# Peroxide quantification in Thalassia testudinum tissue and response to pathogenic Labyrinthula in seagrass collected February 2024 in Tampa Bay, Florida

**Website**: https://www.bco-dmo.org/dataset/970532
**Data Type**: experimental
**Version**: 1
**Version Date**: 2025-09-10

**Project**
» Collaborative Research: VIDA Seagrass: Viral Infection Dynamics Among Seagrass (VIDA Seagrass)

| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
This dataset presents results of peroxide assays and concentrations of hydrogen peroxide from seagrass tissue. While seagrass serve as a vital foundation species in many coastal ecosystems, investigations of marine host-pathogen interactions continue to be underexplored and provide insights into the biological factors leading to seagrass die-offs. Infection of the marine subtropical seagrass Thalassia testudinum (Banks ex König) by pathogenic Labyrinthula sp. was found to induce alterations to the host's oxidative metabolism over the early stages of infection (monitored over a 72-hr time course). In planta hydrogen peroxide levels provided supporting evidence of a coordinated hypersensitive response in the host plant following infection by the etiological agent of seagrass wasting disease, pathogenic Labyrinthula sp.

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## Coverage

**Location**: Tampa Bay, Florida
**Spatial Extent**: **N**:28 **E**:-82.4 **S**:27.5 **W**:-82.7
**Temporal Extent**: 2024-02-16

## Dataset Description

### Background

Seagrasses represent a diverse and highly productive functional group of marine angiosperms that provide an array of vital ecosystem services to coastal communities, including sediment stabilization, carbon sequestration, improvement of water quality, mitigation of ocean acidification, and habitat provision for commercially and recreationally important species (Duarte et al., 2013; Fourqurean et al., 2012; Hendriks et al.,

2014; Lefcheck et al., 2019; Heck et al., 2003). While abiotic stressors, in isolation or in combination, have been shown to cause a negative impact on seagrass beds, there continues to be much less data available on how biological factors, such as seagrass wasting disease (SWD), contribute to population declines (Sullivan et al., 2018).

Heterotrophic protists of the genus *Labyrinthula*, family Labyrinthulaceae, play an etiological role in SWD, contributing strongly to epidemic events (Sullivan et al., 2013). Recent years have provided substantial insights into the seagrass-*Labyrinthula* pathosystem. However, *T. testudinum* has remained relatively understudied when compared to the information available on SWD interactions in other seagrass species (Eisenlord et al., 2024, Dawkins et al., 2018; Brakel et al., 2019; Graham et al., 2023; Groner et al., 2021; Duffin et al., 2021; Pagenkopp Lohan et al., 2024). Although such work has started to establish the foundational relationships between key abiotic factors and occurrences and severity of SWD, reports on the physiological and cellular mechanisms underpinning host immune responses in seagrasses are very limited in scope.

Seagrasses convergently evolved from terrestrial angiosperms and underwent aquatic recolonization in their lineage over ~75 Ma. This process included a number of genomic changes to attain the structural and cellular adaptations required to live in a marine environment (Olsen et al., 2016). Despite these changes, it still seems highly plausible that select components of the seagrass immune system would remain analogous to their well-studied terrestrial counterparts (Janssen & Bremer, 2004; Lee et al., 2018; Castel et al., 2024).

While the hypersensitive cell death process is considered to be a major element of terrestrial plant disease resistance, its occurrence and associated characteristics have not been described in detail in any seagrass species, to our knowledge. Turtlegrass, *T. testudinum*, has been selected for investigation given its characteristics as a dominant, long-lived, tropical/subtropical seagrass that serves as an important foundational species (van Tussenbroek, 2007). Taking into consideration the continued decline of seagrasses due to wasting disease, this work is both timely and critical in addressing the paucity of data surrounding innate immune responses in marine vascular plants, specifically *T. testudinum*.

