Metabolomics data from experiments with bacterial cultures, Gradients 3, 4, and 5 cruises in the North Pacific (R/V Kilo Moana KM1906 in 2019, R/V Thompson TN397 in 2021 and TN412 in 2023) and R/V Rachel Carson RC0104 cruise in Puget Sound in 2023

Website: https://www.bco-dmo.org/dataset/997449
Data Type: Cruise Results, experimental
Version: 1
Version Date: 2026-04-22

Project
» Collaborative Research: Resolving the production and fate of nitrogenous metabolites in the surface ocean (Nitrogenous Metabolites)

Contributors	Affiliation	Role
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Abstract
The data consists of results from six metabolomics experiments with either laboratory cultures of marine bacterial isolates Cobetia sp. OBi1 and Ruegeria pomeroyi DSS-3, in situ seawater sampling or stable-isotope probing experiments with isotopically labeled homarine in the North Pacific Ocean and Puget Sound. The six studies are described below. Chromatography and mass spectrometry were conducted as described in the methods section. Additional details can be found under the study accession URLs.

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Coverage

Location: Metabolomics field data were obtained in the North Pacific Ocean and Puget Sound.
Spatial Extent: **N**:57 **E**:-117 **S**:21.25 **W**:-158.3
Temporal Extent: 2019 - 2023

Methods & Sampling

1. Homarine catabolism: *Cobetia* sp. OBi1 comparative metabolomics under homarine and glucose supported growth

Cobetia sp. OBi1 was grown in three conditions: glucose (12 mM C, 0.8 mM NH4, control), homarine (12 mM C, no additional NH4), and glucose + homarine (12 mM C, 1 mM C, respectively with 0.8 mM NH4). Overnight cultures of *Cobetia* sp. OBi1 (100 mL) were grown at 25°C in glucose-amended seawater media. Next, 10 mL subsamples were taken from overnight culture and centrifuged for 15 minutes at 2800 g, and resuspended in the experimental growth media homarine, glucose, or glucose+homarine, in triplicate. These samples were incubated for 1 hr at 25°C in a dark shaker before harvesting. Cells were harvested by centrifugation for 15 minutes at 2800 g, and supernatant was collected and filtered through a 0.22 um PES membrane filter. Cell pellets and supernatant samples were stored at -80°C and -20°C, respectively.

For particulate metabolomics, cell pellets were extracted using a combination of mechanical and chemical disruption techniques as described in previous work (Boysen et al 2018). Metabolites from the supernatant were extracted using a cation-exchange-based solid phase extraction technique as described previously (Sacks et al 2022), with 1 mL of supernatant diluted into 10 mL of HPLC grade water. Isotopically-labeled internal standards were added for normalization purposes, as reported in Table S17 of Ferrer-Gonzalez et al 2026.

2. Homarine catabolism: *Ruegeria pomeroyi* DSS-3 comparative metabolomics under homarine and glucose supported growth

Cultures of *Ruegeria pomeroyi* DSS-3 were revived from cryostocks onto ½ YTSS agar plates and incubated at 30 °C for 6 days. Single colonies were inoculated into 11 mL of glucose minimal media (GMM) and grown overnight at 30 °C with shaking at 200 rpm. GMM was prepared using a modified L1 minimal medium with glucose 12 mM C as the sole carbon source. All cultures were maintained in sterile 15 mL assay tubes. Overnight cultures were diluted to an optical density of 0.1 at 600 nm (OD_{600}) in fresh GMM, incubated for 11–12 h, and amended with glucose (4 mM C, 0.8 mM NH4, control), homarine (500 nM C, no additional NH4), or glucose + homarine (1 mM C, 2 mM C, respectively with 0.8 mM NH4). Homarine additions were staggered across time points to ensure consistent incubation durations. At each time point, samples were collected for cell counts and particulate metabolites. For cell counts, 1 mL of culture was fixed with glutaraldehyde (final concentration 1%) in labeled cryovials, held at 4 °C for 20 minutes, and then stored at –80 °C. Particulate metabolites were collected by filtering cultures through combusted glass fiber filters using a vacuum manifold set to 8 psi; filters were wrapped in combusted foil and flash-frozen in liquid nitrogen. The experimental cultures were sampled after two hours in biological triplicate, as well as the three control conditions: glucose-only controls, glucose plus homarine controls (uninoculated), and a filter blank.

