

## Preprint

# GelCam: Visualizing Episodic Sinking Particle Flux via a Polyacrylamide Gel-Based Sediment Trap

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2 **Polyacrylamide Gel-Based Sediment Trap**

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12 particles

13

14

15 **ABSTRACT**

16 While particle-intercepting traps remain a dominant method for quantifying the contribution of  
17 sinking particles to the biological carbon pump, fluxes are typically integrated over days to months.  
18 Observations of time-varying particle flux over shorter durations are very limited. To this end, we  
19 prototyped a camera system called “GelCam” which captures a rapid time-lapse image sequence  
20 of particles that settle into a polyacrylamide gel layer located at the base of a sediment trap tube.  
21 Here, we describe the system design, post image processing, and results from nine deployments  
22 during the EXPORTS campaigns in 2018 and 2021 in the North Pacific and Atlantic ocean.  
23 Because wave-driven oscillations of the surface-tethered traps produced a lateral motion of the  
24 settled particles, we applied a cross-correlation method for tracking individual particles over time.  
25 All particles were subsequently classified into one of six categories based on visual traits, and then  
26 quantified into a particulate organic carbon (POC) flux. Using this image-based approach, we are  
27 able to distinguish differences in depth-based transfer efficiency among groups, and detect a diel  
28 variation in fecal pellet flux. Additionally, the GelCam resolved flux events on timescales shorter  
29 than days, allowing for the investigation of covariance among different particle types over short  
30 timescales. Paired with the direct recovery of samples to quantify carbon content and high  
31 resolution particle images, this approach will enhance our ability to resolve sinking “events” that  
32 occur episodically and may be missed when integrating over a traditional trap deployment.

33

## 34 INTRODUCTION

35 The ocean sink provides a net uptake of approximately  $2.8 \cdot 10^{15}$  g of carbon annually, which  
36 accounts for more than 25% of anthropogenic CO<sub>2</sub> emissions (Friedlingstein et al., 2023). Among  
37 processes of marine carbon sequestration, sinking particles transport  $\sim 10^{15}$  g/yr of carbon from  
38 the surface ocean to the deep ocean (Middelburg, 2011; Muller-Karger, 2005; Henson et al., 2011).  
39 Knowing the biological carbon pump is critical for predicting climate change effects. There are a  
40 few challenges to overcome before we can accurately model the ocean carbon uptake process.  
41 First, the carbon transport is driven by complex biophysical and chemical processes (Nowicki et  
42 al., 2022). Photosynthesis by phytoplankton (Basu and Mackey, 2018), consumption and  
43 production by zooplankton and fish (Cavan et al., 2017), and sinking of marine particles (De La  
44 Rocha and Passow, 2007) contribute to the high uncertainty in modeling the carbon transport.  
45 Additionally, aggregation (Burd and Jackson, 2009; Alldredge and Gotschalk, 1988),  
46 disaggregation (Briggs et al., 2020; Song et al., 2023), and microbial degradation occurring on  
47 particles (Collins et al., 2015), alter the particle size and contribute to the high variability in the  
48 sinking velocity of marine particles (McDonnell and Buesseler, 2010). Moreover, spatial and  
49 temporal variability further complicates the modeling of global biogeochemical cycles (Piao et al.,  
50 2013; SierraI et al., 2007). These challenges along with observational limitations in remote areas  
51 and deep sea environments, substantially constrains our understanding of carbon flux within the  
52 ocean.

53 Sediment traps provide a direct measurement of sinking marine particles, as the downward carbon  
54 flux can be retrieved from the total organic matter captured over a period of time within a fixed  
55 collection area of a tube or a funnel (Buesseler et al., 2007). Traditional sediment trap  
56 measurements provide an integrated carbon flux sample collected over time scales ranging from

57 days to months (Buesseler et al., 2007). Post analyses are necessary for most traditional sediment  
58 trap measurements. For example, Muller and Suess (1979) applied elemental combustion analysis  
59 to measure the organic carbon content in sediment trap samples. Durkin et al. (2021) used  
60 microscopic imaging techniques to analyze particles preserved in the polyacrylamide gel. In  
61 addition to being labor-demanding, the traditional sediment trap method is limited in its ability to  
62 assess episodic variations on timescales of hours.

63 Classifying sinking particle types is important for accurately predicting the carbon flux because  
64 each particle type contributes to carbon transport in its unique way. For example, zooplankton  
65 fecal pellets have been observed to play a major role in controlling carbon flux in some studies  
66 (e.g., (Cavan et al., 2015; Turner, 2002)). More specifically, Durkin et al. (2021) and Steinberg et  
67 al. (2023) emphasized the role of salp fecal pellets in the Subarctic Northeast Pacific Ocean.  
68 Additionally, the importance of large marine snow particles is highlighted in other studies, by  
69 which aggregation enhances the transport of organic matter (Alldredge and Silver, 1988; Fowler  
70 and Knauer, 1986). Contributions of phytoplankton (Durkin et al., 2016), mini fecal pellets  
71 (Gowing and Silver, 1985), and physical subduction (Omand et al., 2015) that includes particles  
72 cannot be ignored as well. However, the composition of sinking particle flux varies in space and  
73 time, leading to challenges in their classification. Developing a time-resolved particle  
74 classification system for sediment trap samples is needed. Using an imaging system, such as  
75 optical sediment traps, not only simplifies sample processing but enables the evaluation of flux  
76 components across different particle types.

77 To better understand and constrain temporal variability in particle fluxes and identify the  
78 ecological sources that drive these variations, we have developed a novel, low-cost, and easy-to-  
79 deploy instrument, named “GelCam”. This instrument allows for a rapid evaluation without

80 extensive post analysis, and chronological diagnosis of particle identities. Here we demonstrate  
81 the GelCam design and analyze episodic particulate organic carbon fluxes from ten datasets  
82 collected from two NASA EXPORTS (EXport Processes in the Ocean from Remote Sensing)  
83 field campaigns. The image post-processing contains a particle tracking algorithm and particle  
84 classification, which provides the arrival time and morphology of each particle. The GelCam with  
85 the framework of imaging processing can be applied to quantify the particle flux using two  
86 methods, with one based on cumulative particle areas and the other one based on the particle  
87 tracking and classification results. Here we model the particulate organic carbon from particle  
88 images and compare the fluxes between GelCam and other methods. We also present the diel  
89 changes of fecal pellet fluxes and investigate flux events attributable to different particle types.  
90 We finally discuss the benefits of using the GelCam to extract quantitative carbon flux and  
91 facilitate modeling of the biological carbon pump.

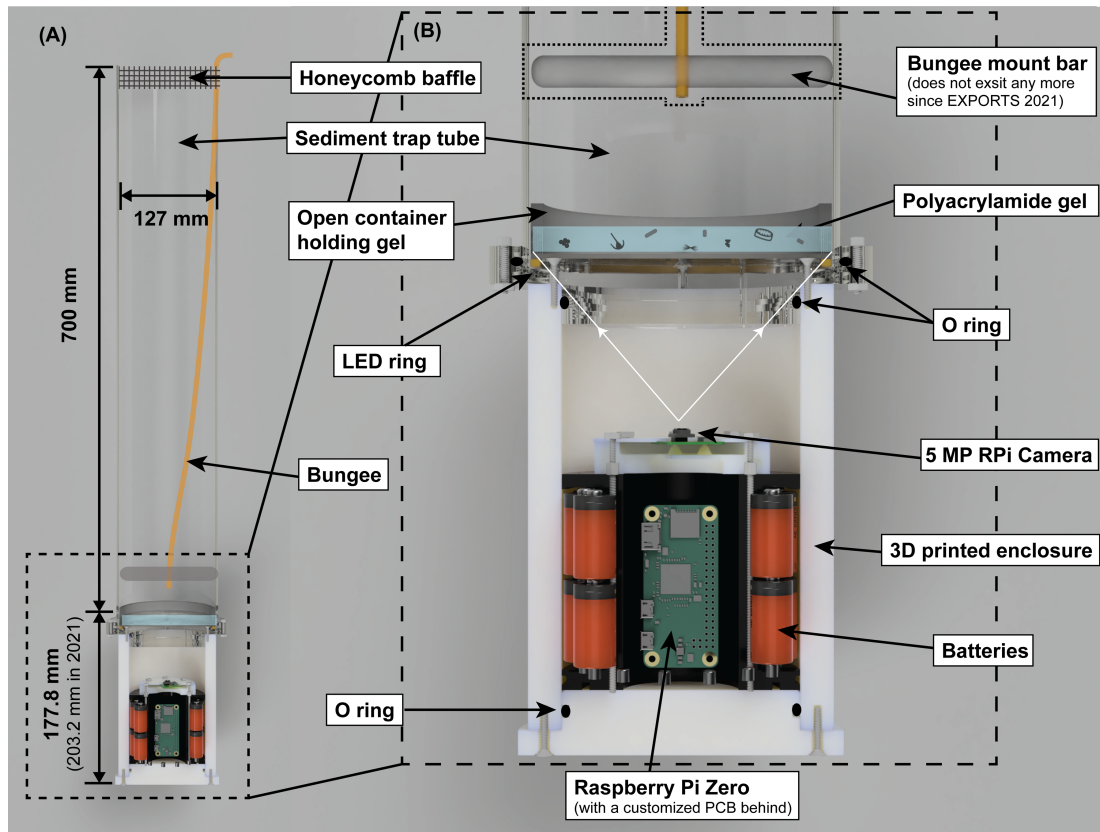
92

## 93 **MATERIALS AND PROCEDURES**

### 94 **GelCam design**

95 The GelCam is a time-lapse camera system designed to pair with a particle-intercepting sediment  
96 trap and it has two iterations that have already been deployed. In the current design, we used a  
97 cylindrical trap with an outer diameter of 127 mm, and a total length of 700 mm (Figure 1 (A)).  
98 A lid closure method used a bungee that was mounted to either a horizontal bar or to the side wall  
99 of the tube (since 2021). The open bottom of the tube was attached to the camera system with  
100 opposed flanges and an o-ring face seal. Before sealing the camera to the tube, a shallow container

101 with a transparent bottom was filled with a ~10 mm thick layer of polyacrylamide gel and placed  
102 at the base of the trap, and served to retain deposited particles.



103  
104 **Figure 1.** Schematic diagram of the GelCam system: (A) overview of the sediment trap with the  
105 GelCam attached to the bottom, and (B) detailed cross-section view within the enclosure.

106  
107 The GelCam housing was composed of an optically clear one inch thick acrylic top lid, a  
108 cylindrical acetal resin (Delrin) tube and a acetal resin bottom lid (Figure 1(A)). The housing  
109 length was 177.8 mm during EXPORTS 2018. An upgrade in 2021 increased the length to 203.2  
110 mm to provide more inner space and a larger field of view (FOV). The top lid contained a potted  
111 LED ring with a width of 4 mm and an outer diameter of 120 mm using clear epoxy resin, located  
112 on the upper surface to eliminate direct light or reflections and allowing only diffuse, scattered  
113 light to enter the camera. Brackets fabricated by 3D printing techniques and designed to secure

114 the batteries and electronics, were integrated within the housing. We used 7 packs of 12 V  
115 (nominal) stacks, each consisting of 4 CR123A batteries, yielding 134 Watt-hrs. The batteries  
116 were used to power a Arduino Nano micro controller, a Raspberry Pi Zero WiFi single-board  
117 computer, a Raspberry Pi camera module, and the LED ring. The low-power Arduino was used  
118 to duty cycle the R-Pi Zero, enabling us the sustain weeks-long deployments with photos every  
119 20 minutes. The camera was mounted facing upwards toward the gel container with a viewing  
120 axis aligned with the central axis of the sediment trap, enabling us to image nearly the entire base  
121 of the gel-filled cup.

