Prey Engulfment as the Dominant Pathway of Methylmercury Uptake in a Heterotrophic Dinoflagellate Experiment

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Data Type: experimental
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Project
» Collaborative Research: Transformations and mercury isotopic fractionation of methylmercury by marine phytoplankton (Phytoplankton MeHg)

Contributors	Affiliation	Role
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Abstract
These data include all relevant analyses from methylmercury (MeHg) uptake experiments utilizing the heterotrophic dinoflagellate Oxyrrhis marina (urn:lsid:marinespecies.org:taxname:109902), varying salinity, temperature, and dissolved organic matter (DOM) source – including heat-killed O. marina. The goal of these experiments was to determine whether uptake from the dissolved phase or prey engulfment would contribute more to cellular MeHg accumulation. All experimental treatments were triplicated and had a control with no added MeHg. These studies support the following hypotheses: MeHg uptake is higher in the presence of phytoplankton prey, uptake is significantly lower at a low salinity (11 compared to 17 and 34), and temperature (at 12, 15, and 22°C) has no effect on MeHg uptake.

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Coverage

Location: Laboratory experiments conducted at the Department of Marines Sciences, University of connecticut, Groton, CT 06340
Temporal Extent: 2019-06 - 2021-02

Methods & Sampling

Methods are detailed in Myer et al. (2025).

All materials and supplies that were in contact with plankton cultures were sterilized, and all glassware was acid-washed and combusted. Experiments utilized both filtered and artificial seawater. Natural seawater was collected from Long Island Sound, filtered, autoclaved, and refrigerated at 18°C. Artificial seawater (ASW) was

prepared using MilliQ water with added salts. ASW was filtered and also refrigerated at 18°C. Plankton culture methods are detailed in Myer et al. (2025).

Eight-hour plankton (*Oxyrrhis marina* (urn:lsid:marinespecies.org:taxname:109902)) exposure experiments were carried out in triplicate vessels (Pyrex glass bottles) for each treatment. Experimental variables included temperature, salinity, and organic matter type, with variations in dissolved organic carbon (DOC) and prey (*Isochrysis galbana* (urn:lsid:marinespecies.org:taxname:573884)). One additional treatment involved using heat-killed *O. marina*, following modified methods from Zhong & Wang (2009).

Temperature experiments were conducted at 12, 15, and 22 °C. Salinity was manipulated through dilutions of ASW with MilliQ water (11 and 17) from a stock solution (34). The low DOC treatment was prepared using ASW with a final concentration of 130 µM DOC, and the high DOC treatment was prepared from filtered natural seawater with a final concentration of 210 µM DOC.

The experiment that tested the control of organic matter on MeHg uptake used higher MeHg spike concentration (5 ng/L) and *O. marina* cell concentration (5000 cells/L), than the consecutive salinity and temperature experiments (MeHg: 1.7 ng/L; *O. marina*: 3000 cells/L).

MeHg was spiked before the addition of *O. marina* to allow for equilibration. In the experimental treatments utilizing *I. galbana* as prey, these cells were added at the same time as the MeHg spike. Experimental flasks were inoculated with *O. marina*, starting the experiment. Cells and water were collected immediately (t_0 = 0 hours), and then again at 4 and 8 hours. At these time points, 85 mL was filtered sequentially using an acid-washed vacuum filtration tower using 10, then 3, and lastly 0.2 µm MilliporeSigma Isopore Polycarbonate Membrane Filters. All filters were placed into new 15 mL Falcon tubes and stored briefly in a dark cooler, prior to freezing (-20 °C), and until acid digestion and MeHg analysis. 1mL of unfiltered water was sampled from each bottle and preserved with Lugol's solution for cell counting. For DOC analysis, 30 mL of 0.2 µm filtered seawater was collected and acidified with hydrochloric acid, then refrigerated at 18°C. For dissolved MeHg analysis, filtrate was collected into 125 mL PETE bottles and acidified with trace metal HCl. For the DOC experiment, bulk solution was collected for analysis and dissolved MeHg was calculated.