For metabolite extractions, a one phase extraction was performed with 40:40:20:0.01 methanol:acetonitrile:water:formic acid solution as the extraction solvent (Canelas et al 2009). Filters were placed in 15 mL Teflon tubes with pre-chilled extraction solvent, incubated at -20 ºC for 10 minutes, bead beaten with silica beads, and centrifuged. The solvent was then collected, transferred into glass tubes, and the procedure was repeated three times while keeping samples cold as much as possible. Samples were dried down under nitrogen gas, reconstituted in 400 uL of H2O, and stored at -80 ºC until analysis by LC-MS. Isotopically-labeled internal standards were added for normalization purposes, as reported in Table S17 of Ferrer-Gonzalez et al 2026.

3. Homarine Catabolism: In situ metabolomics from KM1906, surface samples along a transect in the North Pacific

Metabolite samples were collected during the Gradients 3 cruise (KM1906) in the North Pacific in 2019. Briefly, 10L of water was collected from the shipboard flow-through underway sampling system and particulate metabolites were sampled by filtering the seawater using peristaltic pumps onto 142 mm diameter, 0.2 um pore size PTFE Omnipore filters, flash frozen in liquid nitrogen, and stored at -80 C until analysis. Dissolved metabolites were sampled by collecting the filtrate in 50 mL acid washed polypropylene Falcon tubes.

For particulate metabolites, cell pellets were extracted using a combination of mechanical and chemical disruption techniques as described in previous work (Boysen et al 2018). Dissolved metabolites were extracted using a cation-exchange-based solid phase extraction technique as described previously (Sacks et al 2022). Following extraction, metabolites were dried down under N2 gas, reconstituted in water with isotope labeled internal standards, and analyzed using liquid chromatography mass spectrometry. Homarine concentrations were quantified by comparison to a 2H3-homarine internal standard.

4. Homarine catabolism: Marine microbial isotope-tracing metabolomics experiments for cruise TN397 in the Fall of 2021 at two different stations in the North Pacific

Stable-isotope probing was performed to track homarine degradation products in natural marine microbial communities from three locations (Figure 2D of Ferrer-Gonzalez et al 2026). Seawater was collected into acid-

washed 10-l polycarbonate carboys from a trace metal clean stayfish system suspended at a depth of 8 m and prefiltered through 100 µm nylon mesh. Seawater incubated with isotopically-labeled 2H3-homarine was analyzed for the compounds enriched in our model organisms (as described in Ferrer-Gonzalez et al 2026), with the isotopic labels.

Nine treatment bottles were spiked with 500 nM 2H3-homarine with nine control bottles receiving no additions. Bottles were incubated in blue-shaded temperature and light-controlled incubators designed to mimic mixed-layer conditions of the sampling location. Triplicate bottles with and without homarine addition were harvested at 2, 24, and 48 hours. The experiment was repeated with the same treatments at a second location.

For particulate metabolomics, cell pellets were extracted using a combination of mechanical and chemical disruption techniques as described in previous work (Boysen et al 2018). Metabolites from the supernatant were extracted using a cation-exchange-based solid phase extraction technique as described previously (Sacks et al 2022), with 1 mL of supernatant diluted into 10 mL of HPLC grade water. To prevent confusion using the isotope labels, we used a subset of isotopically-labeled internal standards, as reported in Table S17 of Ferrer-Gonzalez et al 2026.

5. Homarine catabolism: Marine microbial isotope-tracing metabolomics experiments for cruise TN412 in the Winter of 2023 at two different stations in the North Pacific

Stable-isotope probing was performed to track homarine degradation products in natural marine microbial communities from three locations (Figure 2D of Ferrer-Gonzalez et al 2026). Seawater was sampled from a trace metal clean stayfish system suspended at a depth of 8 m and prefiltered through 100 µm nylon mesh. Seawater incubated with isotopically-labeled 13C715N-homarine was analyzed for the compounds enriched in our model organisms (as described in Ferrer-Gonzalez et al 2026), with the isotopic labels.

Triplicate samples were collected into acid washed 2 L polycarbonate bottles, spiked with 90 nM of 13C7-15N-labeled homarine, and incubated in temperature and light-controlled incubators for 5 different timepoints (6, 12, 24, 48 and 96 hours). Triplicates of spiked and unspiked samples were filtered as quickly as possible, no more than 30 minutes (T0, and unamended control samples, respectively).

6. Homarine catabolism: Marine microbial isotope-tracing metabolomics experiments for cruise RC0104 in the Summer of 2023 in Puget Sound

Stable-isotope probing was performed to track homarine degradation products in natural marine microbial communities from three locations (Figure 2D of Ferrer-Gonzalez et al 2026). Seawater incubated with isotopically-labeled 13C715N-homarine was analyzed for the compounds enriched in our model organisms (see other studies within this project), with the isotopic labels.

