Adenosine triphosphate and microbial biomass measurements from the Chesapeake Bay, sampled aboard RV Fay Slover on April 11, 2018

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Project
» Adenosine triphosphate as a master variable for biomass in the oceanographic context (ATP biomass indicator)

Contributors	Affiliation	Role
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Abstract
This dataset contains values from instruments and Niskin bottle samples from a research expedition on the RV Slover on April 11, 2018. Six stations were sampled with five bottle samples each from the coastal ocean to tributaries of the Chesapeake Bay. The variables are latitude, longitude, station number, depth, sigma-t, beam attenuation, relative chlorophyll fluorescence, salinity, temperature, total ATP (dissolved and particulate), carbon of prokaryotic microbes (microscope estimates), and carbon of eukaryotic microbes (microscope estimates).

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Coverage

Location: Neritic system off the Chesapeake Bay, the Chesapeake Bay and a tributary
Spatial Extent: **N**:37.00831167 **E**:-75.66680167 **S**:36.90872833 **W**:-76.22801167
Temporal Extent: 2018-04-11

Methods & Sampling

Field measurements and bottle collections: Seawater was collected on the research vessel Fay Slover using 5 L Niskin bottles along a gradient from the open ocean near the Chesapeake light tower into the Chesapeake Bay and the James River, in April 2018 and June 2019 (Table 1, Fig. 1). Conductivity, temperature, and depth were measured using a SBE 32 CTD equipped with a Wetlabs fluorometer and a Wetlabs transmissometer (650 nm).

Field collection of ATP: ATP samples were processed using the hot-water extraction method described by Bochdansky et al. (2021). Three types of ATP samples were collected: 1) pATP in 5 ml seawater filtered through 0.2 mm pore size polycarbonate filters (Isopore GTTP, 25 mm diam.), 2) dissolved ATP (ATP in the filtrate of 0.2 mm filtration), and 3) 0.5 ml ATP in whole water without filtration. Polycarbonate filters were chosen over GF/F filters because of their higher retention efficiency (Taguchi and Laws, 1988; Lee et al., 1995)

and because the volumes filtered were small, to ensure filtration times were kept to a minimum even with the use of the smaller pore size. Five milliliters of each depth were immediately and simultaneously filtered using a filtration manifold (25 mm-diameter filters, stainless steel screen supports) preloaded with the polycarbonate filters. The filters were placed in 15 ml polypropylene centrifuge tubes (FalconTM) within seconds after the water passed. The dissolved ATP was captured in 15 ml polypropylene centrifuge vials placed underneath the filtration manifold and inside the vacuum flasks. The dissolved ATP samples (i.e., the 0.2 mm filtrates) were immersed in a boiling water bath for ~15 minutes immediately after filtration to sterilize the water and inactivate ATPases and then frozen at -80 °C until analysis.

In April 2018, approximately 4.5 ml of boiling ultrapure water was quickly added to each centrifuge tube. The tubes were stoppered and transferred to a beaker with boiling water for ~15 minutes for extraction of intracellular ATP and inactivation of ATPases. After extraction, the tubes were cooled to room temperature and then kept frozen at -20 °C for transport to the laboratory. Samples were subsequently kept at -80 °C until analysis.

In June 2019, instead of boiling on board, filters, filtrates, and whole water samples were collected as above but immediately frozen in liquid nitrogen, transported to the laboratory at -20 °C, and stored at -80 °C in the lab. Hot-water extraction was then performed on the still frozen samples (i.e., without prior thawing) in the laboratory (see Bochdansky et al. 2021 for details). The shock freezing-boiling treatment breaks up cells more efficiently than boiling alone, which results in higher extraction efficiencies (Bochdansky et al. 2021).

In 2018, 500 ml of water from each bottle was collected and extracted in a boiling hot-water bath before the samples were cooled to room temperature, and then frozen at -20 °C. In 2019, 500 ml of water from each bottle was placed in 15 ml centrifuge tubes and shock-frozen in liquid nitrogen. These unfiltered samples thus contained both particulate and dissolved ATP and were labeled total ATP (tATP). All samples were brought to the laboratory in a -20 °C freezer and subsequently stored at -80 °C in the laboratory. For analysis of the 500 ml shock-frozen samples, boiling hot water was added to the samples and extracted for ~15 minutes in a boiling-water bath.

