UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

February 14, 2013

Enbridge Energy Partners, LP
c/o Mr. Rich Adams
Vice President, Operations
Superior City Centre
Second Floor
1409 Hammond Ave.
Superior, Wisconsin 54880

Re: Centre for Offshore Oil, Gas and Energy Research (COOGER) Laser *In-Situ* Scanning and Transmissometry (LISST) Data for Evaluating the Efficiency and Potential Ecological Effects of *In-situ* Sediment.

Dear Mr. Adams:

Enclosed is the report entitled Centre for Offshore Oil, Gas and Energy Research (COOGER) Laser *In-Situ* Scanning and Transmissometry (LISST) Data for Evaluating the Efficiency and Potential Ecological Effects of *In-situ* Sediment (LISST Report). The LISST Report was prepared for the United States Environmental Protection Agency (U.S. EPA) by Mr. Scott Ryan and Dr. Kenneth Lee.

This transmittal fulfills the information request to the U.S. EPA made by Mr. John Bohrmann on February 1, 2013 for LISST data collected by Mr. Scott Ryan during the 2012 Agitation Study between July 24, 2012 and August 8, 2012.

If you have any questions regarding this transmittal, please contact me immediately at (231) 301-0559.

Sincerely,

Ralph Dollhopf
Federal On-Scene Coordinator and Incident Commander
U.S. EPA, Region 5

Attachment (1)

cc: K. Peaceman, U.S. EPA, ORC
C. Mikalian, U.S. EPA, ORC
M. DeLong, MDEQ
Records Center, U.S. EPA, Reg. V

Laser *In-Situ* Scanning and Transmissometry (LISST) Data for evaluating the Efficiency and Potential Ecological Effects of *In-situ* Sediment Agitation

Prepared for

U.S. Environmental Protection Agency (EPA)

by

Scott A. Ryan[1] and Kenneth Lee[2*]

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Background

Scientific experiments were conducted under the direction of the US EPA under a multi-disciplinary program to quantify the efficiency of the "agitation toolbox" techniques performed in response to the July 2010 Enbridge Line 6B release in Marshall, MI. During a set of controlled sediment agitation experiments conducted along the Kalamazoo River between July 23, 2012 and August 8, 2012, laser *In-Situ* Scattering and Transmissometry (LISST) analysis were conducted to provide scientific support required for data analysis and interpretation.

Methods

During the agitation experiments sediment size distribution analysis was conducted with a LISST-100X (Type C) particle analyzer (Sequoia Scientific Inc., Seattle, WA). This instrument is an optical device that measures the size and volume of particles in a given sample based on the physical properties of light as it is scattered by the particles. In order to achieve this, a laser beam is directed through the sample chamber and any of this light scattered by particles present within the sample is focused by a specialized lens onto a series of detector rings numbered 1 through 32 (Fig. 1). Light intensity readings for the 32 discrete rings is processed with the manufacturer-provided inversion algorithm to automatically calculate volume concentrations (in µL/L) for 32 particle size bins, along with the output for 10 other parameters including laser transmission, sensor power, laser reference sensor in calibrated units, pressure, temperature, computed optical transmission over path, and beam-attenuation. The 32 particle size bins measured by the LISST-100X are logarithmically spaced across the analytical range, the upper size in each bin being 1.18 times that of the lower (Table 1).

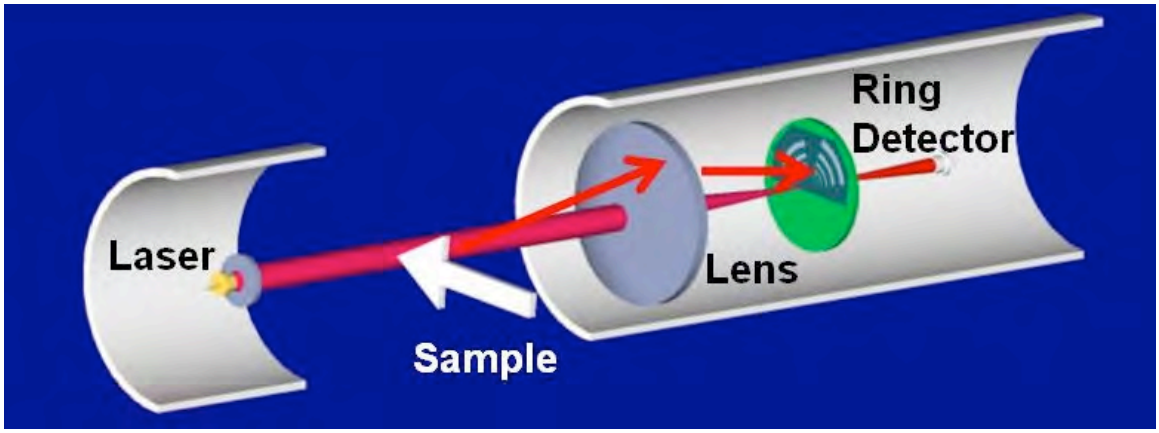


Figure 1: Basic illustration of the LISST-100X optics system showing the laser. Source: Sequoia Scientific

Table 1: The minimum, mean and maximum limit of each size bin in microns for the LISST-100X particle counter.

Size Bin #	Min	Mean	Max
1	2.50	2.73	2.95
2	2.95	3.22	3.48
3	3.48	3.79	4.11
4	4.11	4.48	4.85
5	4.85	5.28	5.72
6	5.72	6.23	6.75
7	6.75	7.36	7.96
8	7.96	8.68	9.40
9	9.40	10.24	11.09
10	11.09	12.09	13.08
11	13.08	14.26	15.44
12	15.44	16.83	18.22
13	18.22	19.86	21.50
14	21.50	23.43	25.37
15	25.37	27.65	29.93
16	29.93	32.63	35.32
17	35.32	38.50	41.68
18	41.68	45.43	49.18
19	49.18	53.61	58.04
20	58.04	63.26	68.48
21	68.48	74.65	80.81
22	80.81	88.08	95.36
23	95.36	103.94	112.52
24	112.52	122.65	132.77
25	132.77	144.72	156.67
26	156.67	170.77	184.87
27	184.87	201.51	218.15
28	218.15	237.78	257.42
29	257.42	280.58	303.75
30	303.75	331.09	358.43
31	358.43	390.68	422.94
32	422.94	461.01	499.07

At each station (representative experimental site) a vertical LISST profile was conducted within the experimental enclosure before agitation (pre-agitation). Following agitation treatment, extremely levels of turbidity within the enclosures precluded *in-situ* LISST operation; therefore, discrete sampling, dilution, and subsequent bench-top analysiswas required. For each of these post-agitation samples, discrete samples were collected at up to three depths (top, middle, and bottom, depending on water depth) using horizontal Van-Dorn samplers at times 0, 15 min, 30 min, 1 h, 2 h, 4 h, and 6 h (designated as T-0, T-15min, T-30min, T-1h, etc.). Single subsamples were drawn from from Van-Dorn for LISST analysis into 250 mL Nalgene bottles. LISST analysis was conducted mmediately following sample collection in the field using a full-path mixing chamber fitted directly to the LISST optics. In light of the high turbidy levels encountered as a result of sediment agitation, immediately before analysis, it was routinely necessary to dilute the samples between 10 and 400 times using two graduated cylinders (1 × 100 mL, 1 × 1 L). A complete standard operating procedure (SOP) for the LISST can be found in the appendix of this report.

In addition to direct sample dilution before analysis, dilution within the experimental enclosure also had to be considered as water from outside the enclosure was used during agitation. The enclosure dilution factor was calculated using water column depth and "inside freeboard," the distance from the surface of the water to the top of the enclosure. These measurements were obtained throughout each experiment. While making these calculations, missing values were estimated using the following rules.

1. When the pre-agitation "inside freeboard" measurement was missing, "outside freeboard" was used instead.
2. When "inside freeboard" measurements were unavailable immediately following agitation, the next nearest post-agitation data point was used.
3. When a missing "inside freeboard" measurement had valid measurements both before and after it, a rate of change over time was calculated and the missing point filled in accordingly.
4. When the T-6h "inside freeboard" measurement was missing, the T-4h value was used instead.

Enclosure dilution factor was ultimately calculated by

$$1 + ((IF_{pre} - IF_t) / D_{pre})$$

where IF_{pre} is pre-agitation "inside freeboard", IF_t is "inside freeboard" at time 't,' and D_{pre} is pre-agitation water depth. Table 2 shows the enclosure dilution values as calculated for all stations sampled. All data plots in this report show LISST data which has been adjusted for both the sample and enclosure dilution factors.

Table 2: Enclosure dilution calculations with estimated values in red.

		Station					
		DeltaZ	DeltaEE	DeltaH	MP21_5	MP14_8	MP5_5
		Depth (ft)					
Sampling Time (h)	0	2.2	1.5	1.5	1.6	1.5	1.5
		Inside Freeboard Measurement (ft)					
	-1	0.730	1.900	1.400	2.880	2.680	1.500
	0	0.390	1.160	0.880	2.170	1.920	0.900
	0.25	0.390	1.160	0.880	2.245	2.020	0.965
	0.5	0.440	1.160	0.880	2.320	2.120	1.030
	1	0.510	1.183	0.970	2.410	2.160	1.080
	2	0.510	1.230	1.000	2.490	2.240	1.140
	4	0.570	1.280	1.030	2.580	2.320	1.210
	6	0.680	1.280	1.030	2.790	2.390	1.270
		Enclosure Dilution Factor					
	0	1.155	1.493	1.347	1.444	1.507	1.400
	0.25	1.155	1.493	1.347	1.397	1.440	1.357
	0.5	1.132	1.493	1.347	1.350	1.373	1.313
	1	1.100	1.478	1.287	1.294	1.347	1.280
	2	1.100	1.447	1.267	1.244	1.293	1.240
	4	1.073	1.413	1.247	1.188	1.240	1.193
	6	1.023	1.413	1.247	1.056	1.193	1.153

Potential Sources of Error

Instrument Error

A series of 21 field blanks were collected over the 6 days that agitation experiments were conducted. These blanks were collected with the intention of identifying the level of potential instrument errors present within the agitation experiment dataset. Table 3 shows the summarized field blank data.

Table 3: Analysis of the 21 field blanks that were collected; TPC = total particle concentration.

	LISST Particle Size Bin																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Mean (µL/ L)	0.025	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.002	
SD (µL/ L)	0.076	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.004	
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	TPC
Mean (µL/ L)	0.001	0.014	0.005	0.003	0.005	0.005	0.003	0.004	0.005	0.005	0.003	0.004	0.021	0.065	0.274	0.406	0.861
SD (µL/ L)	0.002	0.060	0.009	0.003	0.009	0.012	0.007	0.006	0.014	0.015	0.007	0.004	0.030	0.112	0.396	0.449	0.882

Mean particle concentrations calculated using all 21 field blanks show that values are quite low across the board in this dataset. These minor deviations from baseline are most likely attributed to instrument noise and give little reason for concern.

Error Associated with Sample Dilution

Due to the high levels of turbidity associated with the experimental agitation process, it was necessary to dilute samples prior to LISST analysis. To estimate the level of potential error in the LISST data associated with this sample dilution procedure, a dilution series experiment was conducted.

A sample collected from the top 18.3 cm (0.6 ft) of the water column at T-0, at station Delta EE, was diluted 10×, 20×, and 30×. From these results, the mean, standard deviation (SD), and the relative standard deviation were calculated. The relative standard deviation, SD (%), is expressed in percent and is obtained by dividing the standard deviation by the mean, and multiplying by 100.

While mean and SD are certainly useful, SD (%) seems to be the most telling statistic from this exercise. As can be seen in Table 4, SD (%) seems to be greatest in the very small and very large particle size bins, with the most prominent trend being that of progressively increasing error through the largest particle size bins.

Table 4: Dilution series results - station Delta EE, T-0, 'top'.

	LISST Particle Size Bin															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
10x Dil. (µL/ L)	59.99	39.87	23.34	13.24	9.45	11.16	17.45	21.84	22.97	25.79	26.28	25.37	24.91	22.88	21.59	19.55
20x Dil. (µL/ L)	38.07	28.19	18.98	12.46	9.73	11.21	16.40	20.46	21.29	23.38	24.41	23.91	23.98	22.56	21.34	19.56
30x Dil. (µL/ L)	36.02	27.02	18.51	12.46	10.01	11.71	17.09	21.11	21.75	23.62	24.42	23.84	23.81	22.19	20.61	18.40
Mean (µL/ L)	44.69	31.69	20.28	12.72	9.73	11.36	16.98	21.13	22.01	24.27	25.04	24.37	24.23	22.55	21.18	19.17
SD (µL/ L)	13.29	7.10	2.66	0.45	0.28	0.31	0.53	0.69	0.87	1.33	1.08	0.86	0.59	0.35	0.51	0.67
SD (%)	29.73	22.41	13.13	3.52	2.89	2.70	3.13	3.28	3.95	5.48	4.30	3.55	2.44	1.53	2.41	3.50
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
10x Dil. (µL/ L)	17.61	15.70	13.75	11.97	10.04	7.87	5.80	4.25	3.29	2.45	1.64	1.04	0.60	0.37	0.32	0.47
20x Dil. (µL/ L)	17.60	15.64	13.61	11.89	10.23	8.57	7.17	6.48	6.91	8.01	9.01	9.32	8.19	6.26	4.63	3.62
30x Dil. (µL/ L)	15.96	13.55	11.27	9.53	8.03	6.74	5.79	5.69	7.14	10.53	15.36	19.46	19.63	16.44	11.52	6.71
Mean (µL/ L)	17.06	14.96	12.88	11.13	9.43	7.73	6.25	5.47	5.78	7.00	8.67	9.94	9.47	7.69	5.49	3.60
SD (µL/ L)	0.95	1.22	1.39	1.39	1.22	0.92	0.79	1.13	2.16	4.13	6.87	9.23	9.58	8.13	5.65	3.12
SD (%)	5.57	8.17	10.83	12.47	12.92	11.93	12.69	20.66	37.32	59.03	79.18	92.85	101.11	105.72	102.97	86.69

On the basis of these results, it is reasonable that during the dilution process larger particles were most likely lost due to their increased potential for settling to the bottom of the vessel during sample processing (i.e. before and during pouring). Despite every effort to homogenize the contents of each graduated cylinder before pouring, increased error in the higher particle size range remains an inevitability of the process.

Error Associated with Sample Splitting

When dealing with highly sediment-laden water, pouring from the 250 mL Nalgene subsample bottle into a graduated cylinder before dilution was also a potential source of error. Due to the fact that a large portion of the sediment within each 250 mL subsample would settle almost instantaneously after shaking, obtaining a homogeneous aliquot proved to be a difficult task. In addition, many of the 'bottom' samples were sediment-water slurries containing a number of relatively large and loosely consolidated sediment conglomerates. While pouring during sample splitting operations, the number of these conglomerates included in a single aliquot was entirely random.

Tables 5 and 6 show the results of triplicate sample analyses on 'bottom' samples of differing sediment concentration: one taken from MP14.8 (T-0, high sediment content, 400× dilution) and the second from MP21.5 (T-4h, low sediment content, 10× dilution). Triplicate analysis of the high-sediment sample collected at MP14.8 (Table 5) shows moderate levels of error in particle size bins 1 and 2 (very small particles), and 30, 31 and 32 (very large particles), and fairly high levels of error between bins 13-15 (midsize particles). Results from the low-sediment sample collected at MP21.5 show fairly low SD (%) throughout the lower particle size range, with increased relative standard deviation values in bins 21 and higher – extremely low total particle concentrations in these large particle size bins likely contributed to the excessively high SD (%) values.

Table 5: Triplicate analysis - station MP14.8, T-0, 'bottom', 400× dilution.

	LISST Particle Size Bin															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mean (µL/L)	71.7	75.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	200.9	281.3	462.1	602.8
SD (µL/L)	38.0	28.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	348.0	303.4	243.6	0.0
SD (%)	53.0	37.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	173.2	107.9	52.7	0.0
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Mean (µL/L)	602.8	602.8	874.8	863.9	821.6	744.5	641.1	540.9	475.9	412.1	357.8	324.2	299.3	274.9	270.1	334.3
SD (µL/L)	0.0	0.0	149.7	145.6	127.7	102.1	75.3	52.9	33.8	12.8	8.9	31.9	55.7	73.6	102.8	167.9
SD (%)	0.0	0.0	17.1	16.9	15.5	13.7	11.8	9.8	7.1	3.1	2.5	9.8	18.6	26.8	38.1	50.2

Table 6: Triplicate analysis - station MP21.5, T-4h, 'bottom', 10× dilution.

	LISST Particle Size Bin															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mean (µL/L)	13.7	9.1	5.1	2.8	1.9	2.3	3.7	5.0	6.6	5.7	6.9	6.2	5.3	4.0	3.0	2.0
SD (µL/L)	0.2	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.3	0.4	0.4	0.4	0.3	0.2
SD (%)	1.8	0.3	1.9	3.9	4.6	3.7	2.2	1.4	2.0	0.3	4.0	6.3	8.5	9.9	10.3	9.9
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Mean (µL/L)	1.3	0.9	0.6	0.4	0.3	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.0
SD (µL/L)	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.3	0.3	0.2	0.1	0.0
SD (%)	7.8	5.4	8.4	18.4	31.6	47.5	65.4	83.7	102.1	122.5	141.4	152.6	156.9	156.9	157.8	152.9

Despite the seemingly large potential for error illustrated above, it should be noted that for most intents and purposes the data provide should be considered reliable enough for its intended purpose. The cases above are extreme examples which were selected in order to test the maximum potential for analytical error. Samples which were heavily diluted before analysis also tended to be the ones which were most difficult to subsample, and subsequently have the highest potential for error. Particle concentrations in these samples tended to be extremely high and easily distinguishable from the remainder of the dataset. For samples such as these, attempting to conduct detailed analysis using data from the largest particle-size bins should be avoided due to the error inherent in the dilution process. For the purposes of this report, when particle concentration is presented in a figure without accompanying particle size data, an alternate figure will also be shown which only includes data from bins 1-25 (2.5-157µm); this selection represents LISST particle size bins with SD (%) values <50% in the dilution series analysis (Table 4).

Results and Discussion

Delta EE

Figures 2 and 3 show total particle concentration (TPC) vs. depth and adjusted TPC (particle sizes <157μm) vs. depth for each sampling interval at Delta EE. TPC increased dramatically (primarily in the near bottom sample) post-agitation and had mostly re-settled by T-1h. Although the absolute particle concentration is lower in the adjusted TPC plot, overall trends remain the same. It should be noted that the relatively higher TPC value for the T-15min sample as compared to the T-0 sample is likely due to sampling error caused by the triggering of the Van-Dorn bottle while it was in accidental contact with bottom. . TPC vs. Time data in Figures 4 and 5 appear much the same as Figures 2 and 3 – there is a large spike in TPC post-agitation, which mostly settles by T-1h.

Figures 6 and 7 show particle concentration data for a series of 8 particle size groups (PSGs) spanning the 2.5-500 μm range of the LISST-100X. These data show that the most predominant particle size range present in the post-agitation samples were in the middle of the analytical size range - the two predominant PSGs covering 21.5-80.8 μm. It should be noted at this point that PSGs 7 and 8 represent particle size ranges which had SD (%) values >50% in the dilution series (Table 4).

Colored 3-D contour plots (Figures 8-11), which show particle size vs. time with particle concentration support the information provided in the above PSGs data set. The plots showed that the bulk of the suspended particulate matter (SPM) present after agitation appears to fall in the middle of the size range, ~10-150 μm, with slower settling of the smaller size fractions (10-30 μm) over time (particularly in the 'top' and 'middle' samples). Note that z-axis range is different on each contour plot in order to obtain the best possible resolution.

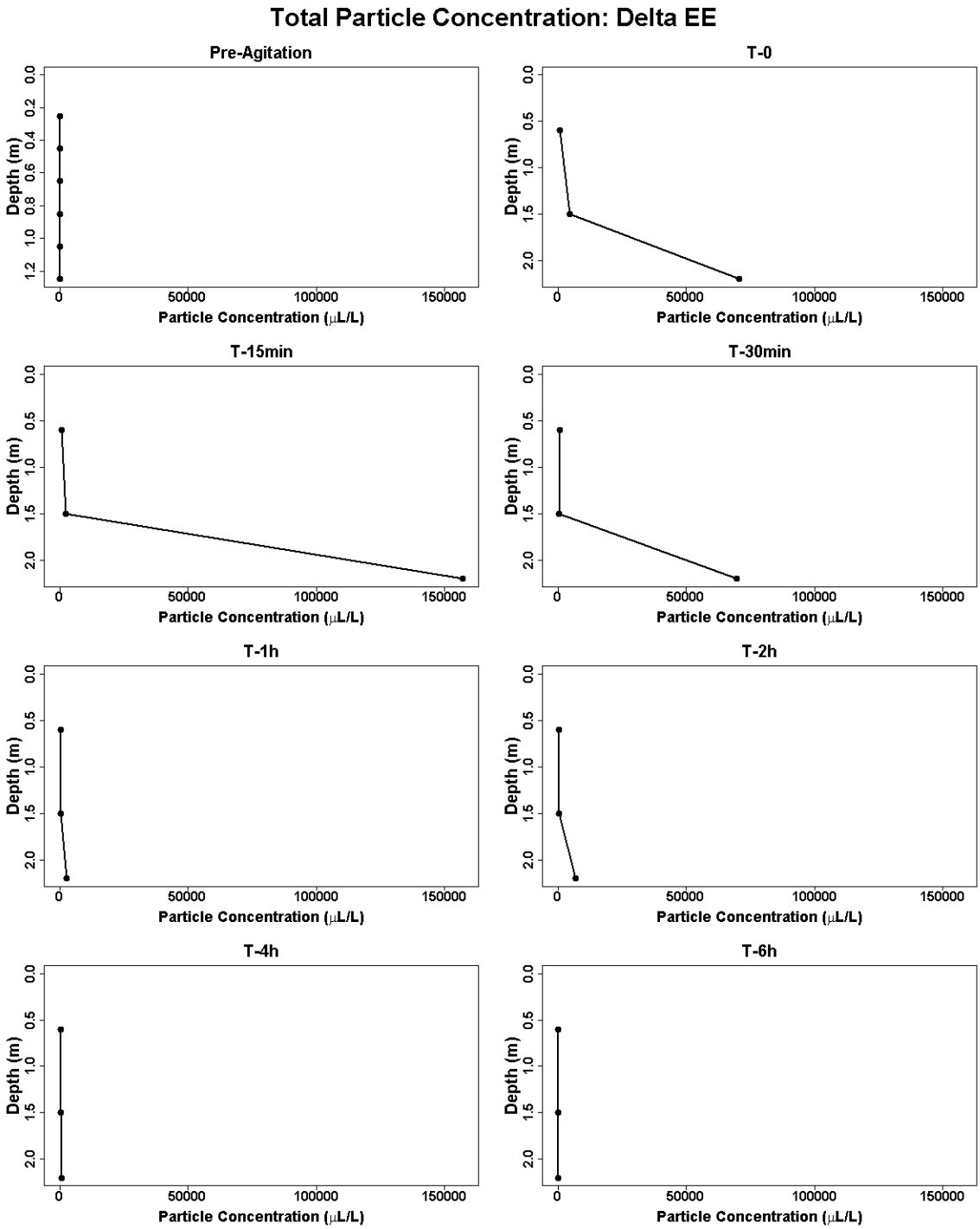


Figure 2: Total particle concentration data for station Delta EE.

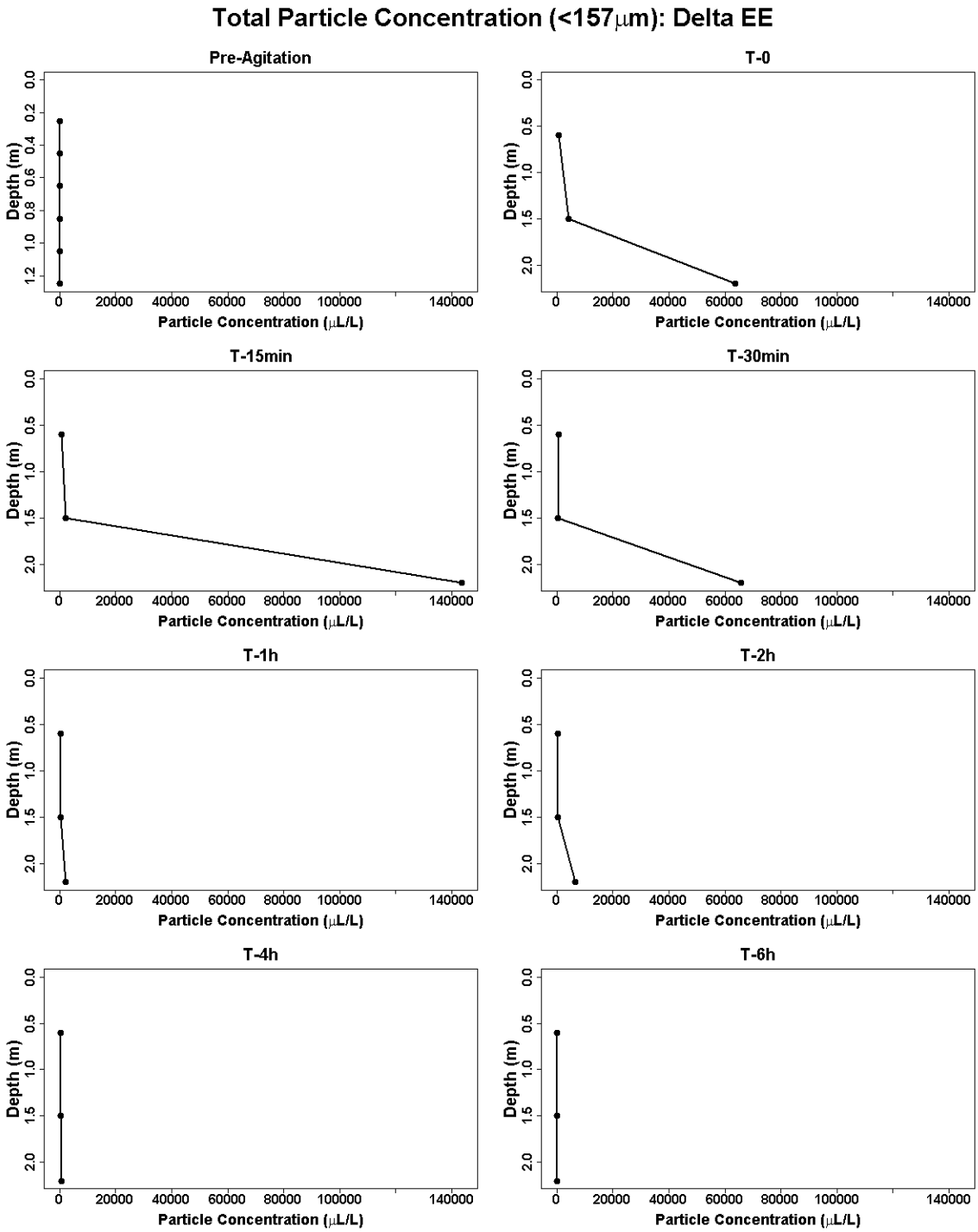


Figure 3: Total particle concentration (particle size <157 μm) data for station Delta EE.

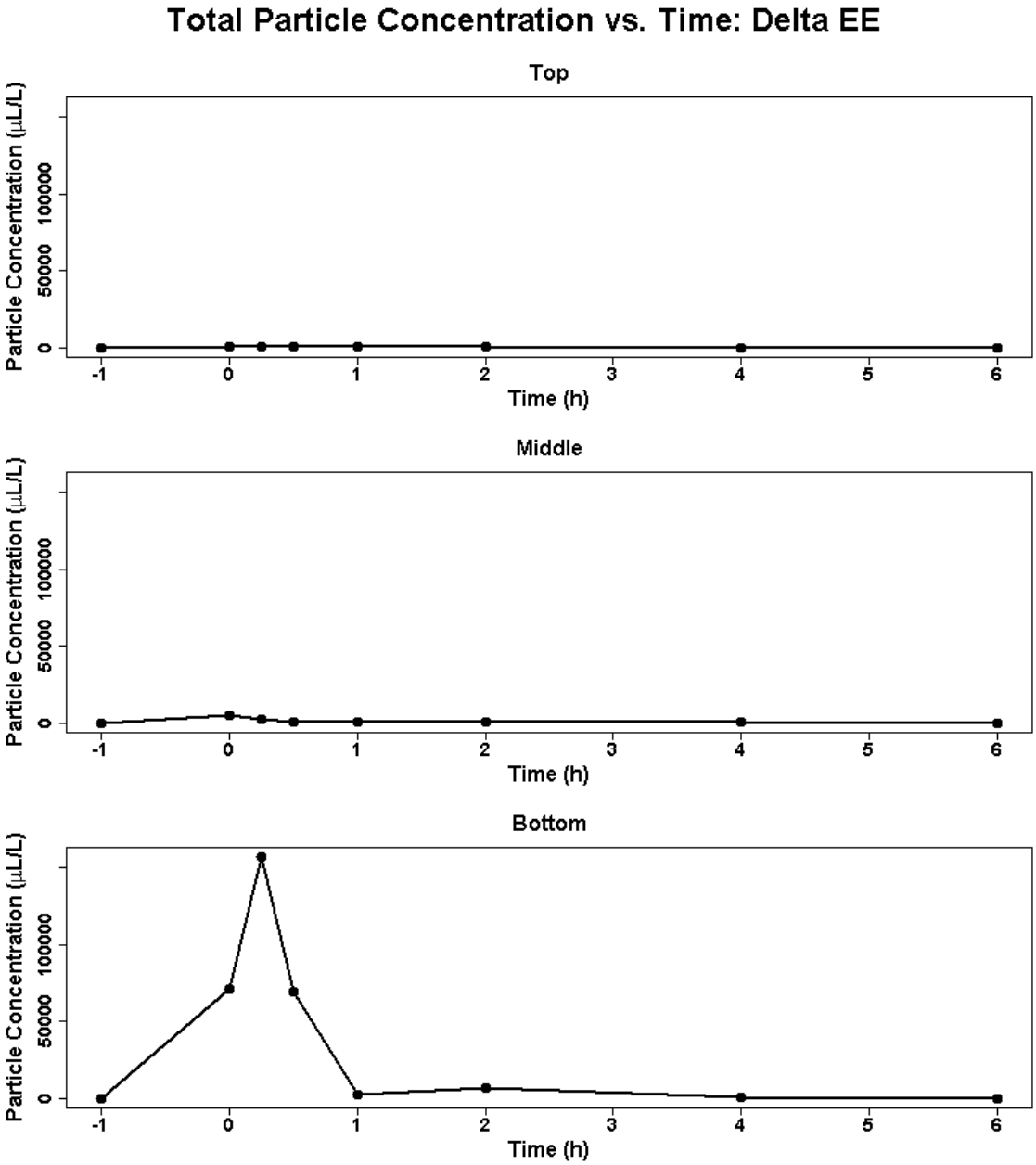


Figure 4: Total particle concentration vs. time for station Delta EE.

