# Molecular and microscopic characterization of sinking particles at the Bermuda-Atlantic Time Series Study station (BATS)

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## Background

The biological carbon pump is mediated mainly by large (> 0.5mm) particles, called "marine snow" that sink out of the euphotic zone to the deep ocean (Fowler and Knauer 1986). Marine snow can be comprised of aggregates of phytoplankton cells, detritus, and/or fecal matter. At BATS, while the size of sinking particles (Durkin et al. 2015) and the plankton communities associated with bulk particle organic carbon (POC) flux (Amacher et al. 2013) have been described, the characteristics of these sinking particles is yet to be known.

The use of DNA-based methods complemented with microscopy allow for the study of the relative contribution of plankton taxa to different particle types and help elucidate their predominant methods of transport to depth, such as through direct formation of aggregates, or by grazing and formation of sinking fecal pellets.

## Objectives

- Determine the different particle types that contribute to flux at different depths.
- Characterize sinking particles using microscopy.
- Describe the community associated with different sinking particle types.

## Methods

### Sampling

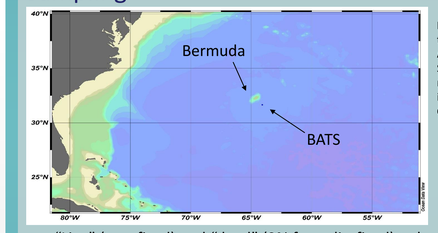


**Figure 1**. Location of the Bermuda Atlantic Time Series Study station (BATS) relative to Bermuda in the Sargasso Sea. Credit: Ali Freibott

- "Live" (non-fixed) and "dead" (2% formalin-fixed) polyacrylamide gels were deployed within particle interceptor trap tubes at 150 m, 200 m, and 300 m depths at the Bermuda-Atlantic Time Series Study station (BATS) (Figs. 1,2) during cruises AE1718/BATS339 and AE1809/BATS345a in September 2017 and March 2018, respectively.

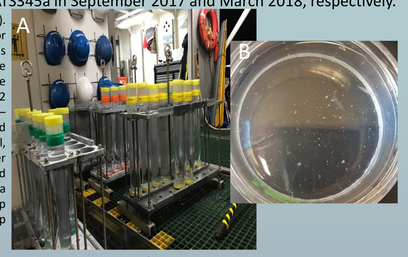


**Figure 2**. (A). Particle interceptor trap tube systems with polyacrylamide gel jars to be deployed for 72 hours. Two traps – one "live" gel, and one "dead" gel, were deployed per depth. (B) Imaged surface area of a "live" 150 m gel trap following trap recovery.

### Particle flux image analyses

- The entire surface area of all gels were imaged upon trap recovery and 10 nonoverlapping images picked for subsequent determination of particle areas and ellipsoidal volumes using ImageJ software.
- Particles were categorized and their organic carbon content was determined from volumes using conversion factors from Alldredge 1998 and Gonzalez and Smetacek 1994.

### Microscopy

- "Dead" particles were stained with DAPI and polysaccharide-specific lectin concanavalin-a then mounted onto microscope slides for visualization using epifluorescence and confocal microscopy.

### DNA-based community analyses

- Bacterial taxa from individually-picked particles were determined by Illumina MiSeq of the V4 region of the 16S rRNA gene, respectively.

