Infectivity assays and ultrastructural characterization of Thalassia testudinum agroinfected with Turtle grass virus X (TGVX) in laboratory aquaria, February-May 2024

Website: https://www.bco-dmo.org/dataset/982240
Data Type: experimental
Version: 1
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Project
» Collaborative Research: VIDA Seagrass: Viral Infection Dynamics Among Seagrass (VIDA Seagrass)

Contributors	Affiliation	Role
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Abstract
This dataset documents molecular, phenotypic, and ultrastructural data generated during the first successful agroinfection of a marine angiosperm (Thalassia testudinum) using an infectious cDNA clone of Turtle Grass Virus X (TGVX), a positive-sense single-stranded RNA potexvirus. The dataset comprises three primary types of image data: Multiplex RT-PCR gel images, used to confirm TGVX presence before and after agroinfection. Photographs of infected and control plants, capturing visible symptoms such as leaf wrinkling, curling, and deformation. Transmission electron microscopy (TEM) images depicting viral cytopathology, including virus-like particles, putative replication organelles, disrupted chloroplast ultrastructure, and other infection-associated features at the cellular level. Each image is indexed in an accompanying file inventory table (file_inventory_table.xlsx), which provides detailed metadata including filename, experimental treatment condition (e.g., mock, pre-inoculation, post-inoculation), imaging method, descriptive annotations of visual features, scale bars (where applicable), and publication status. This dataset includes the full set of TEM images generated during the agroinfection experiments, representing all observed cytopathological features across experimental replicates. All experiments were carried out under controlled aquarium conditions from February to May 2024. This standalone dataset supports research on marine virology, virus-host interactions, and seagrass ecosystem health by enabling visual and molecular examination of TGVX infection dynamics in a key tropical seagrass species.

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Coverage

Location: Tampa Bay, Florida, USA
Spatial Extent: **N**:27.758566 **E**:-82.607178 **S**:27.583468 **W**:-82.635578
Temporal Extent: 2024-02-01 - 2024-05-31

Methods & Sampling

Site

Study sites were located in Tampa Bay, Florida, USA. The viral genome was sourced from naturally infected Thalassia testudinum at Terra Ceia Aquatic Preserve (27.5838°N, 82.6161°W), a shallow coastal seagrass meadow. Experimental host plants were collected from Lassing Park in St. Petersburg (27.7538°N, 82.6281°W), another nearshore seagrass habitat.

Sample Collection and Preparation

Young Thalassia testudinum plants were collected from Lassing Park, St. Petersburg, Florida, USA (27.7538°N, 82.6281°W) on March 4th, 2024. Collected specimens were transported in ambient seawater to the Knight Oceanographic Research Center Aquarium (University of South Florida), transplanted into 6-cm diameter pots with beach sand, and maintained in 10-liter aquaria. Artificial seawater was prepared using Instant Ocean at a salinity of 30 PSU (±0.5). The aquaria system was maintained at 30°C (±0.5) with constant aeration and a 16:8 light:dark photoperiod.

Prior to agroinfection, plants were screened for potexviruses using multiplex RT-PCR with specific primers targeting the TGVX coat protein (MBL5: 5′-CACAGATGAAGAGCTGACC-3′ and MBL6: 5′-TTCGATGAAGTAAGTGGCGG-3′) and degenerate primers for the potexvirus replicase gene (Potex-5/Potex-2RC), as described by van der Vlugt and Berendsen (2002). Mitochondrial nad5 gene primers (nad5-F/nad5-R; Menzel et al. 2002) were included as internal control.

Viral Source and Clone Construction

TGVX genomic RNA was extracted from naturally infected T. testudinum leaves collected at Terra Ceia Aquatic Preserve, Tampa Bay, Florida (27.5838°N, 82.6161°W) on February 5th, 2024 (Van Bogaert et al. 2019). Viral RNA was isolated following the protocol of Sánchez-Navarro et al. (2013) and amplified using the SuperScript IV One-Step RT-PCR System (Invitrogen) with genome-specific primers (MBL1 and MBL2) designed based on the full TGVX genome (GenBank accession MH077559; Van Bogaert et al. 2019). The full-length cDNA (6.3 kb) was assembled into a pLX mini binary vector (Pasin et al. 2018) using a BsmBI-based directional cloning strategy. Resulting constructs were transformed into Escherichia coli DH5α, screened by colony PCR, and validated via whole-plasmid sequencing with Oxford Nanopore technology.