## Methods & Sampling

### METHODOLOGY

### *T. testudinum* Collection and Maintenance

*Thalassia testudinum* shoots were collected from Boca Ciega Bay and Lassing Park in Tampa, Florida (27.7493° N, 82.6300° W; 27.7927° N, 82.7657° W) on February 16th, 2024. Harvested seagrass ramets included the blades, sheath, and at least a 5 cm section of horizontal rhizome, and all plants collected appeared healthy through visual assessments, lacking indications of stress or lesions. The seagrass ramets were cleaned of epiphytes, transplanted within 24 hours of collection, and maintained in saltwater aquaria. Salinity was maintained at ~32, with diel temperatures of 26-28˚C, and light intensity of 110-112 µmol/$m^2$/s, under a 12:12 hour L:D graduated photoperiod (Aqueon® OptiBright® LED Lights) and constant flow. These growing parameters have been previously described as ideal for *T. testudinum* maintenance in aquaria and reflect natural conditions of the collection site (Bishop et al., 2017). The substrate consisted of sediment collected from the harvest site, supplemented with aquaria live sand (CaribSea® Aragonite); terracotta pots and plastic garden planters were used to house the seagrass within the aquaria.

### *Labyrinthula sp.* Culture and Infection Method

Cultures of *Labyrinthula* sp. “E” isolate 8B, a known pathogenic strain, were utilized for all infection trials. This strain has been in culture in our laboratory for over 18 years and its morphology and pathogenicity has been well documented (Bishop et al., 2017; Martin et al., 2016; Trevathan-Tackett et al., 2015). *Labyrinthula* sp. was maintained in serum-seawater agar (SSA) media with autoclaved *T. testudinum* blade clippings to encourage growth. The SSA media consisted of 500 mL of 0.22 µm filtered seawater, salinity of 25 from Instant Ocean Sea Salt, supplemented with 6 g agar, 0.05 g each of peptone and nutritional yeast, and 0.5 g dextrose, 5 mL horse serum, and 12.5 mL antibiotic solution containing 1.25% of both streptomycin and penicillin. All chemicals were sourced via Sigma-Aldrich (St. Louis, MO, USA).

Seagrass infection vectors, of 1 cm in length, were boiled or autoclaved to surface sterilize and placed onto agar growth plates. Once the *Labyrinthula* sp. growth completely encompassed the vector (typically 5-7 days), it was used in infection experiments. Infections proceeded with a healthy 4 cm, fresh weighed blade onto which an infection vector was carefully attached using a Tygon tubing clamp. The full length of the infection vector was placed in direct contact with the healthy blade to encourage *Labyrinthula* sp. transfer and infection.

Sterile seagrass “mock vectors” of 1 cm in length were attached in the same manner to the healthy blades in all control samples. All samples were maintained in individual transparent 50 mL high clarity conical falcon tubes (Thermo Fisher Scientific™, Waltham, MA, USA), containing 45 mL of aquaria water, 26-28˚C, under a light intensity of 100 µmol/$m^2$/s on a 12:12 L:D cycle (GLO® Linear Fluorescent Lighting System) and pulled for assessment at their respective timepoints of interest (24-hrs, 48-hrs, or 72-hrs). Our preliminary experiments showed that most of the physiological and biochemical changes occurred in the host within the first 72-hrs post infection. Thus, a 72-hr time limit was selected for the majority of experiments reported herein. Infected blades that did not require immediate processing were stored at -80˚C for later analysis.

Previously frozen time-course sample blades were placed in liquid nitrogen and ground to a powder using a FastPrep-24® Tissue Homogenizer (Irvine, CA, USA). At first, each sample was placed in a 2 mL lysing matrix tube containing two steel beads without buffer and homogenized at a speed of 6.0 m/s for 60 s. Then, 300 µL of 1x phosphate buffered saline solution (4 mM, pH 7.6) was added to each sample, and the homogenization step was repeated twice until the mixture exhibited a texture of fine paste. Samples were subsequently centrifuged at 17,000 x g for 10 min, and the resulting supernatants were used for hydrogen peroxide and capase-3 assays and normalized using a BCA protein assay kit (Thermo Fisher Scientific™) according to manufacturer’s instructions.

