EV and Viral-Like Particle Abundances and Size Distributions by NTA from CTD Water Samples on R/V Atlantis Cruise AT50-08

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Project
» EAGER: Mechanistic Study of Extracellular Vesicle Production by Marine Microalgae using Advanced Imaging Technologies (Marine Microbial Extracellular Vesicles)

Abstract
These data report nanoparticle tracking analysis (NTA)–derived abundances and size distributions of extracellular vesicles (EVs) and viral-like particles (VLPs) purified from seawater collected during R/V Atlantis cruise AT50-08 in February 2023 in the Eastern Tropical North Pacific oxygen minimum zone (OMZ). EVs and VLPs were concentrated and purified from CTD-rosette water samples collected at selected depths between ~50 and 600 m, then analyzed by NTA to quantify total particle concentrations (particles mL^{-1}) and size distributions (nanometer-scale diameter classes) for each sample. The dataset includes sample identifiers that link each NTA measurement to its corresponding CTD cast, bottle, depth, and station, facilitating integration with hydrographic, chemical, and microbiological data from the same cruise. These measurements provide a quantitative view of EV and viral particle distributions across strong oxygen and nutrient gradients, and support studies of microbial interactions, particle-mediated carbon and nutrient cycling, and the physical–biogeochemical structure of OMZ water columns.

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Coverage

Location: Eastern Tropical Pacific, Minimum Oxygen Zone (OMZ)
Temporal Extent: 2023-02-10 - 2023-03-15

Methods & Sampling

Overview and intent

Extracellular vesicles (EVs) and viral particles were enriched onboard from CTD-rosette seawater to generate a clean, concentrated fraction suitable for downstream particle characterization (e.g., NTA), imaging (e.g., EM), and molecular assays. For each sampled depth, 100 L of seawater were processed the same day of collection and reduced to a final 1 mL EV/virus-enriched eluate, which was then subdivided into 10 × 100 µL aliquots, snap-frozen in liquid nitrogen, and stored at −80 °C.

Sample collection and handling

Seawater was obtained from Niskin bottles mounted on a CTD rosette. For each depth, the targeted volume (100 L) was transferred into cleaned, clearly labeled carboys. From the moment of collection, the sample was handled as a cold, light-protected matrix: carboys were kept in cold, dark conditions and moved promptly into processing to minimize changes in particle integrity and community composition before enrichment.

Sequential pre-filtration to remove larger material

To reduce the burden of large particulates and most intact cells while preserving EVs and viruses in the filtrate,

seawater was sequentially passed through 0.8 µm and then 0.45 µm filtration. Briefly, the 100 L depth sample was first filtered through a 0.8 µm membrane into a clean reservoir. The resulting filtrate was then immediately filtered through a 0.45 µm membrane, producing the operational <0.45 µm fraction for downstream concentration. Throughout filtration, care was taken to maintain low shear and operate within manufacturer-recommended flow and pressure ranges to avoid unnecessary stress on vesicles and viral particles. Filtrates and reservoirs were kept cold during handling. The outcome of this stage was a <0.45 µm feed stream for tangential flow filtration (TFF), with the volume remaining approximately 100 L aside from minor handling losses.

Tangential flow filtration (TFF) for concentration and buffer exchange

The <0.45 µm fraction was concentrated using tangential flow filtration (TFF) equipped with a 100 kDa MWCO cartridge. The filtrate was recirculated per the instrument and cartridge guidance until the sample volume was reduced from ~100 L to approximately 50–100 mL of retentate. In addition to concentrating particles, the TFF step was used to transition the sample into a buffer compatible with downstream EV column clean-up. Buffer exchange was performed by adding multiple retentate-equivalent volumes of the chosen EV/virus-compatible buffer (e.g., filtered seawater or PBS) to the retentate and reconcentrating, repeating as needed in line with manufacturer recommendations. During TFF, the retentate was maintained cold, and operating conditions were managed to avoid excessive transmembrane pressure, foaming, or vigorous agitation. This stage produced a 50–100 mL TFF concentrate enriched for EVs and viruses, compatible with the column.