Agroinfection Assays

The pLX-TGVX plasmid and an empty vector (mock control) were transformed into Agrobacterium tumefaciens strain C58C1. Bacterial suspensions were prepared in induction buffer composed of 10 mM MES (pH 5.6), 10 mM $MgCl_2$, and 200 µM acetosyringone, and adjusted to an optical density of 0.6 at 600 nm. Plants were agroinfiltrated on the abaxial side of two leaves using a needleless syringe. At 17 days post-infiltration, systemic infection was confirmed by RT-PCR detection of the TGVX coat protein from upper, non-inoculated leaves.

Experimental treatments were categorized as mock-inoculated, pre-inoculation (baseline control), and post-inoculation (TGVX-infected).

Microscopy and Symptom Analysis

Phenotypic changes were documented using photography, capturing leaf deformation, curling, and wrinkling in both infected and control plants.

Transmission electron microscopy (TEM) was used to visualize virus-like particles and evaluate cytopathological effects. Leaf tissue was processed using the leaf-dip method and standard fixation, embedding, and ultrathin sectioning protocols (Brandes and Wetter 1959; Alvarado et al. 2019). Observed abnormalities included chloroplast swelling, loss of thylakoid grana, formation of replication organelles, and laminar aggregates, consistent with potexvirus infections.

TEM analysis was performed on both published and unpublished samples to support figure-based interpretation as well as broader ultrastructural screening.

Image Data and Inventory

The dataset includes three categories of image data:

1. RT-PCR gel electrophoresis images: Bands confirming presence/absence of TGVX coat protein and potexvirus replicase.

2. Phenotypic photographs: Images showing visible plant morphology differences between mock and infected conditions.

3. TEM images: Visual evidence of cytopathology caused by TGVX infection, including filamentous virus-like particles, swollen or disorganized chloroplasts, reduced thylakoid grana, membrane-bound replication organelles, and laminar aggregates.

Each image file is cataloged in the file inventory table (file_inventory.csv), which includes:

- Filename and folder name
- Experimental treatment group (mock, pre-inoculation, post-inoculation)
- Image category (RT-PCR, phenotype, TEM)
- Short description of observed features
- Scale bar (when applicable)
- Publication status

The TEM component of this dataset is complete and includes all ultrastructural images generated during the agroinfection experiments. RT-PCR and phenotype images correspond to figures published in the associated manuscript, while some TEM images remain unpublished but are provided here to support broader reuse and interpretation.

Terms and parameter definitions (used in files within TGVX_agroinfection_turtlegrass_files.zip)**:**

Plant_ID, "Unique identifier assigned to each individual *Thalassia testudinum* plant used in the experiment", unitless, NA
Treatment, "Type of inoculation applied to the plant; pLX-TGVX = virus clone treatment, Mock = empty vector control", unitless, NA
Days_Post_Agroinfection, "Time elapsed since agroinfiltration was performed on the plant", days, N/A
TGVX_RT-PCR_Result, "Detection of TGVX RNA in plant tissues using coat protein-specific primers; Positive = viral RNA detected, Negative = not detected", unitless, NA
Infection_Symptoms, "Visual symptoms associated with viral infection; Yes = symptoms present, No = no symptoms", unitless, NA
VLP_Observed_TEM, "Presence of virus-like particles as observed by transmission electron microscopy; Yes = VLPs seen, No = not observed", unitless, NA
Chloroplast_Alterations, "Presence of cytopathological changes in chloroplasts due to viral infection; Yes = observed, No = not observed", unitless, NA
RO_Presence, "Detection of viral replication organelles in tissue sections via TEM; Yes = present, No = absent", unitless, NA
Grana_Reduction_Level, "Qualitative classification of thylakoid grana reduction in infected chloroplasts; None, Moderate, or Severe", unitless, NA