MeHg analyses were performed using the Tekran 2700 Automated Methylmercury Analysis System. Particulate MeHg analysis followed previously established procedures modified from the EPA Method 1630 (Hammerschmidt & Fitzgerald, 2005). Briefly, filters were digested with nitric acid overnight at a 60 °C. The subsequent digest was diluted with MQ water, neutralized with potassium hydroxide (KOH), and buffered to a pH in a range of 4.0-4.5 with 2 M acetate. 30 µL of sodium tetraethylborate (NaBET4) was used to volatilize the MeHg within the sample into methylethylmercury (MeEtHg). Calibration was prepared based on a five-point standard curve using MeHgCl standard solution (Alfa Aesar). The detection limit was 0.012 ng/L. The quality of measurements was determined based on routinely analyzed standard solutions and sample replication. The RSD for sample replicates was 20% (n=2-4 per sample; n=20 total).

Seawater was processed following previously established methods (Munson et al., 2014). The day before analysis, seawater was spiked with sulfuric acid (H2SO4, TraceMetal Grade, Fisher Chemical; final concentration: 1% vol./vol.) for overnight digestion. A small volume of cold (4 °C) 2.5% L-ascorbic acid was added to the solution, which was buffered using 8N KOH and 2N acetate, prior to ethylation with NaBET4. The detection limit for the seawater analytical runs was 0.007 ng/L, and the RSD was 9% for sample replicates (n=3 per sample, n=15 total). MeHg in water samples was validated using standard addition to experimental samples in triplicate. The recovery of the standard spike to samples was 109 ± 15% (n=2-3 per sample; n=11 total).

Cells were enumerated using a stereo microscope (Fisher brand, 5.0x magnification) and a Sedgwick-Rafter cell counting chamber. For each 1 mL sample of *O. marina*, three columns were chosen randomly, counted, and averaged. *I. galbana* cells were enumerated using the Multisizer Coulter Counter II. Cells were counted in duplicate, and if counts differed by ≥10%, a third count was taken and averaged.

Data Processing Description

Data was compiled in Excel. For BCO-DMO, titles and values were standardized for clarity. Missing data was standardized to "nd." Cell counts were all converted to cells/L.

DOC experiments analyzed bulk MeHg in seawater, so particulate values were subtracted from bulk values to determine dissolved MeHg.

Additional calculations, including MeHg content per cell, volume concentration factors (VCFs), and K_d are

featured within Table 1 of the Myer et al. (2025).

BCO-DMO Processing Description

- Imported "Data - Dataset3NSF 1634048.csv" into the BCO-DMO system, replacing "nd." no data key with blanks
- Renamed fields to comply with BCO-DMO naming conventions, removing units, special characters, and spaces
- Upon request from the submitter, "OM" was rounded to the whole unit and "Dissolved_MeHg_concentration" and "Filter_MeHg_concentration" were rounded to two decimal places
- Exported file as "982183_v1_methylmercury_uptake_exp.csv"
- Species name Oxyrrhis marina (urn:lsid:marinespecies.org:taxname:109902) and Isochrysis galbana (urn:lsid:marinespecies.org:taxname:573884) verified as current accepted form on 2025-08-29, using the WoRMs World Registery of Marine Species database.

Problem Description

Two major issues occurred, which resulted in low or even no MeHg recovery. These values are included within the dataset but were not used in any final calculations or statistics within the publication.

Firstly, there were multiple filter samples with low recovery due to issues while running the Tekran 2700 auto-sampler. These included a power outage, incorrectly placed septa on sample vials, and similar issues which resulted in MeEtHg escaping. The following filter samples were impacted:
Salinity experiment:
· 17 salinity (med), replicate 1, t=8h
Heat-killed experiment:
· Replicate 3, t=0h
Organic matter (OM) sources experiment:
· ASW + Prey, replicate 2, t=0h
· ASW + Prey, replicate 3, t=4h

Secondly, some dissolved MeHg water samples were not preserved adequately. As a result, MeHg within these samples degraded before analysis.