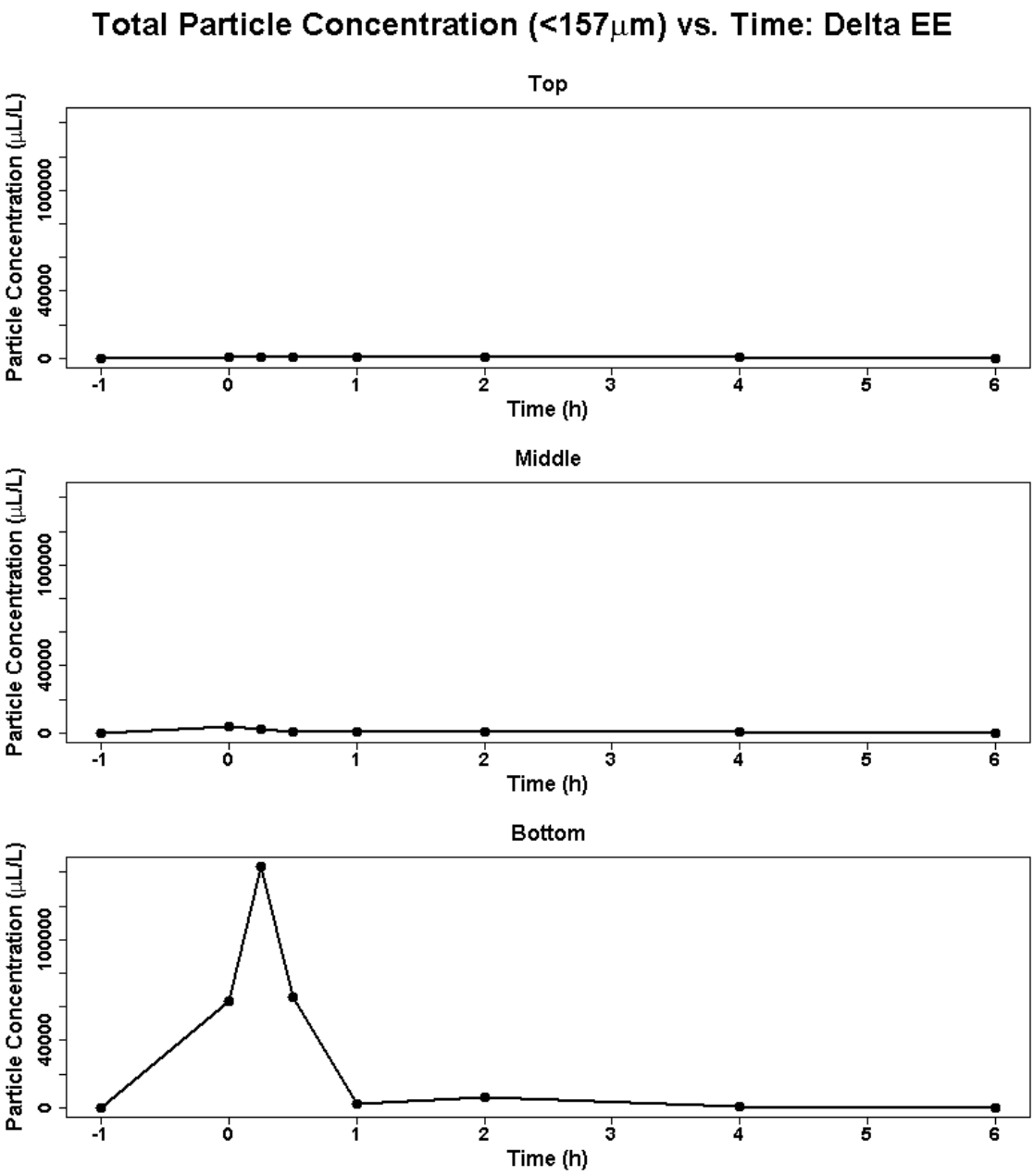


Figure 5: Total particle concentration (particle size <157 μm) vs. time for station Delta EE.

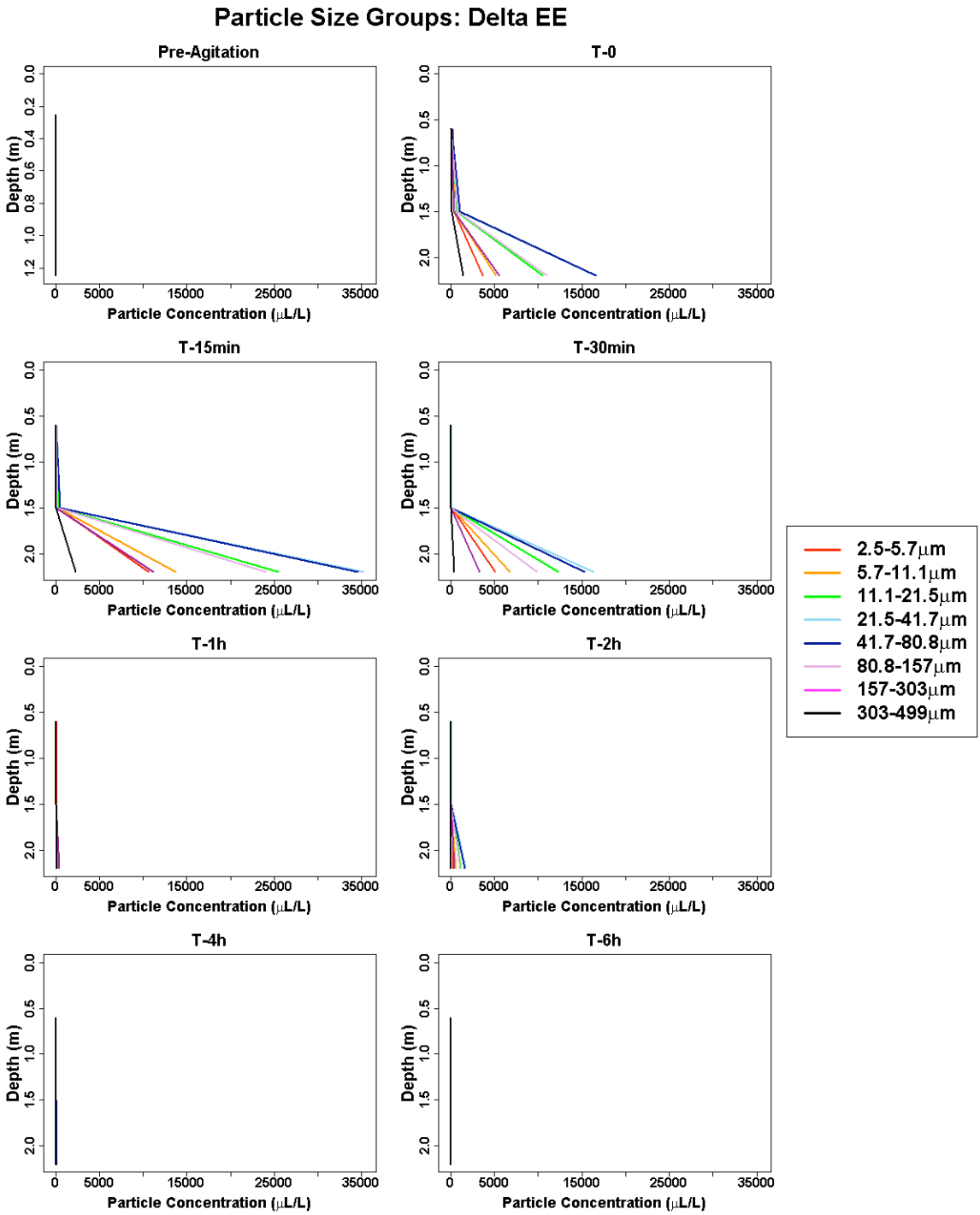


Figure 6: Particle Size Group concentrations for station Delta EE.

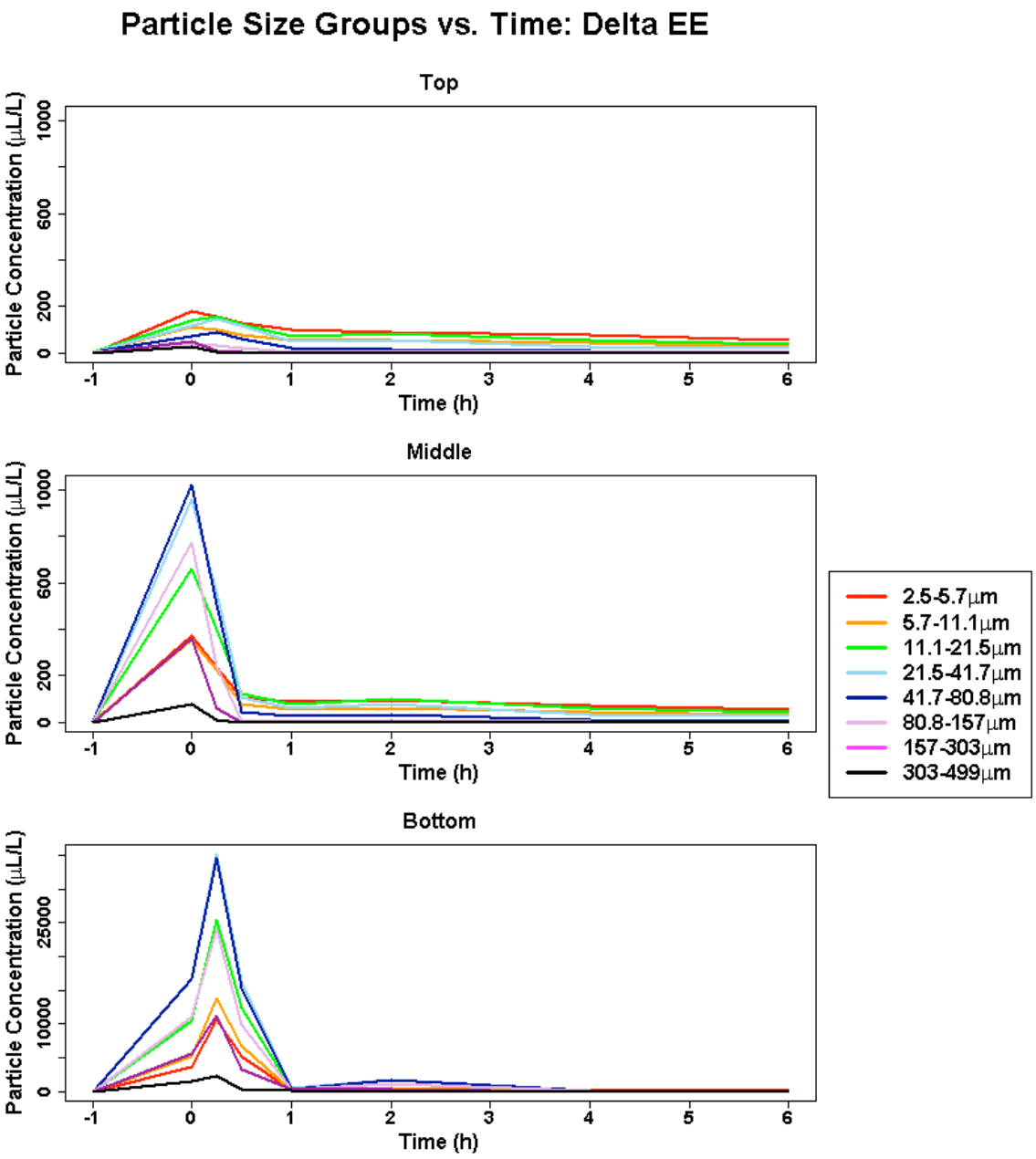


Figure 7: Particle Size Group concentrations vs. Time for station Delta EE.

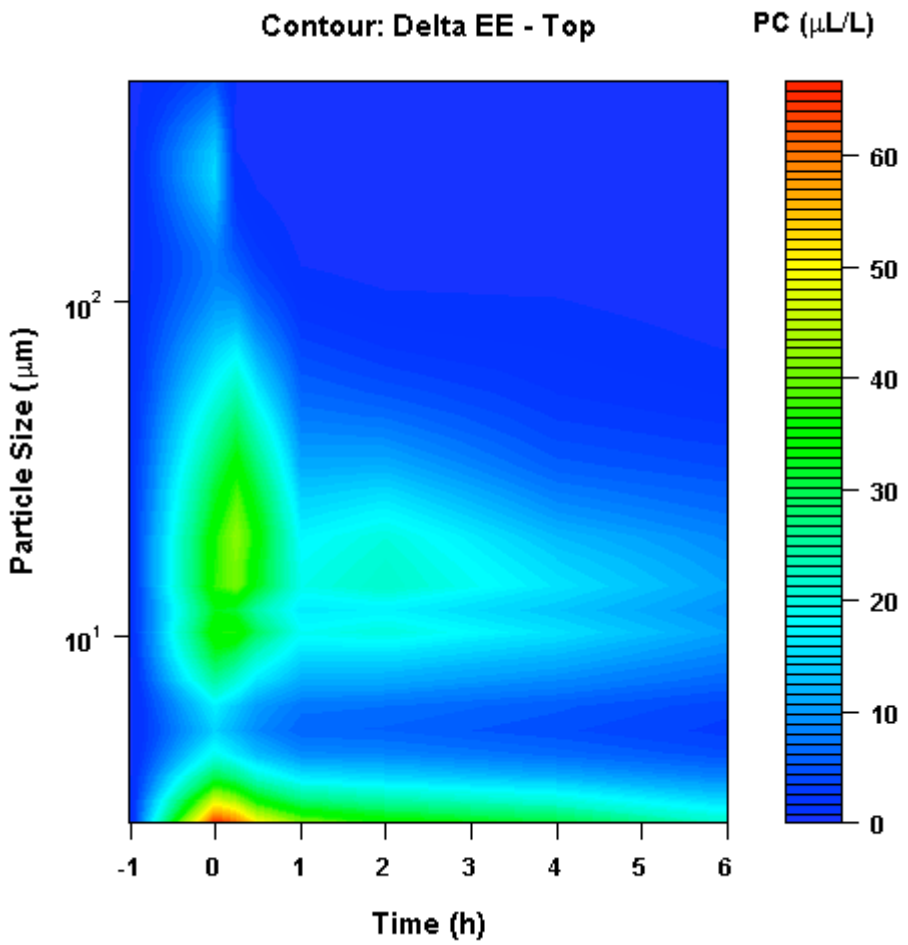


Figure 8: 3D contour plot for the Delta EE 'top' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

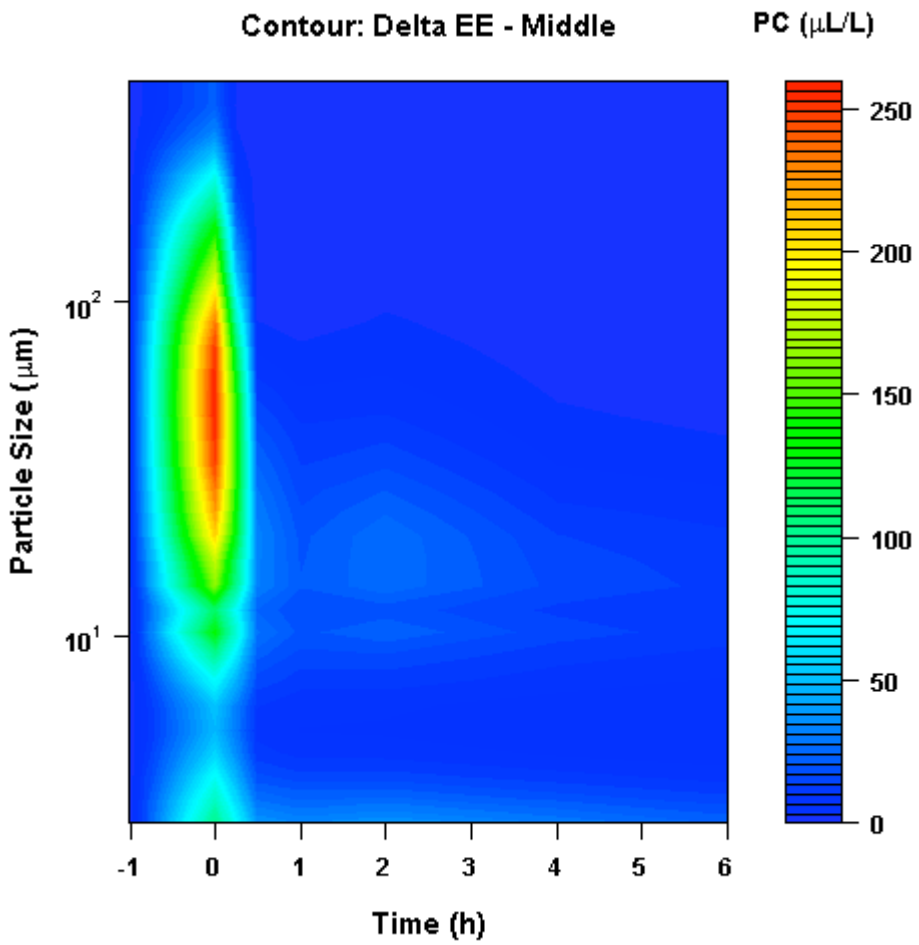


Figure 9: 3D contour plot for the Delta EE 'middle' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

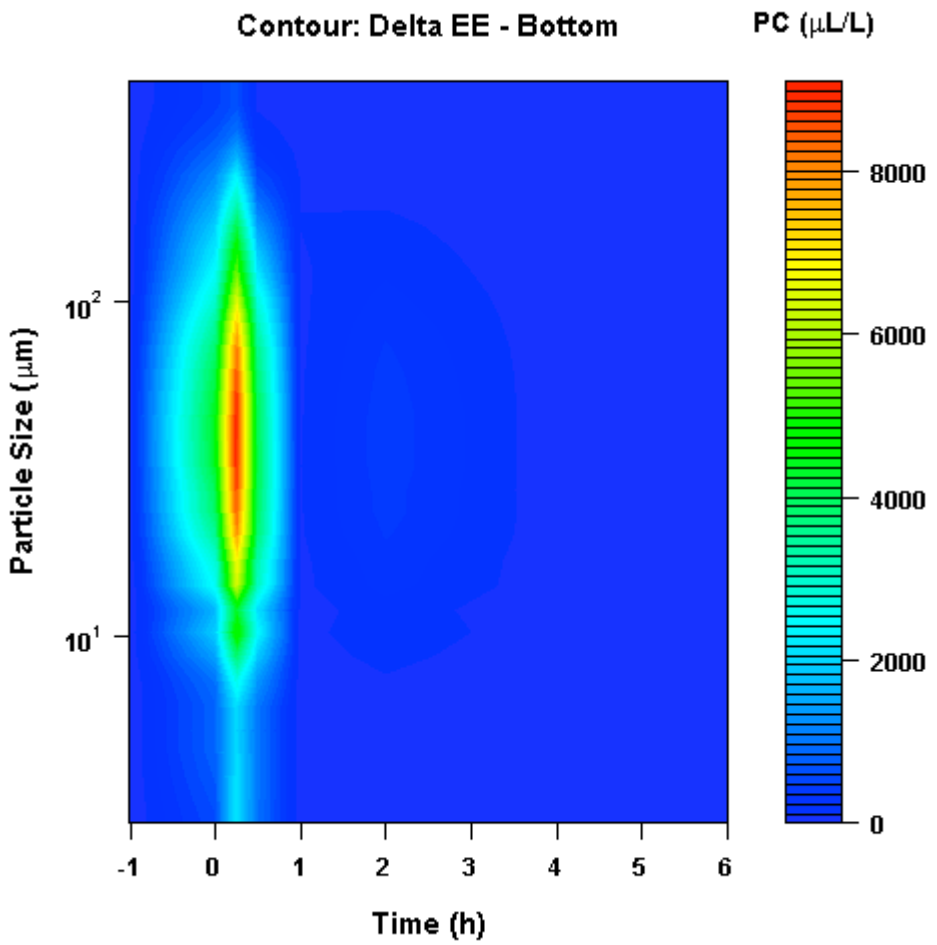


Figure 10: 3D contour plot for the Delta EE 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

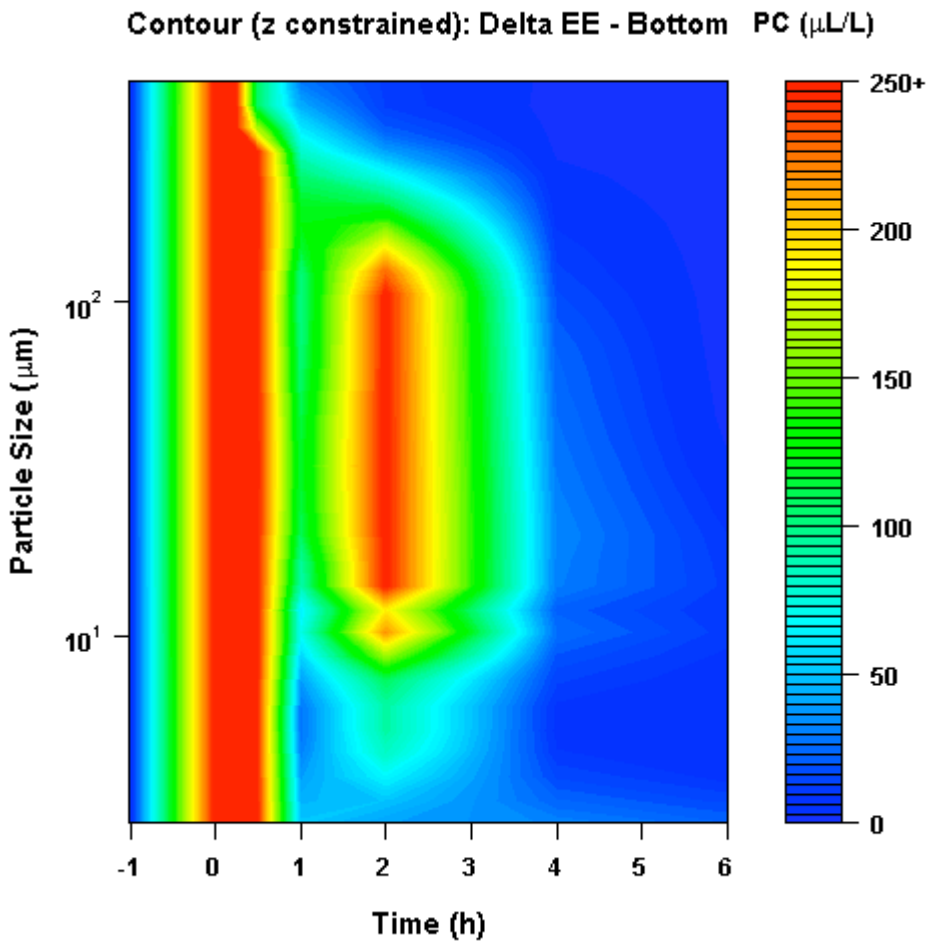


Figure 11: 3D contour plot for the Delta EE 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with constrained colored contours representing particle concentration (z-axis). In this figure, the maximum z-value has been limited to 250 µL/L in order to achieve better resolution at low particle concentrations.

Delta H

TPC plots for station Delta H (Figures 12-15) show a similar trend to those of Delta EE. There was a significant spike in SPM following agitation, primarily affecting the bottom of the water column, but settling significantly by T-1h. PSG plots (Figures 16 and 17) and contours (Figures 18-21) show that the bulk of the sediment falls in the small-medium size range (2.5-100 μm) with the smaller particles (2.5-30 μm) settling more slowly over time.

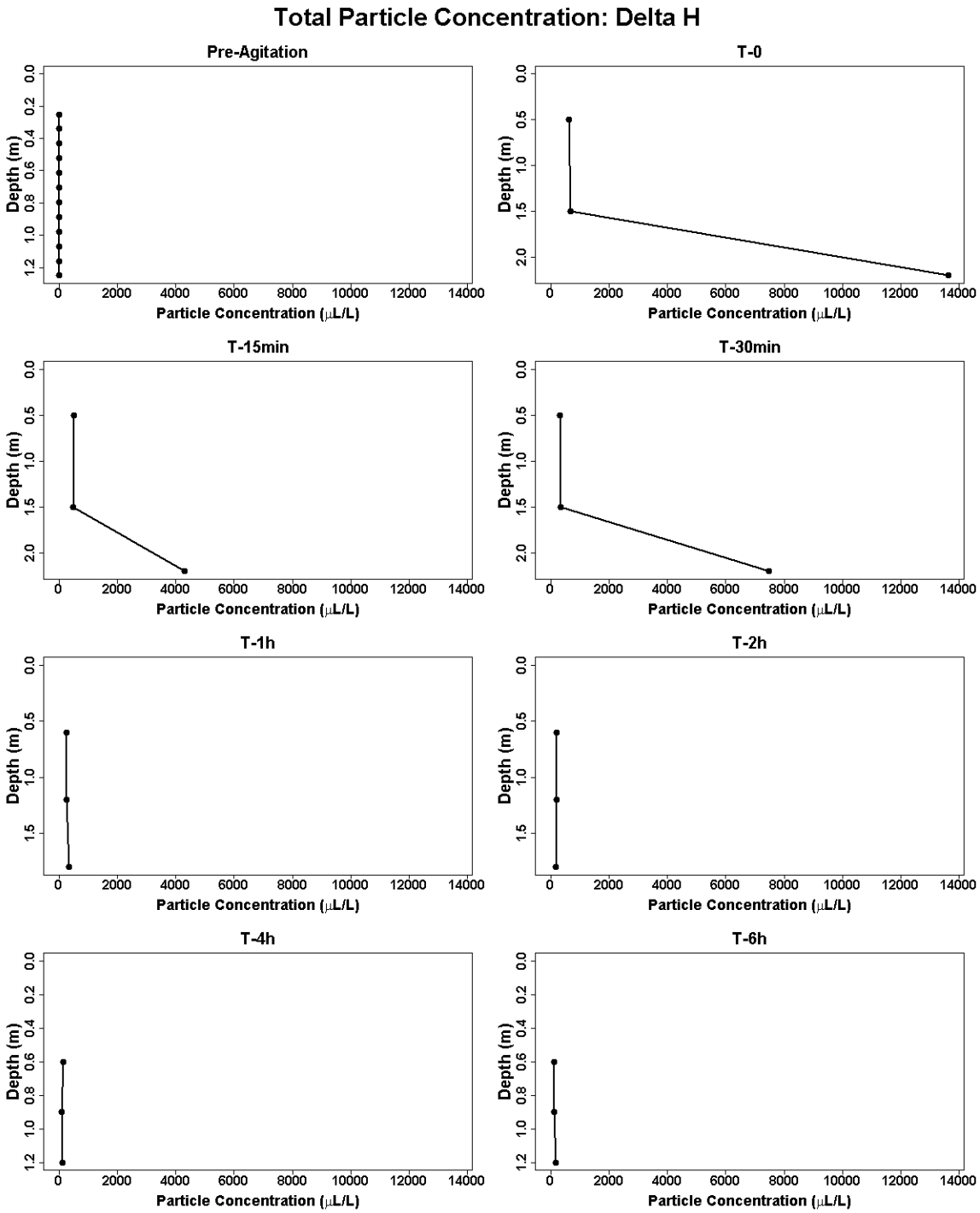


Figure 12: Total particle concentration data for station Delta H.