Experiments using 13C7,15N-homarine-homarine were performed on research cruise RC0104 in the Summer of 2023 at two different stations in Puget Sound (described in Table S8 and displayed in Figure 2 of Ferrer-Gonzalez et al 2026). The preparation of the 13C7-15N-labeled homarine is described in detail in Ferrer-Gonzalez et al 2026. Seawater was collected through a trace metal clean stayfish system suspended at a depth of 8 m prefiltered through 100 µm nylon mesh. Triplicate samples were collected into acid washed 2 L polycarbonate bottles, spiked with 90 nM of 13C7-15N-labeled homarine, and incubated in temperature and light-controlled incubators for 5 different timepoints (6, 12, 24, 48 and 96 hours). Triplicates of spiked and unspiked samples were filtered as quickly as possible, no more than 30 minutes (T0, and unamended control samples, respectively). All particulate samples (4 L) were collected using peristaltic pumps onto Durapore® 0.22 µm, 47 mm, hydrophilic PVDF membrane filters, flash frozen in liquid nitrogen, and stored at -80°C.

Chromatography and Mass Spectrometry

Metabolomics data were acquired by liquid chromatography paired with high resolution mass spectrometry (LC-MS) on a ThermoOrbitrap Q-Exactive HF Mass Spectrometer (QE). Samples were introduced via Hydrophilic Interaction Liquid Chromatography (HILIC) in both positive and negative modes using polarity switching. For HILIC, a SeQuant ZIC-pHILIC column (5 um particle size, 2.1 mm x 150 mm, from Millipore) was used with 10 mM ammonium carbonate in 85:15 acetonitrile to water (Solvent A) and 10 mM ammonium carbonate in 85:15 water to acetonitrile (Solvent B) at a flow rate of 0.15 mL/min. This column was compared with a Waters UPLC BEH amide and a Millipore cHILIC column; the pHILIC showed superior reproducibility and peak shapes. The column was held at 100% A for 2 minutes, ramped to 64% B over 18 minutes, ramped to 100% B over 1 minute, held at 100% B for 5 minutes, and equilibrated at 100% A for 25 minutes (50 minutes total). The column was maintained at 30 C. The injection volume was 2 µL for samples and standard mixes. When starting a batch, the column was equilibrated at the starting conditions for at least 30 minutes. To improve the performance of the HILIC column, we maintained the same injection volume, kept the instrument running water

blanks between samples as necessary, and injected standards in a representative matrix in addition to standards in water. After each batch, the column was flushed with 10 mM ammonium carbonate in 85:15 water to acetonitrile for 20 to 30 minutes. For mass spectrometry data acquisition, polarity switching was used with a scan range of 60 to 900 m/z and a resolution of 60,000. MS parameters were as follows: capillary temperature was 320 C, the H-ESI spray voltage was 3.3 kV, and the auxiliary gas heater temperature was 100 C. The S-lens RF level was 65. Sheath gas, auxiliary gas, and sweep gas flow rates were maintained at 16, 3, and 1, respectively.

Data Processing Description

Custom analysis scripts used in this study are available on GitHub at https://github.com/IngallsLabUW/Homarine_Catabolism_MS and archived at Zenodo (Heal et al., 2026).

BCO-DMO Curation Notes

No data processing was done for this dataset.

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Related Publications

Boysen, A. K., Heal, K. R., Carlson, L. T., & Ingalls, A. E. (2018). Best-Matched Internal Standard Normalization in Liquid Chromatography–Mass Spectrometry Metabolomics Applied to Environmental Samples. Analytical Chemistry, 90(2), 1363–1369. doi:10.1021/acs.analchem.7b04400
Methods

Canelas, A. B., ten Pierick, A., Ras, C., Seifar, R. M., van Dam, J. C., van Gulik, W. M., & Heijnen, J. J. (2009). Quantitative Evaluation of Intracellular Metabolite Extraction Techniques for Yeast Metabolomics. Analytical Chemistry, 81(17), 7379–7389. https://doi.org/10.1021/ac900999t
Methods

Ferrer-González, F. X., Heal, K. R., Sacks, J. S., Romero-Maysonet, Y., Finch, A. H., Carlson, L. T., Coe, L. S. Y., Bartolek, Z., Luthy, C. B., Gaffney, M. P., Angier, S. K., Flynn, S. E., Bachmann Gómez, C. I., Dunn, J. M. L., Bay, K. J., Yamamoto, L. A., Tien, M. Z., Armbrust, E. V., Durham, B. P., et al. (2026). Conserved pathway for homarine catabolism in environmental bacteria. Nature Microbiology. https://doi.org/10.1038/s41564-026-02313-7
Methods