The following water samples were impacted:
Temperature experiment:
· 12 °C (low), replicate 1, t=8h
· 12 °C (low), replicate 3, t=8h
· 22 °C (high), replicate 1, t=8h

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Data Files

File	
982183_v1_methylmercury_uptake_exp.csv	(Comma Separated Values (.csv), 5.34 KB) MD5:c1bebde385b3d3670d834b2d4e63107a
Primary data file for dataset ID 982183, version 1	

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Related Publications

Hammerschmidt, C. R., & Fitzgerald, W. F. (2005). Methylmercury in Mosquitoes Related to Atmospheric Mercury Deposition and Contamination. Environmental Science & Technology, 39(9), 3034–3039. https://doi.org/10.1021/es0485107

Methods

Munson, K. M., Babi, D., & Lamborg, C. H. (2014). Determination of monomethylmercury from seawater with ascorbic acid-assisted direct ethylation. Limnology and Oceanography: Methods, 12(1), 1–9. doi:10.4319/lom.2014.12.1
Methods

Myer, P. K., Mason, R. P., & Baumann, Z. A. (2025). Prey engulfment as the dominant pathway of methylmercury uptake in a heterotrophic dinoflagellate. Marine Environmental Research, 210, 107348. https://doi.org/10.1016/j.marenvres.2025.107348
Results

Zhong, H., & Wang, W.-X. (2009). Controls of Dissolved Organic Matter and Chloride on Mercury Uptake by a Marine Diatom. Environmental Science & Technology, 43(23), 8998–9003. https://doi.org/10.1021/es901646k
Methods

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Parameters

Parameter	Description	Units
Treatment	Experimental variable	unitless
Temperature	Room temperature where the experiment was conducted	degrees Celsius
Salinity	Salinity of the experimental seawater (measured before inoculation)	PSU
OM	Measured volume of Organic Matter (OM)	Micromoles per liter (µmol/L)
Replicate	Experimental replicate, each variable was tested in triplicate + a control	unitless
Hours_of_exposure	Hours elapsed since the start of the experiment (t=0h marked by *O. marina* inoculation)	hrs
Dissolved_MeHg_concentration	Dissolved (<0.2 µm) methylmercury (MeHg) concentration	ng/L
Filter_MeHg_concentration	Particulate (>10 µm) methylmercury (MeHg) concentration	ng/L
Cell_count	Number of cells per liter within each sample bottles at t=0 h	cells/L

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Instruments

Dataset-specific Instrument Name	Multisizer Coulter Counter II
Generic Instrument Name	Coulter Counter
Dataset-specific Description	I. galbana cells were enumerated using the Multisizer Coulter Counter II.
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

Dataset-specific Instrument Name	Atereo microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	O. marina cells were enumerated using a stereo microscope (Fisher brand, 5.0x magnification) and a Sedgwick-Rafter cell counting chamber.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	Refractometer
Generic Instrument Name	Refractometer
Dataset-specific Description	Salinity was confirmed using a refractometer.
Generic Instrument Description	A refractometer is a laboratory or field device for the measurement of an index of refraction (refractometry). The index of refraction is calculated from Snell's law and can be calculated from the composition of the material using the Gladstone-Dale relation. In optics the refractive index (or index of refraction) n of a substance (optical medium) is a dimensionless number that describes how light, or any other radiation, propagates through that medium.

Dataset-specific Instrument Name	Shimadzu Total Organic Carbon/Total Nitrogen Analyzer
Generic Instrument Name	Shimadzu Total Organic Carbon Analyzer TOC-VCPH
Dataset-specific Description	DOC was measured using a Shimadzu Total Organic Carbon/Total Nitrogen Analyzer.
Generic Instrument Description	The Shimadzu Total Organic Carbon Analyzer TOC-VCPH is a PC-controlled, total organic carbon analyzer (high-sensitivity model), designed to measure total carbon (TC), inorganic carbon (IC), total organic carbon (TOC), and non-purgeable organic carbon (NPOC); an optional accessory enables the measurement of particulate organic carbon (POC) and total nitrogen (TN) as well. The instrument uses the 680 degrees Celsius combustion catalytic oxidation method to analyze aqueous samples, and optionally solid and gas samples.