Organism identifiers (name, Life Science Identifier (LSID), ncbi_txid):
Thalassia testudinum, urn:lsid:marinespecies.org:taxname:374720, NCBI:txid55497
Agrobacterium tumefaciens, urn:lsid:marinespecies.org:taxname:1501465, NCBI:txid358
"Escherichia coli DH5[alpha]", , NCBI:txid668369
"Turtle grass virus X", , NCBI:txid2292642

Data Processing Description

Molecular data (RT-PCR results) were processed by visualization on agarose gels stained with ethidium bromide and photographed using the Syngene Ingenius 3 imaging system. Digital images were cropped and labeled using Microsoft PowerPoint 365 to organize figures in a clear and consistent manner.

Transmission electron microscopy (TEM) images were acquired using Gatan DigitalMicrograph software and exported as uncompressed TIFF files. Brightness and contrast levels were uniformly adjusted using GIMP 2.10.32 to enhance visual clarity without altering scientific information. Scale bars were standardized across micrographs using ImageJ (Fiji distribution).

Phenotypic data (visual leaf symptoms) were documented photographically, and images were organized and annotated in Microsoft PowerPoint 365, then exported as TIFF files.

Sequencing reads for validation of the TGVX genome assembly were basecalled and assembled using Oxford Nanopore (Eurofins), then aligned to the reference TGVX genome (GenBank accession MH077559) using SnapGene v8.1.1. Sequence identity and coverage were quantified, and the assembled genome was deposited in GenBank (accession number PP887953).

No statistical analyses or graphical outputs were performed at this stage. All data files and processed images are available in both original and presentation-ready formats.

BCO-DMO Processing Description

* Files within submitted file set under "TGVX_agroinfection_turtlegrass/" were packaged into TGVX_agroinfection_turtlegrass_files.zip and attached to this dataset. The associated file descriptions and metadata were uploaded as supplemental file file_inventory.csv.

* Organism names in this dataset were matched to Life Science Identifiers (LSIDs) using the World Register of Marine Species (WoRMS) on 2025-11-20 and added to the Methods&Sampling metadata section. Identifiers at NCBI were also added.

* Collection metadata included in Sampling_Metadata_Table/Thalassia_Plants_Sampling_Metadata.xlsx with the zip file package was also extracted as csv and attached as a supplemental file directly: Thalassia_Plants_Sampling_Metadata.csv
** Coordinates converted to individual Lat,Lon columns in decimal degrees
** Date format changed to ISO 8601 format.

Problem Description

No major problems or gaps were encountered during data collection or processing. All RT-PCR assays and microscopy procedures were completed as planned. Minor variability in phenotypic symptom expression was observed among agroinfected plants, consistent with natural biological variation. Environmental parameters in aquarium systems (salinity and temperature) were maintained within target ranges (30 PSU ±0.5 and 30 °C ±0.5 °C, respectively), though minor daily fluctuations occurred, which were corrected during routine 24-hour monitoring. No instrument malfunctions or software errors were reported.

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Data Files

File	
TGVX_agroinfection_turtlegrass_files.zip	(ZIP Archive (ZIP), 222.34 MB) MD5:2fc33fdac9238071c671c17bdf5749b5
A zip package containing image and sequence data from Thalassia testudinum agroinfected with Turtle Grass Virus X (TGVX). Includes sampling metadata, the TGVX genome (FASTA), RT-PCR gel and phenotype images, TEM micrographs showing viral cytopathology, and vector schematics. TEM virion, infected, and mock-control images are also included. See the list of files included in this package, along with a description and metadata for each file in the file_inventory.csv	

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Supplemental Files

File	
file_inventory.csv	(Comma Separated Values (.csv), 8.60 KB) MD5:d741193a5d0e184bd56dff6ec342f5de