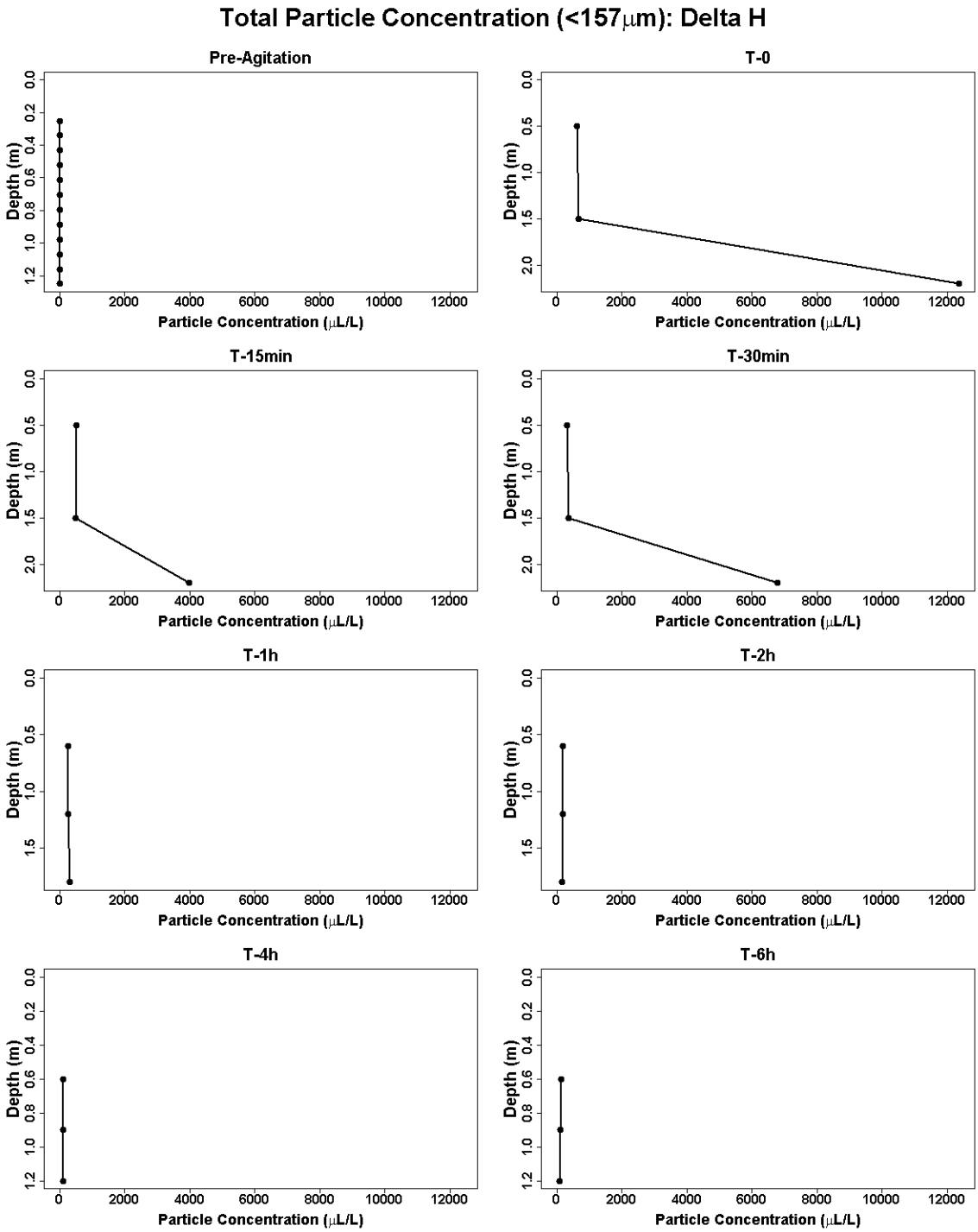


Figure 13: Total particle concentration (particle size <157 μm) data for station Delta H.

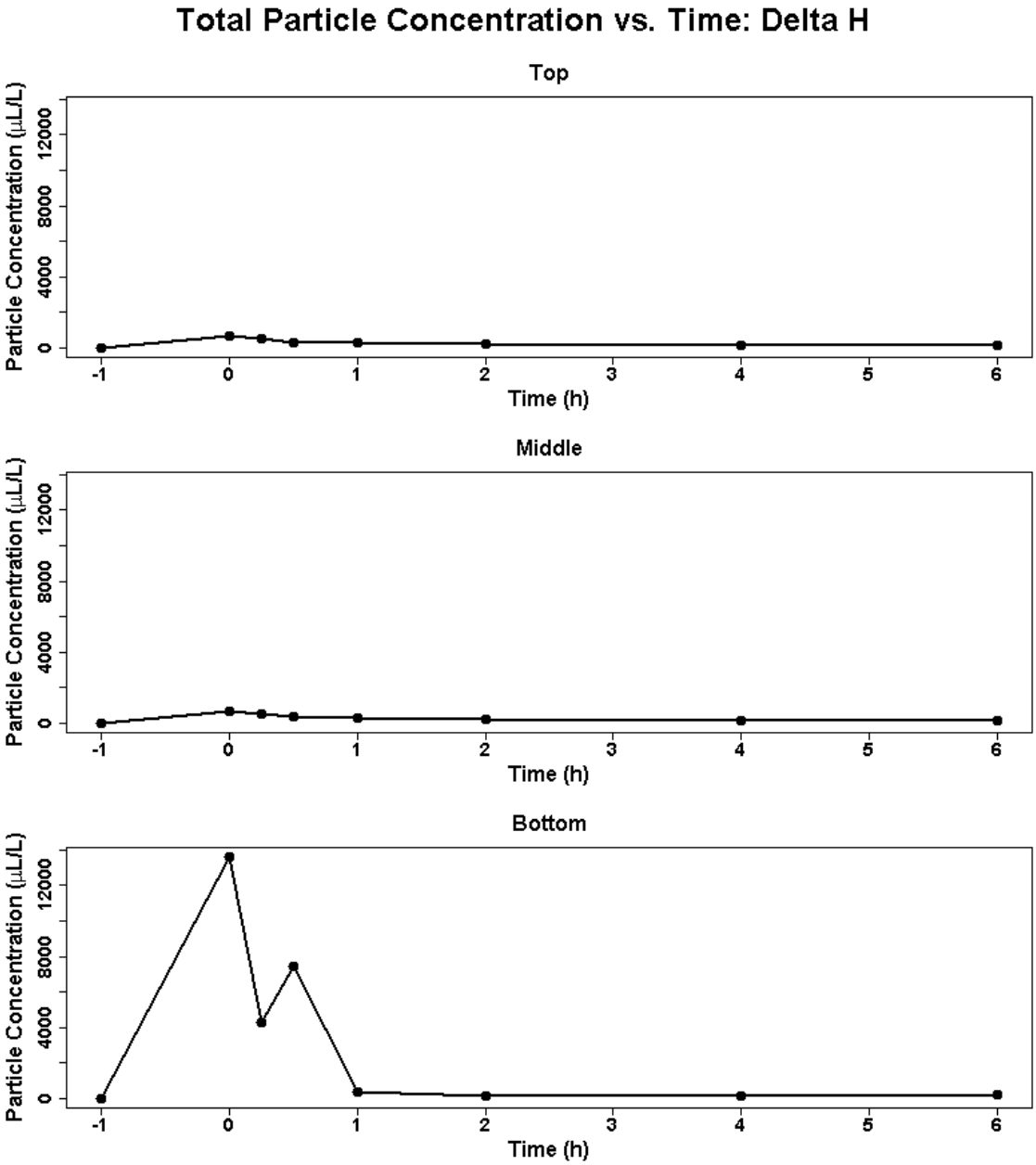


Figure 14: Total particle concentration vs. time for station Delta H.

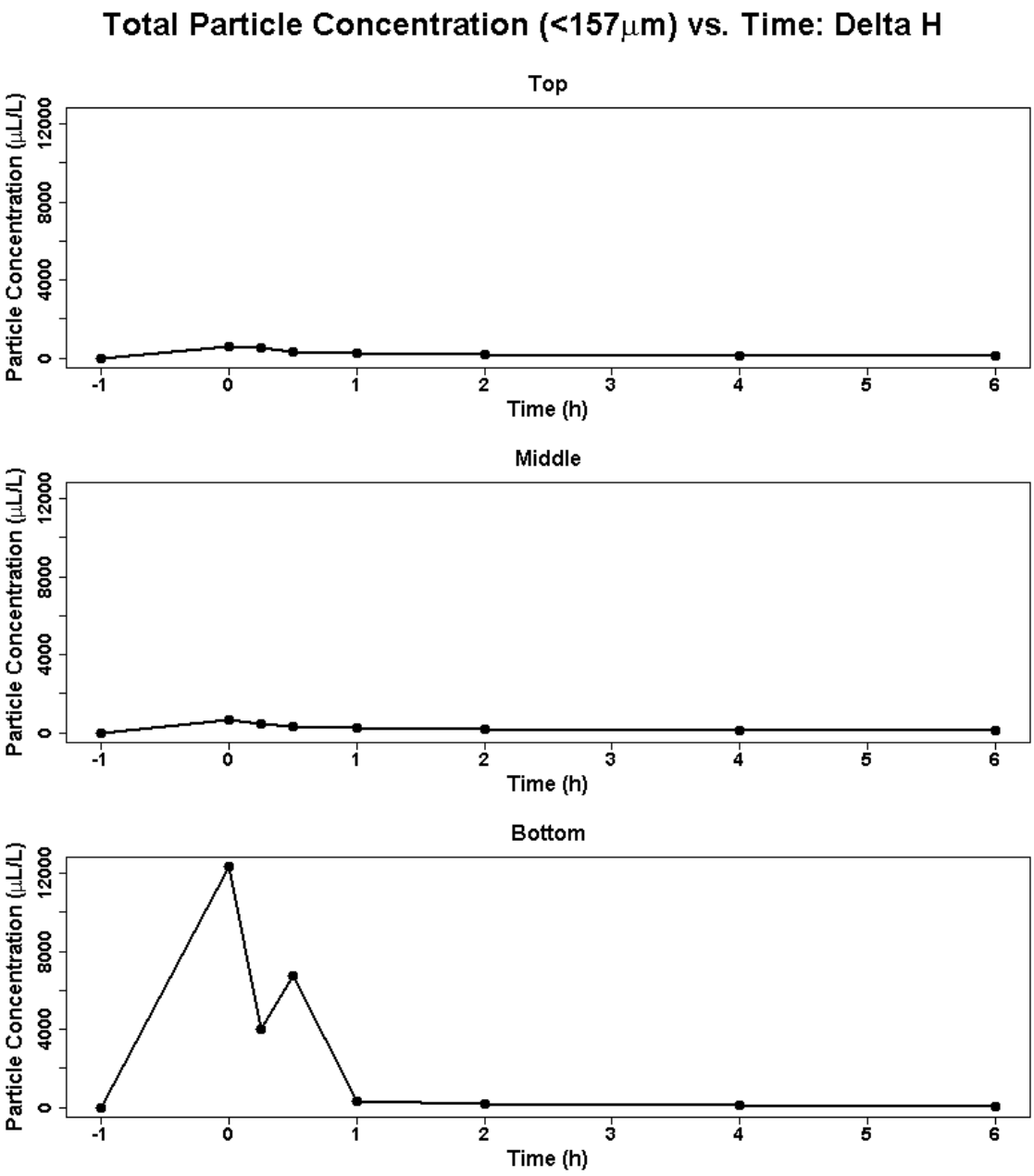


Figure 15: Total particle concentration (particle size <157 μm) vs. time for station Delta H.

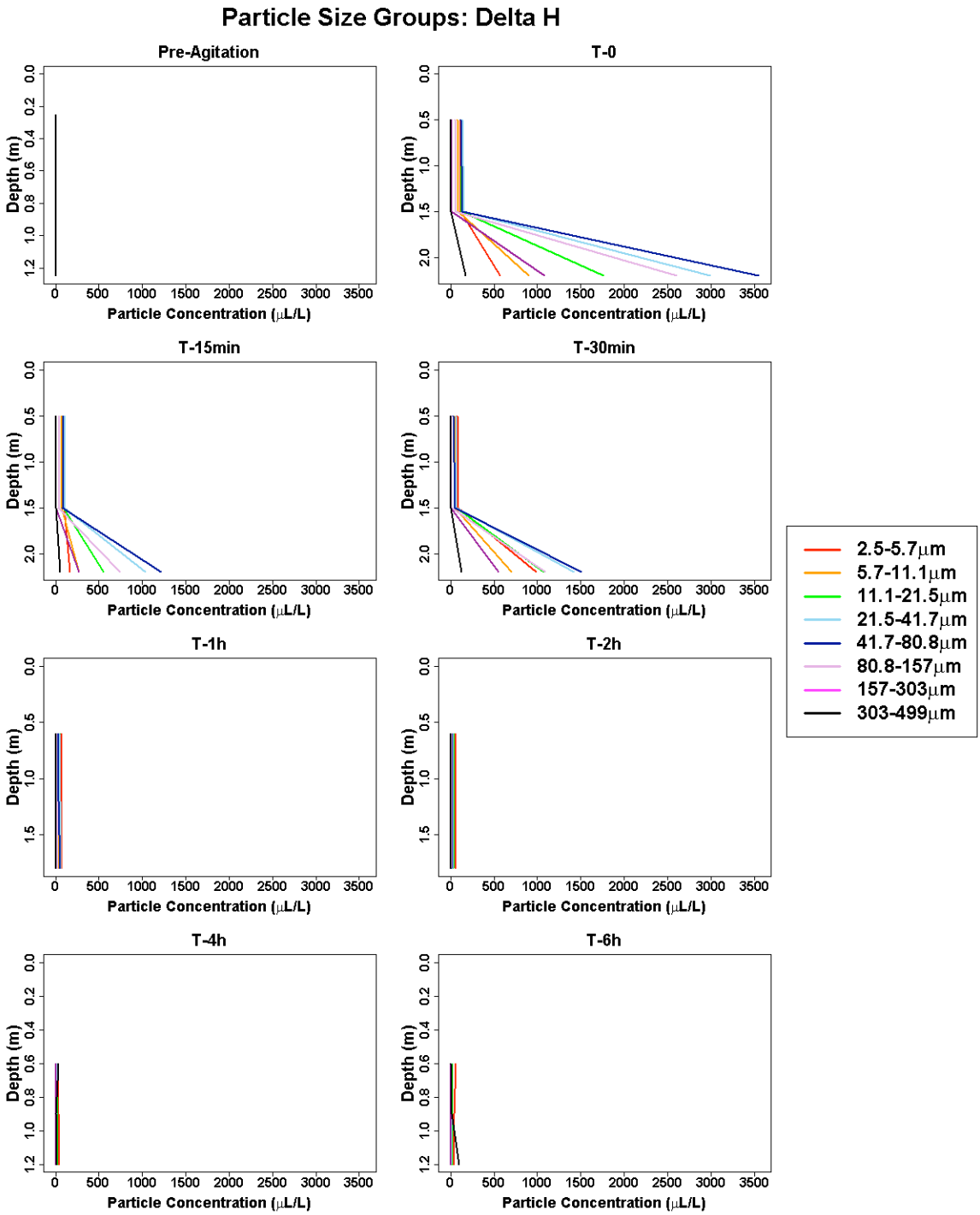


Figure 16: Particle Size Group concentrations for station Delta H.

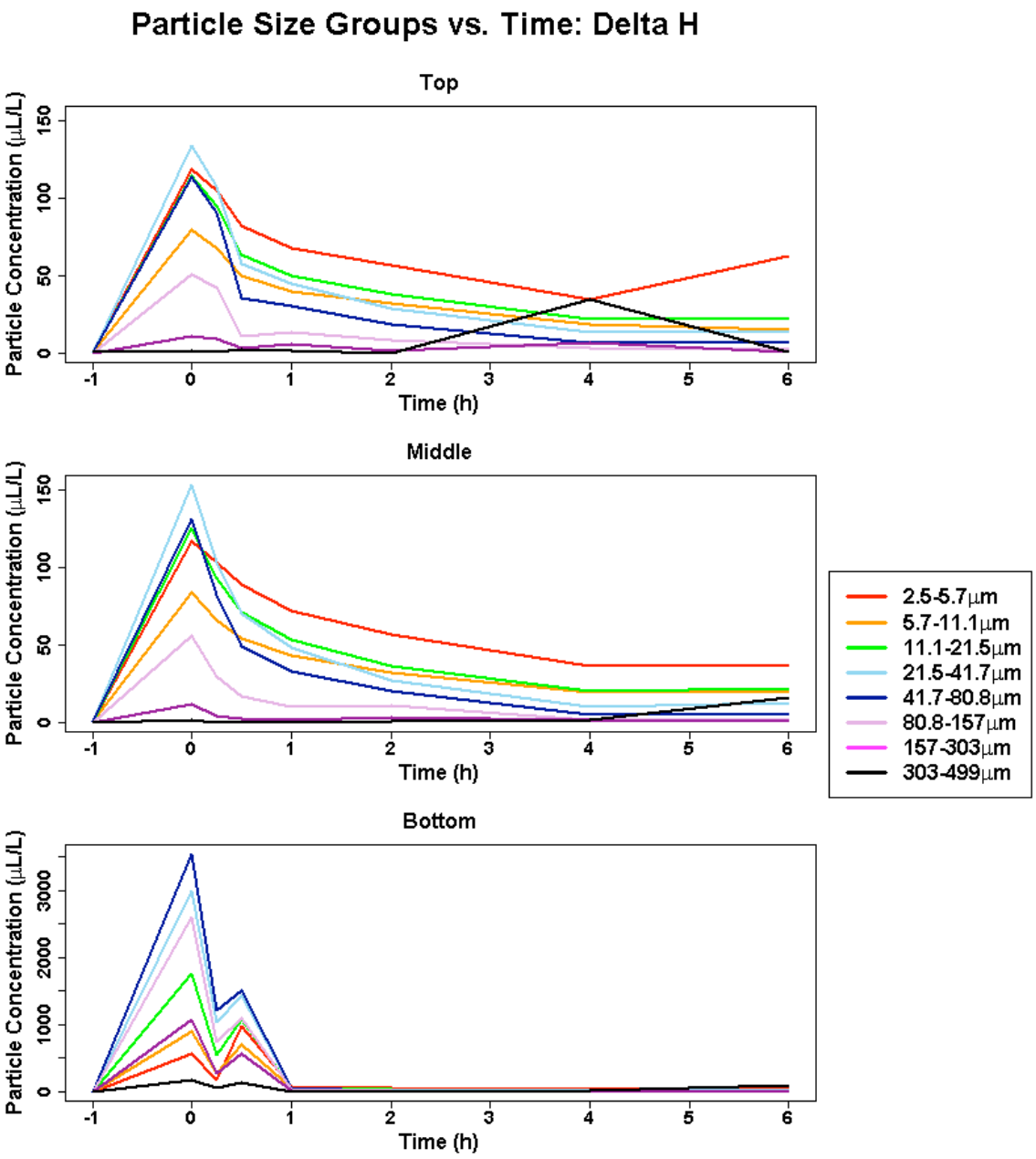


Figure 17: Particle Size Group concentrations vs. time for station Delta H.

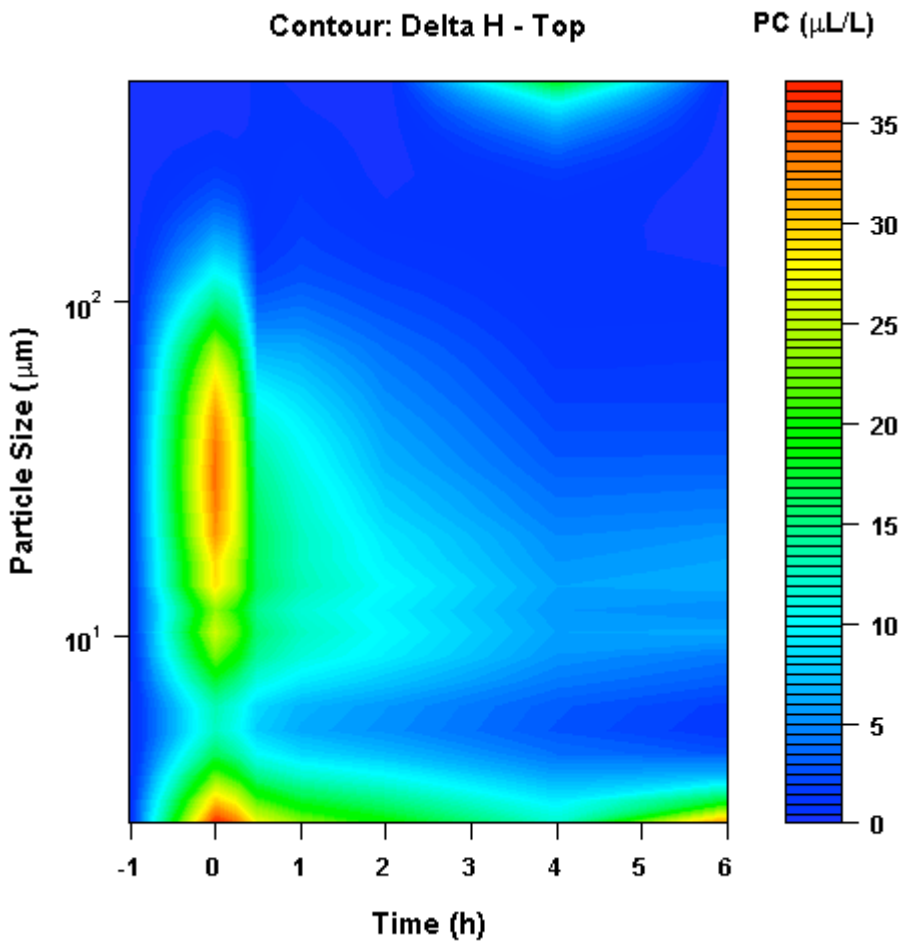


Figure 18: 3D contour plot for the Delta H 'top' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

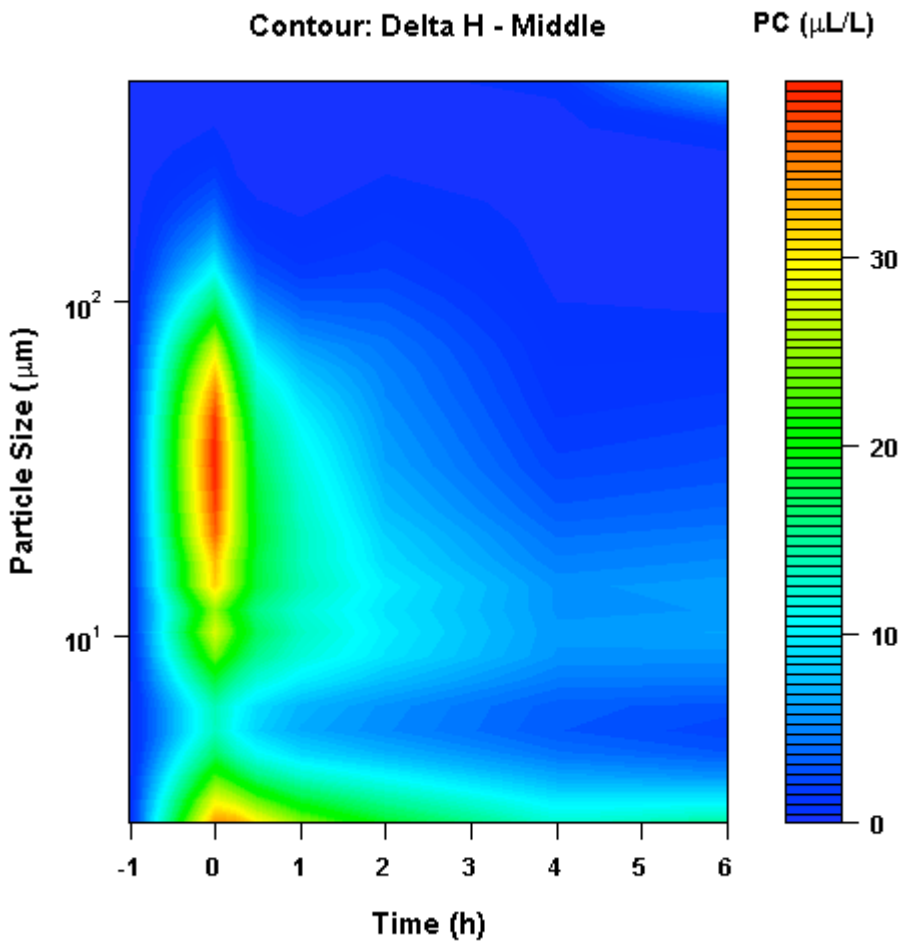


Figure 19: 3D contour plot for the Delta H 'middle' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

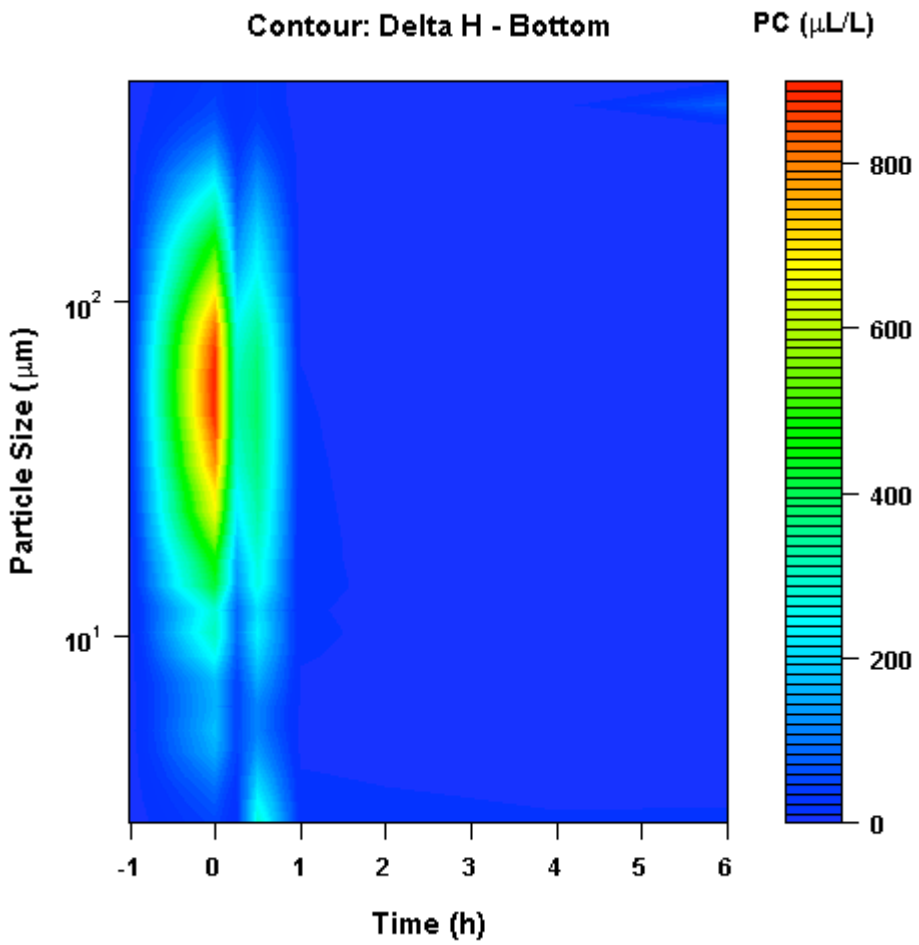


Figure 20: 3D contour plot for the Delta H 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

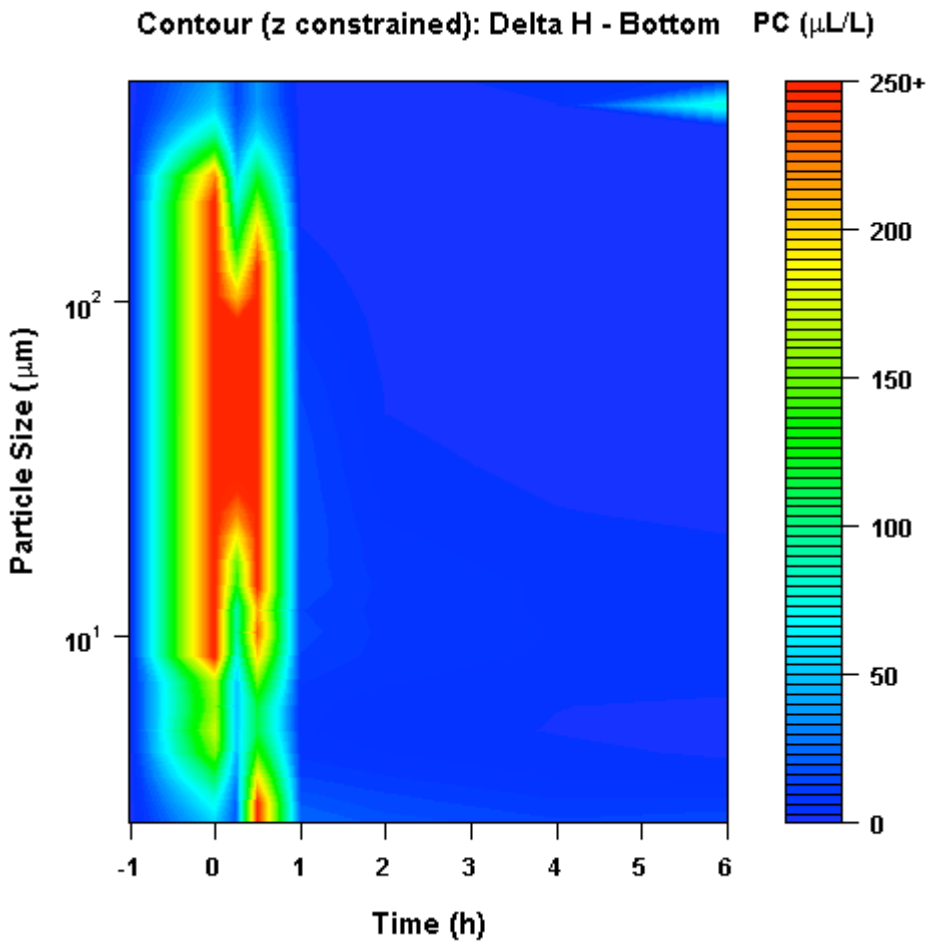


Figure 21: 3D contour plot for the Delta H 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with constrained colored contours representing particle concentration (PC, z-axis). In this figure, the maximum z-value has been limited to 250 µL/L in order to achieve better resolution at low particle concentrations.