Particulate and total ATP samples were topped up to 5 ml with ultrapure water using the gradations on the centrifuge tubes and mixed with a vortex mixer. The 500 ml whole-water samples were also diluted to 5 ml with ultrapure water to reduce salt effects that strongly decrease the luminescence yield. It should be noted that the whole-water extraction method used here (for total ATP) requires sufficiently high ATP levels to produce a signal. This was possible because samples were taken in the mesotrophic coastal ocean and in a eutrophic estuary. Such a small amount of water (500 ml) would be insufficient in oligotrophic or deep-sea environments. Hot-water extraction is only one of two methods proposed by Bochdansky et al. (2021). Many of our subsequent collections were based on chemical extraction using phosphorus benzalkonium chloride (P-BAC) instead. Both methods give highly consistent results, with the values from the chemical extraction method exceeding that of the hot water extraction by 20% (Bochdansky et al. 2021). The hot-water method used here has the advantage that measurements of dissolved ATP can be added easily to the protocol as hot water for the inactivation of ATPases is already at hand.

Laboratory analysis of ATP

Fifty microliters of each sample (in triplicates) were transferred to 6 ml pony scintillation vials (Research Products International), and received 3 ml of ultrapure water, and 50 µL of CellTiter-Glo 2.0 (Promega Corporation). Internal standards were used by spiking a fourth vial with 50 µL of samples with 50 µL of 0.0164 µM ATP standard. Using internal standards instead of separate calibration curves corrects for matrix effects that change the luminescence signal caused by the presence of ions, acids, and organic material (Bochdansky et al. 2021). Luminescence was analyzed in a PerkinElmer Liquid Scintillation Analyzer with a single photon counting protocol of 1 minute each. The counter was programmed to cycle samples five times in sequence. We determined that values from the second cycle were the most consistent and were thus subsequently used for all analyses.

ATP was calculated using the formula: (see Bochdansky et al. 2021)

, (equation 1)

where [ATP] is the concentration of the internal standard as determined by spectrophotometry (i.e., 16.4 nM), the photon counts per minute for the sample, the average value of 4 to 6 blanks (50 ml of Celltiter Glo 2.0 reagent added to 3 ml ultrapure water only), the average counts per minute for the standard vials of the same sample type, the average of triplicate values for each sample, *Vstd* the volume of the standard added to the scintillation vial in ml, *R* the ratio between the volume of the extract (numerator) and the volume of sample filtered (denominator), and *Vextr* the volume of the extract added to the scintillation vial (in ml; 50 ml of the water extract).

Particulate carbon and nitrogen

During the June 2019 cruise, between 75 ml and 250 ml of seawater (less in the inshore and more in the offshore stations) was filtered onto pre-combusted (450 °C, 4 hours) GF/F filters. The filters were stored frozen (-20 °C) and later dried at 50 °C for approximately 2 days. Filters were then rolled in a tin wrap and pressed into pellets to be analyzed in a Europa 20-20 isotope ratio mass spectrometer (IRMS) equipped with an automated N and C analyzer.

Microscopic analysis of biomass

2018 samples: For bacteria abundance, 20 ml of the Niskin bottle sample was added to a 50 ml Falcon tube and preserved with 2% (fin. conc.) formaldehyde. Within 24 hours, 5 ml of the formaldehyde sample (in duplicates) was filtered onto a 25 mm diameter, 0.2 mm pore size, black polycarbonate filter (Isopore type GTBP, Millipore Corp.). The filter was put onto a slide and one drop of Vectashield Antifade Mounting Medium with DAPI (H1200, Vector Laboratories) was applied. A cover slip was added and the slide was stored at -20 °C.