This table lists all files included in the zip package TGVX_agroinfection_turtlegrass_files.zip along with associated metadata. It includes the following parameters:

filename: The name of each data file in the archive.
folder_name: The directory path or folder grouping for each file, identifying its data type (e.g., TEM_TGVX, Phenotype, RT_PCR).
treatment: The experimental condition linked to the file, such as infected, mock-inoculated, or pre-inoculation.
feature: A short description of what the file depicts or represents (e.g., virions, RT-PCR gel, chloroplast structure).
scale_bar: The image scale shown in micrometers (µm) or nanometers (nm); "NA" if not applicable.
notes: Additional descriptive information about the file, such as observed features or experimental context.
related publication: The source or publication status for each file, including DOI or repository (e.g., mBio, NCBI).

Thalassia_Plants_Sampling_Metadata.csv	(Comma Separated Values (.csv), 670 bytes) MD5:6d993cc8772b4585a7960b848a30f48a

Sample metadata including:
Sampling Location,Latitude, Longitude,Sampling Date (Exact),Purpose,Plant Tissue Collected,Collection Method,Preservation/Transport,Notes

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Related Publications

Alvarado-Marchena, L., Furman, B. T., & Breitbart, M. (2025). Construction and characterization of an infectious cDNA clone of turtle grass virus X from a naturally infected Thalassia testudinum plant. MBio, 16(1). https://doi.org/10.1128/mbio.02828-24
Results

Brandes, J., & Wetter, C. (1959). Classification of elongated plant viruses on the basis of particle morphology. Virology, 8(1), 99–115. https://doi.org/10.1016/0042-6822(59)90022-4
Methods

Lim, S. J., Rosario, K., Kernbach, M. E., Gross, A. J., Furman, B. T., & Breitbart, M. (2023). Limited potexvirus diversity in eastern Gulf of Mexico seagrass meadows. https://doi.org/10.1101/2023.12.11.571111
Results

Menzel, W., Jelkmann, W., & Maiss, E. (2002). Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. Journal of Virological Methods, 99(1–2), 81–92. https://doi.org/10.1016/s0166-0934(01)00381-0
Methods

Pasin, F., Tseng, X.-A., Bedoya, L. C., Heydarnejad, J., Deng, T.-C., García, J. A., & Chen, Y.-R. (2018). Streamlined generation of plant virus infectious clones using the pLX mini binary vectors. Journal of Virological Methods, 262, 48–55. https://doi.org/10.1016/j.jviromet.2018.09.007
Methods

Sánchez-Navarro, J. A., Zwart, M. P., & Elena, S. F. (2013). Effects of the Number of Genome Segments on Primary and Systemic Infections with a Multipartite Plant RNA Virus. Journal of Virology, 87(19), 10805–10815. https://doi.org/10.1128/jvi.01402-13 https://doi.org/10.1128/JVI.01402-13
Methods

van der Vlugt, R.A., Berendsen, M. Development of a General Potexvirus Detection Method. European Journal of Plant Pathology 108, 367–371 (2002). https://doi.org/10.1023/A:1015644409484
Methods

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Methods

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Related Datasets

IsRelatedTo

Alvarado-Marchena,L., Furman,B. and Breitbart,M. (2024). Turtle grass virus X, complete genome. GenBank accession number PP887953 (version PP887953.1) [GenBank].
https://www.ncbi.nlm.nih.gov/nuccore/PP887953.1/

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Eppendorf 5424
Generic Instrument Name	Centrifuge
Dataset-specific Description	Microcentrifuge: Eppendorf 5424 – used for pelleting and separation steps in nucleic acid extraction.
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	JEOL JEM-1400
Generic Instrument Name	Electron Microscope
Dataset-specific Description	Transmission Electron Microscopy (TEM): JEOL JEM-1400 – used for imaging of virus-like particles and cytopathology at 80 kV.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of electrons behaving as waves.