MP21.5

TPC plots for station MP21.5 (Figures 22-25) show a similar trend to previous stations with a significant spike in SPM following agitation, but much quicker settling with TPC values nearing their T-6h baseline at T-15min. PSG plots (Figures 26 and 27) and contours (Figures 28-31) show primarily very small particles (~2.5 μm) in the middle and upper section of the water column, but particles of this size are much less prevalent in the near-bottom sample. Aside from these very small particles, the majority of the sediment at MP21.5 fell between 8 and 300 μm in diameter, with what seems to be an increasingly larger representation of the 50-300 μm particles with depth. Contour plots also seem to indicate size-fractioned settling, with large particle sizes settling faster than small ones.

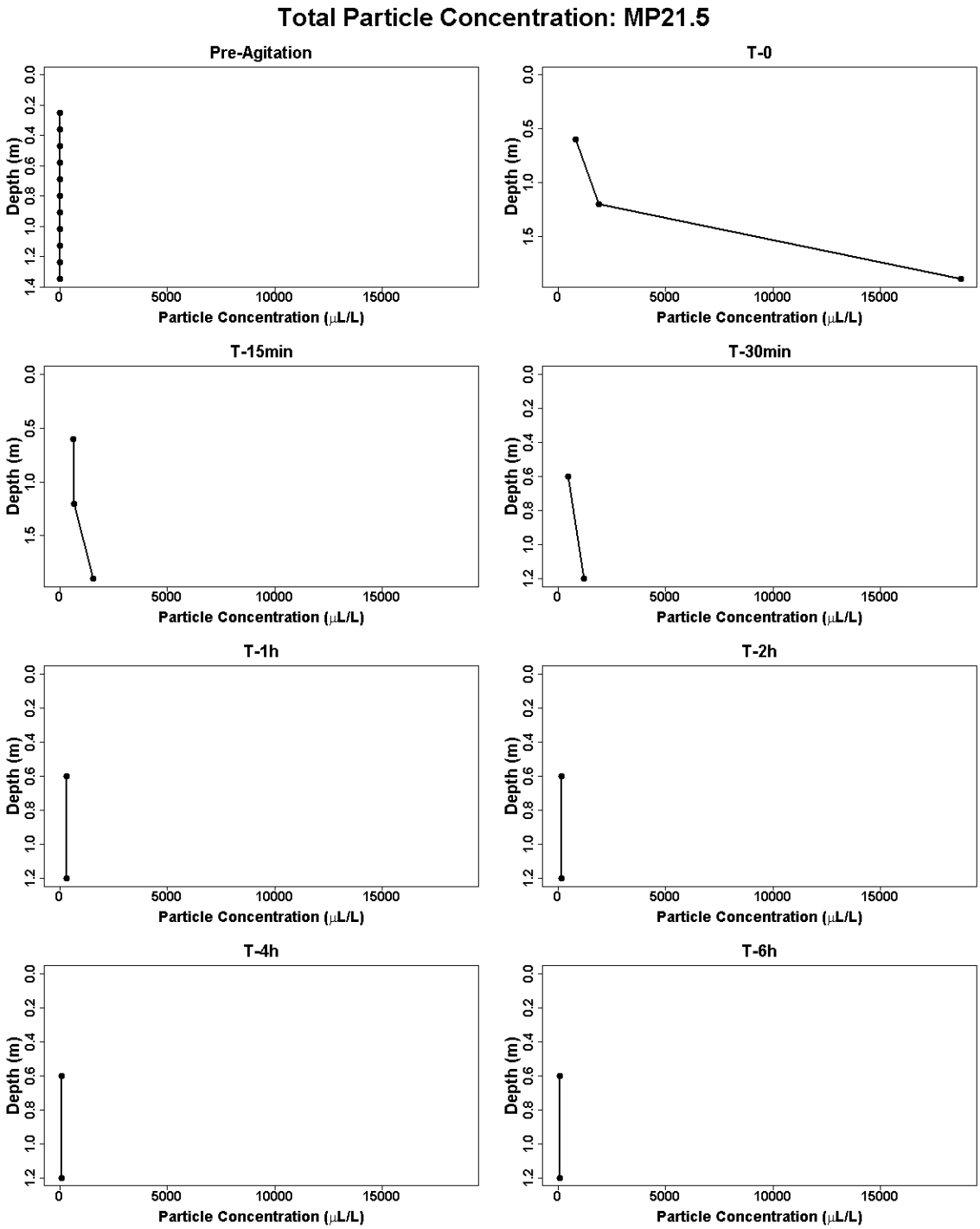


Figure 22: Total particle concentration data for station MP21.5.

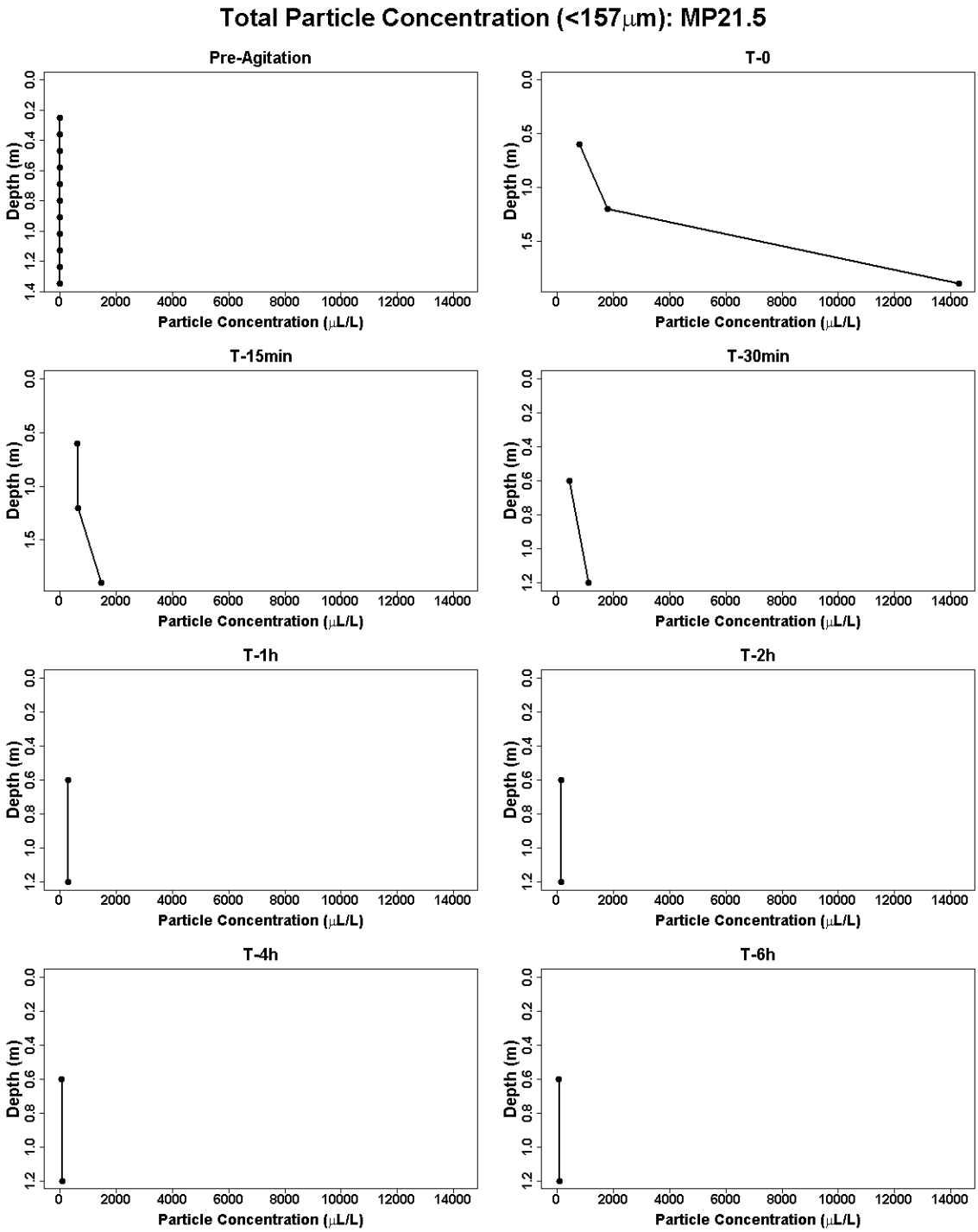


Figure 23: Total particle concentration (particle size <157 μm) data for station MP21.5.

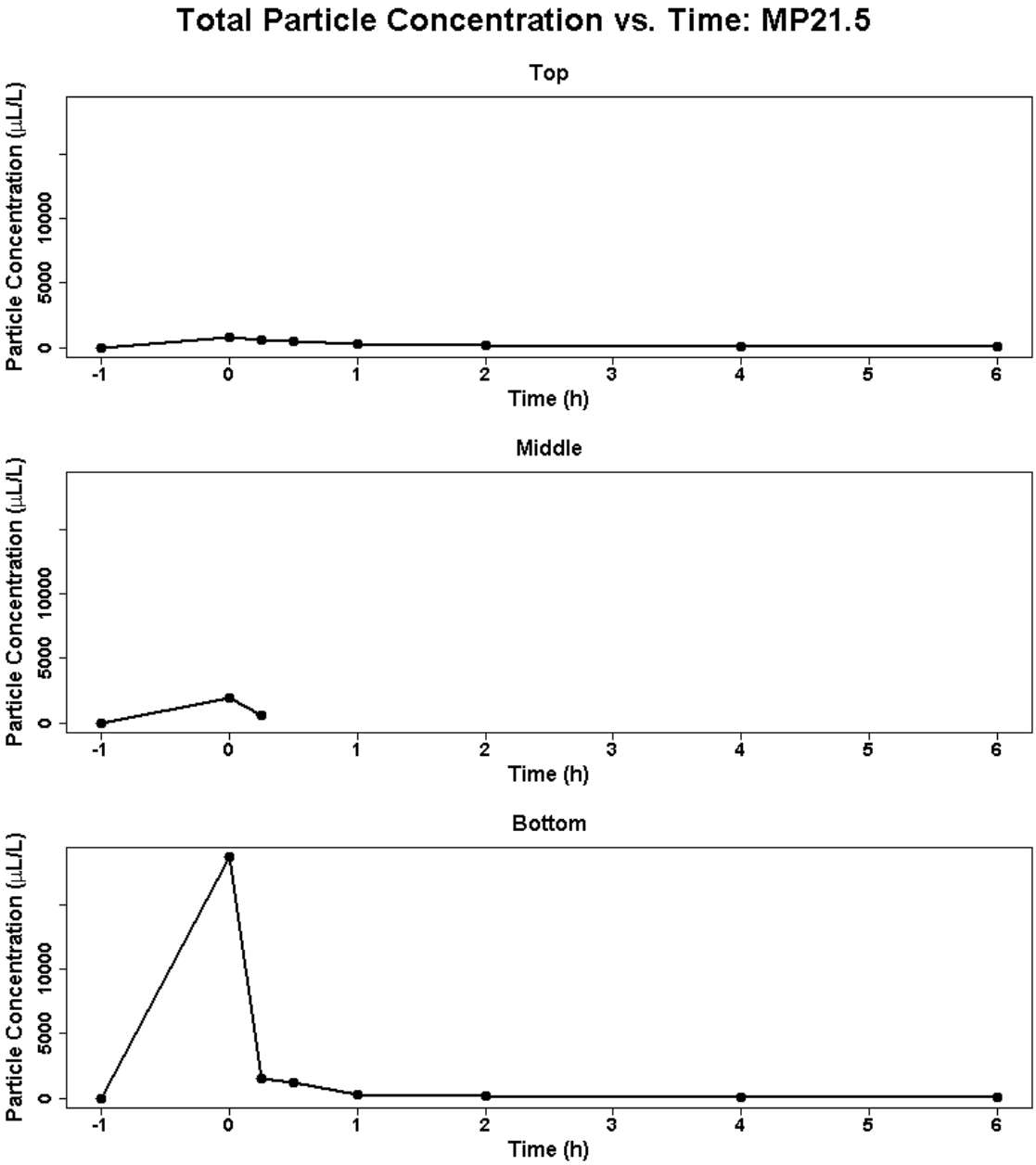


Figure 24: Total particle concentration vs. time for station MP21.5.

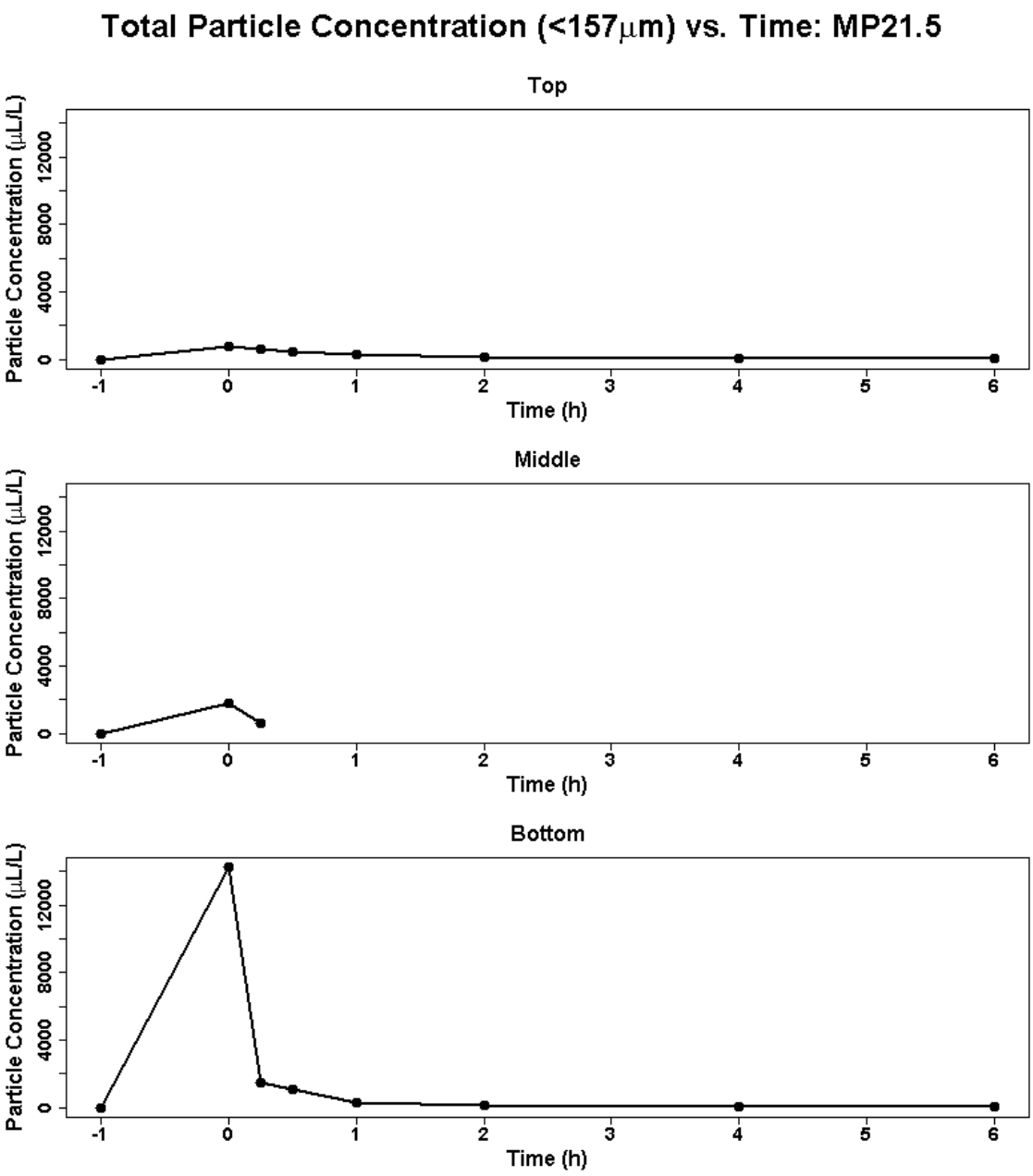


Figure 25: Total particle concentration (particle size <157 μm) vs. time for station MP21.5.

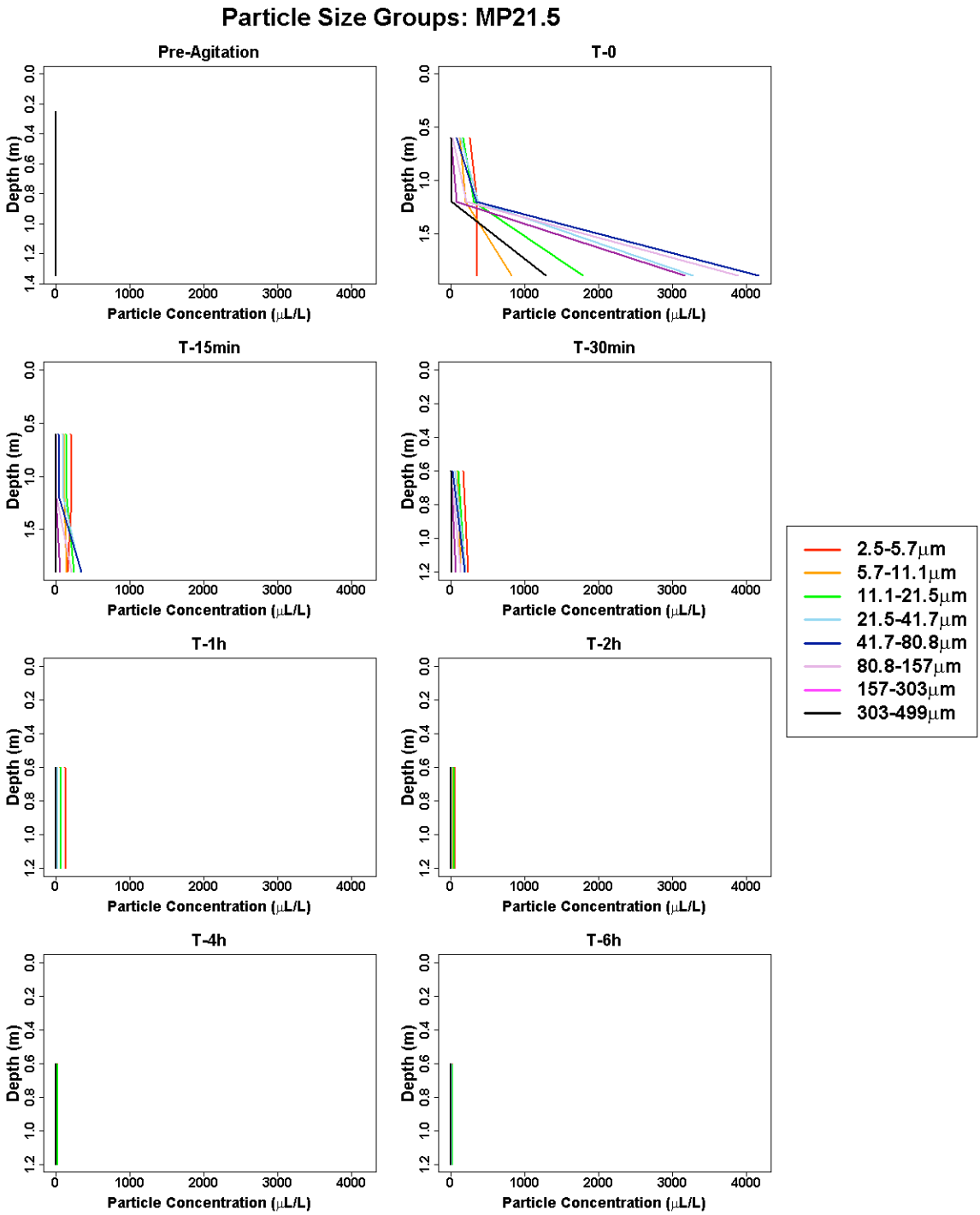


Figure 26: Particle Size Group concentrations for station MP21.5.

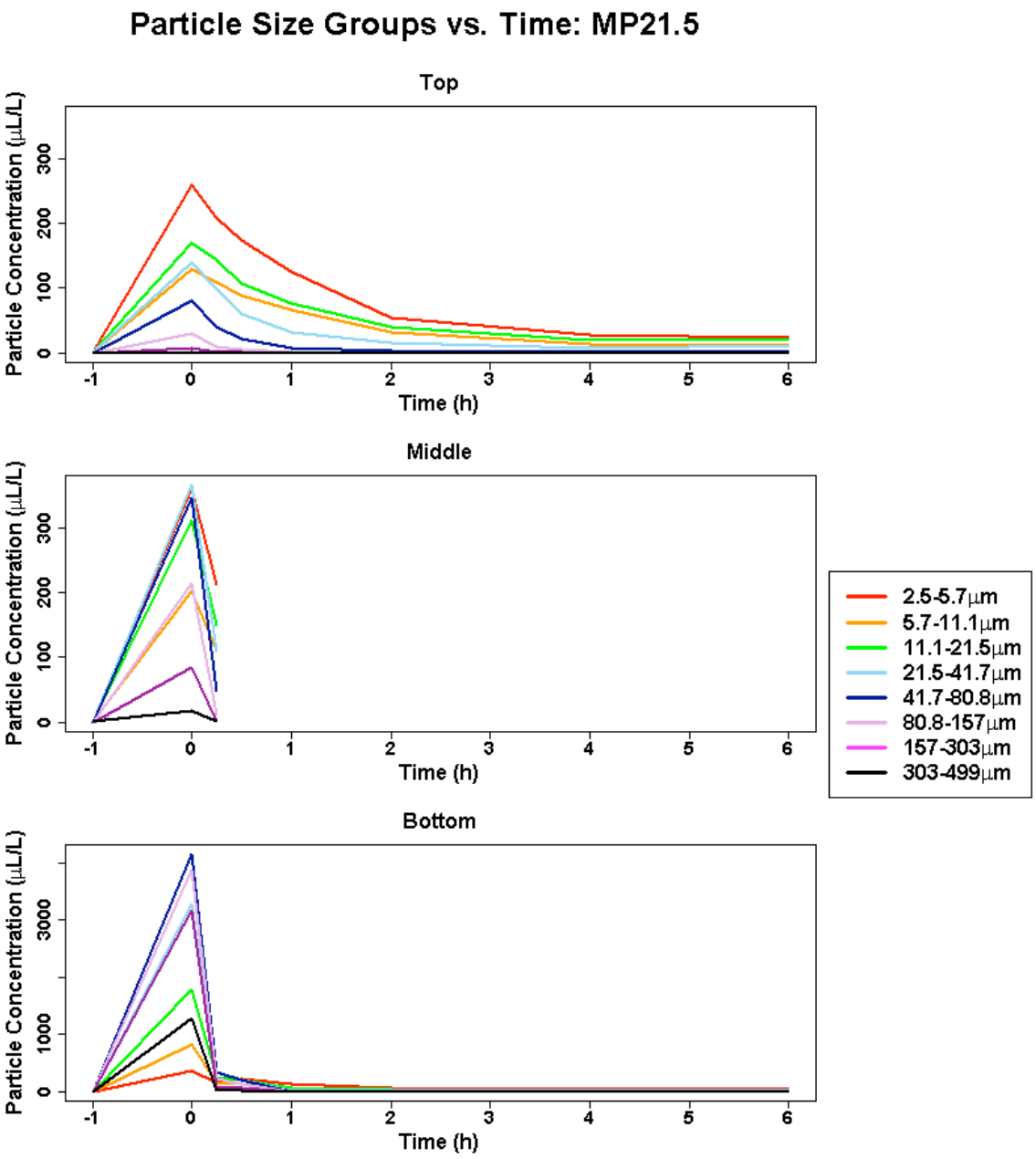


Figure 27: Particle Size Group concentrations vs. time for station MP21.5.

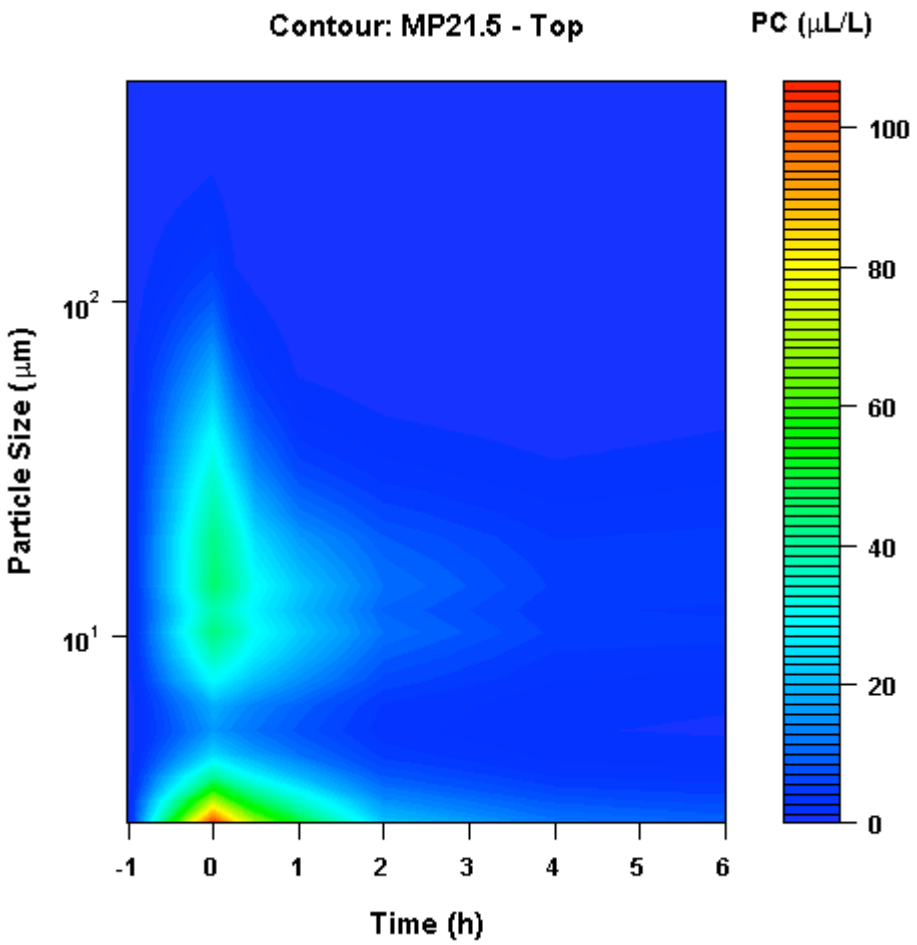


Figure 28: 3D contour plot for the MP21.5 'top' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

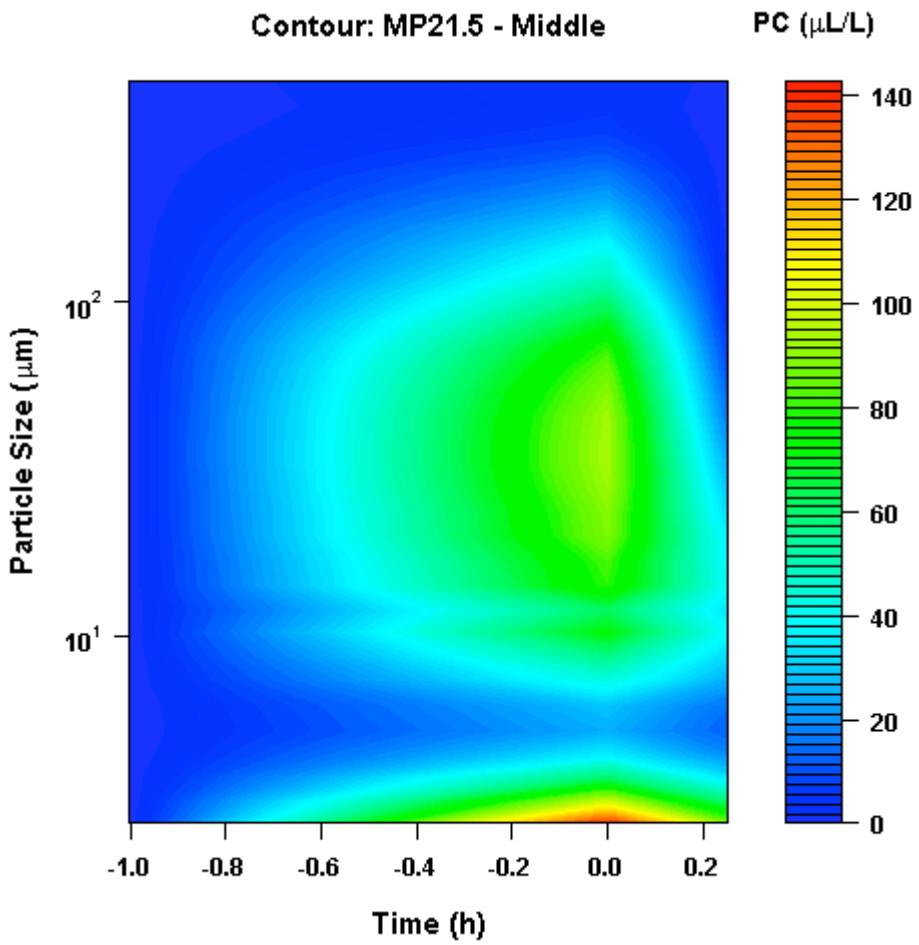


Figure 29: 3D contour plot for the MP21.5 ‘middle’ sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