122 The camera module had a sensor resolution of  $2592 \times 1944$  pixels. The lens used in the 2018  
123 expedition provided a FOV of approximately  $55.2 \text{ mm} \times 41.4 \text{ mm}$  which translated to a pixel  
124 resolution of  $21.3 \mu\text{m}$  per pixel. The taller housing used since 2021 expanded the FOV but  
125 provided a smaller pixel resolution of  $25.5 \mu\text{m}$  per pixel. We calibrated the cameras before each  
126 deployment, with an optical depth trained on the deepest layer of the gel where the particles  
127 accumulated. The Arduino Nano was programmed to duty cycle the R-Pi at 20 minute intervals.  
128 However due to uncertainties in the timer built into the ATMEGA328P micro controller, the actual  
129 time interval varied by deployments within a few minutes. Fortunately, we also integrated a  
130 highly accurate real time clock, which logged the time stamp for each image. Later versions of  
131 this system have replaced the Arduino-based micro controller for a more accurate and lower  
132 power option. Images were archived on a 64GB SD card within the R-Pi Zero. This configuration  
133 had sufficient power to take photos at 20 minute intervals for 8 days. The operational depth rating  
134 of the housing was 300m.



## 135 **Cruises and sampling platform**

136 Gelcams were deployed in the subarctic North Pacific (NP) and North Atlantic (NA) during two  
137 EXPORTS cruises. The former cruise conducted three deployments aboard the *R/V Roger Revelle*  
138 near Ocean Station Papa (50°N, 145°W) during three repeating sampling cycles identified as  
139 Deployment 1, 2, and 3, from August 15 to September 5 2018 (Table 1) (other NASA EXPORTS  
140 papers referred the deployment duration as “epoch”). The deployment plan is presented by Siegel  
141 et al. (2021) and sediment trap details are presented by Estapa et al. (2021). On a surface-tethered  
142 trap (STT) array, we installed the GelCam at three near-surface traps, corresponding to 95, 145,  
143 and 195 m in Deployment 1. Weather-induced damage required subsequent repairs, causing a 10  
144 m increase in depth during Deployment 2 and 3. The second EXPORTS campaign conducted  
145 measurements on the *R/V James Cook* on May 5-8, 2021 near the Porcupine Abyssal Plain  
146 Sustained Observatory site (49°N, 16°W). Durkin et al. (2021) described the preparation of the  
147 sediment trap tube and polyacrylamide gel in detail.

148 During EXPORTS NP, all GelCam cameras were functional, at all three depths across three  
149 deployment durations, obtaining 9 total records. GelCams deployed during Deployment 1 and 3  
150 collected more than 400 images each within more than six days of deployments. Gelcams during  
151 Deployment 2 recorded about 360 images. During EXPORTS NA, housing failures resulted in  
152 only one successful record collected at 125 m depth. This measurement recorded 169 images  
153 within four days. Additionally, the camera system infrequently encountered a compression issue  
154 while transmitting the data to the SD card, leading to anomalous images being saved. These  
155 corrupted image files could not be used for any image processing. A manual check identified 25  
156 images with quality issues out of 3,846.

157 **Table 1.** Summary of sampling locations. \*The North Atlantic deployment applied a hardware  
 158 upgrade of a larger FOV and eliminated the bungee mount bar.

Deployment	Cruise	Location	Dates	Depths	# of images
NP Deployment 1 STT 1, 2, 3	R/V Revelle	Subarctic North Pacific 50.1°N, 145.1°W	Aug. 15-21 2018 Deployment 1	95 m 145 m 195 m	445 410 443
NP Deployment 2 STT 1, 2, 3	R/V Revelle	Subarctic North Pacific 50.4°N, 145.1°W	Aug. 24-28 2018 Deployment 2	105 m 155 m 205 m	369 368 341
NP Deployment 3 STT 1, 2, 3	R/V Revelle	Subarctic North Pacific 50.6°N, 144.9°W	Aug. 31-Sep. 5 2018 Deployment 3	105 m 155 m 205 m	442 446 413
NA Deployment	R/V James Cook	Subarctic North Atlantic 48.9°N, 15.0°W	May 5-8 2021	125 m	169*

159

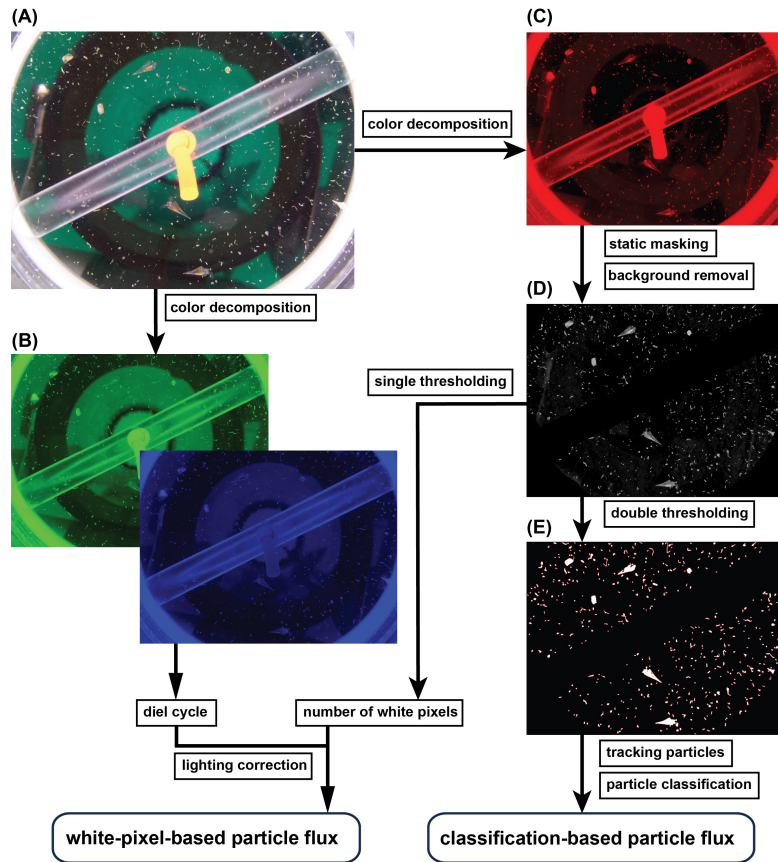
160 **Imaging processing procedures**

161 *Color decomposition to remove ambient light variations*

162 An image processing pipeline was developed for the GelCam images. Each raw image (see  
 163 example in Figure 2(A)) shows the LED near the outer edge of the gel cup (large white circle), the  
 164 bar holding the bungee (EXPORTS 2018 only), and darkened areas of the background where the  
 165 clear tube was mounted to the STT frame and near the top at the lid attachment. Here we created  
 166 a static mask to exclude the LED ring, the bungee mount bar, and other bright background objects  
 167 from the FOV. Areas of very bright pixels represented the LED ring near the edge and the orange  
 168 bungee in the middle. At the same time, less bright areas of the bungee mount bar were outlined  
 169 and then masked out by two fitted straight lines. During daytime, ambient sunlight caused portions  
 170 of the background to appear in a green-blue hue, even at depths up to 205 m. By decomposing the  
 171 images into the green, blue, and red channel (Figure 2(B) and (C)), we found that the red channel  
 172 was highly effective at filtering out the ambient light, since at the depth of the traps, all of the

173 ambient red wavelengths have been absorbed by seawater. We then converted the red channel  
174 image to a gray scale for region of interest (ROI) identification.

175 The temporal variations in the blue and green channel were very effective at illustrating the day-  
176 and-night cycle in ambient sunlight. Since we were interested in diurnal variation of particle flux,  
177 it was crucial that we account for any variations in particle detection likelihood or contouring that  
178 might arise due to the variations of background light. As particles occupied less than 7.5% of the  
179 field of view, the blue and green pixel intensity (averaged over each image) provided a reliable  
180 representation of the overall ambient light changes over time (Figure 3). The variations were very  
181 low during nighttime, while noticeable peaks were observable around mid-day. A strong  
182 correlation was found between the lighting variations within the green and blue channels and  
183 concurrent measurements of surface light levels obtained from a Photosynthetically Active  
184 Radiation (PAR) sensor deployed on a WireWalker (Suppl. Mat., Figure S3). The example  
185 sequence of a single particle over time shown in Figure 3 highlights the RGB color variations  
186 between night and day, with the background color turning green during the daylight hours. The  
187 day-night pattern was not evident in the normalized red channel, and so by using only the red  
188 channel for particle edge detection, we markedly reduced the sensitivity to background light  
189 variations. Selection of the red channel also had the effect of eliminating the color variation  
190 associated with the trap tube components that were far away from the illumination source, such  
191 as the tube bracket and top lip (see the darker concentric rings in Figure 2(A) compared to (C)).



192

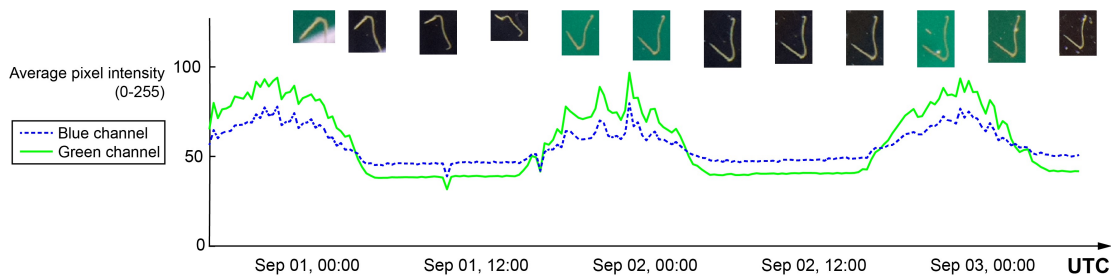
193 **Figure 2.** Pipeline of image pre-processing procedures, with (A) original captured image, (B)  
 194 decomposed images in green and blue channels, (C) red channel image, (D) masked grayscale  
 195 image after background removal, and (E) binarized image with particles outlined. The  
 196 semitransparent mount bar with the orange bungee did not exist during EXPORTS 2021.

197

### 198 ***Background removal***

199 The next step in image preparation was masking of the static parts of the image that contained  
 200 strongly contrasting tube parts close to the illumination source, such as the bungee bar and knot  
 201 (Figure 2(C)). Here we used a proper orthogonal decomposition (POD) method, which has been  
 202 proven effective in particle image velocimetry (PIV) (Mendez et al., 2017) and particle tracking

203 (Song and Rau, 2022). In our current configuration, we observed particle movements and  
204 rotations driven by local circulations and small-scale flows acting on the sediment trap. Such  
205 movements made the application of POD-based background removal possible. We implemented  
206 this by applying a static mask first to all images in the red channel, followed by a singular value  
207 decomposition. Subsequently, we eliminated the first principal component and reconstructed the  
208 image frames. Figure 2 (D) shows the resulting background-removed product of the masked red-  
209 channel image in (C). Using this method, particles were successfully preserved while the  
210 background was eliminated.



211  
212 **Figure 3.** Example day-and-night cycle represented by the average pixel intensities in green (solid  
213 line) and blue (dotted line) channels, combined with one example tracking series at a time interval  
214 of roughly 6 hours (16 frames) during Deployment 3 STT 1. See Figure S3 in supplement materials  
215 for correlations with PAR signals.

216

### 217 *Particle contouring*

218 Next we developed a double-threshold method to convert particles in the grayscale image to  
219 binary form. First, we computed the Otsu threshold (Otsu, 1979) for each image frame after the  
220 background removal. We then averaged these thresholds to establish a global black-and-white  
221 cutoff. The first round of binarization was mostly effective, but we found that using a global

222 threshold did not completely outline particles with looser structures, such as aggregates, which  
223 had segmented shapes. To remedy this, we set an additional but lower threshold by subtracting  
224 three standard deviations of the pixel intensities from the global threshold. The lower cut-off  
225 value retained relatively dimmer pixels, often bridging between aggregate segments, although it  
226 also included some background noise. Here we retained pixels that passed the lower threshold if  
227 they connected with pixels that had passed the first global threshold. Lastly, we applied an area  
228 filter with a 50 pixels minimum (*ESD* (equivalent spherical diameter)  $\sim 170 \mu\text{m}$ ), to exclude  
229 noise features and small particles that lacked sufficient morphological details for classification.  
230 We compiled a table assigning each particle a unique index and summarizing the images and  
231 morphology of particles in all image frames. Figure 2 (E) shows an example of the particles that  
232 passed our binarization method, outlined in red.