A colorimetric hydrogen peroxide assay was conducted on the seagrass *Thalassia testudinum* to assess *in planta* levels of a reactive oxygen species, indicating changes in host oxidative metabolism following *Labyrinthula* sp. infection. The Peroxide Assay Kit was utilized according to the manufacturer’s protocol (Sigma-Aldrich, 2019). and the absorbance at 585 nm was subsequently measured for both standards and samples. The assay uses the chromogenic Fe3+-xylenol orange reaction (FOX), in which a purple complex is formed when Fe2+ is oxidized to Fe3+ by peroxides present in the sample, generating a colorimetric (585 nm) result, proportional to the level of peroxide present (Sigma-Aldrich, 2019; from Peroxide Assay Kit (MAK311) Technical Bulletin).

Hydrogen peroxide ($H_2O_2$) standards (labeled A–H) were prepared at known micromolar concentrations of 0, 3, 6, 9, 12, 18, 24, and 30 µM using ultrapure water and 3% hydrogen peroxide standard. Fresh hydrogen peroxide standard was prepared on the day of the assay, following kit dilution instructions and using Sigma-Aldrich kit reagents to generate the final 30 µM $H_2O_2$ standard. The labeling scheme for the time series unique sample blades, where n = 5 for each group, is as follows: 24-hr control (samples 1-5), 24-hr infected (samples 6-10), 48-hr control (samples 11-15), 48-hr infected (samples 16-20), 72-hr control (samples 21-25), and 72-hr infected (samples 26-30). Sample extracts were treated and put into a 96-well plate for analysis with a Biotek Synergy Plate Reader.

To determine protein content, a Thermo Scientific™ BCA Protein Assay Kit (Product No. 23225) was used according to manufacturer instructions. The kit outlines a dilution of bovine serum albumin (BSA), which generated a nine-point linear regression corresponding to standard protein concentrations of 0-2000 ug/mL. The plate was read at 562 nm absorbance, and the standard curve was utilized to determine the protein concentrations of all seagrass samples.

**Data Processing Description**

Net Absorbance (Net A) values were determined by subtracting the blank values from the Gross Absorbance values to remove the background signal. The average peroxide assay blank for Standard A, where $H_2O_2$ = 0 µM, was 0.33 Absorbance units (AU). Then $H_2O_2$ standards (labeled A-H) were plotted with Net Absorbance versus $H_2O_2$ concentration. A linear regression equation was derived from the best-fit line of the standard curve, and subsequently applied to the sample absorbances to quantify hydrogen peroxide ($H_2O_2$) concentrations in *Thalassia testudinum* samples. Assay equation: $y = 0.0258x + 0.1946$.

Protein concentrations in the seagrass blades, determined via the BCA assay, were used to normalize hydrogen peroxide measurements, thereby controlling for variability in cell density and extraction efficiency among samples. The corrected $H_2O_2$ concentration values are reported in picomoles $H_2O_2$ per microgram of protein.

Supplementary figures are included to display the standard curve (Figure 1) as well as the final *in planta* hydrogen peroxide concentrations (Figure 2), providing evidence of a host hypersensitive response due to infection.

**BCO-DMO Processing Description**

- Imported data from source file "Larson Ross BCO-DMO Data.xlsx"
- Removed non-data rows and added columns for the 'key' information
- Added new column for Duration in hours of experimental treatment
- Added new column for Treatment type, either control or infected
- Rounded values to 3 and 5 digits
- Modified parameter (column) names to conform with BCO-DMO naming conventions. Replaced spaces and special characters with underscores

- Created a Statistics table
- Created PDF formats of Supplemental figures, provided caption descriptions, and updated axes labels

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## Data Files

| File | |
|---|---|
| **970532_v1_peroxide_assay_thalassia_testudinum.csv** | (Comma Separated Values (.csv), 2.72 KB) MD5:a60ffaa6f4cae40a25319bdb2bbb6376 |
| Larson and Ross 2025 Peroxide assay; Primary data file for dataset ID 970532, version 1<br>Parameter (column) descriptions are listed below in the Parameters section | |