Sacks, J. S., Heal, K. R., Boysen, A. K., Carlson, L. T., & Ingalls, A. E. (2022). Quantification of dissolved metabolites in environmental samples through cation-exchange solid-phase extraction paired with liquid chromatography–mass spectrometry. Limnology and Oceanography: Methods, 20(11), 683–700. Portico. https://doi.org/10.1002/lom3.10513
Methods

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Related Datasets

IsRelatedTo

Sosa, O. A., Ingalls, A. E., Heal, K. (2026) **CTD profiles of temperature, oxygen, salinity, fluorescence, beam attenuation and transmission, PAR, and pH collected in the Salish Sea on R/V Rachel Carson cruises RC0078, RC0104, and RC0138 during 2022, 2023 and 2025.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-05-28 doi:10.26008/1912/bco-dmo.997362.1 [view at BCO-DMO]
Relationship Description: Datasets all related to same publication (Ferrer-González et al, 2026).

Sosa, O. A., Ingalls, A. E., Heal, K. (2026) **Nutrients and dissolved organic carbon bottle data from R/V Rachel Carson RC0078 cruise in the Salish Sea in June 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-05-28 doi:10.26008/1912/bco-dmo.997299.1 [view at BCO-DMO]
Relationship Description: Datasets all related to same publication (Ferrer-González et al, 2026).

Sosa, O. A., Ingalls, A. E., Heal, K. (2026) **Sequence data accession numbers originating from seawater homarine addition experiments conducted on R/V Rachel Carson RC0078 cruise in the Salish Sea in June 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-05-28 doi:10.26008/1912/bco-dmo.997408.1 [view at BCO-DMO]
Relationship Description: Datasets all related to same publication (Ferrer-González et al, 2026).

Software

Heal, K., Ferrer-González, F. X., Finch, A. H., Sacks, J. S., Sosa, O. A., & Ingalls, A. E. (2026). Homarine_Catabolism (Version v1.0.1) [Computer software]. Zenodo. https://doi.org/10.5281/ZENODO.20027925 https://doi.org/10.5281/zenodo.20027925

References

Metabolomics Workbench. (2025). *PR002738*. Metabolomics Workbench. https://doi.org/10.21228/M8R54Z

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Cells were harvested by centrifugation for 15 minutes at 2800 g, and supernatant was collected and filtered through a 0.22 um PES membrane filter.
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	Hydrophilic Interaction Liquid Chromatography (HILIC)
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Instrument Description: Samples were introduced via Hydrophilic Interaction Liquid Chromatography (HILIC) in both positive and negative modes using polarity switching.
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	
Generic Instrument Name	Incubator
Dataset-specific Description	Cultures of Ruegeria pomeroyi DSS-3 were revived from cryostocks onto ½ YTSS agar plates and incubated at 30 °C for 6 days.
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	ThermoOrbitrap Q-Exactive HF Mass Spectrometer (QE)
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Instrument Description: Acquisition description: Metabolomics data were acquired by liquid chromatography paired with high resolution mass spectrometry (LC-MS) on a ThermoOrbitrap Q-Exactive HF Mass Spectrometer (QE).
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	peristaltic pumps
Generic Instrument Name	Pump
Dataset-specific Description	All particulate samples (4 L) were collected using peristaltic pumps onto Durapore® 0.22 µm, 47 mm, hydrophilic PVDF membrane filters, flash frozen in liquid nitrogen, and stored at -80°C.
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	Shipboard flow-through underway sampling system
Generic Instrument Name	Pump - Surface Underway Ship Intake
Dataset-specific Description	Briefly, 10L of water was collected from the shipboard flow-through underway sampling system and particulate metabolites were sampled by filtering the seawater using peristaltic pumps onto 142 mm diameter, 0.2 um pore size PTFE Omnipore filters, flash frozen in liquid nitrogen, and stored at -80 C until analysis.
Generic Instrument Description	The 'Pump-underway ship intake' system indicates that samples are from the ship's clean water intake pump. This is essentially a surface water sample from a source of uncontaminated near-surface (commonly 3 to 7 m) seawater that can be pumped continuously to shipboard laboratories on research vessels. There is typically a temperature sensor near the intake (known as the hull temperature) to provide measurements that are as close as possible to the ambient water temperature. The flow from the supply is typically directed through continuously logged sensors such as a thermosalinograph and a fluorometer. Water samples are often collected from the underway supply that may also be referred to as the non-toxic supply. Ideally the data contributor has specified the depth in the ship's hull at which the pump is mounted.