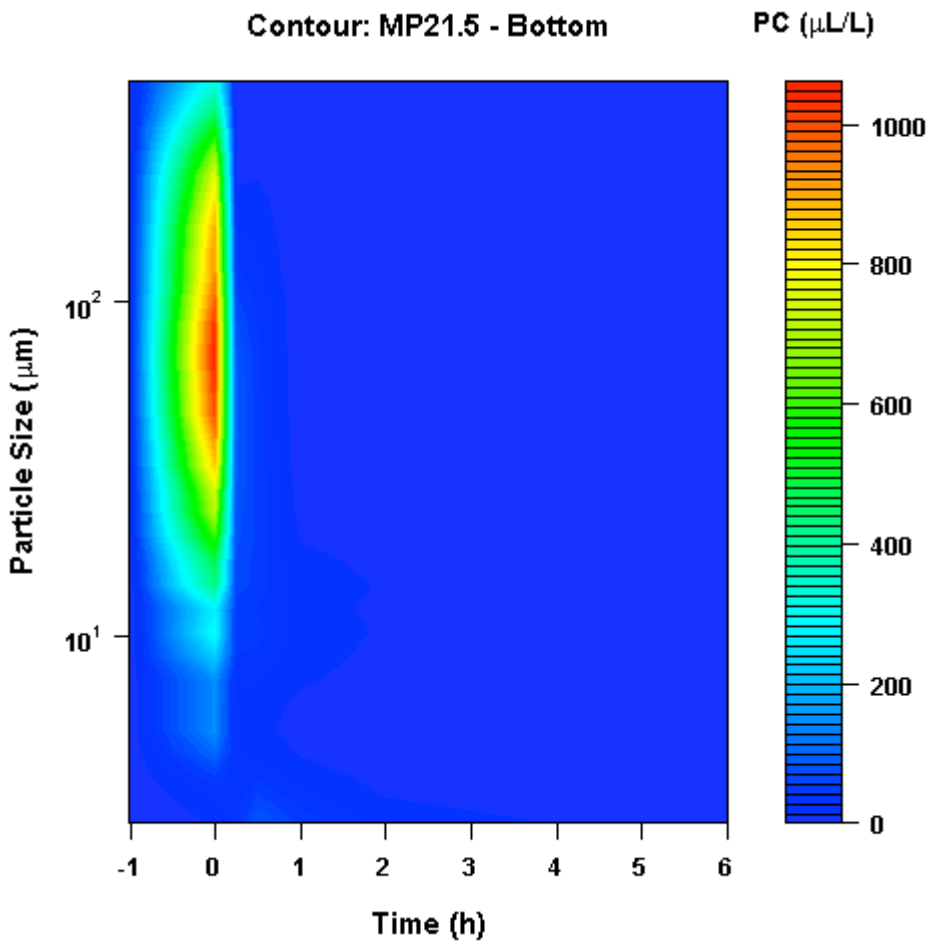


Figure 30: 3D contour plot for the MP21.5 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

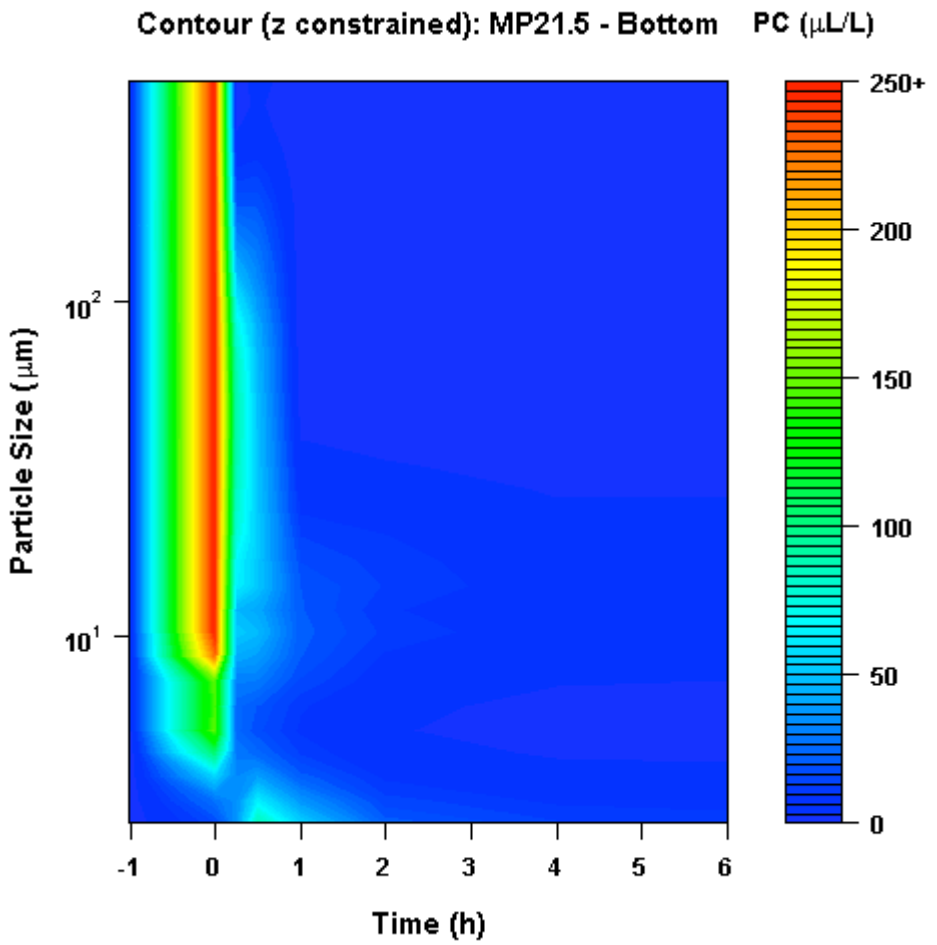


Figure 31: 3D contour plot for the MP21.5 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with constrained colored contours representing particle concentration (PC, z-axis). In this figure, the maximum z-value has been limited to 250 µL/L in order to achieve better resolution at low particle concentrations.

Delta Z

Total particle concentration data collected at station Delta Z (Figures 32-35) is fairly unique in that the post-agitation increase in SPM was much smaller than seen at other stations (TPC topping out at only ~500 µL/L), but this spike was observed almost uniformly throughout the water column. Settling trends remained consistent with other stations, with the majority of the post-agitation SPM settling by T-1h. PSG (Figures 36 and 37) and 3D contour plots (Figures 38-40) show a relatively high concentration of particles between 2.5 and 100 µm, with another peak at the large end of the analytical spectrum (>400 µm), although analytical results in this large size range are unpredictable due to the dilution process. Size fractionated settling also seems to have occurred to a significant degree during this experiment as elevated small particle concentrations (<30 µm) were detected over time. A constrained z-axis contour plot was not generated for this station as maximum particle concentrations did not exceed 250µL/L.

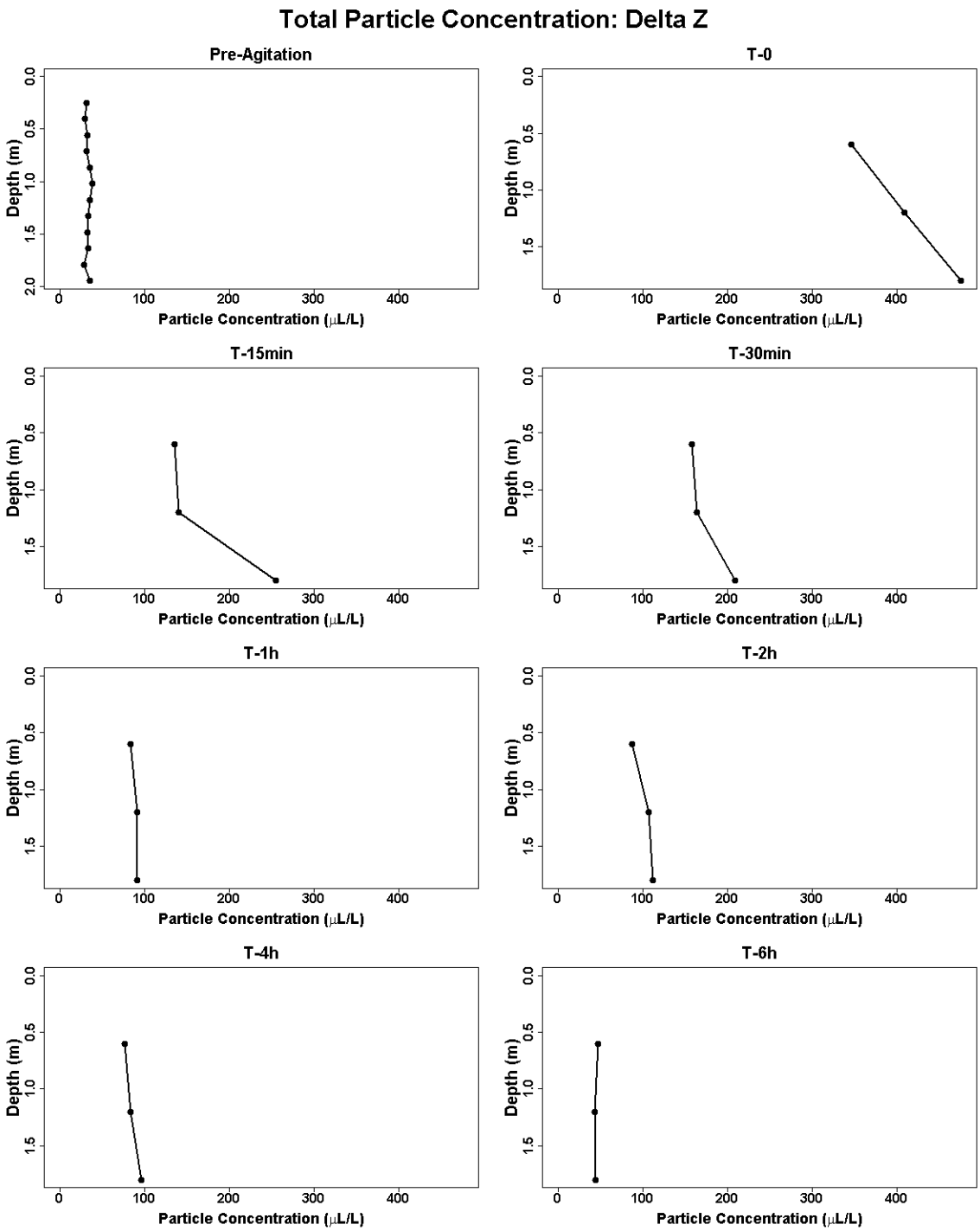


Figure 32: Total particle concentration data for station Delta Z.

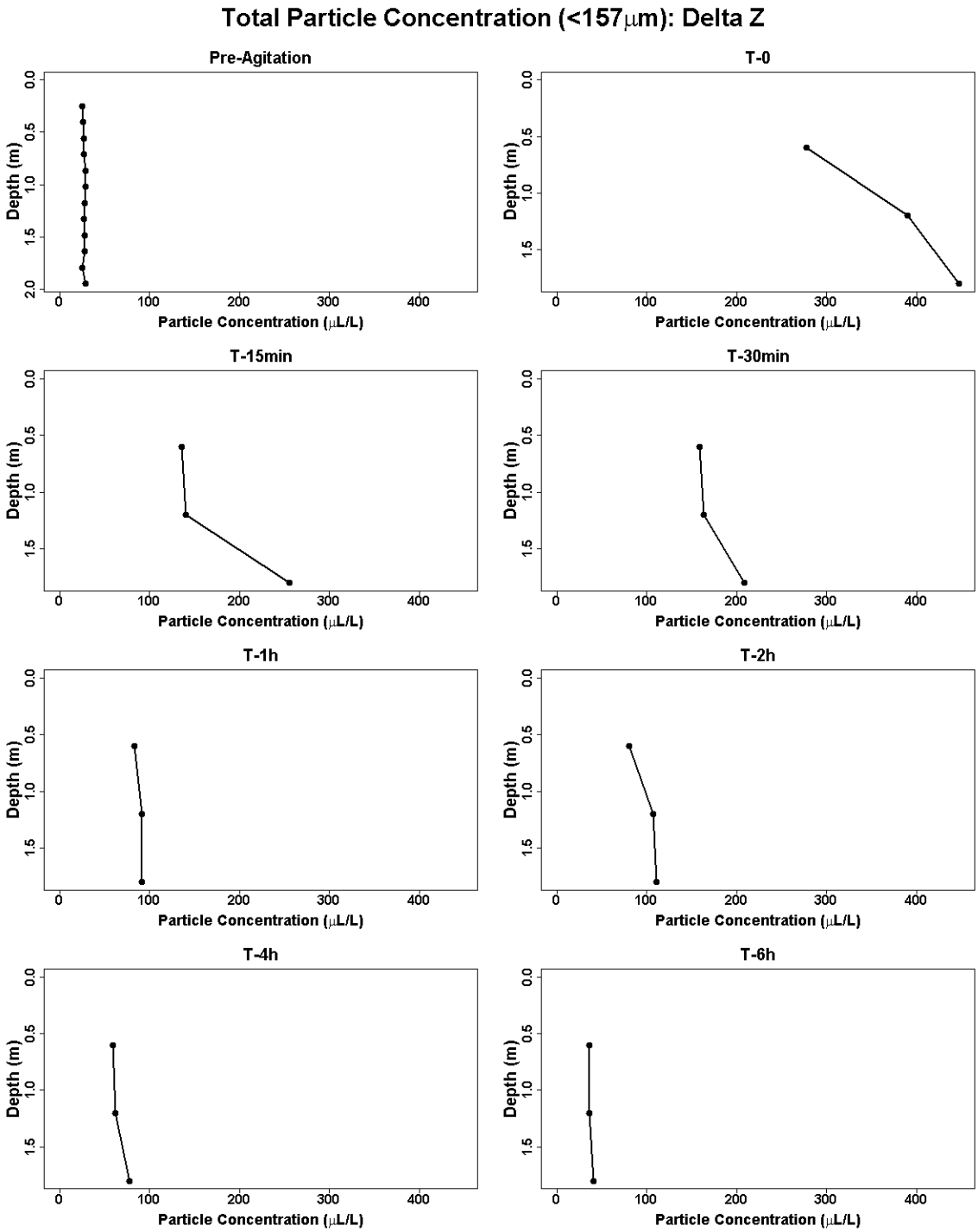


Figure 33: Total particle concentration (particle size <157 μm) data for station Delta Z.

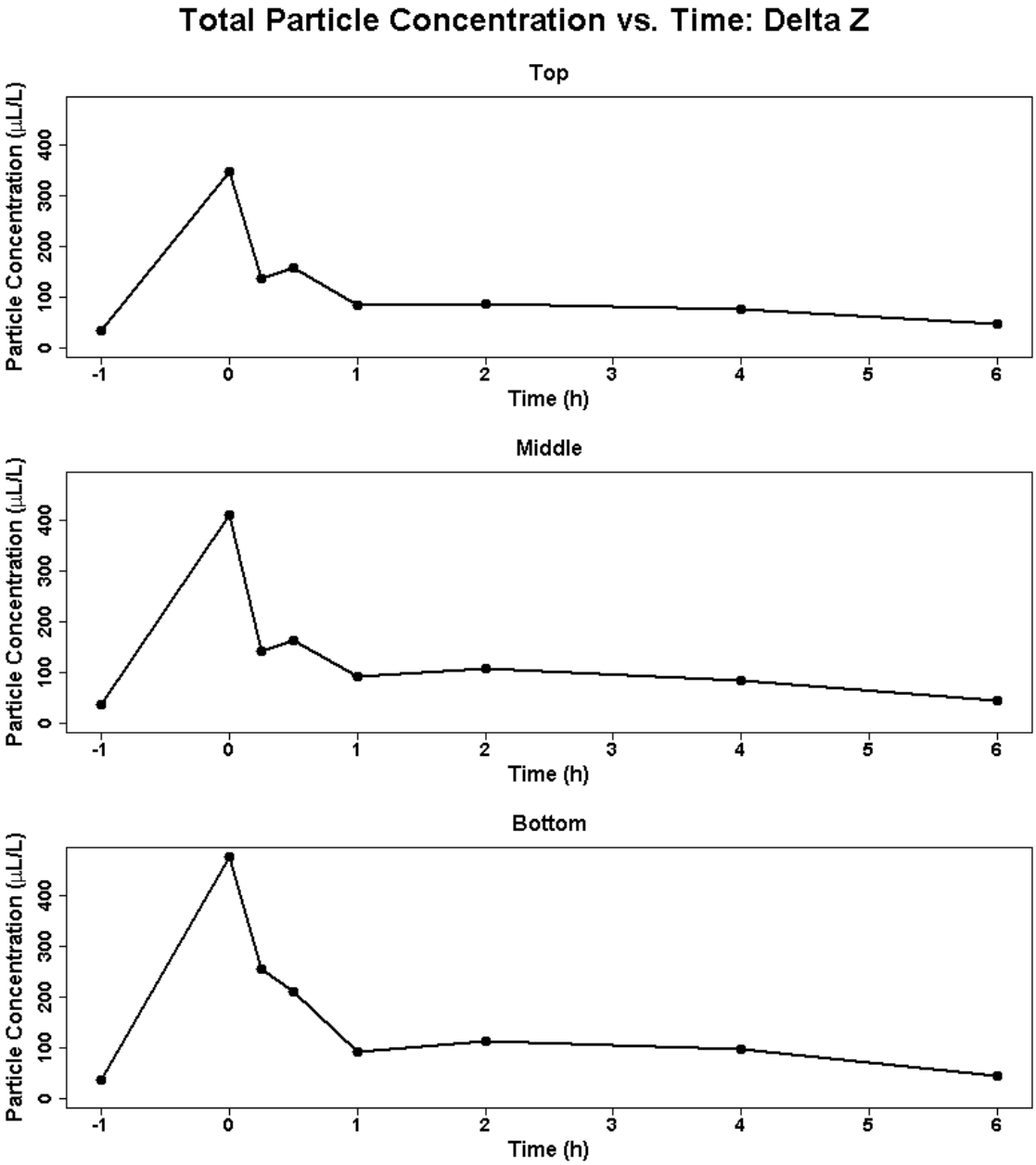


Figure 34: Total particle concentration vs. time for station Delta Z.

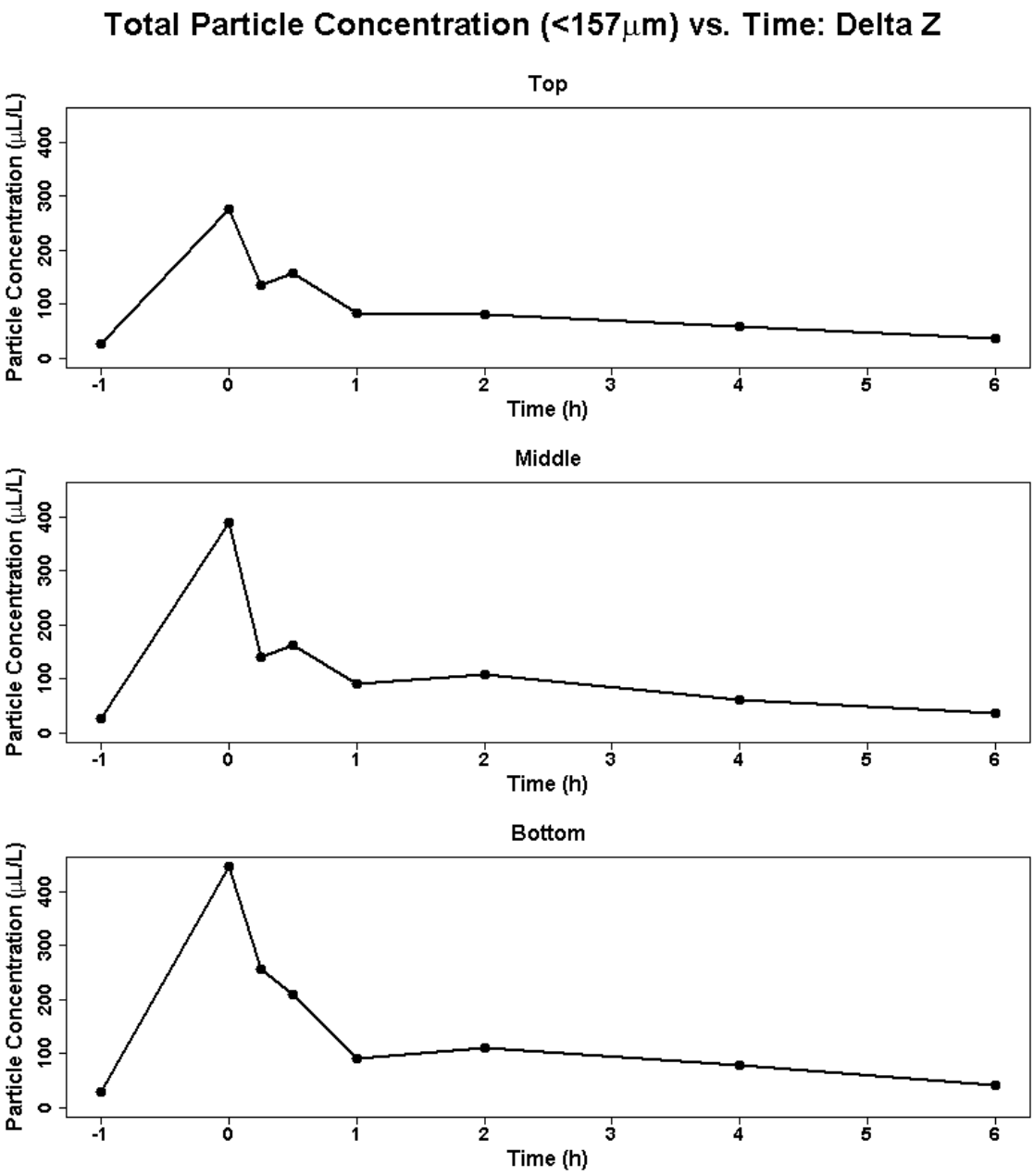


Figure 35: Total particle concentration (particle size <157 μm) vs. time for station Delta Z.

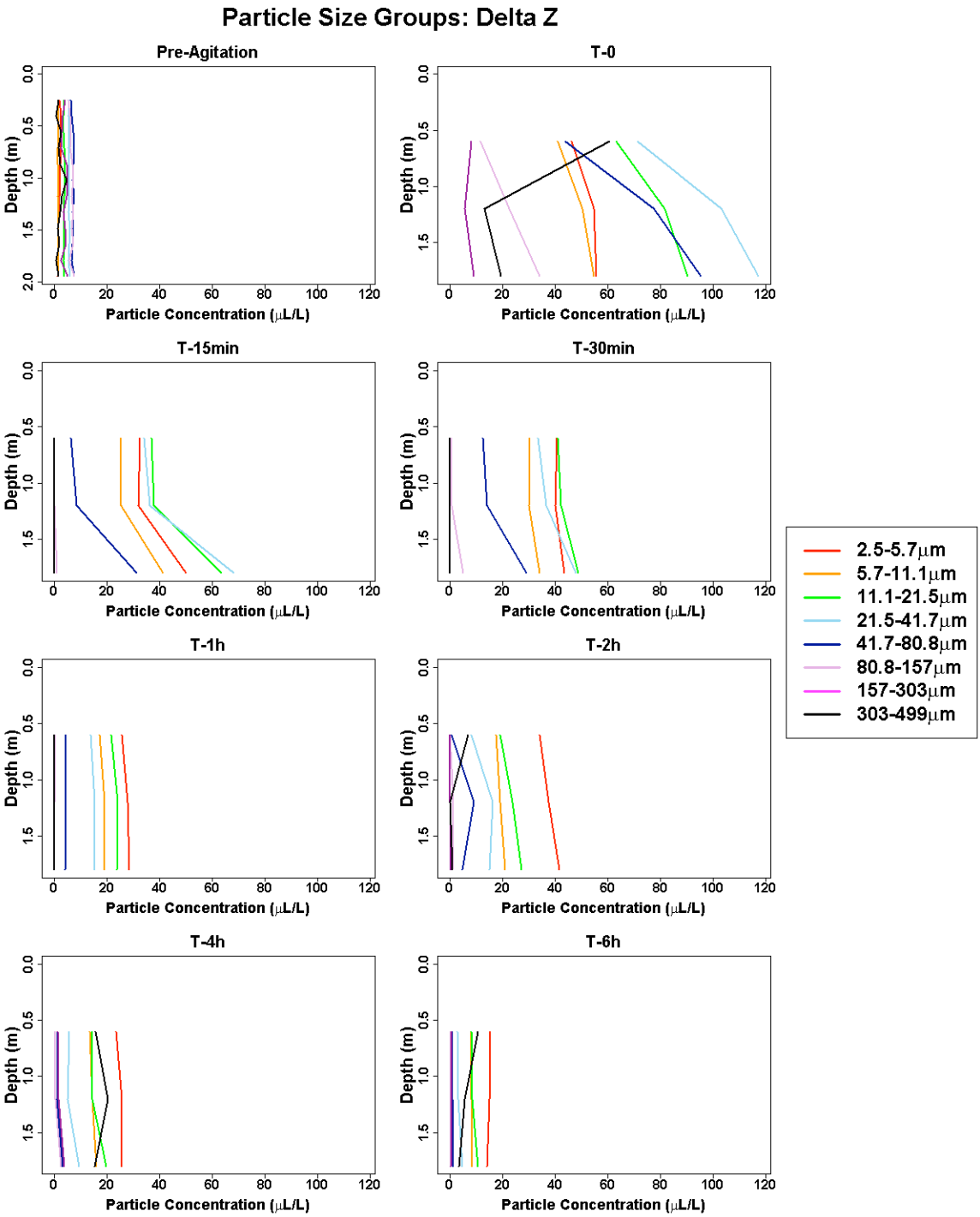


Figure 36: Particle Size Group concentrations for station Delta Z.

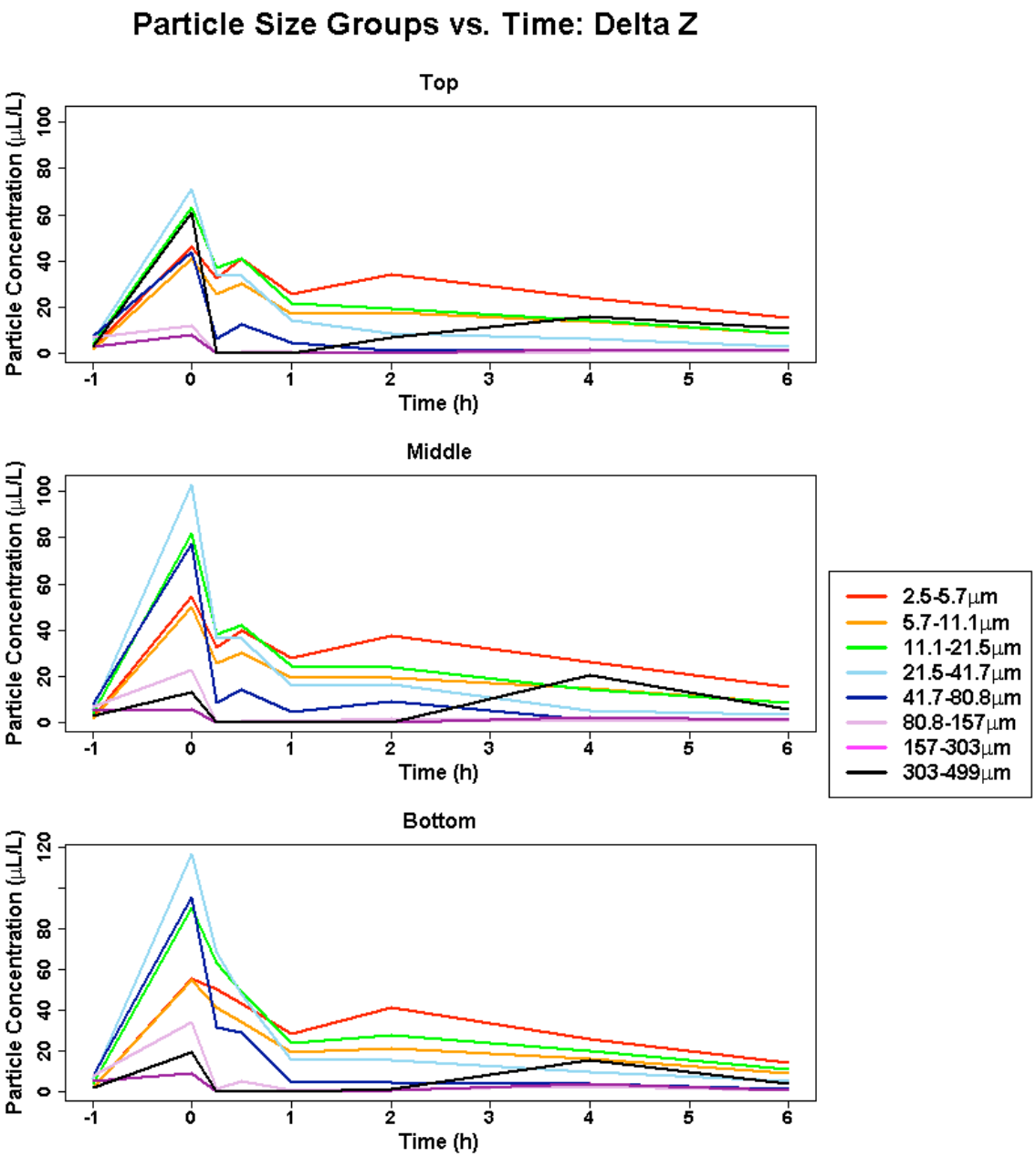


Figure 37: Particle Size Group concentrations vs. time for station Delta Z.