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## Supplemental Files

| File | |
|---|---|
| **Figure1_Larson_and_Ross_Peroxide_Assay_Standard_Curve.pdf** | (Portable Document Format (.pdf), 507.39 KB) MD5:4c349a87f83e84f752902ce9eac28681 |
| Figure 1. Standard curve for Larson and Ross Peroxide Assay (2025) | |
| **Figure2_Larson_and_Ross_Peroxide_Assay_treatments.pdf** | (Portable Document Format (.pdf), 477.52 KB) MD5:d378509014b251bfc1fc02364fd9aeb8 |
| Figure 2. In planta H2O2 concentrations in T. testudinum tissue following Labyrinthula sp. infection at 24-, 48-, and 72-hours | |
| **Statistics_peroxide_assay.csv** | (Comma Separated Values (.csv), 576 bytes) MD5:116ac0c31182635ccd04a0257203199b |
| Average hydrogen peroxide concentration values and standard deviation. This includes actual values and 'positive shifted' values (+3) to aid in visualization in graphs.<br>Sample_group = grouping of 5 samples with the same treatments;<br>Sample_description=Description of the sample treatments (control vs. infected) and time duration;<br>Avg_normalized_H2O2_conc_pmols_per_ug_protein= average value of the sample group's peroxide concentrations normalized to protein in picomoles H2O2 per microgram protein;<br>Average_plus_3_ pmols_H2O2_per_ug_protein = average value + 3 of the sample group's peroxide concentration normalized to protein (used for graphing/visualization)<br>Standard_deviation = Standard deviation of the sample group's normalized peroxide values | |

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## Related Publications

Bishop, N., Martin, D., & Ross, C. (2017). Effects of multi-stress exposure on the infection dynamics of a Labyrinthula sp.-turtle grass pathosystem. Marine Ecology Progress Series, 581, 119–133. https://doi.org/10.3354/meps12318
*Related Research*

Brakel, J., Jakobsson-Thor, S., Bockelmann, A.-C., & Reusch, T. B. H. (2019). Modulation of the Eelgrass – Labyrinthula zosterae Interaction Under Predicted Ocean Warming, Salinity Change and Light Limitation. Frontiers in Marine Science, 6. https://doi.org/10.3389/fmars.2019.00268
*Related Research*

Castel, B., El Mahboubi, K., Jacquet, C., & Delaux, P.-M. (2024). Immunobiodiversity: Conserved and specific immunity across land plants and beyond. Molecular Plant, 17(1), 92–111. https://doi.org/10.1016/j.molp.2023.12.005
*Related Research*

Dawkins, P., Eisenlord, M., Yoshioka, R., Fiorenza, E., Fruchter, S., Giammona, F., Winningham, M., & Harvell, C. (2018). Environment, dosage, and pathogen isolate moderate virulence in eelgrass wasting disease. Diseases of Aquatic Organisms, 130(1), 51–63. https://doi.org/10.3354/dao03263
*Related Research*

Duarte, C. M., Losada, I. J., Hendriks, I. E., Mazarrasa, I., & Marbà, N. (2013). The role of coastal plant communities for climate change mitigation and adaptation. Nature Climate Change, 3(11), 961–968. https://doi.org/10.1038/nclimate1970
*Related Research*

Duffin, P., Martin, D. L., Furman, B. T., & Ross, C. (2021). Spatial Patterns of Thalassia testudinum Immune Status and Labyrinthula spp. Load Implicate Environmental Quality and History as Modulators of Defense Strategies and Wasting Disease in Florida Bay, United States. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.612947
*Related Research*

Eisenlord, M. E., Agnew, M. V., Winningham, M., Lobo, O. J., Vompe, A. D., Wippel, B., Friedman, C. S., Harvell, C. D., & Burge, C. A. (2024). High infectivity and waterborne transmission of seagrass wasting disease. Royal Society Open Science, 11(8). https://doi.org/10.1098/rsos.240663
*Related Research*

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., Apostolaki, E. T., Kendrick, G. A., Krause-Jensen, D., McGlathery, K. J., & Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. Nature Geoscience, 5(7), 505–509. https://doi.org/10.1038/ngeo1477
*Related Research*