### 233 ***Particle tracking***

234 We implemented a particle tracking method to continuously monitor the particle motion within  
235 the gel and measure the particle flux. The surface tethered sediment trap experienced fairly strong  
236 vertical and rotational wave-driven motions during deployment. For the first two deployments in  
237 the North Pacific, the gel cup was freely able to rotate within the tube, and this appeared to  
238 enhance the rotational movements of the gel. Thus, the particles experienced horizontal motion  
239 relative to the camera throughout the deployments (See supplementary videos). The high  
240 viscosity of the gel helped to keep these motions fairly smooth, with strong auto-correlation  
241 between the particle motions that were fairly straightforward to track by eye and with our bulk  
242 motion routines. We first utilized particle image velocimetry (PIV) to capture the bulk movement  
243 between two consecutive image frames. We used the open-source PIVlab code (Thielicke and

244 Stamhuis, 2014) to implement a single PIV pass to detect the velocities associated with the bulk  
245 motion. A square evaluation window with a size of  $320 \times 320$  ( $x \times y$ ) pixels was selected based  
246 on the maximum distant particles seemed to travel within the 20 min image interval. This window  
247 size also ensured sufficient bright pixels for cross correlation in the first few frames. We set the  
248 window overlap at 50% in both  $x$  and  $y$  directions. Following this, a two-dimensional Gaussian  
249 regression model (Nobach and Honkanen, 2005) provided a sub-pixel estimation. Additionally,  
250 we applied a universal outlier detection after the single pass to eliminate inaccurate vectors  
251 (Westerweel and Scarano, 2005). The PIV analysis yielded a bulk velocity field of  $15 \times 11$  vectors.  
252 To approximate particle movement between frames, we applied a two-dimensional interpolation  
253 method to each particle's centroid position.

254 Next, we performed another cross-correlation method to precisely track each particle's movement  
255 between adjacent frames. Here we created two interrogation windows,  $w_1$  and  $w_2$ , from two  
256 successive frames. We defined the first interrogation window,  $w_1$ , as a rectangular box around a  
257 particle indexed  $i$  after the contouring. Each side of the window had a minimum distance of 8  
258 pixels from the particle's edge. The second interrogation window,  $w_2$  in the following frame, was  
259 centered on the guessed position, based on the particle's estimated centroid after interpolating the  
260 PIV vectors. We expanded this window size by 16 pixels for large particles (if the longest particle  
261 dimension was greater than 16 pixels), and by 8 pixels if the particle was small (less than 16  
262 pixels). We then conducted a cross correlation search within  $w_2$  to find the best match. The  
263 location that generated the best correlation, with a correlation coefficient  $> 0.7$  and a distinct peak  
264 in the correlation plane, was allocated as position  $i'$  in the next frame. If this location matched a  
265 particle  $j$  in the second frame ( $j = i'$ ), we linked the two particle indices  $i$  and  $j$  in our tracking  
266 series. Conversely, we considered particles without matches as new arrivals. Particle images in

267 Figure 3 illustrate one tracking example of a long fecal pellet that applied this algorithm. Despite  
268 challenges including translation, deformation, re-orientation, and background lighting variations,  
269 our method effectively identified the same particles over consecutive images. To verify, we  
270 randomly selected and manually examined 500 particle sequences (out of 43,286 total).

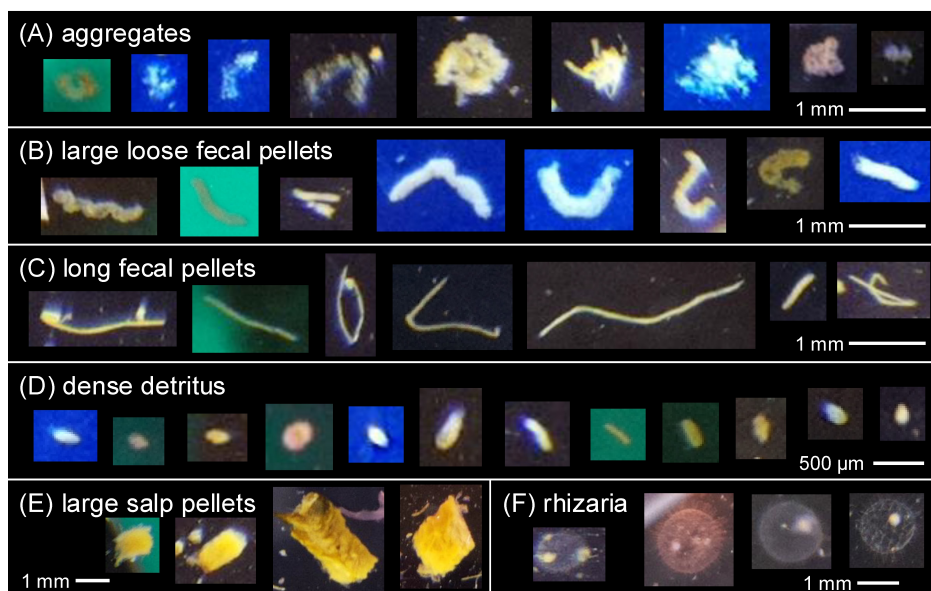
271 In some cases, two particles overlapped and crossed each other (28% of the total). The overlap  
272 issue became increasingly problematic in the later deployment stage as more particles appeared  
273 in the FOV. In these cases, we flagged cases where two or more particles, such as  $i_1$  and  $i_2$ ,  
274 corresponded with the same match  $j$  in the next frame. Moreover, we also ran our tracking  
275 algorithm in a reverse chronological order to detect “separation” events, the reverse of  
276 overlapping. In addition to 500 randomly chosen particle sequences, we manually examined 186  
277 overlapping cases. After applying the algorithm above, the tracking methods successfully  
278 detected and differentiated the overlapping particles in all but two cases. This result convinced us  
279 that our approach to distinguish overlapping particles was successful.

280 The final challenge we needed to resolve were cases where the particles moved past a masked  
281 feature such as the bungee bar. The bar, shown in Figure 2 (A), resulted in particles leaving and  
282 the re-entering the FOV over durations that were longer than the consecutive frame interval. Here,  
283 we extended the duration of predicted centroids over 12 hours using the PIV vectors. Unpaired  
284 particles were defined as those appearing in the last of the tracking series without any matches  
285 since then. We compared the distance between the centroid of a newly arrived particle and  
286 predicted locations of unpaired particles on their estimated trajectories, based on the new arrival  
287 time. If the distance was less than 32 pixels, this newly arrived particle would undergo the same  
288 cross correlation method with other unpaired particles. Based on the same criteria described above,



289 two particles were considered as a match if the correlation coefficient was high with a unique peak  
290 in the correlation plane.

291 Not all particles were traceable from their arrival through the entire deployment to the last image,  
292 and some, such as swimmers (organisms that actively entered the trap and were not part of the  
293 passive flux) were discounted from the particle flux counts. Some particles drifted off-frame, while  
294 others abruptly disappeared or had an intensity that faded below the threshold. To deal with this,  
295 we used a labeling system that indicated whether the particle disappeared at an edge, faded in FOV,  
296 or was manually identified as a swimmer. Of the 43,286 tracked particles, 10,169 were tracked  
297 from arrival to the final frame, 7,439 moved out of the FOV, 132 were categorized as large  
298 swimmers. The rest of the tracking sequences were labeled as particles faded in FOV, weak cross-  
299 correlations, and small areas. Additionally, some particles were found to enter the FOV from the  
300 sides, suggesting that they could be re-entering the image after arriving and leaving at an earlier  
301 time. Fourteen percent of the total particles were flagged as a potential re-entry. Detailed diagnoses  
302 with labels can be found in Table S1 in the supplemental materials.



303

304 **Figure 4.** Passively sinking particle classes with example sub-images, including (A) aggregates,  
305 (B) large loose fecal pellets, (C) long fecal pellets, (D) dense detritus, (E) large salp pellets, and  
306 (F) rhizaria.

307

### 308 **Particle classification**

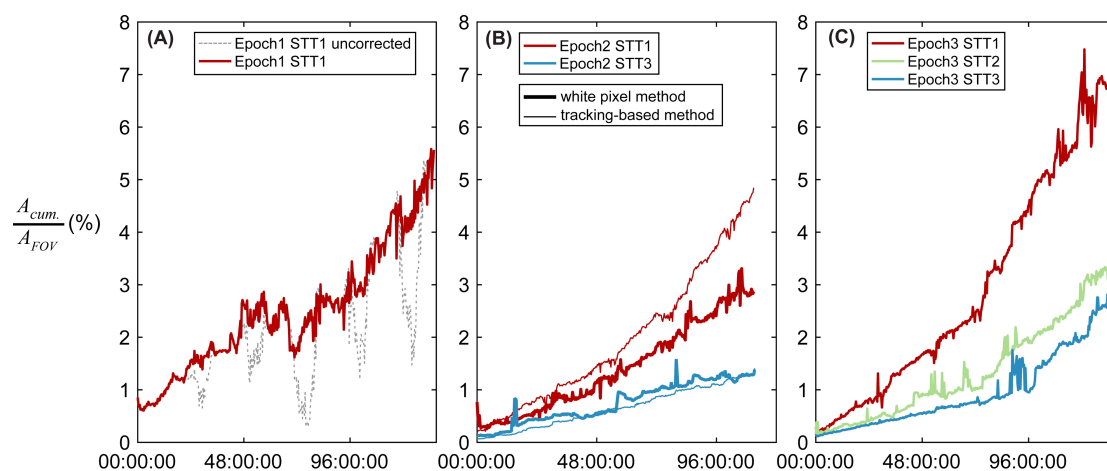
309 Following (Durkin et al., 2021), we classified each unique particle into one of six categories, as  
310 summarized in Figure 4. These particle categories were developed based on detailed microscope  
311 images of the same gels as those presented here. However, due to the image quality and color  
312 variations inherent in the GelCam prototype, the machine learning tools (Amaral and Durkin,  
313 2024) that were developed for the microscope images were not optimal for these ROIs. Because  
314 of the relatively small number of GelCam images involved, and quantity of classified images  
315 needed to train a supervised model, we decided to manually assign each particle an identity based  
316 on their unprocessed colored sub-images. Aggregates had irregular shapes and loose structures  
317 and could have been clusters of phytoplankton cells and other detritus. Large loose fecal pellets  
318 were produced by large zooplankton (e.g. pteropods) and were often elongated with dense coloring.  
319 Their shapes were more regular than aggregates and composed of one or a few distinct sub units.  
320 These might be partially degraded long fecal pellets as well. Long fecal pellets were thin and  
321 elongated with smooth edges. Due to the lower pixel resolution, we classified most small ellipsoid  
322 particles as dense detritus. Following the study that has been published on the presence of salps  
323 (Bruland and Silver, 1981), we categorized their pellets as large and golden-colored, with nearly  
324 rectangular shapes. We also detected a few rhizarians, primarily Phaeodaria. Large zooplankton  
325 were occasionally observed kicking and struggling in the gel layer, and were excluded as

326 swimmers. We differed from the classification suggested by Durkin et al. (2021), in that we did  
 327 not identify any phytoplankton cells or mini pellets, since these usually had a size of less than 50  
 328  $\mu\text{m}$  in ESD (Gowing and Silver, 1985), and were smaller than our detection limit.

329

## 330 ASSESSMENT

### 331 Modeling carbon fluxes



332

333 **Figure 5.** Cumulative particle areas as a function of time for selected samples in NP. Panel (A)  
 334 consists of the number of white pixels from binarized images (dotted gray line) and the lighting-  
 335 corrected cumulative areas (red solid line) during Deployment 1 STT 1. Panel (B) shows  
 336 cumulative particle areas using the white pixel method (thick lines) and using the tracking-based  
 337 method (thin lines) for Deployment 2 STT 1 & 3. Thin lines only focus on passively sinking  
 338 particles classified following Figure 4. Panel (C) uses the white pixel method and compares the  
 339 cumulative particle areas during Deployment 3. See Figure S5 for comparisons of all  
 340 measurements.