EV column clean-up and elution to a final 1 mL product

To further remove soluble components and improve sample cleanliness for downstream analyses, the TFF concentrate was polished using commercial EV columns (Takara or Qiagen, depending on availability and cruise workflow). Columns were equilibrated following the manufacturer's kit instructions, including the specified buffers, volumes, and centrifugation/flow conditions. The TFF concentrate was then loaded onto the column(s); when necessary, multiple columns were used to stay within the recommended sample loading limits. After loading, columns were washed with the supplied wash buffer to reduce carryover of non-target material. Vesicle/virus-enriched fractions were then eluted to a final total volume of 1 mL per depth, either as a single elution or by pooling elutions as appropriate for the kit format and loading strategy. The result of this step was a clean, small-volume EV/virus-enriched eluate suitable for archiving and analytical workflows.

Aliquoting, cryopreservation, and storage

Immediately after elution, the 1 mL product was gently mixed (avoiding vigorous vortexing) and distributed into 10 low-binding tubes at 100 µL per tube. Aliquots were snap-frozen in liquid nitrogen and then transferred to −80 °C for long-term storage. Each tube was labeled with the relevant collection and processing identifiers, including cruise ID, station, cast, depth, date, the designation "EV/virus column eluate," and the aliquot number (e.g., 1/10–10/10), ensuring clear linkage between the archived material and the associated sampling metadata.

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	CTD Rosette with Niskin Bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	The CTD rosette system was used for discrete seawater collection at targeted depths. Niskin bottles mounted on the rosette were triggered to capture water, which was then pooled into pre-cleaned, labeled carboys (100 L per depth). Samples were maintained under cold, dark conditions immediately following collection to preserve particle integrity and minimize biological or chemical alteration prior to processing.
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	ZetaView Evolution Nanoparticle Tracking Analyzer (Particle Metrix, Germany)
Generic Instrument Name	Particle Size Analyzer
Dataset-specific Description	The ZetaView Evolution system was used for nanoparticle tracking analysis (NTA) to quantify particle size distributions and concentrations based on Brownian motion tracking in a video microscopy platform. The instrument supports measurement of particle concentration (typically 10^5–10^9 particles mL^{-1}), size distributions over an approximate range of 10–1000 nm (depending on sample and optical configuration), and zeta potential (−500 to +500 mV) across a pH range of 1–13. The system includes fluorescence NTA (F-NTA) capabilities with up to four excitation lasers and multiple detection channels, enabling detection of labeled particle subpopulations with sensitivity below ~20 AF488-equivalent fluorophores. Available excitation wavelengths include 405, 488, 520, 640, and 660 nm. The instrument also supports colocalization NTA (C-NTA), allowing simultaneous detection of two fluorophores on individual particles, which is particularly useful for characterizing heterogeneous EV and viral populations in complex environmental samples.
Generic Instrument Description	Particle size analysis, particle size measurement, or simply particle sizing is the collective name of the technical procedures, or laboratory techniques which determines the size range, and/or the average, or mean size of the particles in a powder or liquid sample.

Dataset-specific Instrument Name	Low-shear Peristaltic Pump
Generic Instrument Name	Pump
Dataset-specific Description	A low-shear peristaltic pump system was used for sequential pre-filtration to remove larger particulates and most intact cells while retaining extracellular vesicles (EVs) and viral particles in the filtrate. Seawater was passed through 0.8 µm and subsequently 0.45 µm filters using inert tubing, connectors, and clean reservoirs. Flow rates and pressures were maintained within recommended limits to minimize mechanical stress on particles.
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	Tangential Flow Filtration System
Generic Instrument Name	Pump
Dataset-specific Description	Tangential flow filtration (TFF) was used to concentrate and buffer-exchange the
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	EV Column Purification System (Takara / Qiagen)
Generic Instrument Name	Pump
Dataset-specific Description	Final purification of EV- and virus-enriched samples was performed using commercial EV column kits (Takara or Qiagen), operated in spin or flow format depending on workflow constraints. Columns were equilibrated and processed according to manufacturer protocols, including appropriate loading volumes, wash steps, and elution conditions. This step produced a clean, small-volume eluate (1 mL per sample) suitable for downstream analytical applications. Operation required a benchtop centrifuge and/or column rack or manifold.
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