The slides were brought to room temperature in a desiccator to remove condensation before immersion oil was added to the cover slips. Bacteria were enumerated under an Olympus BX61 epifluorescence microscope (100 x oil immersion objective, 2 x loupe, 10 x ocular magnification). ToupView (Toup Tek Photonics) imaging software was used to take images of the bacteria slides. Images were analyzed using a custom macro developed for ImageJ software (National Institutes of Health, https://imagej.nih.gov/ij/index.html). This macro inverted the image, converted it into an 8 bit image, applied a FFT Bandpass Filter, binarized the image, and finally applied a watershed feature that outlined each bacterium. The algorithm automatically counted the number of bacteria and the area of each bacterium on each picture. A fixed value of 20 femtogram (fg) carbon per prokaryotic cell was applied (Ducklow, 2018). We preferred the higher value as cell carbon is typically higher in eutrophic systems than in open and deep ocean environments (Ducklow, 2018; Herndl et al., 2023).

For the enumeration of eukaryotes and measurements of size, two types of analysis were performed: epifluorescence and inverted microscopy. The same slides used for the bacteria counts were also used to count eukaryote microbes directly. The eukaryotes were counted if the bright blue spots were at least twice the size (linear dimension) of the bacteria in the image. If a large red fluorescence due to chlorophyll was noticed but there was no noticeable blue nucleus, it was still counted as a eukaryote. Organisms that were completely inside the picture were counted; if an organism landed on the top or left side of the picture and at least ¾ of the organism was visible, then it was counted too. If the organism landed on the right or the bottom of the image and was cut off, then it was not counted. The total number of eukaryotes was taken for each station and depth and a fixed value of 2,200 fg was applied, assuming that the overwhelming majority of these eukaryotes were nanoplankton (Pomeroy 1974, Fukuda et al., 2007; Sohrin et al., 2010). Given the wide size range in eukaryotes, this step represents a major simplification (see Discussion).

For inverted microscopy, 20 ml sample vials were filled with seawater to the vial shoulder and 6 drops of Lugol's solution were added to produce a tea-colored solution. Once in the lab, samples were mixed by inversion and 10 ml of the sample was measured into a graduated cylinder. Another 4 drops of Lugol's solution were added and then topped off with equal-salinity 0.2 mm-filtered artificial seawater. Once the chamber was topped off, a glass plate was placed on the top to make it air-tight and to hold the water column in the settling chamber. After 24–48 hours of settling, the chamber was drained and replaced with a cover slip.

The slide was then processed under an Olympus CK7 inverted microscope equipped with a digital camera (see above). Organisms on the entire slide were imaged and all of those with the following criteria were analyzed. Organisms that were over 9,000 pixels in area (~ 100 mm2) and had a smooth shape to the structure (indicating a cell membrane) were included. For ciliated cells, only the area inside the cell membrane was measured. Diatom cell chains were measured as one composite organism. Each organism on the image was outlined and the area in pixels was recorded. If two or more organisms were identical, then only one would be

measured and that measurement would be extrapolated to all identical organisms on the image. Any empty-shelled organisms were excluded. Due to time constraints, only approximately every tenth image was examined, and the results extrapolated to the entire sample. The area in pixels was converted to mm2 then the volume was calculated according to Bochdansky et al. (2017a). Volumes were converted into carbon values using equation 2 (Menden-Deuer and Lessard, 2000):

Log10(carbon (pg)) = -0.665+0.939*Log10(volume (mm3) (equation 2)

2019 Samples: Prokaryote samples were fixed with formaldehyde and slides were prepared and stained with DAPI as described above. Prokaryotes within an area of 0.00025 mm2 in thirty random fields were manually counted across the filters for each slide under an Olympus epifluorescence microscope. As with the 2018 samples, bacteria counts were converted to carbon using 20 fg per cell.

For eukaryote biomass estimates, 10 ml of the unfixed sample was filtered through a 0.2 µm GTBP membrane filter as well as a 0.45 µm cellulose nitrate backing filter. When no liquid remained, the pump was switched off and the valves were closed to release the vacuum. One ml of a plasma membrane stain (10.0 µL of CellMask (Invitrogen) in 10 ml of 0.2 mm-filtered 34 ppt artificial seawater) was added to the dry filter and kept for 10 minutes before the valves were opened and the pump was turned on. When the stain was completely filtered, two rounds of 1.0 ml filtered artificial seawater were pipetted evenly over the filters to rinse. Nuclei were counter stained with DAPI and embedded in Vectashield as described above. Slides were kept at -20 °C until microscopic examination.