Dataset-specific Instrument Name	Bio-Rad PowerPac Basic with Bio-Rad Sub-Cell Model 96
Generic Instrument Name	Electrophoresis Chamber
Dataset-specific Description	Bio-Rad PowerPac Basic with Bio-Rad Sub-Cell Model 96 – used for agarose gel electrophoresis.
Generic Instrument Description	General term for an apparatus used in clinical and research laboratories to separate charged colloidal particles (or molecules) of varying size through a medium by applying an electric field.

Dataset-specific Instrument Name	
Generic Instrument Name	Immersion heater
Dataset-specific Description	Temperature Control: Submersible aquarium heaters with digital thermometers – used to maintain stable temperature (~30°C).
Generic Instrument Description	Submersible heating element for water tanks and aquaria.

Dataset-specific Instrument Name	Fisher Scientific Isotemp 650D
Generic Instrument Name	Incubator
Dataset-specific Description	Oven: Fisher Scientific Isotemp 650D – used to polymerize Spurr resin during sample embedding for TEM.
Generic Instrument Description	A device in which environmental conditions (light, photoperiod, temperature, humidity, etc.) can be controlled. Note: we have more specific terms for shipboard incubators (https://www.bco-dmo.org/instrument/629001) and in-situ incubators (https://www.bco-dmo.org/instrument/494).

Dataset-specific Instrument Name	Syngene Ingenius 3
Generic Instrument Name	no_bcodmo_term
Dataset-specific Description	UV Transilluminator: Syngene Ingenius 3 – used for visualization of ethidium bromide-stained nucleic acids.
Generic Instrument Description	No relevant match in BCO-DMO instrument vocabulary.

Dataset-specific Instrument Name	AirClean 600 PCR Workstation
Generic Instrument Name	no_bcodmo_term
Dataset-specific Description	Biosafety Cabinet: AirClean 600 PCR Workstation – used to prevent contamination during RNA and PCR preparation.
Generic Instrument Description	No relevant match in BCO-DMO instrument vocabulary.

Dataset-specific Instrument Name	Leica EM UC6
Generic Instrument Name	no_bcodmo_term
Dataset-specific Description	Ultramicrotome: Leica EM UC6 – used to prepare ultrathin sections for TEM.
Generic Instrument Description	No relevant match in BCO-DMO instrument vocabulary.

Dataset-specific Instrument Name	Glass Aquaria
Generic Instrument Name	no_bcodmo_term
Dataset-specific Description	Glass Aquaria: 10-liter capacity – used for maintaining Thalassia testudinum plants under controlled lab conditions.
Generic Instrument Description	No relevant match in BCO-DMO instrument vocabulary.

Dataset-specific Instrument Name	
Generic Instrument Name	no_bcodmo_term
Dataset-specific Description	Aeration System: Aquarium air pumps with diffusers – used to oxygenate water.
Generic Instrument Description	No relevant match in BCO-DMO instrument vocabulary.

Dataset-specific Instrument Name	GLP24ADJS/19W/LED
Generic Instrument Name	no_bcodmo_term
Dataset-specific Description	Plant Grow Light: GLP24ADJS/19W/LED (Home Depot) – used to simulate natural light photoperiod (16:8 L:D).
Generic Instrument Description	No relevant match in BCO-DMO instrument vocabulary.

Dataset-specific Instrument Name	
Generic Instrument Name	Salinity Sensor
Dataset-specific Description	Salinity Tester: Marine Line salinity meter – used to monitor artificial seawater salinity (target 30 PSU ± 0.5).
Generic Instrument Description	Category of instrument that simultaneously measures electrical conductivity and temperature in the water column to provide temperature and salinity data.

Dataset-specific Instrument Name	Eppendorf BioPhotometer
Generic Instrument Name	Spectrometer
Dataset-specific Description	Spectrophotometer: Eppendorf BioPhotometer – used to quantify nucleic acid concentrations.
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

Dataset-specific Instrument Name	Eppendorf Mastercycler X50a
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Eppendorf Mastercycler X50a – used for RT-PCR amplification
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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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
NSF Division of Ocean Sciences (NSF OCE)	OCE-2219547

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