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Deployments

AT50-08B

Website	https://www.bco-dmo.org/deployment/995472
Platform	R/V Atlantis
Start Date	2023-02-10
End Date	2023-03-16
Description	Project: Collaborative Research: Key Microbial Processes in Oxygen Minimum Zones: From In Situ Community Rate Measurements to Single Cells Chief: Pachiadaki, Maria G Start port: Putarenas, Costa Rica End port: Puntarenas, Costa Rica

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Project Information

EAGER: Mechanistic Study of Extracellular Vesicle Production by Marine Microalgae using Advanced Imaging Technologies (Marine Microbial Extracellular Vesicles)

Coverage: Coastal Western Atlantic waters and Eastern Tropical North Pacific OMZ

NSF Award Abstract:
This EAGER project is a proof-of-concept study on the composition, origins, and dynamics of extracellular vesicles (EVs) produced by marine microalgae in response to various environmental and biotic stressors. EVs are microscopic lipid-encased particles that are released naturally from almost all cell types and are vehicles for a variety of cargo, including genetic material (RNA, DNA), proteins, and lipids. EVs have been variously postulated to serve as a defense against viral attack, a waste disposal mechanism, a stress response, or a means of cell-to-cell communication. Marine microalgae are pivotal players in the global carbon cycle. By better understanding processes that govern their population dynamics and responses to environmental changes, we can develop better predictive models of responses to global climate change. The need to understand these mechanisms is becoming increasingly urgent as climate change becomes more manifest. Very recent findings suggest that EVs play a key role in marine phytoplankton population regulation, but our understanding of their function(s) in planktonic systems is severely limited and fragmentary. This project addresses significant knowledge gaps and explores the potential complexities of marine planktonic EV production. This project provides support and training to a female graduate and undergraduate marine sciences students, who are receiving unique opportunities to master new experimental approaches and state-of-the-art research tools that are extremely rare in marine sciences programs. The project supports high school students in marine sciences studies as a part of the summer science camp (www.sigmacamp.org). A female postdoc is also being trained on the project.

Using the cosmopolitan and geochemically-important microalga E. huxleyi as a model system, this project tests three major hypotheses to enhance our understanding of the purpose(s) of microalgal EV production. (1) Microalgae produce distinctive types of EVs (ectosomes or exosomes) in response to different environmental conditions, and EV types have definitive functions (stress response, viral defense, intercellular communication, waste disposal). (2) EVs' cargo is diverse, so their production and release reflect a complex intercellular communication mechanism. (3) Exosome genesis is a multistage process, and its stages are separated in time. Therefore, algal cells may contain a pool of pre-formed EVs loaded with different cargo that are stored internally, and when induced by a sudden change in external conditions are released through the outer membrane. To adequately test these hypotheses requires using single particle analytical methods in addition to ensemble measurements. The investigators are using an assortment of recently developed methods and original experimental approaches developed by our group to investigate EV compositional variability under selected stress conditions. They use single particle Raman microspectroscopy, pulse-chase Stable Isotope Probing, and LC-MSMS for compositional analysis of EVs, and Cryo-EM and AFM for morphological analyses. If experimental data confirm our suspicions, then phytoplankton EVs represent a novel and essentially overlooked mechanism of extracellular interactions that potentially govern a wide range of globally-important processes.

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-2202723

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