Dataset-specific Instrument Name	Tekran 2700 Automated Methylmercury Analysis System
Generic Instrument Name	Tekran Model 2700 Automated Methyl Mercury Analysis System
Dataset-specific Description	Methylmercury in both particulate and dissolved phases was measured using the Tekran 2700 Automated Methylmercury Analysis System, which utilizes gas chromatography (GC) and cold vapor atomic fluorescence spectroscopy (CVAFS).
Generic Instrument Description	The Tekran 2700 is a fully integrated Gas Chromatography Cold-Vapor Atomic Fluorescence Spectrophotometer (GC-CVAFS) automated Methyl Mercury analysis system. The 2700 can analyze distilled waters, extracted or distilled tissues and solids, and allows direct analysis of suitable water samples. The system is pre-programmed to run EPA Method 1630, however it offers complete method customization including: GC column temperature ramping; programmable analysis cycle settings; high temperature purge cycles; and choice of trap and GC column. It can also interface with ICP-MS or other analytical instruments. The sample analysis cycle is less than 7 minutes per sample. It has a minimum detection limit of 0.002 nanograms per liter (ng/L). The system has IR trap heating and active cooling. See: https://www.tekran.com/products/laboratory/tekran-model-2700-automated-m...

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Project Information

Collaborative Research: Transformations and mercury isotopic fractionation of methylmercury by marine phytoplankton (Phytoplankton MeHg)

Coverage: Antarctic Peninsula

NSF Award Abstract:
The accumulation of mercury (Hg) in seafood is a public health concern. The presence of Hg in seafood depends to a large degree on the air-sea exchange of Hg, with atmospheric deposition leading to accumulation of Hg in the ocean. The pathways to seafood start with the uptake of Hg by phytoplankton from seawater where is has always been assumed to accumulate to be eaten by grazers and passed on to larger organisms. This project challenges this assumption with preliminary data that suggests certain phytoplankton species can transform Hg to volatile forms (mercury vapor & dimethylmercury) that are lost to the atmosphere, a processes that removes Hg from the ocean rather than simply concentrating it into the ecosystem and seafood. This process, which has not been studied before, could dramatically alter our view of the Hg cycle in

the ocean. The researchers funded by this project will look for the specific phytoplankton species that are capable of volatilizing Hg and quantify the rates at which they do so. They will also examine the suspected role of associated sulfur and selenium compounds in the process, as well as quantifying the changes in the Hg isotopic values for potential use as chemical tracers of the source of Hg in the ecosystem and food supply. These results should allow oceanographers to better quantify and refine our knowledge of Hg cycling in the ocean. The project will support participation of graduate students, a postdoctoral scientist, and incorporation of new information directly into courses taught by the researchers. Funding will also support continuing activities by the participants in activities that disseminate information on mercury and its effect on public and environmental health.

Biogeochemical cycling of mercury (Hg) in the ocean may be more complex than previously assumed. New evidence has challenged the idea that methylmercury (MeHg) merely accumulates in phytoplankton and undergoes little to no transformation before being passed into the food web. This project aims to more fully elucidate the mechanisms behind the intracellular transformation of MeHg to volatile Hg and dimethylmercury (Me2Hg) that can be lost to the atmosphere, as well as to evaluate the range of algal taxa that can perform this transformation using directed culture work. Additionally, the PIs will investigate evidence that thiols, organic selenium (Se) compounds, and sulfides are required to facilitate these reactions within the phytoplankton, and specific pathways will be investigated and quantified through this research. Stable Hg isotopic data has been used to track Hg sources and pathways in marine systems and its fractionation during these MeHg transformations will also be quantified for future field study of marine Hg. The investigators hypothesize that coccolithophorids and other haptophytes capable of these intracellular reactions may account for a significant portion of the production of volatile Hg in the ocean. If this turns out to be the case, understanding and quantifying these volatilization processes may significantly alter our current understanding of the overall biogeochemical cycling of Hg in the ocean.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1634048

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