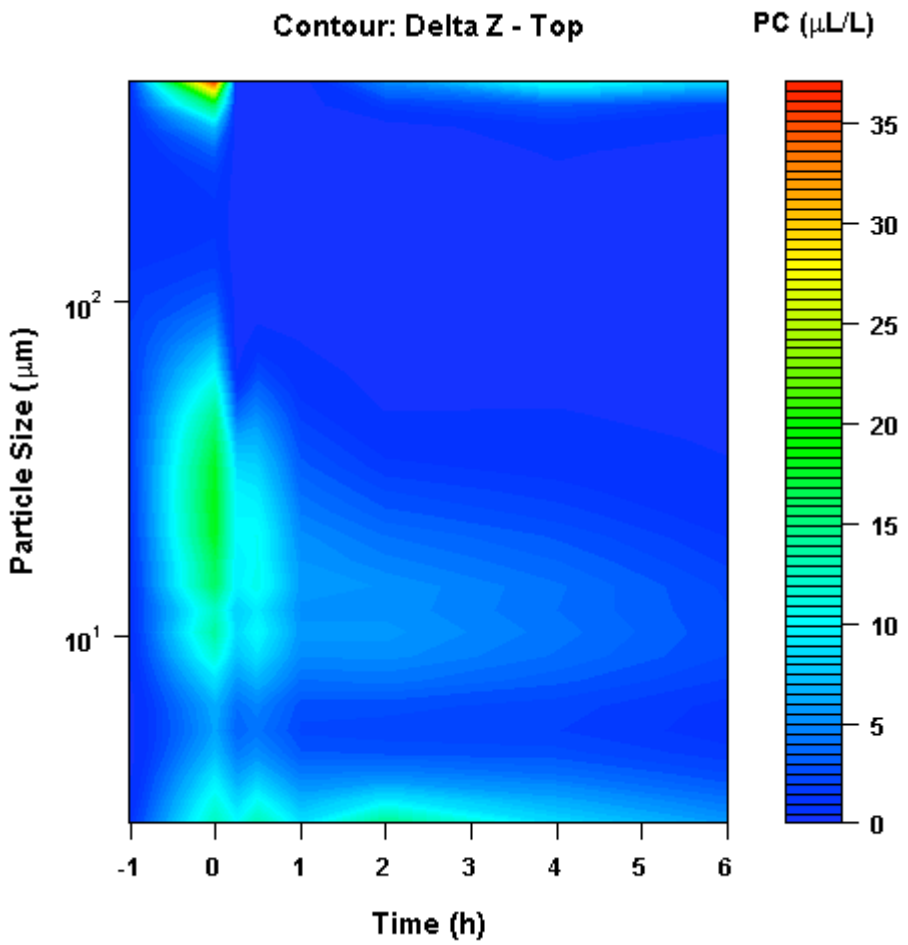


Figure 38: 3D contour plot for the Delta Z 'top' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

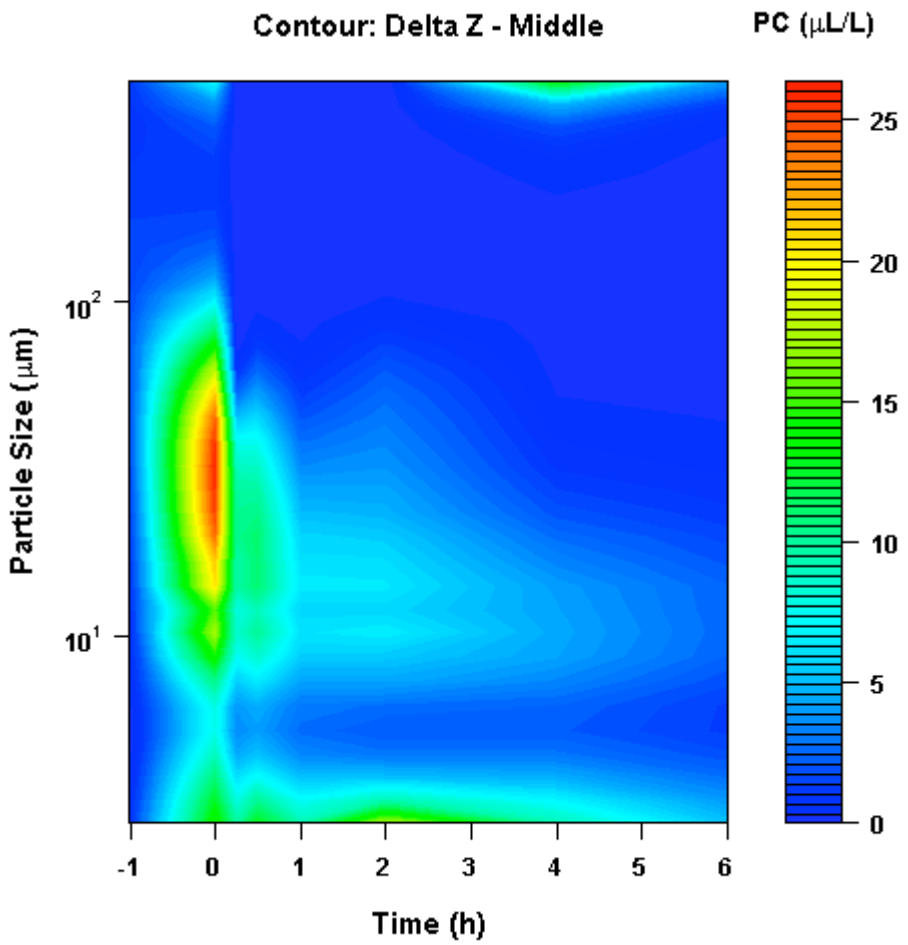


Figure 39: 3D contour plot for the Delta Z 'middle' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

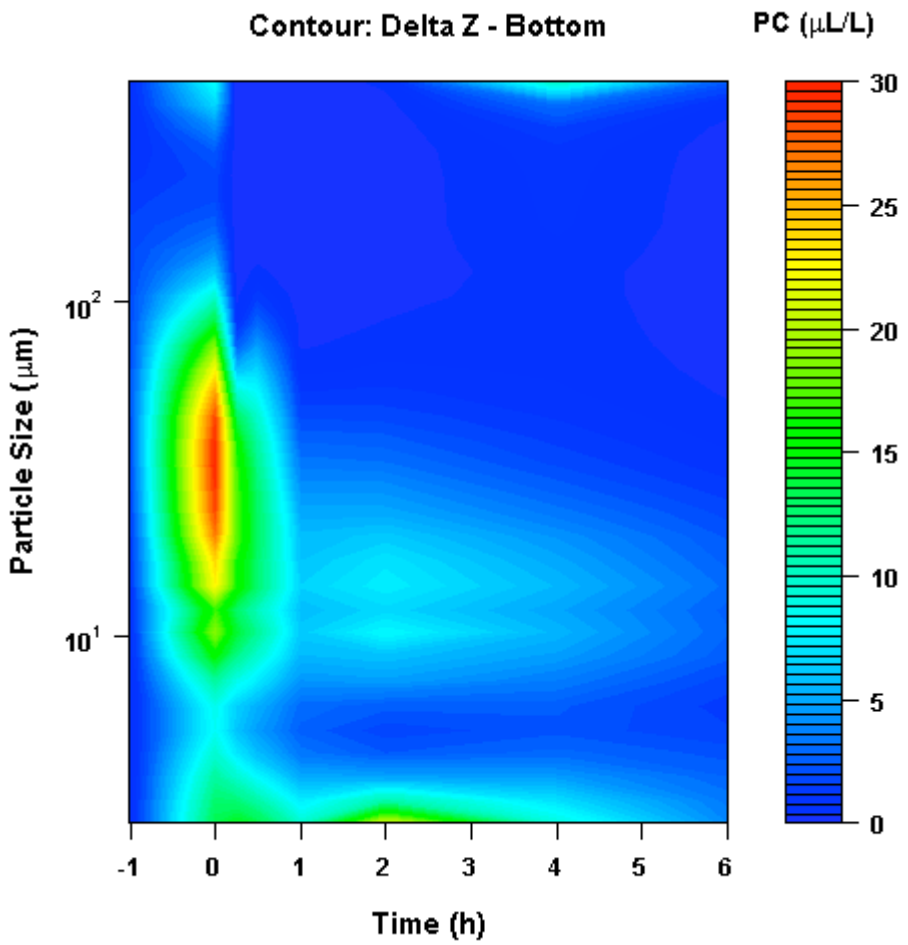


Figure 40: 3D contour plot for the Delta Z 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

MP14.8

TPC plots for station MP14.8 (Figures 41-44) show a spike in SPM following agitation and significant settling by T-15min. Similar to the case with station MP21.5, PSG (Figures 45 and 46) and contour plots (Figures 47-50) show that very small particles (2.5-21.5 μm) dominate the SPM composition in the middle and upper section of the water column, but are not as significant near the bottom. Looking at the larger particle size range, most of the larger SPM detected at MP14.8 was between 40 and 120 μm in diameter, with the larger particles in that range becoming more prevalent with depth.

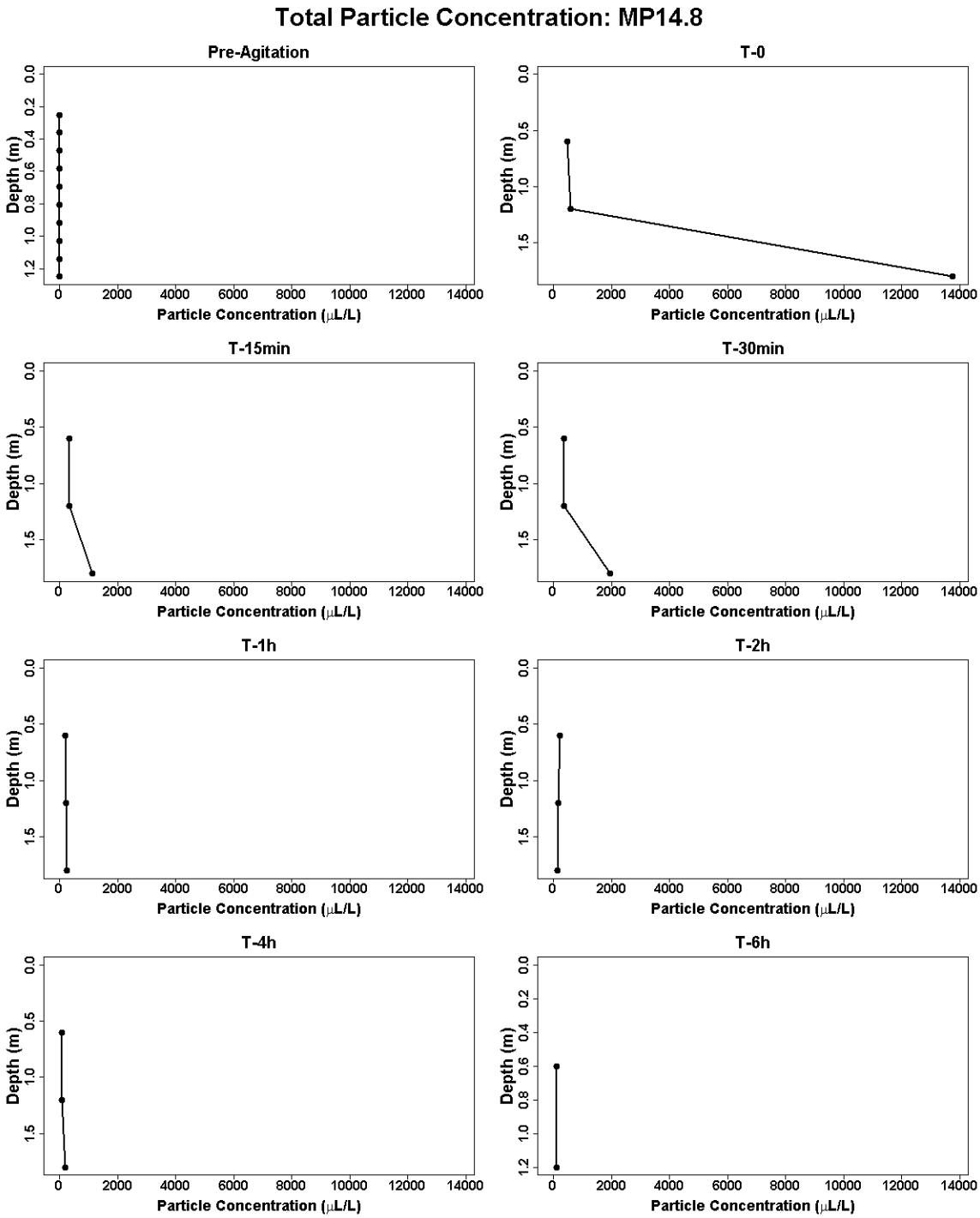


Figure 41: Total particle concentration data for station MP14.8.

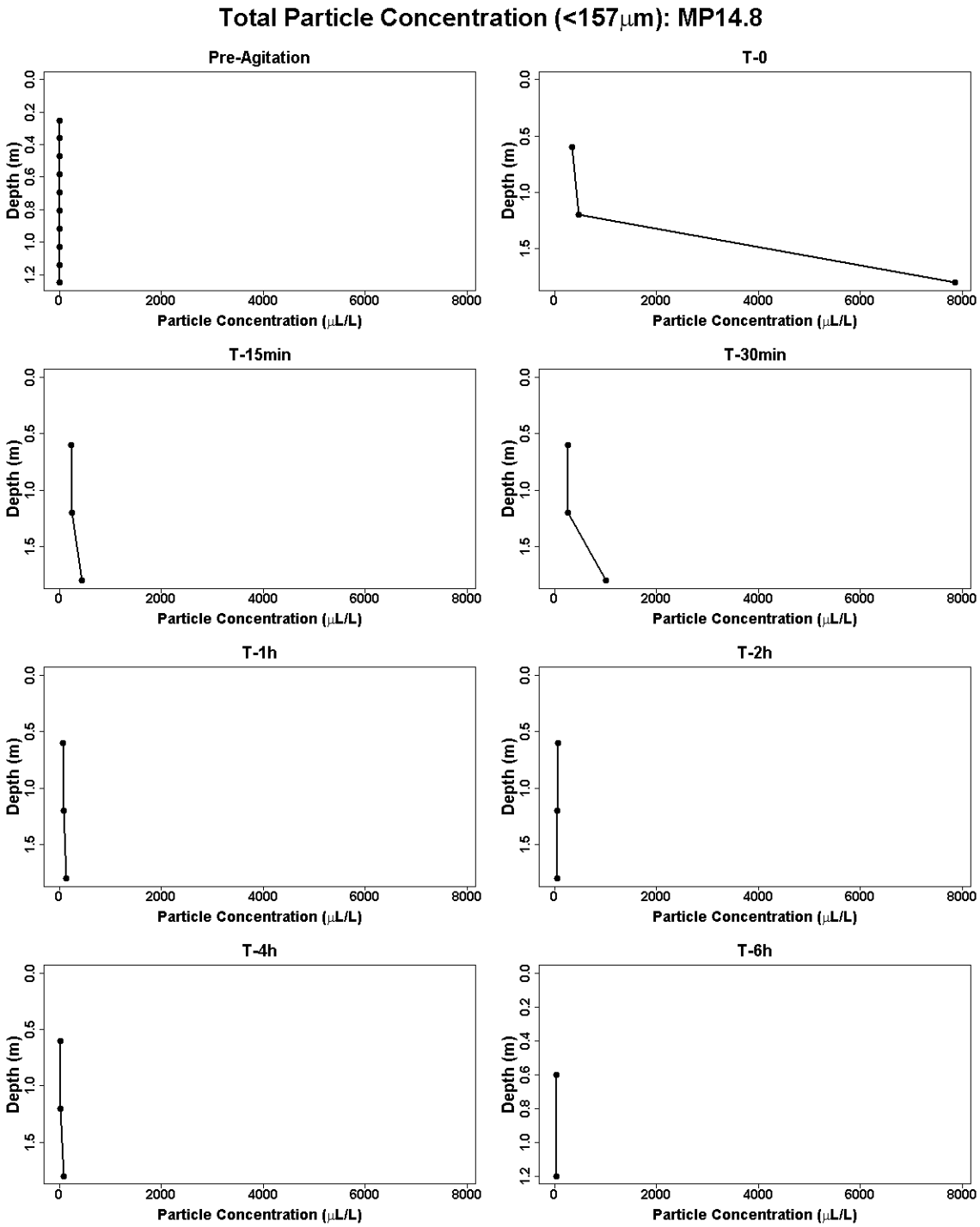


Figure 42: Total particle concentration (particle size <157 μm) data for station MP14.8.

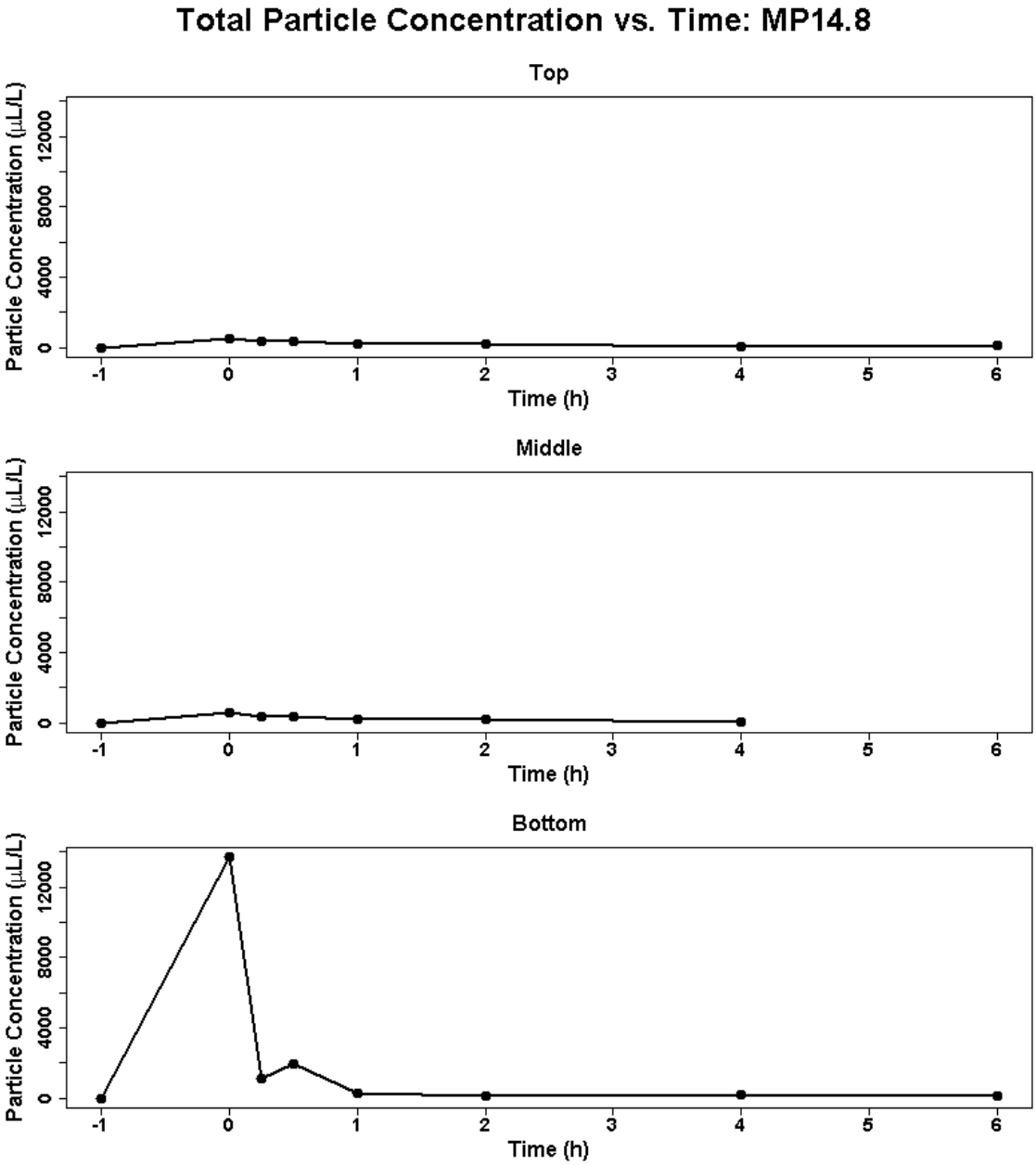


Figure 43: Total particle concentration vs. time for station MP14.8.

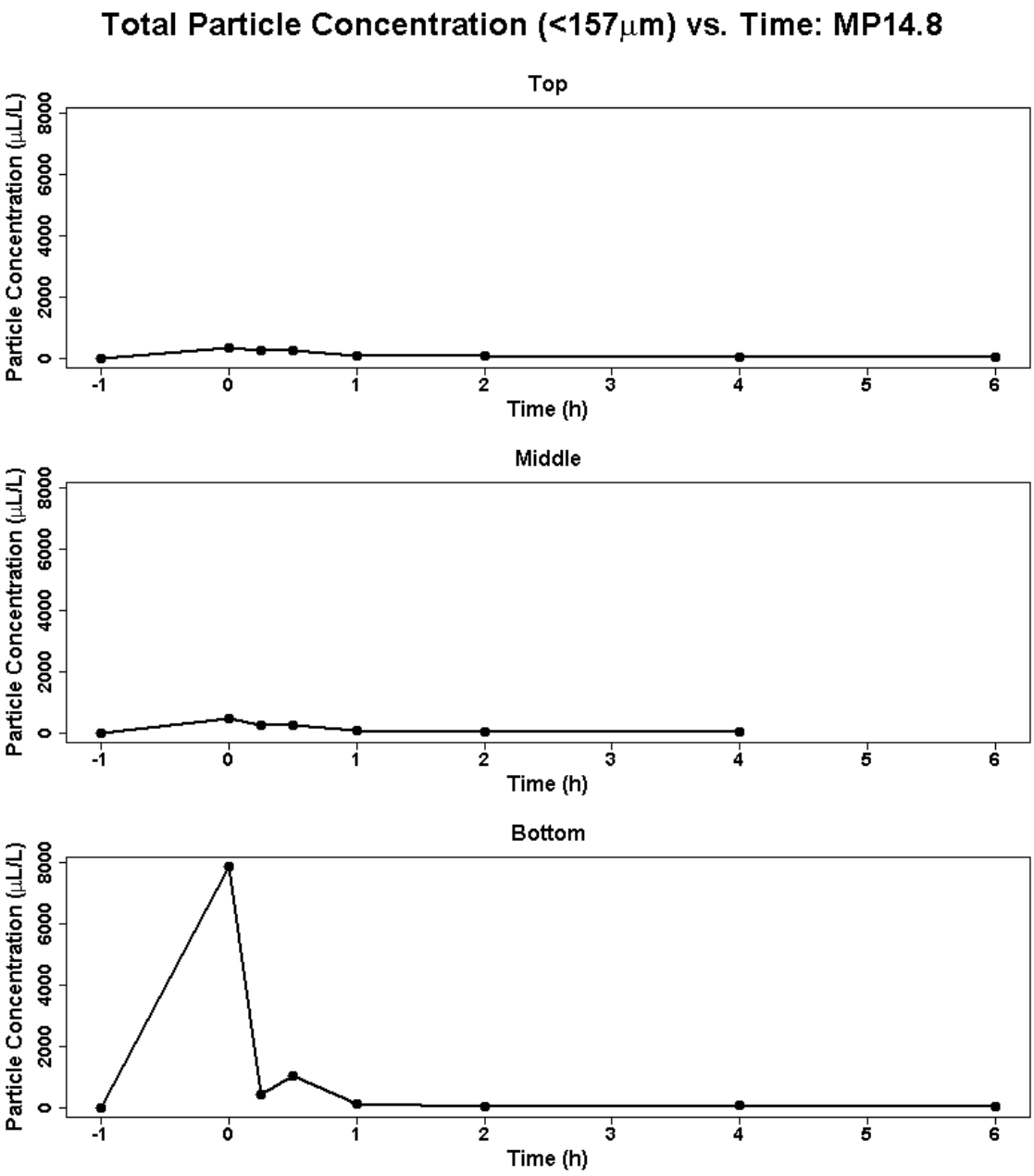


Figure 44: Total particle concentration (particle size <157 μm) vs. time for station MP14.8.

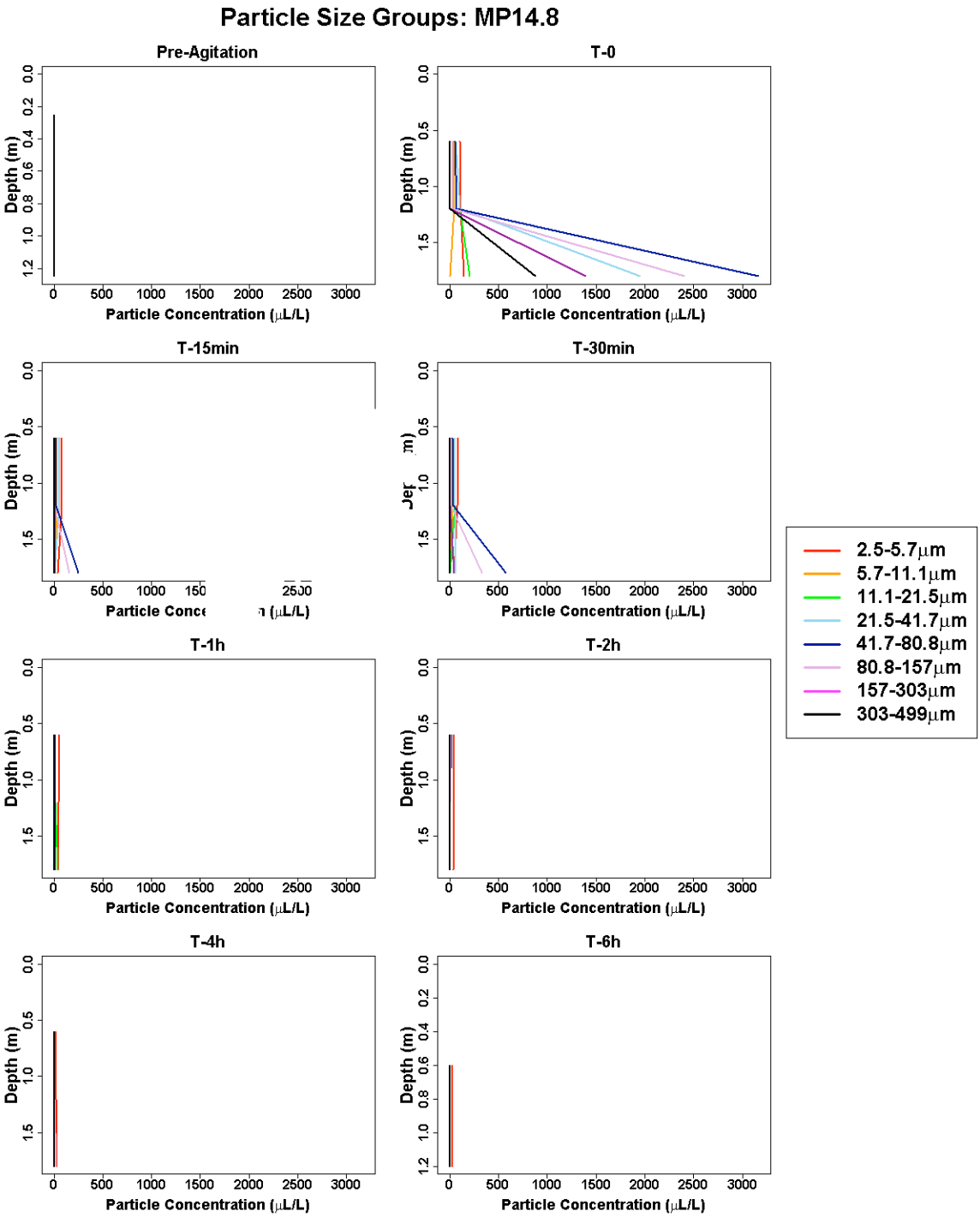


Figure 45: Particle Size Group concentrations for station MP14.8.

