Assessment of Sinking Particle Size Spectra from Marine Snow Catcher Deployments during the first NASA-led EXPORTS field campaign in the North Pacific

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The rationale

The biologically mediated transfer of carbon from the sunlit surface ocean to depth is termed the biological carbon pump and has the capacity to influence the concentrations of atmospheric carbon dioxide over climatologically relevant timescales (Volk and Hoffert, 1985). Predicting the response of the global carbon cycle to changes in climate and biogeochemical conditions is critical and requires a comprehensive understanding of the vertical flux of carbon to the ocean’s interior, where carbon is sequestered away from the atmosphere for decades to millennia (DeVries et al. 2012), regulating anthropogenic climate change. The global carbon export flux in the ocean is highly variable and current estimates show an uncertainty range of 5-12 Pg C yr⁻¹ (Henson et al. 2011 and Boyd and Trull, 2007). Sources and sinks of carbon in the ocean are controlled by both physical and biological processes, which show high complexity and variability. The strength and efficiency of the biological carbon pump depend predominantly on five export pathways (Fig.1): gravitational settling of (1) intact phytoplankton cells, (2) aggregates, and (3) zooplankton byproducts; (4) physical advection and mixing of organic carbon to depth and (5) vertical transport of organic carbon due to migration of zooplankton and their predators (Siegel et al. 2016). These five pathways play a major role in spatially separating the different forms of carbon in the ocean. Gravitational settling particles (1-2-3) are mostly produced in the surface ocean; their fate within the water column is influenced by aggregation and disaggregation processes that change their size: aggregation can increase the removal rate of material in the form of small suspended particles by transforming them into large and possibly rapidly settling particles (Burd and Jackson, 2009). Inversely, disaggregation can increase the concentration of small particles at great depths by deconstructing bigger aggregates but also attenuate their sinking speed. Sinking marine particles are thus subjected to changes in size, shape and composition, which in turn affect their sinking velocity, controlling the distribution of carbon within the water column. Marine particles range from colloidal (submicron diameter) to marine snow (> 0.5 mm) particles. Traditionally, particles > 0.4 μm are considered part of the particulate pool, while anything smaller is considered part of the dissolved pool. Size classification is important as it can give insight on the particles’ ability to sink to depth (suspended vs sinking particles). It is often assumed that particle sinking velocities increase as a function of particle size following Stokes’ law, and that small particles either do not sink or are remineralized within the upper mesopelagic (<500m). However, the presence of small particles has been observed at greater depths (>1000m) challenging this understanding (Dall’Olmo et al. 2014). Moreover, understanding how sinking velocity links to particle size may enable the use of satellite-derived particle size distributions (PSDs) for a mechanistic understanding of the biological carbon pump.

The scope of this study is to investigate how size and sinking velocity are related, by comparing the size distributions of non-sinking, slow-sinking and fast-sinking particles collected in the oligotrophic North Pacific. This work will make up a part of my PhD dissertation.
Methods

Sample collection
Particles were collected as part of the first NASA-led EXPORTS field campaign (https://oceanexports.org) conducted in August and September 2018 in the NE Pacific. Non-, slow- and fast-sinking particles were collected using the Marine Snow Catcher (MSC) at 11 stations with 3 depths between 20 and 500 m. The MSC is a large (100 L) water sampler with a removable base section that contains a tray. It enables the partitioning of particles according to their sinking velocity and not their size. When the MSC is deployed, the terminal apertures of the two sections are kept open to minimize turbulence and are closed at the target depth through a trigger mechanism. Immediately following retrieval, a five-liter t=0 sample is collected from the middle tap and the MSC is secured on the deck in an upright position for exactly 2h to allow the settling of particles. The upper section of the MSC is then drained slowly and the non-sinking fraction is sampled. Subsequently, the upper section of the MSC is removed completely allowing sampling of the base. The overlaying water in the base section is carefully siphoned off and constitutes the slow-sinking fraction, whereas the water in the tray is considered to contain the fast-sinking fraction (Riley et al. 2012 and Giering et al. 2016). After collection, the sinking fraction samples are either analyzed for particulate organic carbon (POC), particulate organic nitrogen (PON), particulate inorganic carbon (PIC), biogenic silica (BSi), lithogenic silica (LSi) and transparent exopolymer particles (TEP) or stored for subsequent analysis through the FlowCAM (Fluid Imaging Technologies, U.S.).

Particle size distribution
As particles are subjected to changes in size due to the biological and physical processes happening in the ocean, their size distribution can give insights on the structuring of the marine environment they were sampled (Sheldon et al. 1972). By definition, particle size distributions (PSDs) give the cumulative particle concentration per unit volume for a unit size interval (Blanco et al. 1994) and they are a useful description of the relationship between particle abundance and size. In the ocean, small particles such as phytoplankton and bacteria are more abundant than larger particles (e.g., marine snow), consequently the total biomass of small particles greatly exceed the one for larger particles. Accordingly, PSDs in marine environments show a monotonically decaying function of the particle size. The interpretation of the slope of the log normalized PSDs and its deviation from linearity is generally used to infer information on the structure of marine ecosystems (Stemmann and Boss, 2012). In situ technologies and measurements used to make local quantitative observations of particles are becoming more available during field campaigns, however there is a need of consensus across methods, instruments calibration, data analysis and quality assessment techniques to facilitate the inter-comparison between different devices and make the observed data integrable into a global modeling framework (Lombard et al. 2019).