341

342 Tracking “new” particle arrivals using the timelapse method enables new insights into carbon  
343 flux variability, but it also raises new challenges. Because this is a new method, with imaging  
344 tools that are still undergoing optimization, we tried two different methods for establishing an  
345 image-based carbon flux proxy. The first - hereafter referred to as the “white pixel method” -  
346 summed up the area of all white pixels in the binarized image. The white-pixel-method aims to  
347 quickly obtain the bulk particle accumulated areas using a single black-and-white threshold. This  
348 method is sensitive to the day-night variations in ambient light. Figure 5 shows the time-varying  
349 percentage FOV coverage, calculated through cumulative particle area divided by the entire FOV.  
350 Brighter ambient light conditions tended to cause an apparent reduction in size, or result in some  
351 particles dropping below the detection limit, which resulted in a drop of white pixel area during  
352 daytime (see gray line, Figure 5(A)). A linear correction was developed that utilized the difference  
353 in light intensity relative to the nighttime blue channel levels. The percentage of pixels that were  
354 “quenched” by the brighter background were then computed based on the interpolated estimate  
355 and the captured particle areas. We correlated the excessive blue light intensity with the  
356 percentage attenuation, allowing for a corrected white pixel number at each daytime time stamp  
357 (see red line, Figure 5(A)). Detailed steps for this method can be found in the supplemental  
358 materials.

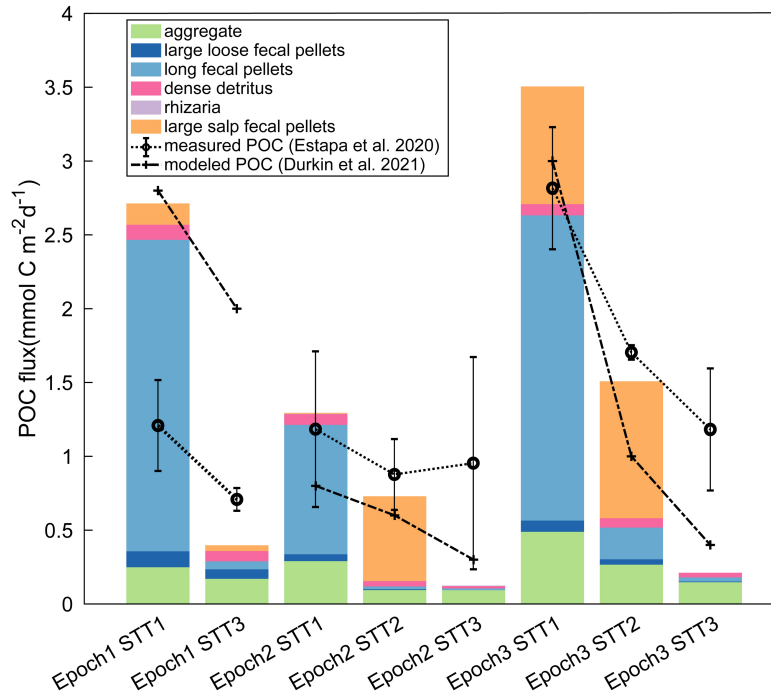
359 While the background lighting correction did appear to correct the daytime dips in cumulative  
360 area computed via the “white pixel” method, we still observed a sharp reduction in area about 70  
361 hours after deployment during Deployment 1 (Figure 5(A)). Review of the raw images revealed  
362 that this occurred because the trap lid bungee broke suddenly during a storm. It obscured part of  
363 the camera view, causing a flattening/decrease in the curve. Consequently, the corrected curve  
364 was composed of two nearly monotonic line segments. Similarly, deployments during

365 Deployment 2 and 3 showed monotonic accumulations (Figure5 B and C), despite some spikes  
366 caused by the arrival of large particles or swimmers. Overall, cumulative particle area flux  
367 decreased with depth (STT 1 was shallowest (95 - 105 m), STT 3 was deepest (195 - 205 m)).  
368 The second method, hereafter referred to as the “tracking-based method”, used a conservative  
369 version of the particle tracking and classification. We did not sum up all tracking series at their  
370 apparent time of arrival. Otherwise particles could be counted more than once due to the  
371 interruption in the tracking sequences, which was also a common issue applying the particle  
372 tracking. Rather, we implemented a frame-based calculation. Given a specific image frame with  
373 time stamp, we first found all identified particles that had been contoured. Next, we used tracking  
374 sequences to trace each particle to its state upon arrival. Using the size and shape upon arrival is  
375 intended to exclude any potential degradation or other morphological changes over time. Next,  
376 following the labeling system described above, we added all particles that left the FOV before  
377 this time stamp and subtracted particles that came into the FOV from the edges. This operation  
378 efficiently corrected the cumulative particle areas influenced by the gel movement. It is likely  
379 that this method could underestimate the particle size for those arriving during the day. However,  
380 the particle size of individual tracked particles did not show a distinct diurnal pattern.  
381 Although it requires more processing time, an advantage of the tracking-based method over the  
382 white pixel method is that it allows an estimate of the time of arrival of particular particle  
383 types/sizes. The particulate organic carbon of each new particle was determined following the  
384 equation  $C = A \cdot V^B$  listed in Durkin et al. (2021), where  $C$  is the carbon per particle ( $mg$ ),  $A$  is the  
385 scaling coefficient,  $V$  is the particle volume ( $\mu m^3$ ), and  $B$  is a unitless exponent. The exponent  $B$   
386 characterizes the porosity of a given type of particle as its size increases. Table S2 in supplemental  
387 materials lists shapes, volumes, and modeling parameters of each particle type. We followed

388 Durkin et al. (2021) closely to model the POC flux. It should be noted that dense detritus were  
389 poorly resolved in our images relative to the microscope images in that paper. This particle type  
390 could be a mixed group of aggregates and fecal pellets.

391 After excluding air bubbles and swimmers, we extracted particle areas from the tracking series  
392 and summed all six classified categories. Figure 5(B) compares the two methods of quantifying  
393 cumulative particle areas in Deployment 2, where the symbolized curves represent the  
394 classification-based integrated areas and the others are the white-pixel-based cumulative particle  
395 areas. The two methods showed great agreement with each other, but the approach based on  
396 tracking and classification generated more smooth responses. Random spikes were eliminated  
397 because the tracking series excluded air bubbles and swimmers. These symbolized curves did not  
398 show any valley, because we only extracted particle areas at the time of arrival. In other words,  
399 using the particle tracking automatically eliminated ambient light induced area variations.  
400 Moreover, this approach accounted for particles leaving and entering the unmasked area, which  
401 also explained the small deviation from using white pixels only. See Figure S5 for measurements  
402 of all stations using both methods.

403 **POC flux compositions and efficiency of carbon flux**



404  
 405 **Figure 6.** Modeled particulate organic carbon (POC) flux by each type during EXPORTS NP. POC  
 406 fluxes are also compared with measured data in formalin-poisoned trap tubes (Estapa et al., 2021)  
 407 and modeled ones based on microscopic images (Durkin et al., 2021).

408  
 409 The tracking based method was used to model the net POC flux for each GelCam deployment  
 410 categorized according to particle type (stacked bars, Figure 6). These results are compared to the  
 411 total carbon fluxes measured directly in the bulk flux tubes (open circles) and modeled POC  
 412 fluxes using microscope images and classification (crosses). The details of these carbon flux  
 413 methods can be found in (Estapa et al., 2021) and (Durkin et al., 2021) respectively. In general,  
 414 the GelCam-modeled POC flux had fairly good agreement with the other methods in the  
 415 shallower traps (STT 1 at 95m and STT 2 at 155m), and tended to underestimate the flux relative  
 416 to the other measurements at STT 3 (at 205m). We suspect that a primary reason for this

417 discrepancy at depth is that the GelCam could not resolve small particles. Particles with an  
418 equivalent diameter smaller than 100  $\mu\text{m}$  could contribute up to 46% to the total POC at STT 3  
419 during the North Pacific EXPORTS cruise (Durkin et al., 2021), whereas the detection limit was  
420 170  $\mu\text{m}$ . Additionally, visual sensors have a difficult time detecting nearly transparent or  
421 disaggregating materials as particles became further degraded with depth.

422 The particle-classified GelCam derived fluxes were primarily composed of aggregates, large loose  
423 fecal pellets, long fecal pellets, and dense detritus during EXPORTS-NP. Long fecal pellets were  
424 dominant at STT 1, contributing 67% to the total POC fluxes. Aggregates, large loose fecal pellets,  
425 and dense detritus were less abundant but still contributed a significant part comparing to rhazaria.  
426 At STT 3, aggregates were the most abundant, and contributed up to 70% of the total carbon. Salp  
427 pellets were observed as one of the dominant particle types in the microscope analysis of the gels  
428 (Durkin et al., 2021; Steinberg et al., 2023). Similarly, we found that large salp pellets dominated  
429 the GelCam-based POC calculations for some deployments. For example, two huge salp pellets  
430 constituted 79% of the total carbon during Deployment 2 STT 2.

431 Transfer efficiency was highly variable across particle types. Here we defined  $T_{100}$  as transfer  
432 efficiency, which is the ratio of carbon flux at 100 m below the reference depth (defined as the  
433 depth of STT 1) and carbon flux at the reference depth (i.e.,  $POC_{STT3}/POC_{STT1}$ ). The transfer  
434 efficiency in the NP were 14%, 9.6%, and 6.0%, respectively. The percentage was lower than the  
435 sediment trap measurements (Estapa et al., 2021) due to the low modeled POC fluxes at STT 3.  
436 Missing small particles could be one main reason. Long fecal pellets and large loose fecal pellets  
437 had very high attenuation ( $> 97.0\%$ ), showing a good agreement with the microscope analysis  
438 (Durkin et al., 2021). During Deployment 2 STT 3, we did not see any large loose fecal pellets and  
439 only 1.3% carbon of long fecal pellets remained compared to STT 1. Aggregates had the highest



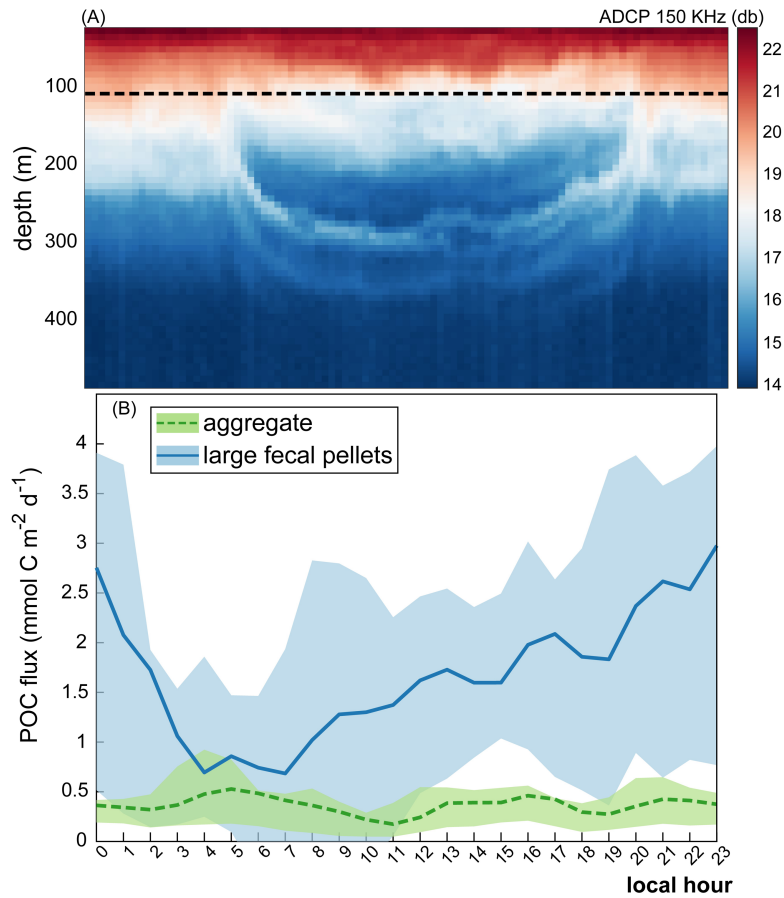
440 transfer efficiencies, which varied from 30% to 68%. One likely reason would be the conversion  
441 from other particle types and aggregation. We did not calculate the transfer efficiency of large salp  
442 fecal pellets due to the low sample number.