## Results

### Bacterial taxa associated with different particle types

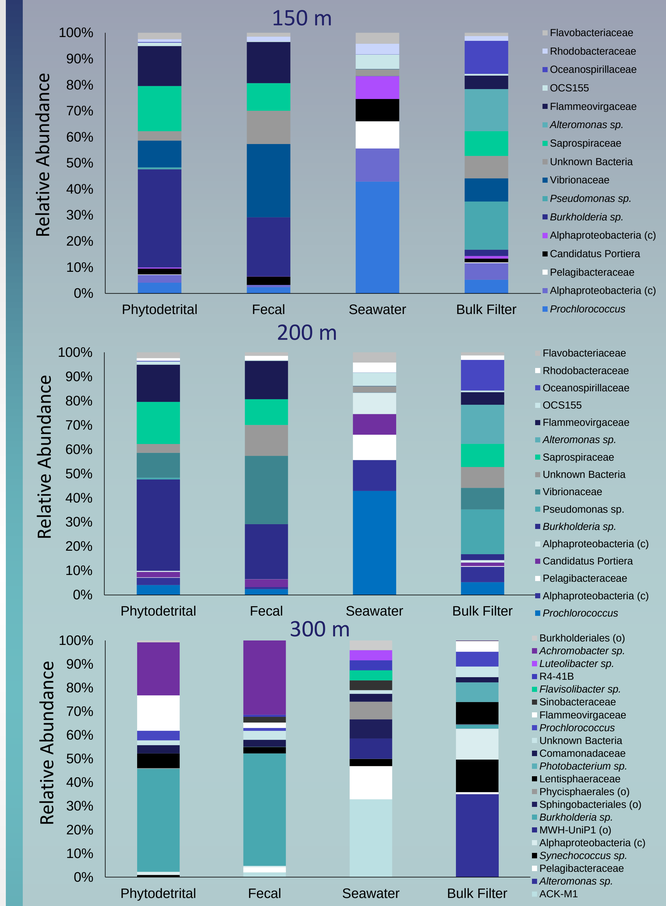


**Figure 3**. Relative abundance of most dominant bacterial taxa within phytodetrital aggregates, fecal pellet-nucleated aggregates, the ambient seawater, and bulk particles at all depths.

- Taxa differ between free-living (seawater) and particle-associated communities.
- The genus *Burkholderia* sp. and family Vibrionaceae were most abundant in phytodetrital and fecal pellet-nucleated particles.

### Microscopic characterization of sinking aggregates

#### Phytodetrital aggregates

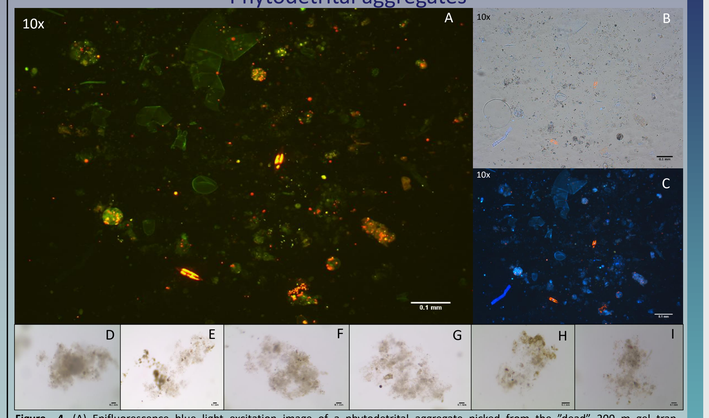


**Figure 4**. (A) Epifluorescence blue light excitation image of a phytodetrital aggregate picked from the "dead" 200 m gel trap. Corresponding brightfield+DAPI (B) and DAPI excitation (C) images. Red: chlorophyll-*a*; yellow: phycoerythrin *(Synechococcus* sp.); green: heterotrophs; blue: DAPI-stained DNA. (D-I) Stereomicroscope images of exemplary phytodetrital particles considered in this study.

#### Fecal pellet-nucleated aggregates

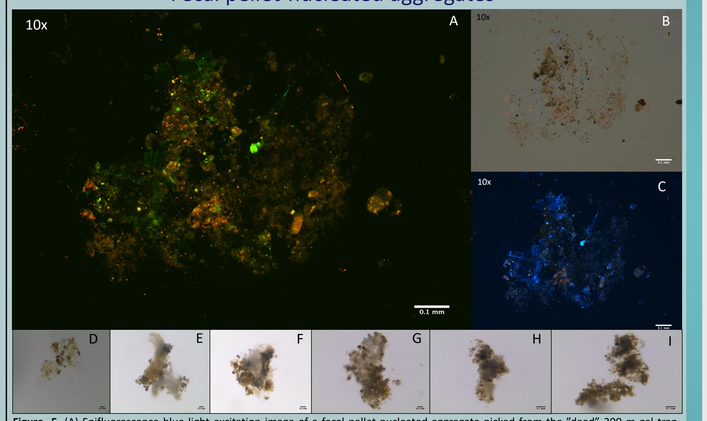


**Figure 5.** (A) Epifluorescence blue light excitation image of a fecal pellet-nucleated aggregate picked from the "dead" 300 m gel trap. Corresponding brightfield+DAPI (B) and DAPI excitation (C) images. Red: chlorophyll-*a*; yellow: phycoerythrin *(Synechococcus* sp.); green: heterotrophs; blue: DAPI-stained DNA. (D-I) Stereomicroscope images of exemplary fecal pellet-nucleated particles considered in this study.