We analyzed the size distribution of the sinking particle fractions this summer during my visit at Passow Lab at Memorial University of Newfoundland using the FlowCAM 8000 Series. The FlowCAM integrates the capabilities of flow cytometry, microscopy, and fluorescence to image particles in moving fluid. Water samples are drawn into a flow cell with a syringe pump, and a digital
camera (1920x1200 pixels - CMOS) photographs the particles as they pass through the flow cell. The flow cell is placed in front of the objective lens and is illuminated by a flash LED light from its rear face (Fig. 3a). The appropriate flow cell size cross section is chosen by considering the objective magnification, the size range of the particles under study and the desired flow rate. The depth of the flow cell constrains the upper limit for the size of the particles that can be analyzed, while the lower limit is constrained by the smallest size resolved by the objective magnification (see Table 1).

<table>
<thead>
<tr>
<th>Objective</th>
<th>Magnification</th>
<th>Flow cell dimension (μm)</th>
<th>Minimum size resolved - ESD (μm)</th>
<th>Maximum size resolved - ESD (μm)</th>
<th>Syringe (mL)</th>
<th>Flow rate range (mL/min)</th>
<th>Pixel size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20x</td>
<td>200x</td>
<td>50X300</td>
<td>1</td>
<td>50</td>
<td>0.5</td>
<td>0.005-10</td>
<td>0.338</td>
</tr>
<tr>
<td>10x</td>
<td>100x</td>
<td>100X700</td>
<td>2</td>
<td>100</td>
<td>1.0</td>
<td>0.01-20</td>
<td>0.7313</td>
</tr>
<tr>
<td>4x</td>
<td>40x</td>
<td>300X1500</td>
<td>5</td>
<td>300</td>
<td>5.0</td>
<td>0.05-100</td>
<td>1.813</td>
</tr>
<tr>
<td>2x</td>
<td>20x</td>
<td>1000X3000</td>
<td>12</td>
<td>1000</td>
<td>12.5</td>
<td>0.125-250</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table 1. FlowCAM general default settings for the four magnifications (200x, 100x, 40x and 20x)

We set the camera to capture images synchronously at a predefined rate (AutoImage mode) because the samples were fixed and contained mostly small particles. Groups of pixels that represent particles were then “segmented” out of each raw image and saved as a separate collage image as shown in Fig. 3b (FlowCAM Manual. Version 3.4).

We ran our sinking particle samples using two magnifications (200x and 40x) to be able to capture the entire size range of the particles (see Table 2). To compute the particle size distributions of each sinking fraction, we grouped the particles in size bins using the octave scale (2^n) and we calculated the minimum number of particles needed in each size bin to compute PSDs with certainty (Alvarez et al. 2011). In total we obtained about 200,000 digital images of single marine particles running all the non- and fast-sinking particle samples. The slow-sinking samples will be analyzed next.

<table>
<thead>
<tr>
<th>Objective</th>
<th>Min size (μm)</th>
<th>Max size (μm)</th>
<th>Bin range</th>
</tr>
</thead>
<tbody>
<tr>
<td>20x</td>
<td>1</td>
<td>50</td>
<td>1 to 5</td>
</tr>
<tr>
<td>4x</td>
<td>5</td>
<td>300</td>
<td>4 to 8</td>
</tr>
</tbody>
</table>

Table 2. Size range and size bin in octave scale used to analyze the EXPORTS sample using two magnifications (200x and 40x)
**Image processing**

A recent study by Giering and Hosking (*submitted*) found that the interpretation of particle size of marine particle images in the context of investigating biogeochemical cycling in the ocean is strongly affected by the choice of particle detection algorithm. For this reason, careful consideration will be placed in choosing the particle detection routine to be applied. I am currently processing the digital images using *scikit-image* in Python ([https://scikit-image.org](https://scikit-image.org)) to be able to detect the particles and subsequently size them.

In order to measure the objects captured by a digital image, the boundaries of the object have to be carefully defined. To do so, it is common to use intensity thresholds that may be implemented by using filtering techniques. An intensity threshold is a gray-scale value used to convert color digital images into black and white ones (i.e., binary images). In a gray-scale, each pixel has an intensity value that ranges from 0 to 255, where 0 equals black and 255 equals white, and the values in between are shades of gray. To produce a binary image, each pixel’s gray-scale (0-255) value is reduced to a simple 0/1 value, where again 0 = black and 1 = white. There are several threshold algorithms available (e.g., Otsu's method) that assign a predefined threshold value so that every pixel having a gray-scale value equal or higher is assigned to binary value of 1, while every pixel below is assigned a binary value of 0 (FlowCAM Manual. Version 3.4). On the other hand, filtering methods such as edge detection aims at identifying points of discontinuities in the image (i.e., sharp changes in image brightness). The filtering methods can be used to implement the threshold algorithms for a better detection of the imaged object. I am currently applying different image detection methods to find the routine that better suit my marine particle images (example in Fig. 4).

![Example of one of the routines I have coded to detect the EXPORTS marine particle imaged. The routine includes: Sobel edge detection algorithm, a morphological operator to close the holes and Otsu threshold algorithm](image)

**Next steps**

Once the particles will be detected and sized, I will test the following two hypotheses:

**H1**: Assuming Stokes' law, we expect the PSD slopes of non-sinking particles to be steeper (‘few large particles’) than the slope of fast-sinking particles (‘many large particles’).

**H2**: Assuming steady state and no lateral advection, particles at depth are a product of sinking particles produced at surface and particle processes during sinking (e.g., bacterial remineralization, zooplankton grazing, aggregation, disaggregation). If the assumptions hold, we expect differences in the shape of the PSDs at different depths.

One important note is that particle size has to be considered in relation with particle type, especially density, which regulates particle sinking velocity. We will use the biogeochemical data (POC, PIC, PON, BSi, LSi and TEP) obtained for each sinking fraction to investigate how size and composition correlate and influence particle sinking velocity.

**Bibliography**