443

#### 444 **Episodic fluxes of fecal pellets**

445 In contrast to traps that use only a final accumulation to estimate a time-integrated carbon flux, the  
446 GelCam provides a unique opportunity to visually examine the time series of particle fluxes during  
447 the trap deployment. One of the more striking patterns that emerged from the particle arrival and  
448 tracking analysis was the observation of diurnal fluctuations in the arrivals of fecal pellets across  
449 deployments and stations in the shallowest trap. Particle fluxes of aggregates (Figure 7(B), green  
450 line) and fecal pellets (Figure 7(B), blue line) at STT 1 were binned into two-hour time intervals  
451 and averaged into local solar time bins (operational time zone). A total of 13 days across three  
452 deployments were used for averaging the hourly fluxes. The fecal pellet fluxes included large loose  
453 and long fecal pellets only. We did not include the dense detritus, rhizaria or salp fecal pellets in  
454 the analysis. It should be noted that we also disregarded the fluxes after the mount bungee broke  
455 during Deployment 1 but kept the data before the interruption. The average fecal pellet flux  
456 reached a maximum of up to  $3 \text{ mmol C m}^{-2} \text{ d}^{-1}$  occurring around 11 PM to 12 AM. After the daily  
457 maximum, the carbon fluxes from fecal pellets reduced quickly and reached less than  $1 \text{ mmol C}$   
458  $\text{m}^{-2} \text{ d}^{-1}$ . In contrast, the aggregate fluxes did not show any distinct diurnal patterns. Because of the  
459 very strong attenuation with depth of the fecal pellet classes, we did not have enough samples to  
460 implement a statistical analysis in STT 2 and STT 3. We also observed diurnal changes in the flux

461 of fecal pellets in the North Atlantic dataset (STT 1 at 125 m; see Figure S6). The GelCam observed  
462 the most fecal pellets during the second night and a consistent flux of aggregates over three days.  
463



464  
465 **Figure 7.** (A) acoustic backscattering strength from the 150 KHz ADCP with a depth range of 25  
466 m to 500 m, shown in decibel (db). The dashed black line represents the deployment depth of  
467 GelCam at 105 m. (B) Hourly fluxes of fecal pellets in blue and aggregates in green at STT 1  
468 during EXPORTS-NP. POC flux was averaged within a two-hour time interval. Mean values are  
469 displayed as center lines. The upper and lower boundaries of the patches represent 75<sup>th</sup> and 25<sup>th</sup>  
470 percentile of the samples.

471

472 The flux increase in fecal pellets occurring at night likely resulted from diurnal migration of  
473 zooplankton that swam to depths shallower than the trap in the upper ocean to feed at night (Cyr  
474 and Pace, 1992; Haney, 1988; Mackas and Bohrer, 1976). In Figure 7(A) the acoustic  
475 backscattering signal analyzed from the 150 KHz acoustic Doppler current profiler (ADCP)  
476 indicated higher near-surface migrator activity during the nighttime compared to day (see the  
477 anomaly of acoustic backscatter in Figure 1 from Maas et al. (2021)). Another interesting finding  
478 is that the time varying flux of fecal pellets in Figure 7 shows a skewed trend between the fecal  
479 pellet flux increase and decrease. The data suggests a sharp drop-off after midnight, with a  
480 minimum at around 6 a.m. (local) and a steady slow increase over the day.

481

#### 482 **Flux events across particle types**

483 Flux events, sometimes called “pulse” events, have high rates of particle flux within a short time  
484 frame. To better model the biological carbon pump, it is critical to quantify these flux events  
485 (Smith et al., 2018). Bauerfind et al. (1994) observed a sharp increase of fecal pellet flux after the  
486 maximum diatom sedimentation. However, this and other studies (e.g., (Bauerfeind et al., 1994)  
487 and (Cao et al., 2024)) have conducted observations of flux events over a long time scale (from  
488 weeks to months). The use of GelCams enables measurements of flux events over timescales  
489 from hours to days. Among the GelCam deployments described in this manuscript, Deployment  
490 3 from the North Pacific campaign (Aug. 31 - Sep. 5, 2018) provided the most complete and best  
491 quality record at all three depths. Here we examine the flux patterns of discrete particle types  
492 from the NP Deployment 3 as a function of time and depth (Figure 8). In order to highlight the  
493 temporal trends over multiple days and de-emphasize the day-and-night variation, we smoothed

494 the flux time series using a moving average of 73 frames, equivalent to approximately 24 hours.  
495 We note that while this approach helped to highlight multi-day trends, it reduced the pattern of  
496 diel variations highlighted in Figure 7.

497 The flux of aggregates showed a decrease over depth consistent with the traditional Martin Curve  
498 pattern due to particle remineralization (Figure 8(A)). On average, the flux was 52% ( $\pm 14\%$  at  
499 STT 2), and 27% ( $\pm 6\%$  at STT3) of the flux at STT 1 (105 m). These ratios stayed fairly constant,  
500 even as the flux roughly tripled at all depths, varying from a minimum of 0.14 up to 1.0 mmol C  
501  $\text{m}^{-2}\text{d}^{-1}$  at STT 1 over the six day deployment. The time series of the deeper fluxes was thus  
502 correlated with the shallowest trap STT1 ( $R^2 = 0.92$  ( $p < 0.05$ ) and  $R^2 = 0.84$  ( $p < 0.05$ ) at STT 2  
503 and STT 3, respectively).

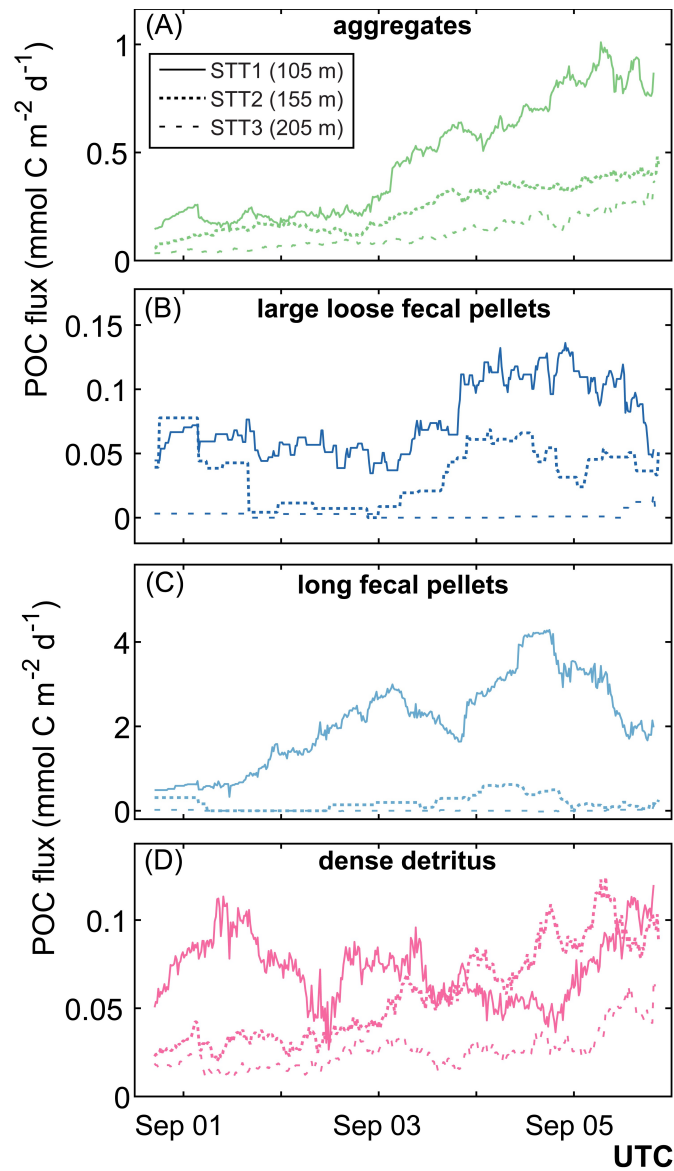
504 The flux of large loose and long fecal pellets also showed an increase between the early and later  
505 stages of the deployment Figure 8(B) and (C)). The depth attenuation of large loose pellets was  
506 on average 46% between STT 2 and STT 1, and nearly 100% by STT 3 with only 8 unique  
507 particles of this type observed at 205 m. The temporal variations of large loose pellets were  
508 correlated between STT 1 and STT 2 ( $R^2 = 0.31$ ,  $p < 0.05$ ) and uncorrelated ( $R^2 = 0.03$ ,  $p < 0.05$ )  
509 by STT3. The correlation between the shallower traps, which both showed a distinctive increase  
510 on Sep. 3 and peak on Sep. 4, lent confidence that we were resolving real short-term variations  
511 in flux that could be tracked across depth (compare the solid and short-dashed lines in Figure  
512 8(B)).

513 The temporal variability in long fecal pellets at STT1 showed a similar pattern, with a gradual  
514 increase from 0.33 mmol C  $\text{m}^{-2}\text{d}^{-1}$ , and a peak of 4.3 mmol C  $\text{m}^{-2}\text{d}^{-1}$  around 6 p.m. on Sep. 4  
515 (Figure 8(C)). We observed a very strong depth attenuation, with a mean loss of 9.4% between  
516 STT 1 and STT 2, and 0.20% by STT 3. The time series of POC fluxes between STT 1 and STT

517 2 were weakly correlated with  $R^2 = 0.26$  ( $p < 0.05$ ), while the higher attenuation at STT 3 caused  
518 a lower correlation of  $R^2 = 0.13$  ( $p < 0.05$ ). Interestingly, we also saw a correlation between long  
519 fecal pellets and large loose pellets with  $R^2$  of 0.39 ( $p < 0.05$ ) at STT 1. It is plausible that there  
520 was cross-over with the labeling of these two categories and they were derived from the same  
521 origins, or that the distinct groups of animals that created the pellets were correlated with  
522 fluctuations of abundance, and thus pellet production rates.

523 The dense detritus category was perhaps the most confounding in terms of temporal variations  
524 (Figure 8(D)). POC flux was overall fairly low, with a range between 0.03 and 0.12 mmol C  
525  $m^{-2}d^{-1}$  at STT1. This was the only category where we observed a sustained period where the flux  
526 at STT2 exceeded that of STT1. With the data we have, it is only possible to speculate about the  
527 reason(s) for this reversal. For example, it could have been due to disaggregation of large particles  
528 into the dense detritus category between STT1 and STT2. Alternatively, there may have been a  
529 source of dense pellets located between the two traps, horizontal advection of patches, or a  
530 temporal lag that could have contributed to temporary inversions in the expected flux attenuation  
531 profile. By STT3, we observed that dense detritus were attenuated by 35% compared to the  
532 shallowest depth.

533 Additionally, we noticed a strong correlation between aggregates at STT 1 and dense detritus at  
534 greater depths ( $R^2 = 0.91$  ( $p < 0.05$ ) and  $R^2 = 0.50$  ( $p < 0.05$ ), respectively for STT2 and STT3).  
535 One likely reason was that the category of dense detritus were actually small aggregates or  
536 fragments. We only observed 6 rhizaria and 36 salp pellets across three depths during the six day  
537 deployment in Deployment 3. Thus correlations based on these categories were less suggestive.  
538 Tables S3 and S4 in supplemental materials show a full list of coefficients of determination  
539 between depths or particle types.



540

541 **Figure 8.** Time variations of POC fluxes across particle types during NP Deployment 3. Fluxes

542 at STT 1, 2, and 3 are represented by solid, dotted, and dashed lines, respectively.