- Phytodetrital aggregates collected were predominantly composed of single phytoplankton cells, smaller aggregates of nano- and pico-phytoplankton, as well as zooplankton molts and appendages adhered within a transparent matrix.
- Fecal pellet-nucleated aggregates were significantly more compact, optically denser, and composed of ovoid fecal pellets and smaller phytoplankton aggregates.

### Contribution of different particle types to flux

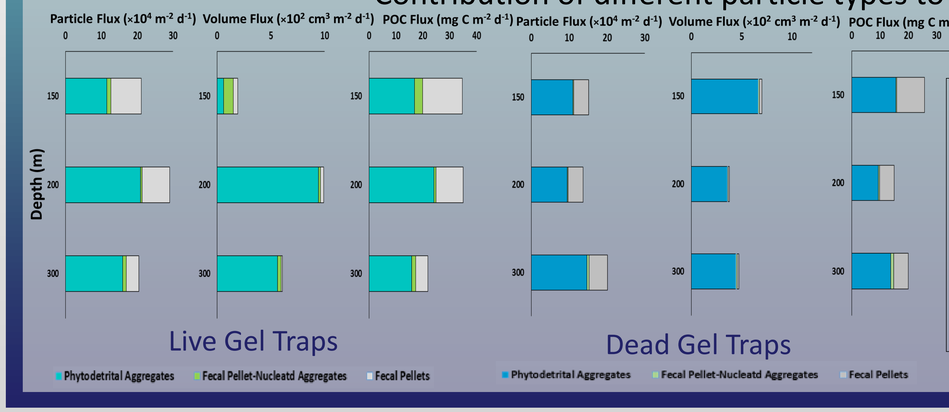


**Figure 6**. Numerical, volume, and POC fluxes of each particle category in live and dead gel traps deployed at depths of 150 m, 200 m, and 300 m for 72 hours.

- Phytodetrital aggregates dominated particle and volume flux and carried most of the POC flux at all depths (≈80%, all fluxes).
- Fecal pellet-nucleated aggregates contributed the least (≈5%) relative to phytodetrital aggregates and fecal pellets (≈15%) to fluxes at all depths
- Relative contribution of fecal pellets to flux decreased with depth.
- Live traps showed higher fluxes of all particle types compared to dead traps.

## Results Cont'd.

### Microscopic characterization of polysaccharides on sinking aggregates

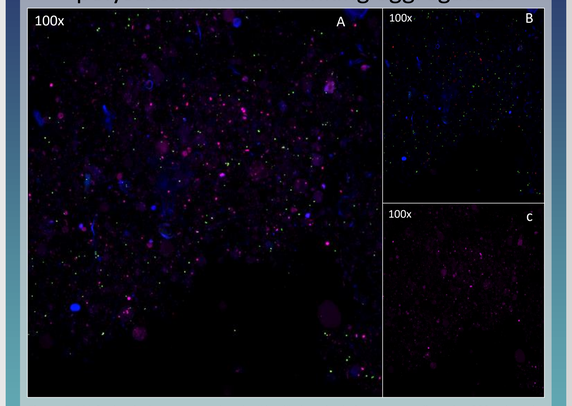


**Figure 7.** (A) Confocal microscopy overlay of a "dead" phytodetrital aggregate collected at 200 m in spring 2018. Magenta: concanavalin-a stained polysaccharide matrix; red: chlorophyll-*a*; green: phycoerythrin (*Synechococcus* sp.); blue: DAPI-stained DNA. (B) Layer showing red, green, and blue fluorescence only. (C) Layer showing the polysaccharidic matrix only.

- The transparent matrix of phytodetrital aggregates were composed of polysaccharidic substances (A,C).
- *Synechococcus* sp. cells were prevalent on phytodetrital particles as shown by phycoerythrin (green) fluorescence (A,B), but not *Prochlorococcus*.

## Conclusion & Future Work

- ✓ Phytodetrital aggregates dominated particle fluxes.
- ✓ These phytodetrital aggregates are composed of a matrix of transparent, polysaccharidic substances, likely transparent exopolymeric particles (TEP).
- ✓ Relative composition of particle-associated and free-living bacterial taxa differ.
- ✓ Future work will include the analysis of eukaryotic taxa within the different particle types.
- ✓ This work is part of a larger effort to study picophytoplankton-bacteria interactions and their role in aggregation.

## References

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