *D. tertiolecta*.

Materials and Methods

Test Sample

The grab samples were collected on August 1, 2022 and were listed as Perdido Bay #1 and Perdido Bay #2. The samples were contained in ½ gallon high density polyethylene containers, which were intact upon arrival. The arrival temperature of the sample met the sample acceptance criteria. The Chain of Custody forms for this sample is attached hereto in Appendix A.

The water quality values fell into expected ranges for pH, salinity, and dissolved oxygen. All other chemical characterization data for the effluent sample upon arrival in the laboratory is provided on the Sample Data Bench Sheet in Appendix B.

The samples were adjusted to a salinity of 20‰ prior to testing. The samples were filtered through a 0.45 µm sterile filter and then frozen at -20°C until test initiation.

Site specifics for toxics sampling May 2023

Site #1 - Elevenmile Creek, just north of Kingsfield Road Bridge 30°34' 26.36N, 87°19' 17.80W

Site #2 - Upper Perdido Bay, just off the beach 30°24' 50.30 N, 87°22' 18.35" W

Sampling was done with a bucket grab of surface water

A summary of the sample received, and Hydrosphere's identification number is presented in Table 1. Sample Information below.

Table 1. Sample Information

Location ID	Date	Time	Hydrosphere ID
Perdido Bay #1	08/01/22	1200	22173A
Perdido Bay #2	08/01/22	1300	22173B

Test Organisms

The saltwater algae, *D. tertiolecta*, was cultured at Hydrosphere Research.

Test Methods

The test method used was a modified version of "U.S. Environmental Protection Agency. The *Selenastrum capricornutum* Printz Algal Assay Bottle Test. EPA 600/9-78-018, July 1978."

Test Methodology

The Algal Growth Potential test is a demonstration of a sample's ability to grow algae. An unknown sample was compared to a laboratory prepared growth media. In this case, the growth media was Saltwater Algal Growth Media (SAGM). The test concentrations were 0 (control) and 100 percent sample.

100 mLs of sample was added to each test replicate. The test sample was then inoculated with rinsed algae at 1,000 cells/mL. The test vessels were stoppered and placed in a special temperature-controlled water bath with constant lighting. The test vessels were swirled by hand twice daily.

Toxicity Test Monitoring

The bioassay test was processed and initiated on September 7, 2022. The samples were checked for dissolved oxygen, total residual chlorine, pH, salinity, alkalinity, and hardness prior to test initiation. Sample temperature was measured daily. The water bath temperature was monitored daily. No other water quality measurements were conducted during the test. On the last day of the test, the samples were filtered through Advantec® GA-55 47mm glass fiber filters to determine gravimetric dry weights.

Test Location

The bioassay test was performed at Hydrosphere Research, 11842 Research Circle, Alachua, FL 32615; telephone number (386) 462-7889.

Statement of Quality Assurance

This report was reviewed by the Hydrosphere Research Laboratory Director to ensure the procedures outlined in the Hydrosphere Research Quality Manual were followed. Testing was conducted using generally accepted lab practices. Hydrosphere Research believes the results are true and accurate and meet all NELAP standards.

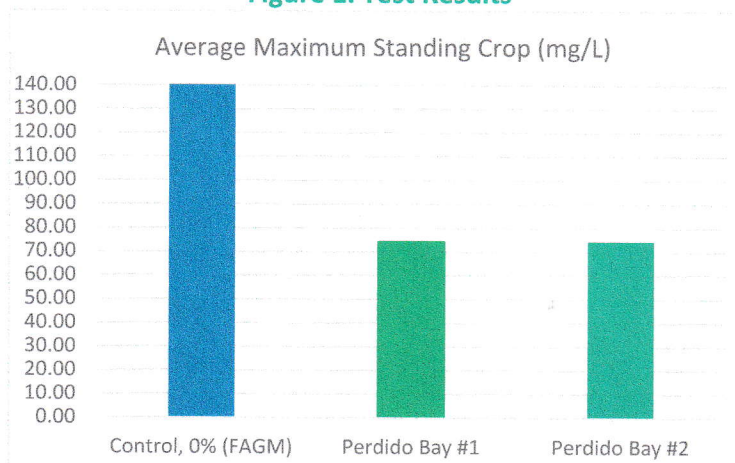
Results & Discussion

All results are measured in Maximum Standing Crop in mg dry weight/L. The raw data, bench sheets, and Maximum Standing Crop calculations are in Appendix B. The test results are presented in Table 2 and Figure 1 below.

Table 2. Test Results

Client Sample	Average Maximum Standing Crop (mg/L)	Standard Deviation
Control, 0% (SAGM)	158.90	3.439
Perdido Bay #1	74.33	5.470
Perdido Bay #2	74.29	15.028

Figure 1. Test Results



The laboratory FAGM control produced an MSC of 158.90 mg/L. The sample provided did not demonstrate significant growth when compared to the laboratory control.

No unusual observations or deviations from standard test protocol were noted. The test results only relate to the sample described in this report. The bioassay tests were initiated within 6-months of the sample collection time. The tests were acceptable tests based on control growth and test conditions.

References

U.S. Environmental Protection Agency. The *Selenastrum capricornutum* Printz Algal Assay Bottle Test. EPA 600/9-78-018, July 1978

Handbook of Analytical Quality Control in Water and Wastewater Laboratories, EPA-600/4-79-019, March 1979.

Chemical and physical parameters reported herein were determined by methods described in "Methods for Chemical Analysis of Water and Waste", EPA 600/4-79-020, March 1983.