543

## 544 **DISCUSSION**

545 GelCams provide a new tool for observing short time variations of particle fluxes occurring

546 within the duration of a typical surface tethered trap deployment. GelCams, when used alongside

547 other flux measuring instruments, can be used to estimate carbon flux, and resolve episodic events  
548 and diel variations among different particle types. The modeled POC fluxes from the GelCams  
549 generally agreed with ground-truth measurements (Estapa et al., 2021; Durkin et al., 2021;  
550 Buesseler et al., 2020), although the current design cannot resolve small particles like the mini  
551 pellets described in other studies (Durkin et al., 2021). The simple, low cost, and open source  
552 design suggests that GelCams could be reproduced and attached to other platforms such as  
553 neutrally buoyant sediment traps.

554 Sediment traps equipped with a GelCam offer several other advantages. By taking a continuous  
555 series of photographs of the trap contents, we can observe if and when swimmers arrive, and any  
556 disruptions or changes that occur to the particles during the time spent in the trap before recovery.  
557 By tracking each individual particle over time, we can qualitatively observe if the particles are  
558 undergoing morphological changes, or if the gel is effective at preserving their size and other  
559 characteristics.

560 We tried two different methods to model the time-resolved particle flux from the image sequences.  
561 The white pixel method and the tracking-based methods agreed with each other in terms of the  
562 cumulative particle areas. We can choose between the two depending on the processing time and  
563 required data products. The white pixel method yielded a quick method to estimate the bulk  
564 particle flux, but was affected by background ambient light variations between day and night. We  
565 proposed a simple method to remove this effect using the red channel of the RGB image, which  
566 yielded a time series with a more monotonic increase in cumulative particle area. We can simply  
567 extract the bulk flux information and time varying fluxes in particle areas, although the carbon  
568 flux will require a further calibration incorporating particle transparency properties. This method  
569 is similar to the attenuation-flux approach employed by Estapa et al. (2024) using optical sediment

570 traps, except the attenuation flux approach sums the attenuation values of each pixel, while the  
571 “white pixel method” here used the binarized images so each pixel had a value of unity (i.e., the  
572 method does not account for light intensities and transparency of particles). While more  
573 computationally- and labor-intensive, the particle tracking method with classification was less  
574 affected by the day-night ambient light variation, and provided information about the specific  
575 time of arrival of individual particles. These classifications allowed us to examine the relative  
576 importance of each particle type in the bulk flux, and short-term patterns (such as diel variation)  
577 and detailed temporal correlations between particle types and depths. The GelCam framework  
578 thus generates a large data set that allows for both individual case studies and statistical analysis.  
579 Recovery and subsequent analysis of the gel samples under an imaging microscope and via  
580 extraction for molecular or mineral analysis can reveal even more biological details and help with  
581 ground truthing (see (Durkin et al., 2021)).

582 The GelCam image series emphasizes the important role of fecal pellets in carbon flux during the  
583 EXPORTS North Pacific campaign. In agreement with previously published results (Durkin et  
584 al., 2021), long fecal pellets were the dominant particle type in the traps near the surface. The  
585 long fecal pellets and large loose fecal pellets showed rapid flux attenuation, with roughly 91%  
586 of the POC flux removed between the top two traps. Aggregates and dense detritus had higher  
587 transfer efficiencies than fecal pellets, where more complex physical and biochemical processes  
588 could significantly influence the results. Microbial degradation potentially played a more  
589 important role (Stephens et al., 2024), as small aggregates or detritus could be fragments of  
590 degraded fecal pellets. Additionally, the particle type of dense detritus could include aggregates  
591 or fecal pellets in small size. Distinguishing between them was a challenge in the GelCam images,  
592 which had a resolution approximately four times lower than the 115× microscope images



593 published in Durkin et al. (2021). Functionally, these particle types may represent particles in a  
594 more advanced stage of microbial degradation compared to the other pellet categories. The  
595 particles that were categorized as aggregates were possibly highly degraded detritus with a loose  
596 structure. It also seems plausible that the aggregates and dense detritus in the deeper traps  
597 originated as fecal pellets at shallow depths. Moreover, physical processes including aggregation  
598 (Burd and Jackson, 2009; Kiørboe, 2001; Alldredge and Silver, 1988) and disaggregation (Briggs  
599 et al., 2020; Song et al., 2023) which can result in morphological changes as the particle sink over  
600 time, likely contributed to the uncertainty in the transfer efficiency of any particular particle class.  
601 Compared to traditional sediment trap instruments, GelCam provided unique insights into the  
602 variability of carbon flux during the trap deployments, providing a time-series with 15 minute  
603 resolution. With this record, we were able to observe diel variations in fecal pellet fluxes in the  
604 shallowest traps in both the North Pacific and North Atlantic. The diel flux peaked at midnight,  
605 likely due to the migration of zooplankton and fish to graze above the trap depth. This finding  
606 was consistent with other diel vertical migration evidence from nets and bio-acoustics (Maas et  
607 al., 2021), showing the swarming of plankton species to the surface ocean at night. A comparison  
608 using aggregates did not show any distinct diurnal changes. Analysis of 14 cumulative days of  
609 fecal pellet flux also revealed a temporal asymmetry, with a rapid three-fold drop in pellet flux  
610 between midnight and 6 a.m. (local) and then a gradual increase over the rest of the day. This  
611 asymmetrical pattern could be attributable to feeding or defecation behaviors, and further studies  
612 are required to elucidate the drivers and if similar patterns are observed in other locations.

613 The temporal correlations we presented in Tables S3 and S4 between trap depths are without any  
614 lag. These analyses thus assume that the processes that dictate these short timescale changes in  
615 flux are occurring simultaneously across depth. In practice however, if particles are sourced from

616 above the shallowest trap, and take some time to sink past and into deeper traps, we might expect  
617 a time lag reflecting the vertical distance between the traps divided by the sinking speed. Previous  
618 studies using particle abundance proxies such as optical backscattering (Dall’Olmo and Mork,  
619 2014; Briggs et al., 2011) and laser optical plankton counters (Petrik et al., 2013; Jackson et al.,  
620 2015) have shown a descending front of particles reaching successively deeper depths over time.  
621 We expected to potentially see similar results in the timing of flux events with the GelCam.  
622 Overall however, these records did not reveal obvious time lags, and so the correlations were  
623 analyzed with zero lag.

624 An exception to this may be qualitatively evident in the GelCam timeseries from STT 1 and 3  
625 from Deployment 3 EXPORTS-NP (see Figure 5(C) and Figure 8(A)). In Figure 5(C), we  
626 observed that the total cumulative particle areas all had a noticeable slope shift, with an increase  
627 at STT 1 (105 m) around 75 hours after the deployment while the slope of STT 3 (205 m) did not  
628 increase until 96 hours. This shift in slope provided a bulk sinking speed of roughly 100 m/day.  
629 The particle classifications suggest that the increase was partially driven by aggregates (Figure  
630 8(A)). Dividing the vertical distance between STT 1 and 3, by the time lag, we achieve a roughly  
631 estimated sinking speed of 50 m/day for aggregates. The estimated sinking speeds of bulk  
632 particles or aggregates fall within the range of another study on the same cruise (Romanelli et al.,  
633 2024) and previous studies (McDonnell and Buesseler, 2010; Fowler and Small, 1972; Trull et  
634 al., 2008; Ploug et al., 2008). The absence of this pattern for other particle types could be  
635 indicative of faster sinking speeds, though our data was not sufficient to make a conclusive  
636 assessment of this hypothesis. We anticipate that future GelCam studies will allow more robust  
637 observations of these dynamics.

638 The GelCam system is a promising tool for providing high temporal resolution flux, but it also  
639 has a number of limitations that could be addressed with future technical improvements. A  
640 primary challenge was the low pixel resolution and variability in illumination, which can be  
641 improved in the future. In this study, we could not determine cumulative mini fecal pellets, as  
642 their size was smaller than one pixel. Additionally, particle classification had high uncertainty for  
643 small particles that just passed the detection threshold. For example, long fecal pellets, or fibers  
644 could be misidentified due to inadequate details. Durkin et al. (2021) noted aggregates as one  
645 dominant population in the Subarctic North Pacific, but many aggregates were lost during the  
646 identification process because of their loose structures and transparent exopolymer particles (TEP)  
647 (Passow, 2002; Azetsu-Scott and Passow, 2004). Inevitably, we could not account for the  
648 contributions of organic carbon from TEP.

649 Salp fecal pellets were found to be abundant during EXPORTS-NP (Durkin et al., 2021;  
650 Stamieszkin et al., 2021), but we could only resolve ones with large sizes due to over-saturated  
651 colors in small particles. We observed very few large salp pellets but these large particles could  
652 substantially affect the modeling of POC fluxes (e.g., Deployment 2 STT 2). Since we only  
653 deployed one GelCam at each depth, with a sampling area of  $< 30\text{cm}^2$ , the limitation could cause  
654 high variability of rhizaria and large salp pellets, collected from different sediment trap tubes.  
655 These rare occurrences compared to large numbers of other particles suggests that the deployment  
656 of larger numbers of GelCams and other particle-intercepting sediment traps could improve  
657 collection statistics. Fortunately the low cost of GelCam could make it possible in the future.  
658 Additionally, smaller salp pellets were usually classified into aggregates or detritus. We could  
659 minimize this uncertainty by resolving particle color in the next development iteration.

660 We only resolved 2D images of particles from the single-camera imaging system. While we could  
661 predict thickness of particles that retained regular shapes, such as long fecal pellets, particles prone  
662 to flattening within the gel would have more biased thickness predictions. These particles, such as  
663 especially delicate aggregates, tend to flatten and extend their projection areas when resting on a  
664 platform, thus leading to the overestimation of thickness based on assumptions of spherical shapes.  
665 In addition to uncalibrated 3D morphological information, 2D images resulted in the overlap of  
666 sub-images. For future improvements, capturing an additional view for 3D volume reconstruction,  
667 or establishing an empirical relationship for aggregate thickness, would be advantageous.

668 Hardware upgrades will enhance the GelCam design in the future. For instance, in EXPORTS-  
669 NA, we encountered images with a blue hue, but new GelCam models will send fixed parameters  
670 to the Pi camera to ensure calibrated white balance. Upgrading to more advanced camera modules  
671 with attached lenses will also generate a higher pixel resolution. Although it is still challenging  
672 to detect mini fecal pellets ( $< 50 \mu m$ ), these improvements can reduce the detection limit of the  
673 imaging system. We are also exploring the integration of GelCam with Lagrangian floats. As  
674 Siegel and Deuser (1997) demonstrated, horizontal bulk flow is normally orders of magnitude  
675 greater than the sinking velocity, so replacing the surface tethered traps (STT) with neutrally  
676 buoyant sediment traps (NBST) will lead to a more precise flux measurement within the water  
677 column. Furthermore, incorporating a swimmer exclusion device (e.g. the “labyrinth of doom”  
678 (Coale, 1990)) could still be favorable. Although we could exclude them during the post  
679 processing, the swimming zooplankton brought more uncertainty in PIV analysis and they may  
680 potentially interact with other particles.

681 The white pixel method can be further developed by building an empirical relation to the POC  
682 fluxes. This idea is not new, as Bishop et al. (2016) estimated the carbon fluxes by empirically

683 relating thickness of particles to their projection areas and converting the particle volume into the  
684 carbon content. Similarly, Estapa et al. (2024) empirically correlated the light attenuation of  
685 particles with the POC fluxes. We did not apply any of these empirical relations, because particles  
686 were illuminated from the side (not behind as required for an attenuation estimate), particle areas  
687 were corrected based on lighting, and particles preserved in the gel layer did not become fully  
688 flattened due to gravity. Building a reliable relation requires an extensive datasets collected by  
689 GelCam and other similar instruments featuring upward facing cameras and side lighting (i.e.,  
690 quasi-darkfield illumination). Alternatively, we could utilize the other tracking-based method for  
691 more accurate POC flux estimation than an empirical relation. The tracking-based and white-  
692 pixel-based methods are interchangeable depending on the specific purpose.