Graham, O. J., Stephens, T., Rappazzo, B., Klohmann, C., Dayal, S., Adamczyk, E. M., Olson, A., Hessing-Lewis, M., Eisenlord, M., Yang, B., Burge, C., Gomes, C. P., & Harvell, D. (2023). Deeper habitats and cooler temperatures moderate a climate-driven seagrass disease. Philosophical Transactions of the Royal Society B: Biological Sciences, 378(1873). https://doi.org/10.1098/rstb.2022.0016
*Related Research*

Groner, M., Eisenlord, M., Yoshioka, R., Fiorenza, E., Dawkins, P., Graham, O., Winningham, M., Vompe, A., Rivlin, N., Yang, B., Burge, C., Rappazzo, B., Gomes, C., & Harvell, C. (2021). Warming sea surface temperatures fuel summer epidemics of eelgrass wasting disease. Marine Ecology Progress Series, 679, 47–58. https://doi.org/10.3354/meps13902
*Related Research*

Heck Hay, K., Hays, G., & Orth, R. (2003). Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series, 253, 123–136. https://doi.org/10.3354/meps253123
*Related Research*

Hendriks, I. E., Olsen, Y. S., Ramajo, L., Basso, L., Steckbauer, A., Moore, T. S., Howard, J., & Duarte, C. M. (2014). Photosynthetic activity buffers ocean acidification in seagrass meadows. Biogeosciences, 11(2), 333–346. https://doi.org/10.5194/bg-11-333-2014
*Related Research*

Janssen, T., & Bremer, K. (2004). The age of major monocot groups inferred from 800+rbcL sequences. Botanical Journal of the Linnean Society, 146(4), 385–398. https://doi.org/10.1111/j.1095-8339.2004.00345.x
*Related Research*

Lee, H., Golicz, A. A., Bayer, P. E., Severn-Ellis, A. A., Chan, C.-K. K., Batley, J., Kendrick, G. A., & Edwards, D. (2018). Genomic comparison of two independent seagrass lineages reveals habitat-driven convergent evolution. Journal of Experimental Botany, 69(15), 3689–3702. https://doi.org/10.1093/jxb/ery147
*Related Research*

Lefcheck, J. S., Hughes, B. B., Johnson, A. J., Pfirrmann, B. W., Rasher, D. B., Smyth, A. R., Williams, B. L., Beck, M. W., & Orth, R. J. (2019). Are coastal habitats important nurseries? A meta-analysis. Conservation

Letters, 12(4). Portico. https://doi.org/10.1111/conl.12645
*Related Research*

Martin, D. L., Chiari, Y., Boone, E., Sherman, T. D., Ross, C., Wyllie-Echeverria, S., Gaydos, J. K., & Boettcher, A. A. (2016). Functional, Phylogenetic and Host-Geographic Signatures of Labyrinthula spp. Provide for Putative Species Delimitation and a Global-Scale View of Seagrass Wasting Disease. Estuaries and Coasts, 39(5), 1403–1421. https://doi.org/10.1007/s12237-016-0087-z
*Methods*

Olsen, J. L., Rouzé, P., Verhelst, B., Lin, Y.-C., Bayer, T., Collen, J., Dattolo, E., De Paoli, E., Dittami, S., Maumus, F., Michel, G., Kersting, A., Lauritano, C., Lohaus, R., Töpel, M., Tonon, T., Vanneste, K., Amirebrahimi, M., Brakel, J., … Van de Peer, Y. (2016). The genome of the seagrass Zostera marina reveals angiosperm adaptation to the sea. Nature, 530(7590), 331–335. https://doi.org/10.1038/nature16548
*Related Research*

Pagenkopp Lohan, K. M., DiMaria, R., Martin, D. L., Hughes, A. R., Peterson, B. J., Boyer, K. E., Stachowicz, J. J., Jorgensen, P., Ruiz, G. M., & Ross, C. (2025). Phylogeography of Labyrinthula species and strains shows high connectivity and low genetic variation across seagrass hosts and geographic locations in North America. Frontiers in Marine Science, 11. https://doi.org/10.3389/fmars.2024.1463968
*Related Research*

Sigma-Aldrich. (2019). Peroxide Assay Kit (Product MAK311) Technical Bulletin. Retrieved July 31, 2025, from https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/532/566/mak311bul.pdf
*Methods*