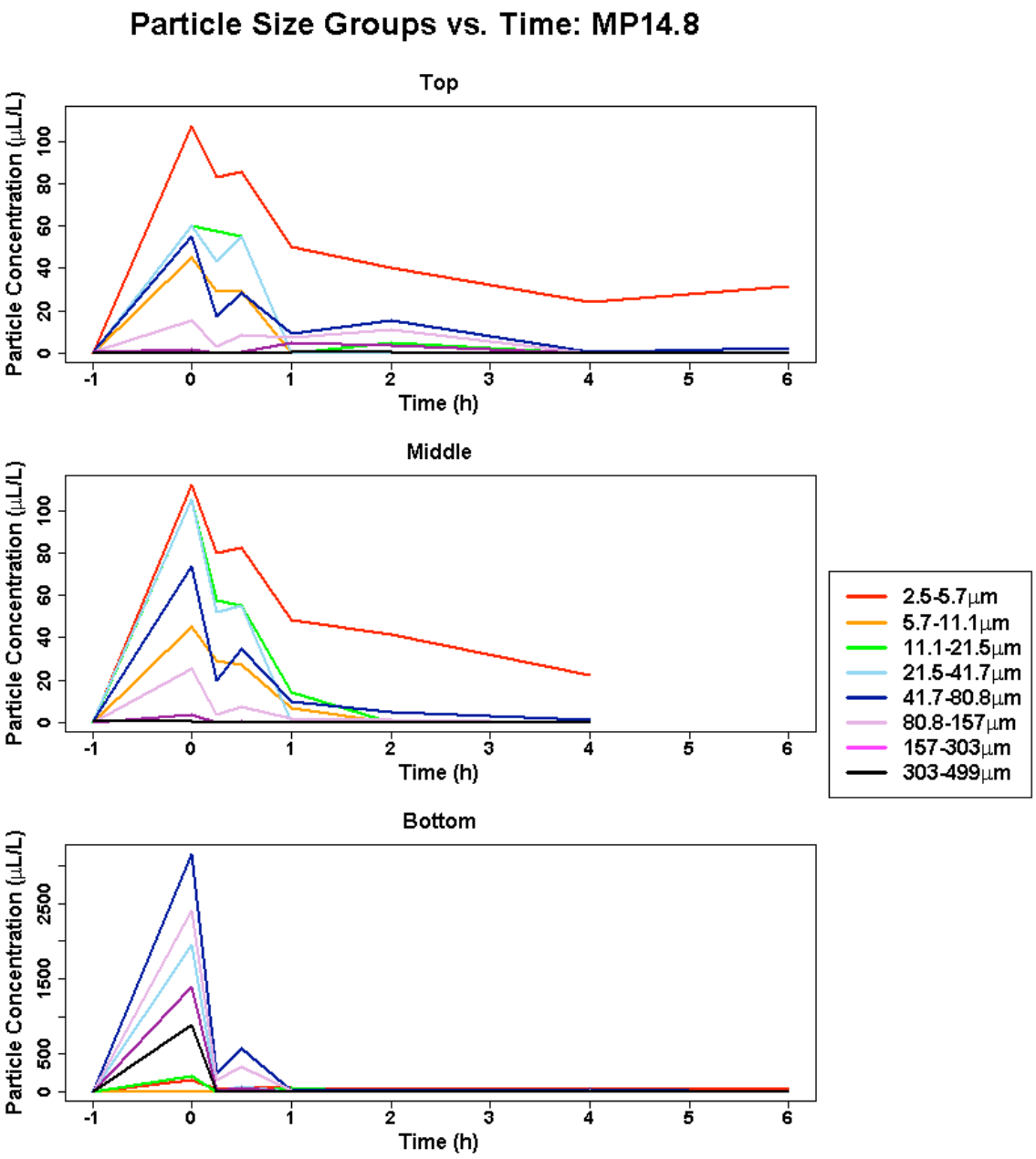


Figure 46: Particle Size Group concentrations vs. time for station MP14.8.

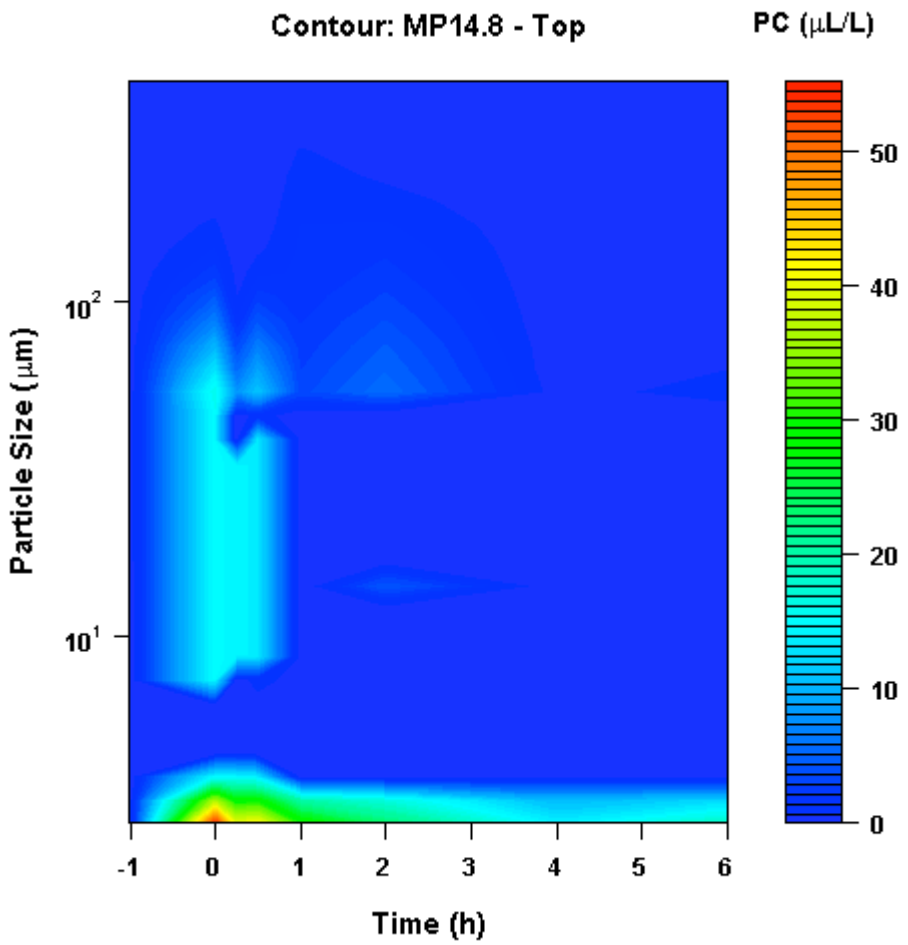


Figure 47: 3D contour plot for the MP14.8 'top' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

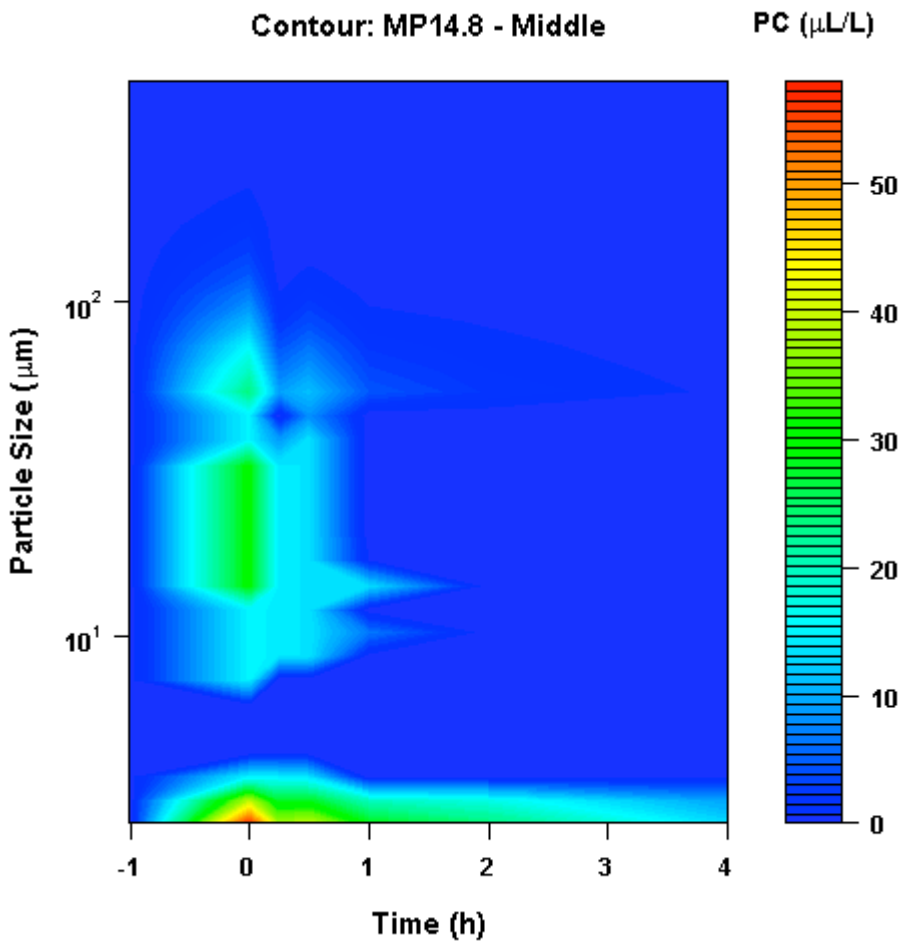


Figure 48: 3D contour plot for the MP14.8 ‘middle’ sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

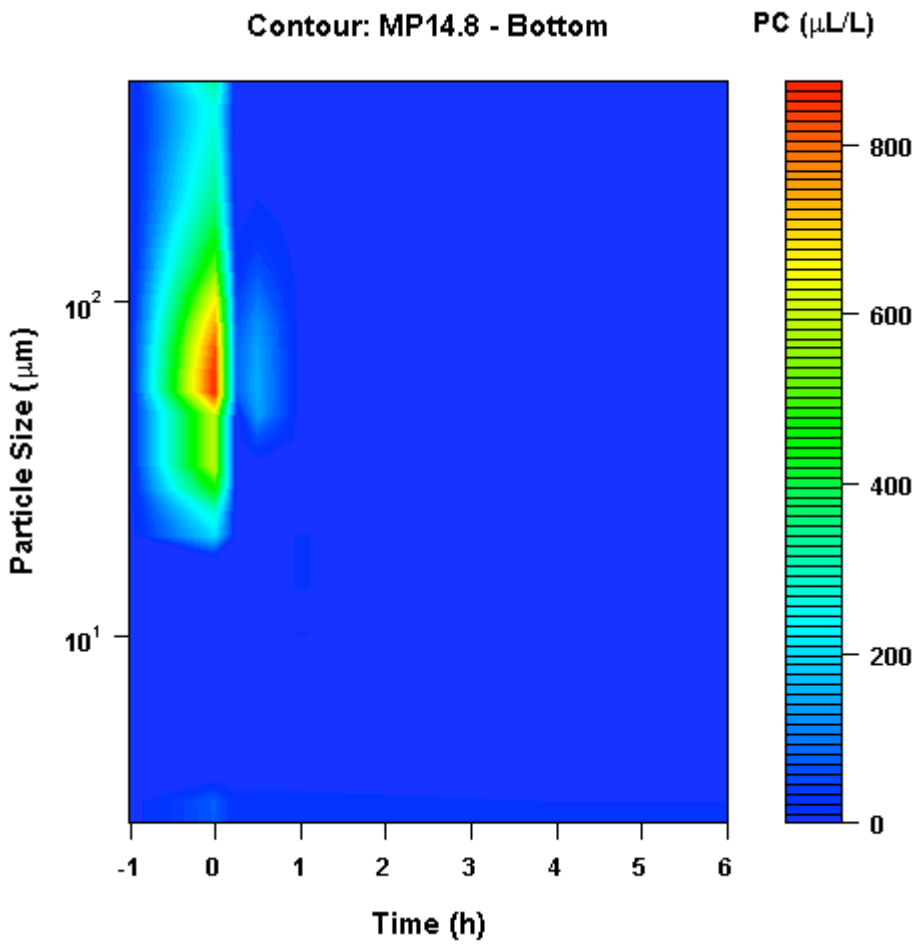


Figure 49: 3D contour plot for the MP14.8 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

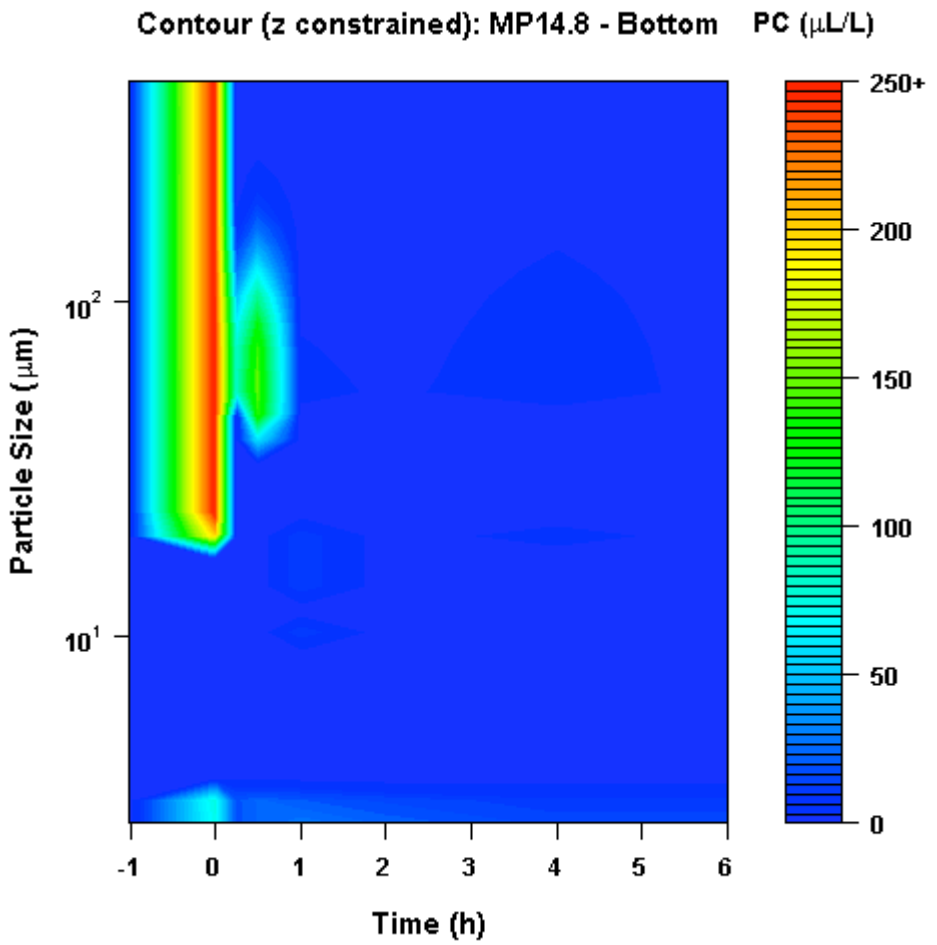


Figure 50: 3D contour plot for the MP14.8 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with constrained colored contours representing particle concentration (PC, z-axis). In this figure, the maximum z-value has been limited to 250 µL/L in order to achieve better resolution at low particle concentrations.

MP5.5

TPC concentrations found during the experiment at MP5.5 show a spike in SPM following agitation and significant settling occurring by T-15min (Figures 51-54). PSG (Figures 55 and 56) and contour plots (Figures 57-60) show that SPM in the top and middle layers of the water column was dominated by particles in the 2.5-5 μm and 9-100 μm ranges, while particles in the 80-300 μm range were most prevalent at the bottom. Once again, small particles (<30 μm) seem to persist in the water column longer than the larger ones.

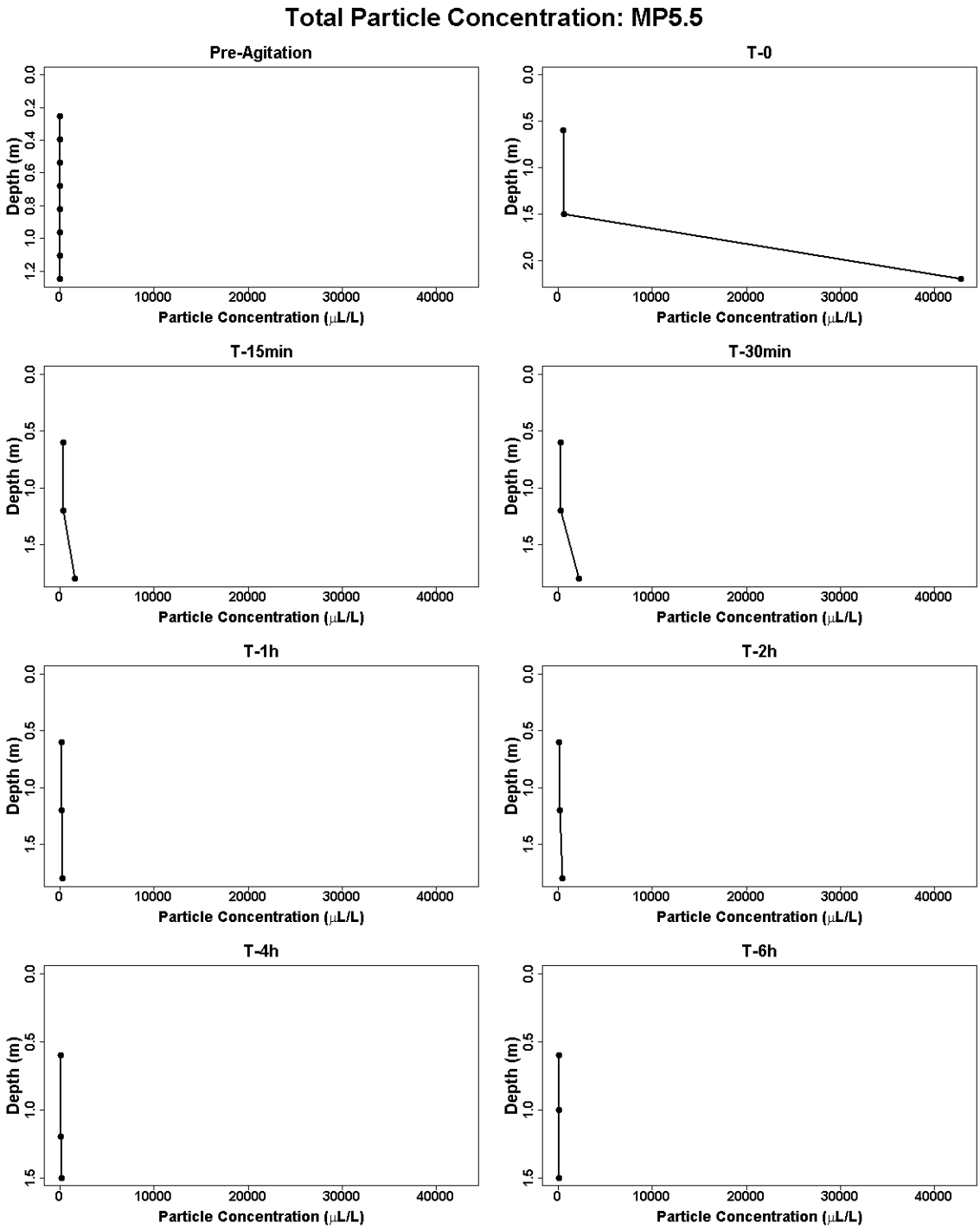


Figure 51: Total particle concentration data for station MP5.5.

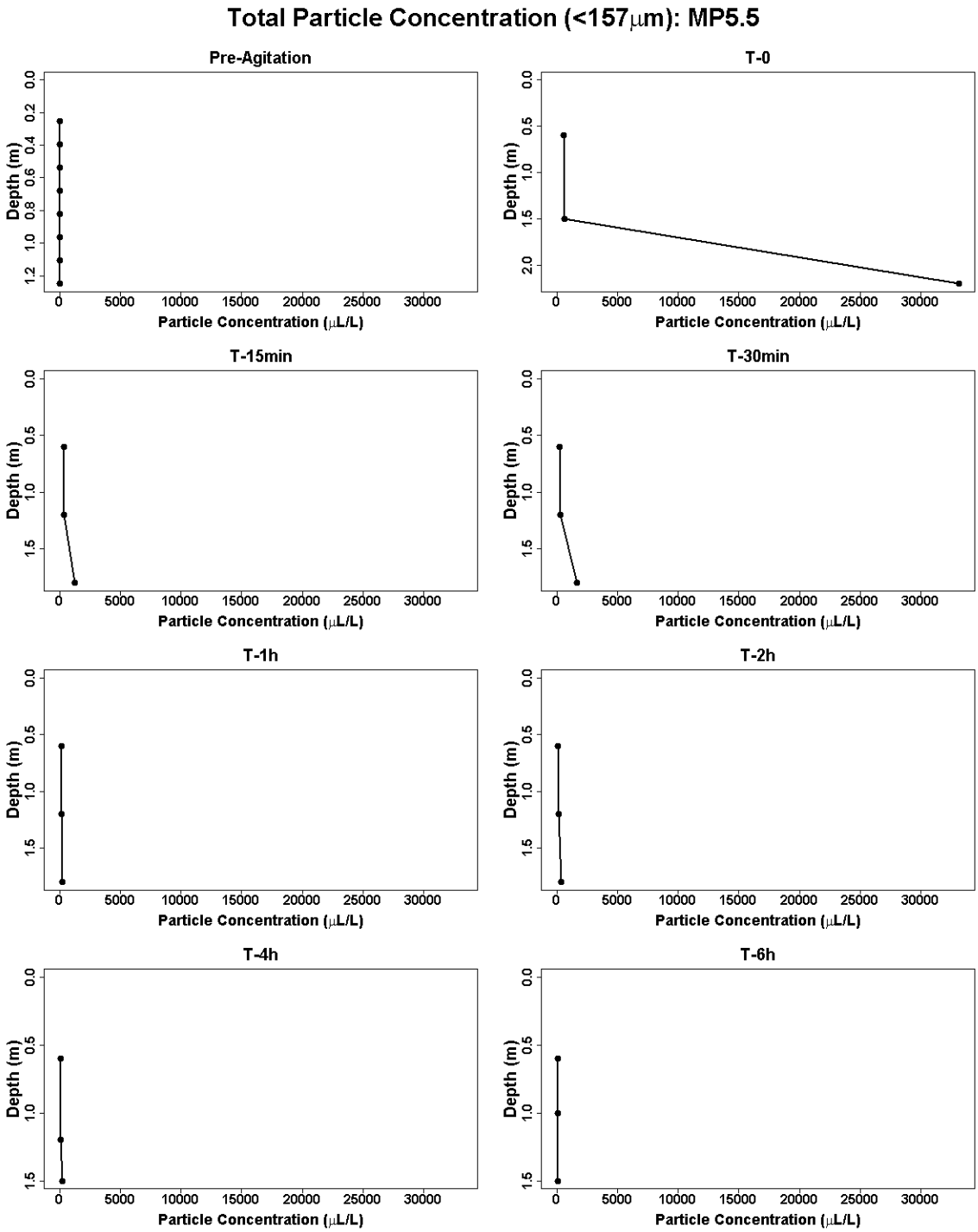


Figure 52: Total particle concentration (particle size <157 μm) data for station MP5.5.

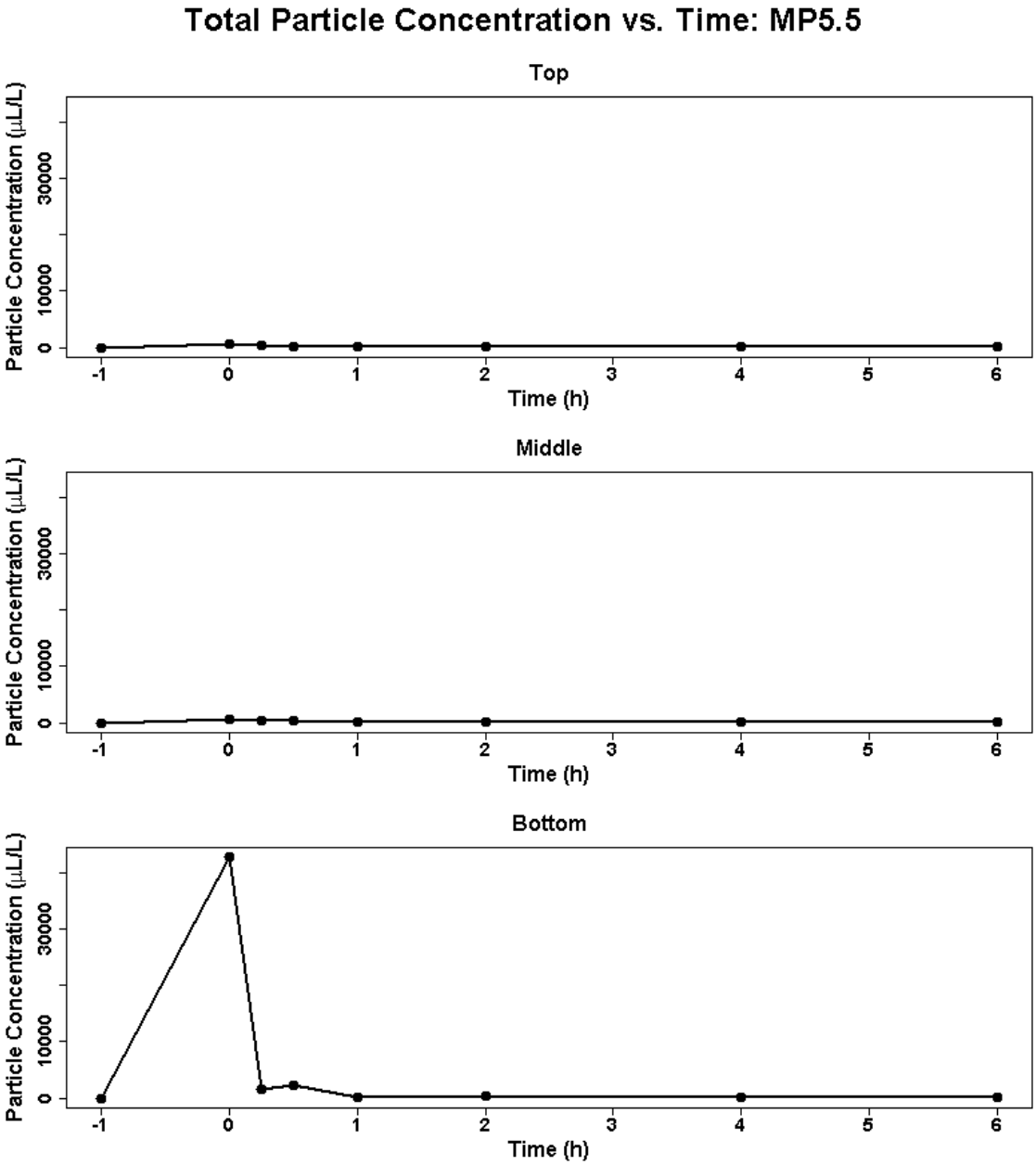


Figure 53: Total particle concentration vs. time for station MP5.5.

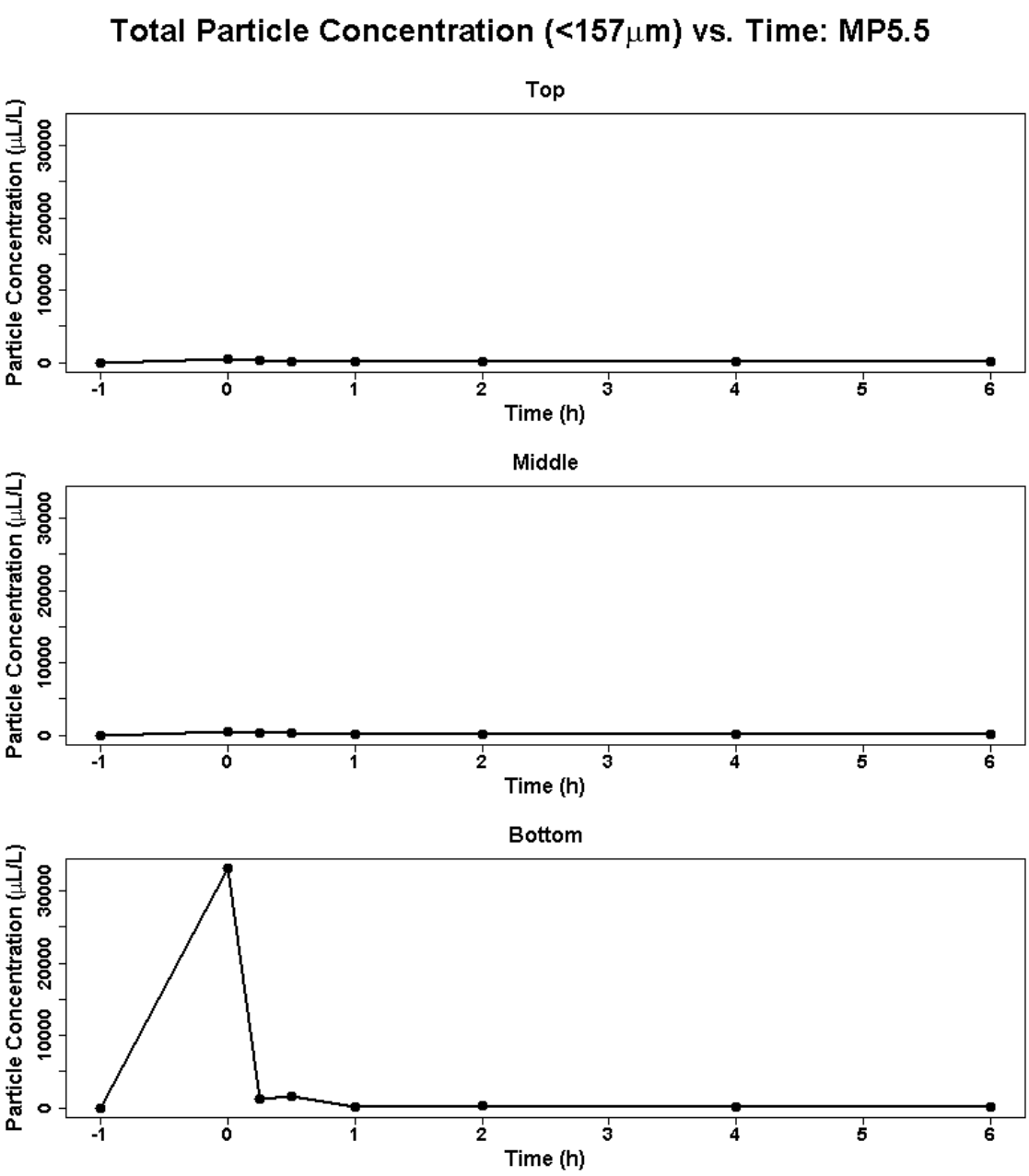


Figure 54: Total particle concentration (particle size <157 μm) vs. time for station MP5.5.