693 Implementing an automated particle classification process will be beneficial, to reduce the labor  
694 of processing sub-images manually. Leveraging machine learning methods will provide  
695 significant insights into the model of carbon export(Irisson et al., 2022; Trudnowska et al., 2021;  
696 Amaral and Durkin, 2024). For example, Davies et al. (2017) applied a deep convolutional neural  
697 network (CNN) to classify particles, including larvae, copepod, and oil droplets, in an in situ  
698 imaging system. However, many classifiers are still constrained by the limited size of available  
699 particle image datasets (Irisson et al., 2022). We believe machine learning methods and GelCam  
700 will have a mutually beneficial relationship in the future. On one side, machine learning will  
701 enhance the particle identification capabilities of the GelCam. More importantly, as a low-cost  
702 but reliable instrument, the GelCam records a wide range of particle types, which will generate a  
703 larger database of particle images and improve the accuracy of machine learning classifiers.

704 Future work can expand the GelCam in more studies of the biological carbon pump. The  
705 polyacrylamide gel layer maintains particle structure during sample collection, therefore we can

706 incorporate more GelCams in various directions to capture the 3D shape. By removing the gel  
707 layer from the sediment trap, we can track marine particles without interfering with natural  
708 biological processes. This adjustment would enable visual quantification of microbial  
709 degradation rates acting on fecal pellets and aggregates. In other words, this modified instrument  
710 will potentially allow observations of time varying morphological changes or even fragmentation.  
711 Additionally, continuing to deploy GelCams with traditional sediment traps at different locations,  
712 seasons, and depths will not only yield estimates of a bulk particle sinking velocity but also  
713 provide more insights into the varying settling behaviors across different particle types.

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722

## 723 **CONFLICT OF INTEREST**

724 None declared.

725

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931 ***Data Availability Statement***

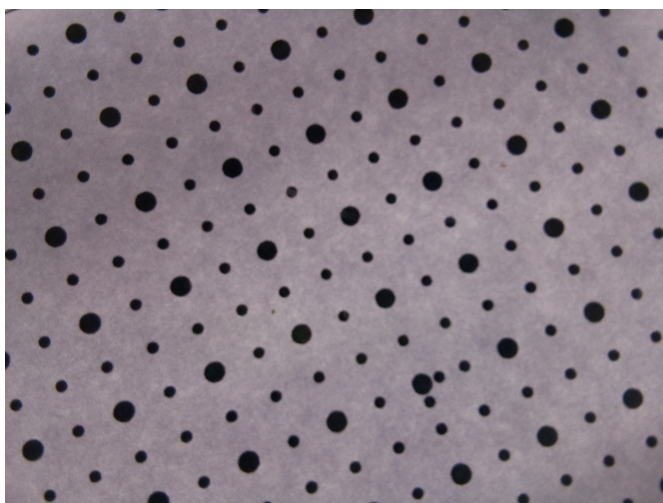
932 The data that support the findings of this study are openly available in in NASA's SeaBASS  
933 archive at [https://oceandata.sci.gsfc.nasa.gov/ob/getfile/dd2fe323be\\_EXPORTS-](https://oceandata.sci.gsfc.nasa.gov/ob/getfile/dd2fe323be_EXPORTS-EXPORTSNP_RR1813_GelCam_20180814-20180909_R1.sb)  
934 [EXPORTSNP\\_RR1813\\_GelCam\\_20180814-20180909\\_R1.sb](https://oceandata.sci.gsfc.nasa.gov/ob/getfile/dd2fe323be_EXPORTS-EXPORTSNP_RR1813_GelCam_20180814-20180909_R1.sb) and  
935 [https://oceandata.sci.gsfc.nasa.gov/ob/getfile/8a0152ccab\\_EXPORTS-](https://oceandata.sci.gsfc.nasa.gov/ob/getfile/8a0152ccab_EXPORTS-EXPORTSNA_JC214_GelCam_20210504-20210509_R1.sb)  
936 [EXPORTSNA\\_JC214\\_GelCam\\_20210504-20210509\\_R1.sb](https://oceandata.sci.gsfc.nasa.gov/ob/getfile/8a0152ccab_EXPORTS-EXPORTSNA_JC214_GelCam_20210504-20210509_R1.sb).



## SUPPLEMENTAL MATERIALS

### Camera calibration

As the camera viewed through layers of acrylics and polyacrylamide gel, we printed a calibration target as shown in Figure S1 in order to measure the magnification ratio and reconstruct undistorted images. The calibration plate consisted of a grid of black dots used for establishing a mapping between the image and the real coordinates. Larger dots had a diameter of 2 mm with a spacing of 10 mm, while the diameter of smaller dots was 1 mm. During the calibration, the target was placed onto the top surface of the GelCam housing, which was also the bottom of the gel layer. Making sure the target was covering the entire field of view, we then captured the image of the calibration target. With a simple binarization, we computed centers of all black dots and mapped their locations with real coordinates. The distortion effects were corrected by fitting a polynomial function. As a result, we could estimate the pixel size in any location of captured images. The average magnification was  $21.3 \mu\text{m}$  per pixel during EXPORTS 2018 and  $25.3 \mu\text{m}$  per pixel during EXPORTS 2021.



**Figure S1.** Calibration image used for calculating magnification and correcting distortion during EXPORTS 2021.

### **Summary of tracking sequences and labeling system**

Table S1 presents the number of binarized particles and tracking results from ten deployments. The shallowest depth, STT 1, recorded the most particles, while measurements in STT 2 observed half as many particles as STT 1. With a time interval of approximately 20 minutes, our tracking algorithm successfully monitored particles for more than ten hours (~30 frames) on average. Excluding fast-moving zooplankton, air bubbles, and other misidentified particles, the tracking duration could be further improved for particles categorized in Figure 4.

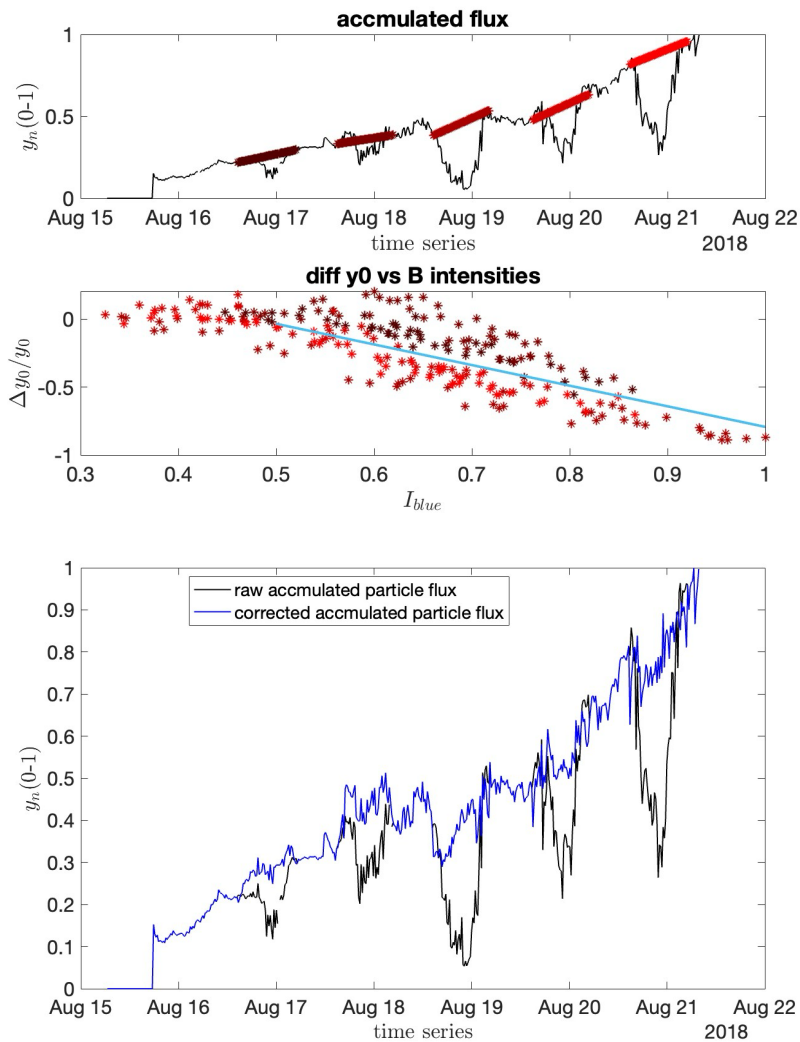
We employed a numerical labeling system to categorize various situations when the particle tracking was ceased. Specifically, the notation “-1” denoted instances when particles were leaving the FOV. Whenever no particle was successfully matched, and the best correlation coefficient was smaller than 0.5, we identified these cases using the label “-2”. The label “-3” was assigned when we could not find a matched particle, particularly when the particle’s area in the preceding frame was less than 100 pixels ( $ESD \sim 240\mu m$ ). Another distinct scenario arose when we failed to track a particle due to its insufficient average gray scale intensity lower than the global Otsu’s threshold. In such cases, we marked the particle with the label “-4”. Occasionally, we encountered moving zooplankton during our analysis, leading to the assignment of a “-5” flag. The notation “-6” represented all other situations where the tracking process was lost or unsuccessful. Lastly, we defined a “-7” where a particle was entering the FOV from the outside. The label “-7” was generated in a same way as “-1”, when we ran the tracking algorithm in a reverse chronological

order. These designated labels enabled a systematic characterization of the diverse outcomes encountered throughout our investigation.

**Table S1.** Summary of particle tracking. Binarized particles represent particles contoured and preprocessed for tracking. Tracking particles include all particle categories with zooplankton and air bubbles, before performing a classification. Average tracking frames correspond to the mean duration of tracking. Each frame represents a time interval of 20 minutes approximately.

Deployments	Contoured particles	Tracking sequences	-1 leaving FOV	-2 weak correlation	-3 small areas	-4 dim particles	-5 large swimmer	-6 others	-7 entering FOV
NP Epoch1	STT1	13,786	3,244	3,827	1,731	937	90	1,926	2,180
	STT2	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	STT3	10,193	1,166	3,062	2,025	235	17	1,687	1,180
NP Epoch2	STT1	3,525	681	456	634	302	14	318	469
	STT2	1,580	240	348	454	62	2	137	207
	STT3	1,404	234	366	347	87	3	152	174
NP Epoch3	STT1	5,423	845	402	862	250	0	30	704
	STT2	3,328	758	571	673	93	4	296	583
	STT3	2,810	108	65	137	15	0	36	396
NA	32,068	1,184	163	89	300	169	2	58	100

## Lighting correction of the white pixel method



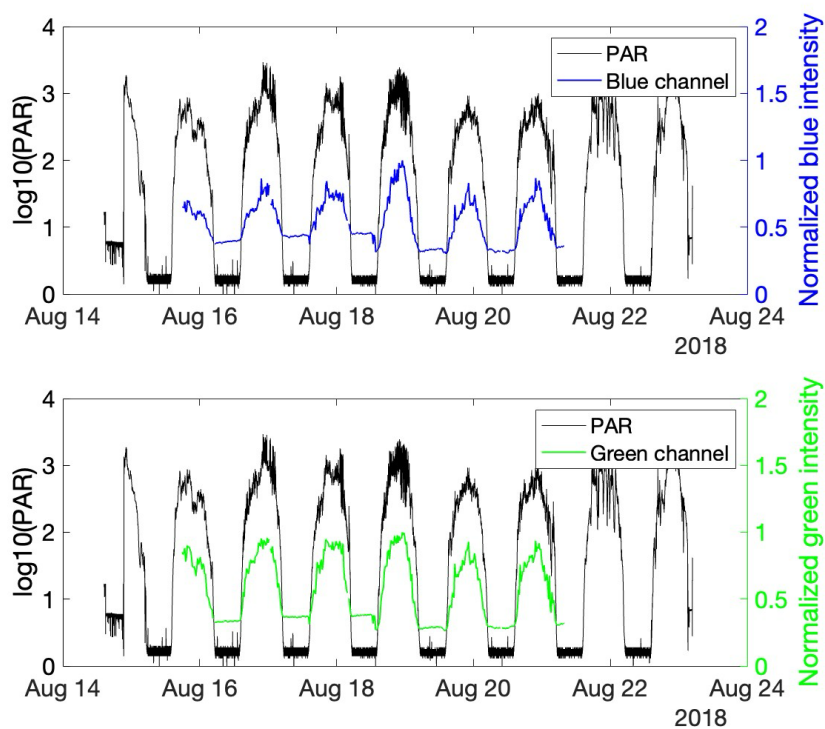
**Figure S2.** Correction of the cumulative particle areas in white pixels. (a) linear interpolation during the nighttime, (b) linear fitting of the percentage attenuation against the blue light intensities, and (c) corrected number of white pixels.