Dataset-specific Instrument Name	dark shaker
Generic Instrument Name	Shaker
Dataset-specific Description	These samples were incubated for 1 hr at 25°C in a dark shaker before harvesting.
Generic Instrument Description	A Shaker is a piece of lab equipment used to mix, blend, or to agitate substances in tube(s) or flask(s) by shaking them, which is mainly used in the fields of chemistry and biology. A shaker contains an oscillating board which is used to place the flasks, beakers, test tubes, etc.

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Deployments

RC0104

Website	https://www.bco-dmo.org/deployment/997367
Platform	R/V Rachel Carson (UW)
Start Date	2023-08-31
End Date	2023-09-06
Description	Project: DON-2023

KM1906

Website	https://www.bco-dmo.org/deployment/997454
Platform	R/V Kilo Moana
Start Date	2019-04-09
End Date	2019-04-29
Description	Project: Gradients

TN412

Website	https://www.bco-dmo.org/deployment/997459
Platform	R/V Thomas G. Thompson
Start Date	2023-01-22
End Date	2023-02-18
Description	Project: Gradients

TN397

Website	https://www.bco-dmo.org/deployment/997457
Platform	R/V Thomas G. Thompson
Start Date	2021-11-18
End Date	2021-12-15
Description	Project: Gradients 4

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Project Information

Collaborative Research: Resolving the production and fate of nitrogenous metabolites in the surface ocean (Nitrogenous Metabolites)

NSF Award Abstract:
Photosynthetic microbes provide food for nearly all other life in the ocean. Their metabolism produces organic molecules called metabolites that can leak out of cells, be intentionally excreted into seawater, or be released during cell death. Once outside the cell, these metabolites are the basis for specific interactions among microbes and determine community structure and activity. Yet, current understanding of metabolites in the ocean is limited by a historical lack of ability to measure them. The work proposed here will expand current knowledge of metabolite structures, concentrations, and production rates using recently developed analytical methods. These methods have already led to the discovery that homarine, a substituted pyridine first found in lobster in 1933, is the most abundant detectable metabolite in microbial communities of the North Pacific

Ocean. While homarine is known as a predator deterrent, osmoprotectant, methyl donor, and antibiofouling agent, studies of its role in microbial community dynamics are lacking. The work proposed will clarify the role of homarine in the ocean's microbial communities. This work will create an open-source metabolite database that will serve the broader field of metabolomics, a growing area in environmental, engineering, and medical sciences. This collaboration will also promote the careers of a graduate student and a postdoctoral researcher as well as an early career professor from an underrepresented group at a primarily undergraduate institution (PUI). Undergraduates from both institutions will contribute to project development and implementation, local cruises on the R.V. Carson, lab work, and dissemination of results. This research will be integrated into a curriculum-based research experience for undergraduates in a 200-level genetics course at the PUI, University of Puget Sound.

The proposed work will carry out field studies and laboratory experiments to test the hypothesis that metabolites are quantitatively significant forms of carbon and nitrogen flowing through microbial communities. The identity, quantity, and production rates of metabolites will also be determined. For homarine, the enzymes and organisms responsible for its transformations will be determined. Specific proposed activities will 1) Quantify nitrogenous metabolite pools and their net production rates (particulate and dissolved) in phytoplankton cultures and in marine surface water communities; 2) Isolate homarine consuming heterotrophic bacteria and use mutagenesis techniques, transcriptomics, and stable isotope assisted metabolomics to annotate genes and characterize the biochemical reactions involved in the degradation of homarine; 3) Carry out incubations of stable isotope labeled homarine in phytoplankton cultures, heterotrophic bacterial cultures sensitive to homarine, and natural communities to quantitatively evaluate the effect of homarine on growth, track homarine through metabolic pathways, and determine the kinetics of homarine uptake; 4) Identify homarine consumers and biochemical pathways for homarine use in the environment by mining existing environmental metatranscriptomes for homarine catabolism genes. The combination of these approaches will provide better understanding of the flow of nitrogen containing metabolites through marine microbial ecosystems. Results from this work will be disseminated through peer reviewed open-source publications as well as presentations to the scientific community and the general public.

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-2125886
NSF Division of Ocean Sciences (NSF OCE)	OCE-2124712

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