Sullivan, B. K., Sherman, T. D., Damare, V. S., Lilje, O., & Gleason, F. H. (2013). Potential roles of Labyrinthula spp. in global seagrass population declines. Fungal Ecology, 6(5), 328–338. https://doi.org/10.1016/j.funeco.2013.06.004
*Related Research*

Sullivan, B. K., Trevathan-Tackett, S. M., Neuhauser, S., & Govers, L. L. (2018). Review: Host-pathogen dynamics of seagrass diseases under future global change. Marine Pollution Bulletin, 134, 75–88. https://doi.org/10.1016/j.marpolbul.2017.09.030
*Related Research*

Trevathan-Tackett, S. M., Lane, A. L., Bishop, N., & Ross, C. (2015). Metabolites derived from the tropical seagrass Thalassia testudinum are bioactive against pathogenic Labyrinthula sp. Aquatic Botany, 122, 1–8. https://doi.org/10.1016/j.aquabot.2014.12.005
*Related Research*

Tussenbroek, B. I. van, Vonk, J. A., Stapel, J., Erftemeijer, P. L. A., Middelburg, J. J., & Zieman, J. C. (n.d.). The Biology of Thalassia: Paradigms and Recent Advances in Research. SEAGRASSES: BIOLOGY, ECOLOGYAND CONSERVATION, 409–439. https://doi.org/10.1007/978-1-4020-2983-7_18
*Related Research*

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Type | Indicates whether standard or sample | unitless |
| ID | Sample identification where letters are standards and numbers are samples | unitless |
| Treatment | Indicates treatment for the experiment (control or infected) | unitless |
| Duration_hours | Duration of the experimental treatment | hours |
| H2O2_concentration | Concentration of hydrogen peroxide; Standard concentrations were measured; Sample concentrations were obtained from linear regressions. | micromolar (umols/L) |
| Gross_A_585nm | Gross absorbance at 585 nanometers | absorbance units (AU) |
| Net_A_585nm | Net absorbance at 585 nanometers obtained by subtracting the assay blank from the gross absorbance | absorbance units (AU) |
| Blank_value | Blank value determined from gross absorbance where H2O2 concentration was zero (Assay 1 = 0.33) | absorbance units (AU) |
| protein_content | Protein content (total protein concentration) | micrograms per milliliter (ug/mL) |
| H2O2_conc_normalized_to_protein | Hydrogen peroxide concentration normalized to protein content | picomoles H2O2 per microgram of protein (pmols/ug) |
| H2O2_conc_plus3 | Hydrogen peroxide concentration value plus 3 to make all the values positive for visualization | picomoles H2O2 per microgram of protein (pmols/ug) |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Saltwater aquaria |
| **Generic Instrument Name** | Aquarium |
| **Dataset-specific Description** | Thalassia testudinum shoots were cleaned of epiphytes, transplanted within 24 hours of collection, and maintained in saltwater aquaria. |
| **Generic Instrument Description** | Aquarium - a vivarium consisting of at least one transparent side in which water-dwelling plants or animals are kept |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Centrifuge |
| **Generic Instrument Name** | Centrifuge |
| **Dataset-specific Description** | Samples were centrifuged at 17,000 x g for 10 min |
| **Generic Instrument Description** | A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | FastPrep-24® Tissue Homogenizer |
| **Generic Instrument Name** | Homogenizer |
| **Dataset-specific Description** | Previously frozen time-course sample blades were placed in liquid nitrogen and ground to a powder using a FastPrep-24® Tissue Homogenizer (Irvine, CA, USA). |
| **Generic Instrument Description** | A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Aqueon® OptiBright® LED Lights |
| **Generic Instrument Name** | LED light |
| **Dataset-specific Description** | Harvested seagrass ramets were exposed to light intensity of 110-112 µmol/m2/s, under a 12:12 hour L:D graduated photoperiod (Aqueon® OptiBright® LED Lights). |
| **Generic Instrument Description** | A light-emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes, releasing energy in the form of photons. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | GLO® Linear Fluorescent Lighting System |
| **Generic Instrument Name** | LED light |
| **Dataset-specific Description** | Sterile seagrass “mock vectors” of 1 cm in length were attached in the same manner to the healthy blades in all control samples. All samples were maintained in individual transparent 50 mL high clarity conical falcon tubes under a light intensity of 100 µmol/m2/s on a 12:12 L:D cycle (GLO® Linear Fluorescent Lighting System) |
| **Generic Instrument Description** | A light-emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes, releasing energy in the form of photons. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Biotek Synergy plate reader |
| **Generic Instrument Name** | plate reader |
| **Dataset-specific Description** | Absorbance of sample extracts and standards was measured with a Biotek Synergy Plate Reader. |
| **Generic Instrument Description** | Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23. |