Eukaryote slides were prepared for depths #1, #3, and #5 at each station (actual depths varied), skipping depths in between (Table 1). Thirty randomly selected image pairs, taken with DAPI and TRITC filter sets, were captured per slide using ToupView Imager software (Fig. 2). DAPI images were taken to confirm that cell nuclei were present. Cell bodies were delineated using ImageJ (Fig. 2) and cell volumes and carbon values calculated as for the Lugol's samples.

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Related Publications

Bochdansky, A. B., Beecher, A. A., Calderon, J. R., Stouffer, A. N., & Washington, N. N. (2024). A comparison of adenosine triphosphate with other metrics of microbial biomass in a gradient from the North Atlantic to the Chesapeake Bay. Frontiers in Marine Science, 11. https://doi.org/10.3389/fmars.2024.1288812
Results

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Parameters

Parameters for this dataset have not yet been identified

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Project Information

Adenosine triphosphate as a master variable for biomass in the oceanographic context (ATP biomass indicator)

Coverage: Global

NSF Award Abstract:
In the ocean, most living organisms are microbes that are too small to be seen by the naked eye. Despite their

small size, microbes play an important role in processes that govern marine ecosystems and food webs. For example, microbes affect the concentrations of nutrients and gases in the water and the atmosphere, thereby exerting a significant impact on the climate globally. Consequently, it is important to know how many microbes there are in any given environment because there is a direct causal connection between living mass and overall biological activity. Determining how “alive” any volume of water is, however, is a difficult task. The gold standard is to count microbial cells under the microscope. This method is extremely time consuming when done well and needs to be performed separately on many different types of microbial cells. In addition, standard microscopic techniques do not reveal whether the cells were alive when they were collected. In contrast, a chemical method based on the amount of adenosine triphosphate (ATP) offers distinct advantages. Notably, ATP is relatively easy to measure, and the method can be widely used because all living cells contain ATP in similar concentrations. This study tests and applies an improved method of ATP analysis to generate data at very high resolution in space and time. One PhD student and six undergraduate students will receive research training and the project fosters international research collaborations with European scientists. This research provides deeper insights into the distribution of live matter in different regions and depths of the world’s oceans.

Decades ago, adenosine triphosphate (ATP) was proposed as a universal biomass indicator. However, its application in the field of oceanography has been limited due to misconceptions regarding cellular ATP concentration. Recent evidence suggests that ATP functions as a hydrotrope requiring homeostatically controlled ATP levels much higher than those solely needed for energy metabolism. ATP occurs in surprisingly stable concentrations in cytoplasm across a wide range of microbes thus representing live cytoplasm volume. This project examines in detail the usefulness of particulate ATP (PATP) as a biomass marker over a large section of the North Atlantic Ocean with special emphasis on mesopelagic and deep-sea environments where chlorophyll is a poor indicator of biomass or associated biological processes. The project uses field collections of marine snow and ambient water in combination with particle cameras to examine the microscale heterogeny of biomass in the water column. Laboratory studies determine factors that may influence the recovery of PATP through filtration and extraction protocols and determine to what extent ATP concentrations potentially deviate from the typical cytoplasm concentration during phosphorus limitation. The improved PATP-biomass method offers numerous operational advantages, especially the fact that it can be employed at high spatial and temporal resolution. Once validated, the PATP biomass method could be widely adopted as a key variable for biomass in routine oceanographic surveys. This project supports graduate and undergraduate students from diverse backgrounds to contribute to laboratory and field research. Public outreach efforts include tours and presentations for middle and high-school students, as well as the general public.

This project is funded by the Chemical Oceanography and Biological Oceanography Programs in the Division of Ocean Sciences.

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-2319114

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