We estimated the total area of deposited particles using grayscale images, which we derived from the red-channel image after the background subtraction. Again we applied the global Otsu's black-and-white threshold. The number of white pixels that exceeded the global Otsu threshold provided

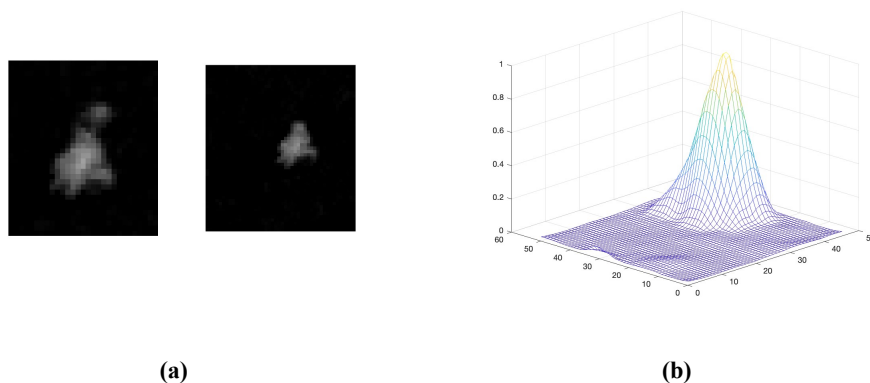
a quick approximation of the total deposited particles. However, the presence of ambient light, especially at shallow depths, posed a challenge, as high ambient lighting intensities could lead to the underestimation of particle sizes due to light scattering effects.

To tackle this challenge, we developed a correction method based on the ambient lighting intensity. As highlighted in the section above, the predominant background illuminations were observed in the green and blue channels. Here we defined excessive blue light intensity as the difference in light intensity from the nighttime blue channel. By definition, the excessive intensity reached the maximum around noon. Based on the observation that particle areas became smaller under stronger ambient light, we assumed that the excessive intensity was linearly correlated with the percentage decrease in binarized particle area. It should be noted that the blue channel was used because the area attenuation was slightly more sensitive than the green light. To calculate the loss percentage in particle area, we interpolated linearly between the end of last night and the start of concurrent nighttime time stamps to estimate unaffected white pixel number. The loss percentage could then be computed based on the interpolated estimation and the captured particle areas. We then correlated the excessive blue light intensity with the percentage attenuation, allowing for a corrected white pixel number at each daytime time stamp. Despite occasional dips in the curve, the implementation of this correction yielded promising results quickly and efficiently, as detailed in below sessions. More detailed steps for this method can be found in supplemental materials.

## Other supplemental figures and tables



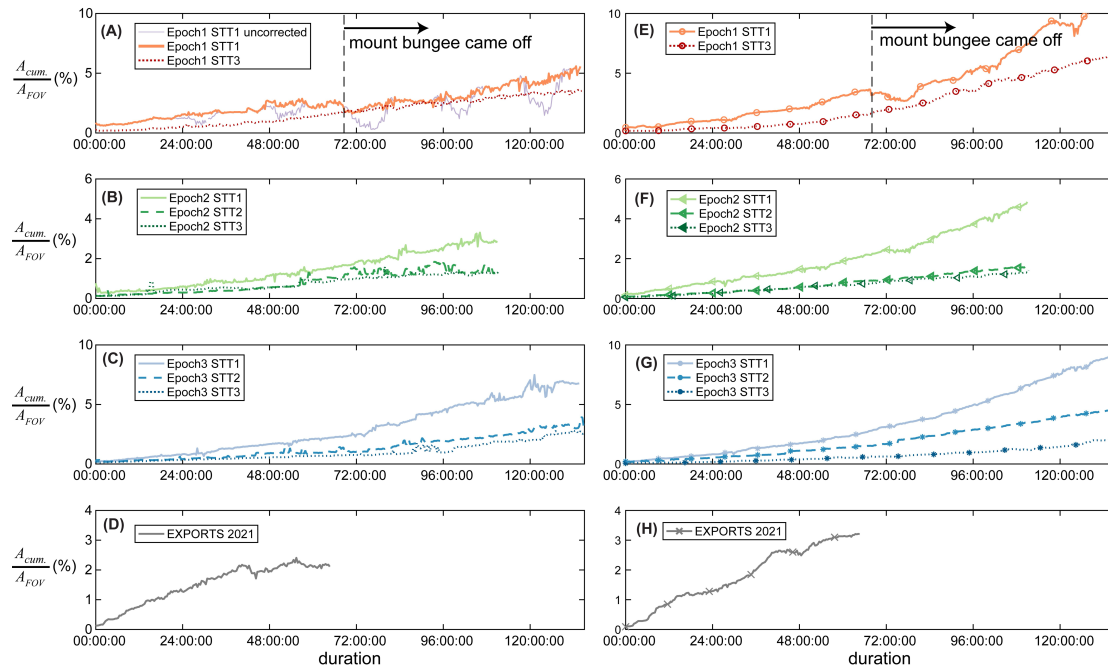
**Figure S3.** Time series of surface PAR and average light intensities in blue (A) and green (B) channels. The average blue/green channel light intensities were normalized by the maximum average across all image frames.



**Figure S4.** (a) interrogation and search windows, and (b) the plane of correlation coefficients.

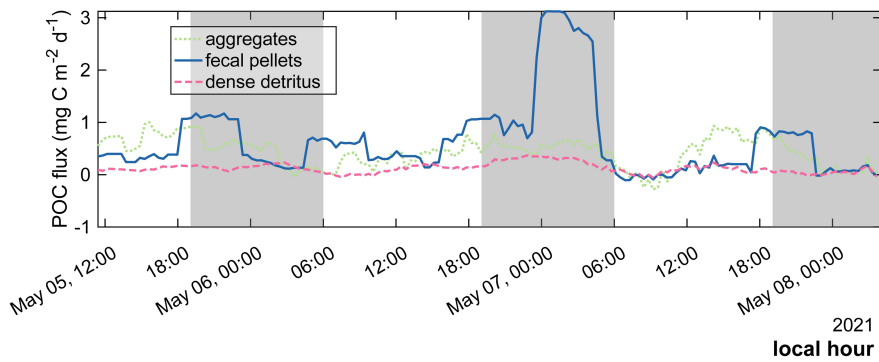
**Table S2.** Parameters that used to model the particulate carbon content of each particle type. We use the same particle types and parameters presented by Durkin et al. (2021) except dense detritus. Here  $ESD$  represents the equivalent spherical diameter obtained from particle projection areas,  $l$  represents the major axis length, while  $w$  is the minor axis length. Carbon mass per particle  $C$  (mg) is calculated by  $C = A \cdot V^B$ , where  $A$  is the scaling coefficient,  $V$  is the particle volume ( $\mu\text{m}^3$ ), and  $B$  is a unitless exponent.

particle type	shape	volume	A	B
aggregates	sphere	$\frac{\pi}{6}ESD^3$	$0.1 \cdot 10^{-9}$	0.80
large loose fecal pellets	cylinder	$\frac{\pi}{4}lw^2$	$0.1 \cdot 10^{-9}$	0.83
long fecal pellets	cylinder	$\frac{\pi}{4}lw^2$	$0.1 \cdot 10^{-9}$	1
dense detritus	sphere	$\frac{\pi}{6}ESD^3$	$0.1 \cdot 10^{-9}$	0.83
salp pellets	cuboid	$\frac{1}{4}lw^2$	$0.04 \cdot 10^{-9}$	1
rhizaria	sphere	$\frac{\pi}{6}ESD^3$	$0.004 \cdot 10^{-9}$	0.939





**Figure S5.** Accumulative fluxes at different deployments. Panels of (A), (B), (C), and (D) use the number of white pixels with the lighting correction method to quantify the time-integrated fluxes, while symbolized curves in (E), (F), (G), and (H) apply the particle classification results. Panel (A) also compares the cumulative fluxes of using the raw images or the ambient light correction in Deployment 1 STT 1. A zero duration on the x axis corresponded to the time when the deployment started. The significant discrepancy between (A) and (E) resulted from the storm during Deployment 1.



**Figure S6.** Time-varying POC fluxes of three particle types (aggregates in green, large loose and long fecal pellets in blue, dense detritus) in EXPORTS-NA. Shaded areas represent the nighttime.

**Table S3.** Coefficients of determination  $R^2$  of different particle types across depths, with p-values in the bracket.

aggregates	STT 1	STT 2	STT 3
STT 1	1 (0)	0.91 (< 0.01)	0.84 (< 0.01)
STT 2	0.92 (< 0.01)	1 (0)	0.85 (< 0.01)
STT 3	0.85 (< 0.01)	0.84 (< 0.01)	1 (0)
large loose fecal pellets	STT 1	STT 2	STT 3
STT 1	1 (0)	0.31 (< 0.01)	0.03 (< 0.01)
STT 2	0.31 (< 0.01)	1 (0)	0.003 (0.28)

STT 3	0.03 (< 0.01)	0.003 (0.27)	1 (0)
long fecal pellets	STT 1	STT 2	STT 3
STT 1	1 (0)	0.26 (< 0.01)	0.13 (< 0.01)
STT 2	0.26 (< 0.01)	1 (0)	0.002 (0.33)
STT 3	0.17 (< 0.01)	0.002 (0.35)	1 (0)
dense detritus	STT 1	STT 2	STT 3
STT 1	1 (0)	0.005 (0.17)	0.07 (< 0.01)
STT 2	0.005 (0.17)	1 (0)	0.55 (< 0.01)
STT 3	0.06 (< 0.01)	0.55 (< 0.01)	1 (0)

**Table S4.** Coefficients of determination  $R^2$  across particle types at the same depths, with p-values in the bracket.

STT 1	aggregates	large loose fecal pellets	long fecal pellets	dense detritus
aggregates	1 (0)	0.59 (< 0.01)	0.50 (< 0.01)	0.005 (0.16)
large loose fecal pellets	0.59 (< 0.01)	1 (0)	0.39 (< 0.01)	0.08 (< 0.01)
long fecal pellets	0.50 (< 0.01)	0.39 (< 0.01)	1 (0)	0.22 (< 0.01)
dense detritus	0.005 (0.16)	0.09 (< 0.01)	0.22 (< 0.01)	1 (0)
STT 2	aggregates	large loose fecal pellets	long fecal pellets	dense detritus
aggregates	1 (0)	0.09 (< 0.01)	0.14 (< 0.01)	0.85 (< 0.01)
large loose fecal pellets	0.09 (< 0.01)	1 (0)	0.39 (< 0.01)	0.09 (< 0.01)
long fecal pellets	0.14 (< 0.01)	0.39 (< 0.01)	1 (0)	0.12 (< 0.01)
dense detritus	0.85 (< 0.01)	0.09 (< 0.01)	0.12 (< 0.01)	1 (0)
STT 3	aggregates	large loose fecal pellets	long fecal pellets	dense detritus
aggregates	1 (0)	0.09 (< 0.01)	0.06 (< 0.01)	0.65 (< 0.01)
large loose fecal pellets	0.09 (< 0.01)	1 (0)	0.35 (< 0.01)	0.17 (< 0.01)
long fecal pellets	0.06 (< 0.01)	0.35 (< 0.01)	1 (0)	0.17 (< 0.01)
dense detritus	0.65 (< 0.01)	0.17 (< 0.01)	0.17 (< 0.01)	1 (0)