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## Project Information

**Collaborative Research: VIDA Seagrass: Viral Infection Dynamics Among Seagrass (VIDA Seagrass)**

**Coverage**: Tampa Bay, Florida

*NSF Award Abstract:*
Seagrasses are marine flowering plants (or angiosperms) that create expansive underwater meadows that form the basis of highly productive and valuable ecosystems in coastal oceans. Unlike terrestrial systems where angiosperms dominate plant diversity, seagrasses are the only flowering plants in marine environments. Based on the profound impacts of viral infections on terrestrial plants, viruses are expected to influence seagrass ecology. However, no prior work has investigated viral infection dynamics in seagrasses or the impact of viruses on seagrass health. This project provides fundamental knowledge about seagrass-virus interactions through field and laboratory studies of Thalassia testudinum (i.e., turtlegrass, a climax species and

key ecosystem engineer), and turtlegrass virus X (TVX), the only seagrass virus currently reported from experimental research. The lack of a seagrass-virus study system has kept the scientific community from learning which factors drive viral infection in marine angiosperms. By establishing the first seagrass-virus study system, a novel virus-host pathosystem for which virtually nothing is known, this project contributes to a more comprehensive understanding of seagrass ecology and serves as a model for investigating the growing number of seagrass viruses discovered through sequencing efforts. This multifaceted project trains one postdoctoral researcher, two graduate students, and six undergraduate students. Dissemination of results and data through open access channels informs the broader community and provides scientists with data for their own research to propel the field of seagrass virology. This project also engages educators and students participating in programs that strive to increase participation from underrepresented groups in STEM fields. Teachers from the Jacksonville Teacher Residency Program are getting involved through development of lessons that dive into seagrass biology. Students from Girls Incorporated, Girl Scouts, and the University of South Florida's Oceanography Camp for Girls are participating as citizen scientists by photographing Tampa Bay's seagrass ecosystems and contributing their observations to the Seagrass Spotter website. This project also increases awareness of seagrass ecosystems and challenges the public perception that all viruses are pathogenic through hands-on activities at the annual St. Petersburg Science Festival.

Seagrass-virus interactions are being investigated through a two-tiered approach involving field studies in Tampa Bay, Florida and microcosm experiments. Field surveys focus on elucidating the nature of turtlegrass-TVX interactions (positive, neutral or negative) and the relationship between turtlegrass genotypic diversity and virus distribution in a natural population where TVX has persisted for at least five years. TVX load is monitored bimonthly over two years to assess how viral load relates to turtlegrass genotype and performance (growth, health, reproductive effort), and abiotic parameters. The investigated turtlegrass meadow contains TVX-positive and negative specimens, thus providing a perfect natural laboratory with homogenous environmental characteristics that allow exploration of the drivers of viral infection. Given that environmental changes may alter host-microbe interactions, complementary microcosm experiments are evaluating turtlegrass responses to TVX infection at the physiological (survival, photochemical capacity, cellular responses) and molecular (transcriptomic) levels in a controlled environment under normal conditions and in the context of salinity changes, an important seagrass stressor. Microcosm experiments also provide the first profiles of seagrass gene expression and measurement of cellular metabolites in response to viral infection. Expected results have direct implications for understanding seagrass production and resilience in the face of global climate change and anthropogenic stress.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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## Funding


| Funding Source | Award |
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| NSF Division of Ocean Sciences (NSF OCE) | OCE-2219548 |


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