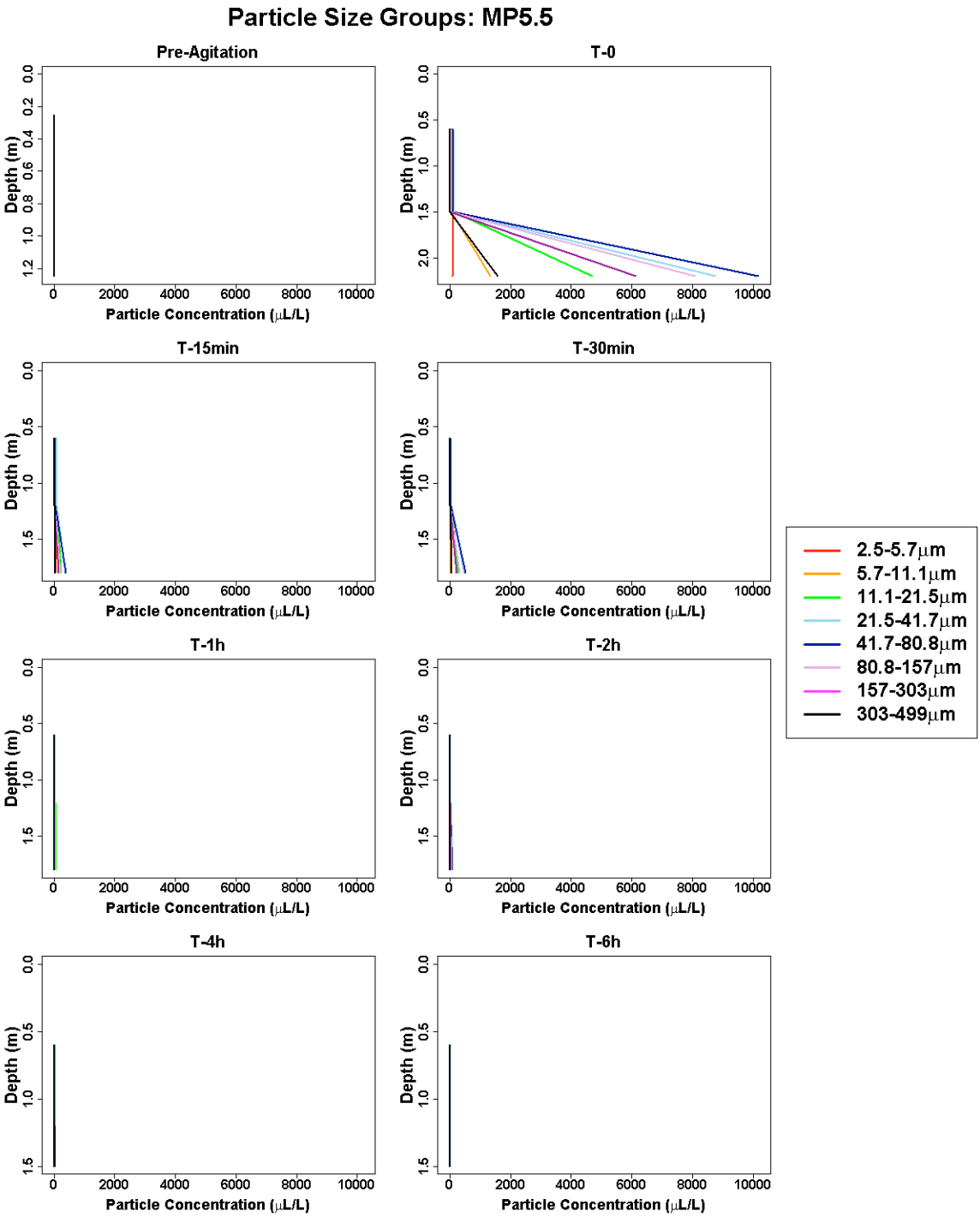


Figure 55: Particle Size Group concentrations for station MP5.5.

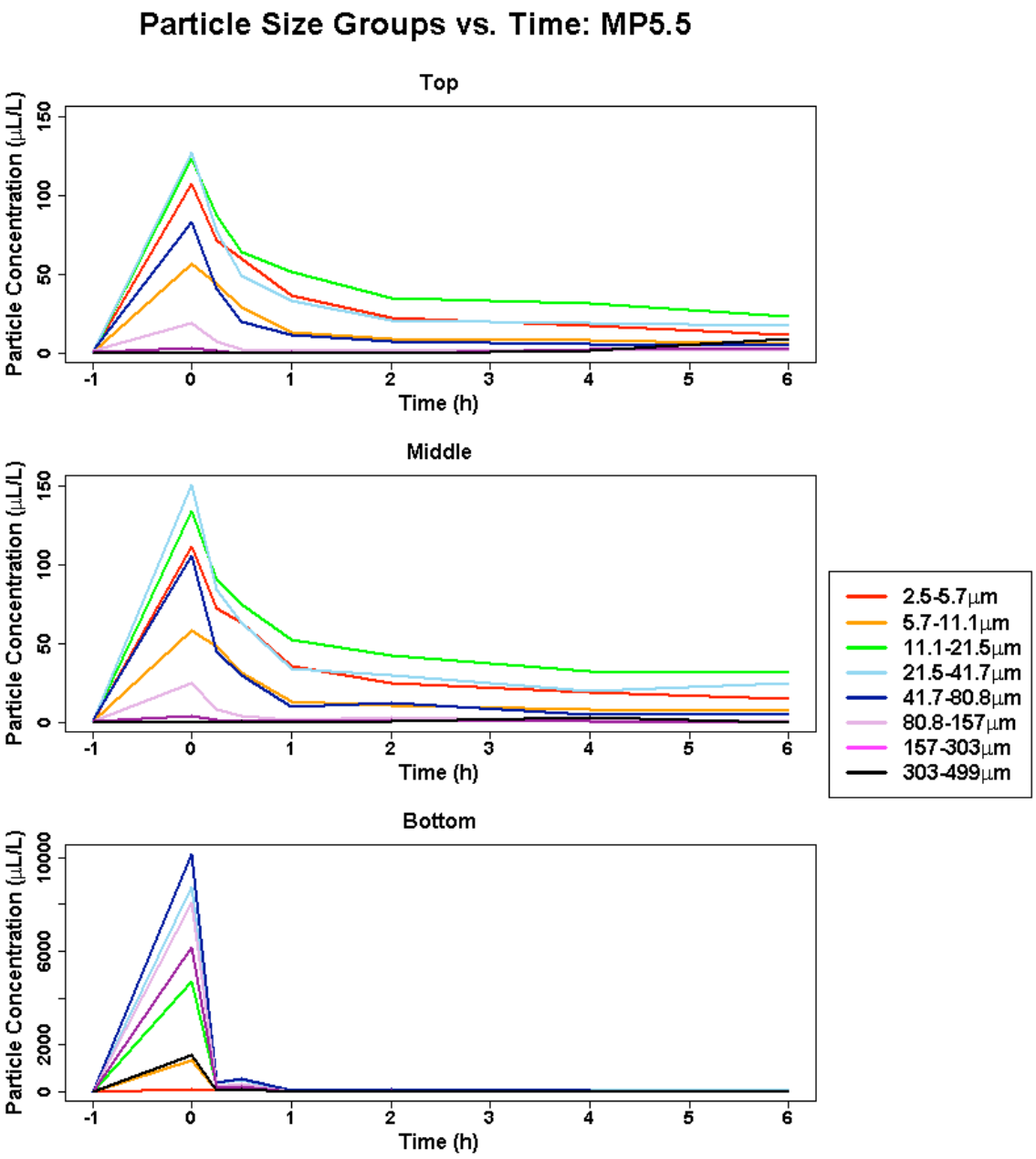


Figure 56: Particle Size Group concentrations vs. time for station MP5.5.

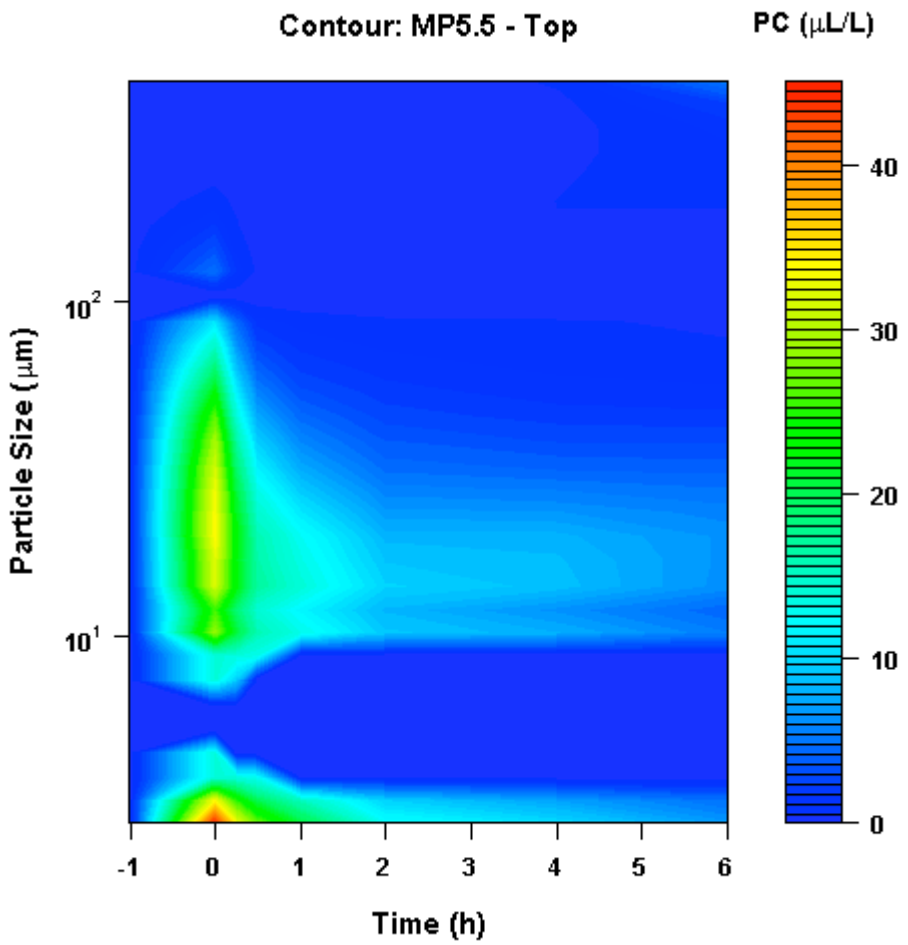


Figure 57: 3D contour plot for the MP5.5 'top' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

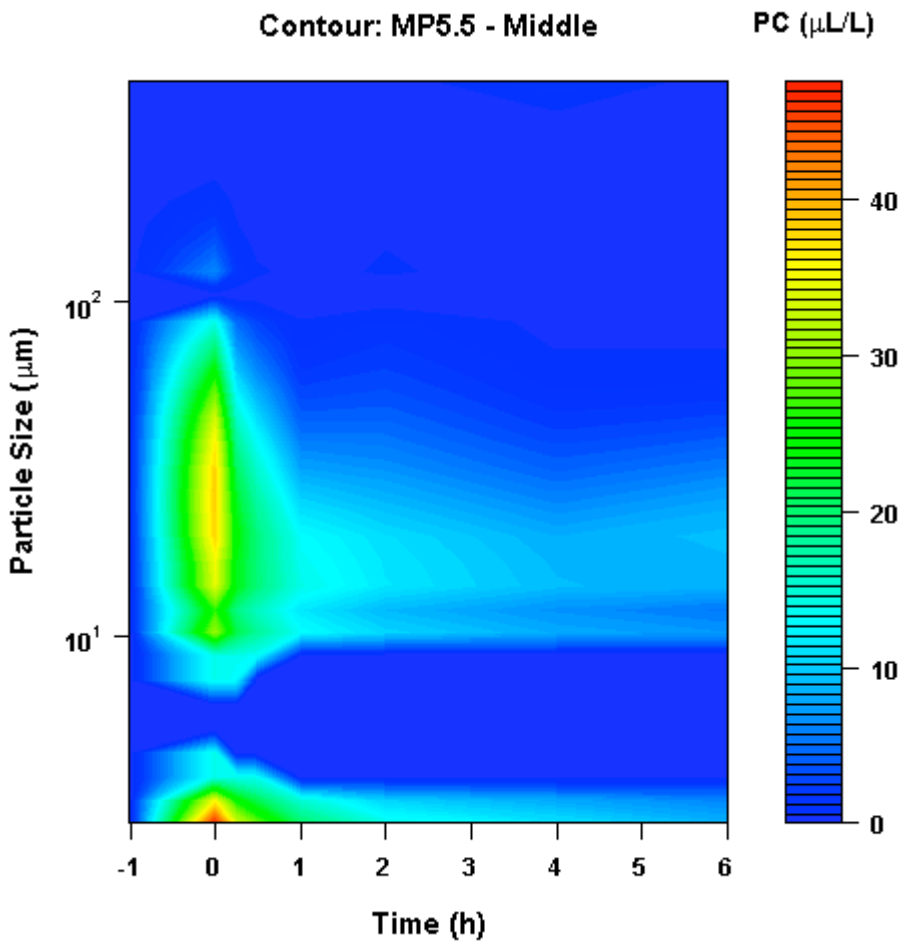


Figure 58: 3D contour plot for the MP5.5 ‘middle’ sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

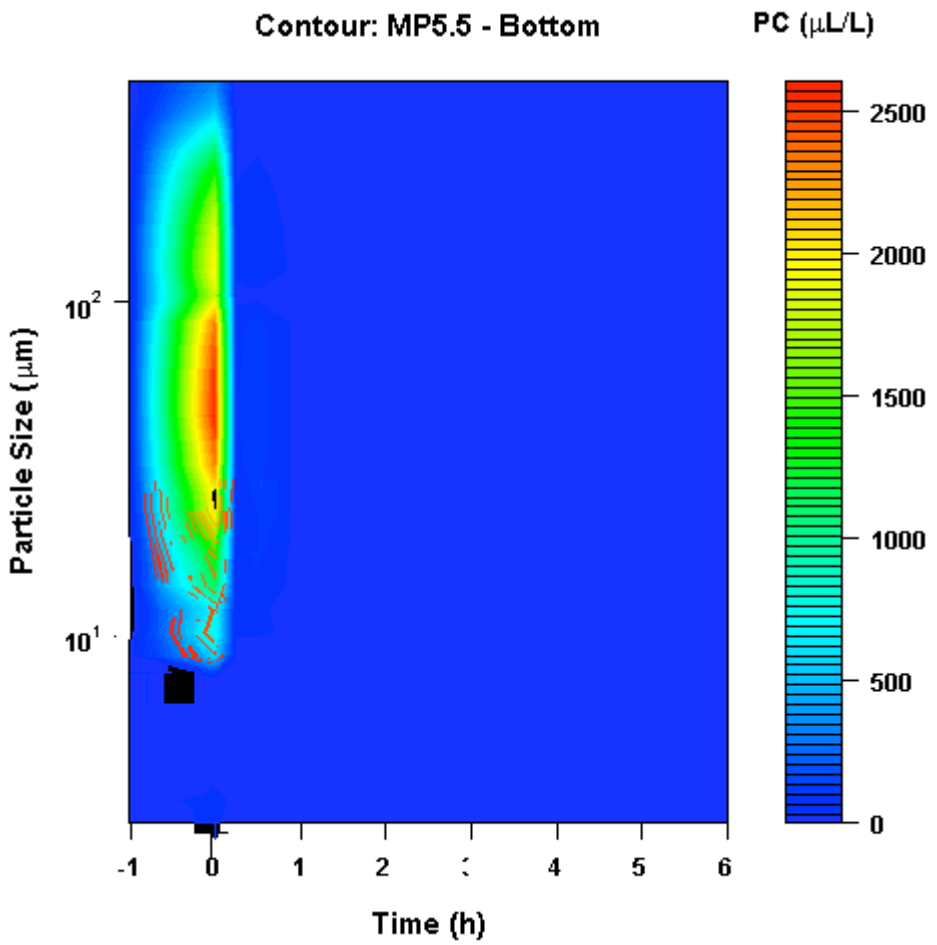


Figure 59: 3D contour plot for the MP5.5 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with colored contours representing particle concentration (PC, z-axis).

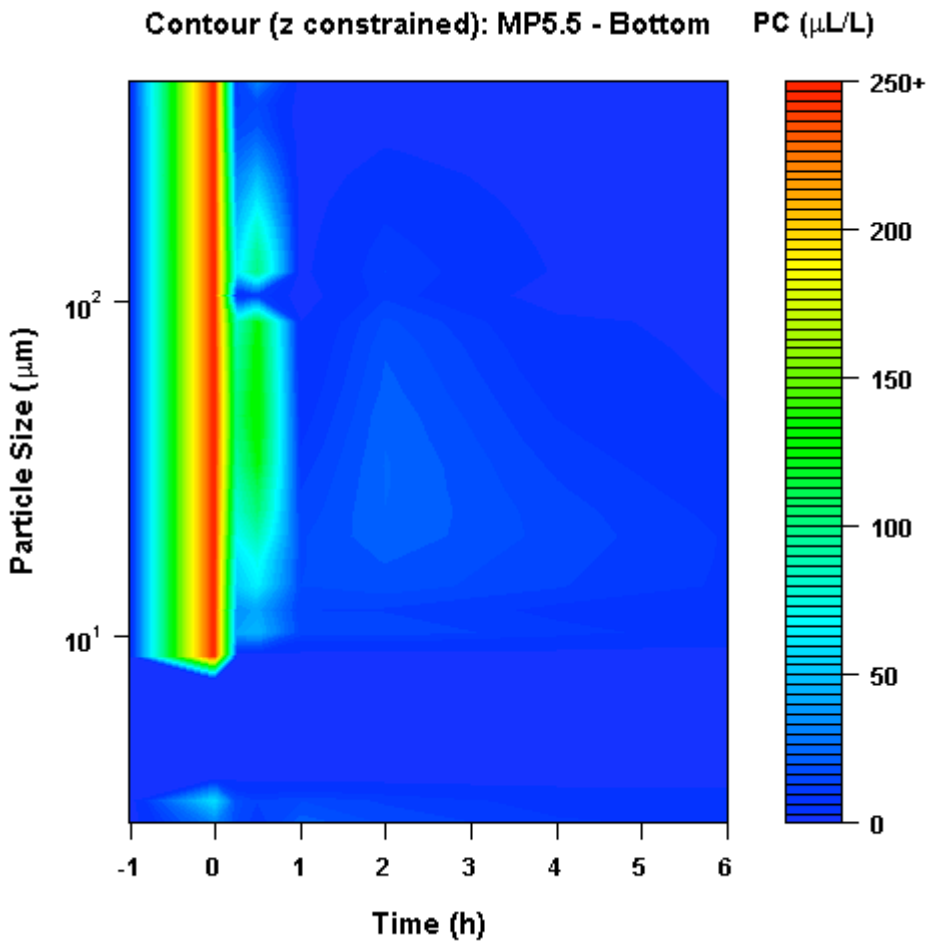


Figure 60: 3D contour plot for the MP5.5 'bottom' sample showing particle size (y-axis) vs. time (x-axis) with constrained colored contours representing particle concentration (PC, z-axis). In this figure, the maximum z-value has been limited to 250 µL/L in order to achieve better resolution at low particle concentrations.

Appendix

LISST-100X Particle Size Analyzer Standard Operation Procedure (SOP)

The Sequoia Laser *In Situ* Scattering and Transmissometry (LISST-100X) instrument uses a laser diffraction technique to determine particle size distribution (PSD). The LISST consists of an optical system producing a collimated laser beam, a detector array, electronics for signal pre-amplification and processing, a data storage and scheduling computer, and a battery.

During sediment agitation experiments, we recommend using an *in situ* deployment in order to capture particle dynamics exactly as they are found within the microcosm, unless the particle concentration is extremely high, in which case discrete samples should be taken near the surface, in the middle and near the bottom of the water column, followed by dilution and bench-top analysis.

List of Materials

- User's Manual with software disk
- LISST-100X instrument
- Communication cable
- External power cable
- Bench top instrument stands
- Allen wrench set
- Pre-moistened lens wipes
- Bucket
- De-ionized (DI) water (should be at ambient temperature)
- Water spray bottle
- LISST horizontal lowering harness
- Rope for lowering the LISST into the water column

In Situ Operating Procedures (pre-agitation)

Startup and background check

1. Remove the communication cable from the case. Connect the 5-pin underwater connector of the communication cable to the LISST-100X, and the 9-pin DB-9 connector to a notebook computer.

2. Install the LISST-100X software including the calibration files specific to the instrument. For ease of operation, create a shortcut to the LISST program on your desktop.
3. Start the LISST software by clicking on the program shortcut.
4. Click *LISST>Connect* (using *LISST* menu or by clicking icon that looks like a traffic light) then select *LISST>Wake up LISST* (or by clicking icon which looks like a sunny field). A dialogue box will then appear counting down from 138 seconds, which is the maximum time required for the instrument to wake up.
5. Wipe the optic windows on the LISST using lens wipes being careful not to scratch or otherwise damage the lenses. Rinse the optics thoroughly using DI water.
6. Submerge the LISST optics in a bucket of DI water in preparation for collecting instrument background.
7. Acquire background scatter profiles by selecting *LISST>Collect Background Scatter Data* (or by pressing the  button on the toolbar) and, when prompted, select the factory background scatter file (looks like factory_zsc_####.asc, with "####" being the serial number of the instrument). Once selected, this factory background will be shown on the displayed axis. The background scatter measurement is critical to good instrument performance and will also check the overall health of the instrument. It will verify that all of the systems are functioning and that the optics are in proper alignment.
8. Press the *BEGIN Collection* button. The software will now acquire 20 samples from the LISST and superimpose the mean collected background scatter data over the factory baseline values (specific to each instrument). If the values are acceptable they should be saved in the daily data storage directory by clicking the *Accept and Save* button and entering a filename (format: YYYY-MM-DD_bkg.asc) when prompted.
9. If there is a problem, an error message will be displayed and in most cases corrective action will need to be taken. eg. cleaning the mixing chamber and optical windows with a pre-moistened wipe, rinsing with de-ionized water, ensuring that the current laser power is close to the factory laser power, etc. Once complete, click the *BEGIN Collection* button again to re-start the background checking process.
10. A new background file should be saved each day.

Programming

1. Connect the LISST-100X to the PC and start the LISST-SOP program.
2. Open the Operating Modes window by choosing *LISST>Operating Modes* or by pressing the  button on the toolbar. Select the *Operating Mode Tab* at the top of the window and choose "Fixed Sample Rate", enter '1'

into the ‘samples are to be an average of’ box, and a sample interval of 1 second.
3. Next, select *Start Conditions* tab in order to configure when the instrument will begin sampling. Under this tab, select “External Mechanical Switch”.
4. Choose the *Stop Conditions* tab and once again select “External Mechanical Switch”.
5. Select *Apply* or *OK* buttons to configure the instrument with the current settings. If the *Apply* button is pressed the program will return to the current window. Returning to the *Instrument Status* window will display a summary of the current settings. If the *OK* button is pressed, when the configuration is complete the user will be prompted to open the *Terminal* window to start the program. To start the program and have it start looking for the Start conditions press the *Run* button on the *Terminal* window.
6. Once the program is confirmed to be running and waiting for the correct start conditions, the LISST-SOP program can be closed and the communications cable can be disconnected.
7. Remember to replace the connector cap before deployment.

Deployment

1. Attach horizontal lowering harness to LISST.
2. Attach rope securely to the horizontal lowering harness, being sure to loop around the metal ring several times in order to distribute pressure evenly.
3. Tie the loose end of the rope to the boat, so the instrument is not lost if the operator loses grip of the rope.
4. Record the station number and a brief descriptor of the work to be conducted (pre/post-agitation, time post agitation, etc).
5. Clean the optical windows on the LISST using a pre-moistened wipe, and rinse with DI water.
6. Using the rope to suspend the instrument, position the LISST optics slightly below the surface of the water and hold there for 30 seconds.
7. Engage the mechanical switch (white) in order to start data logging and confirm that the green LED on the connector end cap is blinking; indicating each time a sample is collected.
8. When ready, record the profile start time and begin lowering the LISST into the water at a slow and steady rate so that an accurate depth profile can be obtained.
9. When the bottom of the profile has been reached, record the time, then retrieve the LISST, disengaging the mechanical switch once it has reached the surface.
10. Once again, clean the optical windows on the LISST using a pre-moistened wipe, and rinse with DI water.
11. Submerge the LISST optics in a bucket of clean (preferably DI) water, and engage the mechanical switch for one minute then disengage the mechanical switch and remove the instrument from the bucket.

Saving data and shutdown

1. Once sampling for the day is complete, connect the 5-pin underwater connector of the communication cable to the LISST-100X, and the 9-pin DB-9 connector to a laptop personal computer.
2. Start the LISST software by clicking on the program shortcut.
3. Press the Stop button in the Terminal window, or the 

button in the main program window.
4. Press the Instrument Query Button  to display the instrument status including the number of samples saved.
5. Select *Offload* from the *LISST* menu or choose the  button from the toolbar. Choose the file(s) to be offloaded (identify using the date and time listed in the *Modified* column) by clicking on the file name on the list. Multiple files can be selected by holding down the CTRL key while clicking on files. Use the SHIFT key to select a range of files.
6. Press the OK button to accept the current selection. A dialog box with a path for storing the downloaded data will appear. Edit the path or press on the Browse button to select the daily data storage directory. Press OK to begin the offloading.
7. When offloading is complete, confirm that data files have been saved to the correct directory.
8. In the log book, record the relevant filenames for the day.
9. Click the *Put LISST to Sleep* button (looks like a night-sky) or select *LISST>Put LISST to Sleep* to put the LISST back into its low power sleep mode.

Bench-top SOP (post-agitation)

Setup

1. Remove the instrument stands and set them up on a flat bench top working surface.
2. Remove the LISST-100X from the case and set it on the stands. The LISST-100X unit may need to be affixed to instrument stands using tape or string/rope if analysis is being done at sea.
3. Remove the full path mixing chamber from the case, attach the flexible tubing and tubing stop clamp. Connect and plug in stirrer controller.

4. Slip the mixing chamber between the optical windows of the instrument such that these windows can be submerged for calibration and analysis; be careful not to damage the rubber o-rings which make the seal between mixing chamber and optical windows. (See Appendix F of the User's manual for more details on chamber installation.)
5. Check and adjust the mixing chamber and the spacer to make sure that the chamber is sealed well to both optical windows and will not leak when filled with water.
6. Add magnetic stir-bar to mixing chamber.
7. Remove the communication cable from the case. Connect the 5-pin underwater connector of the communication cable to the LISST-100X, and the 9-pin DB-9 connector to a laptop personal computer.
8. Install the LISST-100X software including the calibration files specific to the instrument. For ease of operation, create a shortcut to the LISST program on your desktop.

Software configuration

1. Start the LISST software by clicking on the program shortcut.
2. Click *LISST>Connect* (using *LISST* menu or by clicking icon that looks like a traffic light) then select *LISST>Wake up LISST* (or by clicking icon which looks like a sunny field). A dialogue box will then appear counting down from 138 seconds, which is the maximum time required for the instrument to wake up.
3. Click on *File>Settings* then select the *Output* tab and check all of "build a binary particle size file (.psd)", "build an ASCII particle size file (.asc)", and "build and ASCII raw data file (.log)" then click *OK*. This step instructs the LISST software to automatically create a raw LOG file, and processed PSD and ASCII files for each sample run.

Rinsing

Rinsing the full path mixing chamber is a 4-step process.

1. When not in immediate use, mixing chamber should always be left filled with de-ionized water. Drain the contents of the mixing chamber by opening the stop-clamp which is located on the drain tube.
2. Wipe mixing chamber, magnetic stir-bar, and both optical windows well with a pre-moistened wipe. (This step only needs to be performed after running surface samples or other highly oiled water samples.)
3. Perform two "small rinses" with de-ionized water. A "small rinse" entails leaving the stop-clamp opened and pouring only enough de-ionized water into the mixing chamber to fully cover the bottom, then letting it drain completely. Do this twice.

4. Perform one “big rinse” with de-ionized water. Close the stop-clamp and fill the mixing chamber to the top with de-ionized water then open the stop-clamp and let the mixing chamber drain completely.

Checking background

1. Create a new data directory for the day and/or subdirectory for the station as required - refer to example directory structure provided as reference.
2. Connect-to and wake-up the LISST if not already done (as described above) and turn on magnetic stirrer.
3. Rinse (as per instructions above) and fill the mixing chamber with clean de-ionized water.
4. Double check that there are no leaks between the mixing chamber and the optical windows on the LISST.
5. Turn on stirrer and set dial to approximately 120 revolutions per minute (RPM). Because the stirrer motor is controlled by an analog dial, achieving the correct RPM will need to be done through trial-and-error.
6. Acquire background scatter profiles by selecting *LISST>Collect Background Scatter Data* (or by pressing the  button on the toolbar) and, when prompted, select the factory background scatter file (looks like factory_zsc_####.asc, with “####” being the serial number of the instrument). Once selected, this factory background will be shown on the displayed axis. The background scatter measurement is critical to good instrument performance and will also check the overall health of the instrument. It will verify that all of the systems are functioning and that the optics are in proper alignment.
7. Press the *BEGIN Collection* button. The software will now acquire 20 samples from the LISST and superimpose the mean collected background scatter data over the factory baseline values (specific to each instrument). If the values are acceptable they should be saved in the daily root directory (refer to example directory structure) by clicking the *Accept and Save* button and entering a filename (format: YYYY-MM-DD_bkg.asc) when prompted.
8. If there is a problem, an error message will be displayed and in most cases corrective action will need to be taken. eg. cleaning the mixing chamber and optical windows with a pre-moistened wipe, rinsing with de-ionized water, ensuring that the current laser power is close to the factory laser power, or, in some cases, a realignment of the instrument may need to be performed (in most cases, this cannot be done in the field). Once complete, click the *BEGIN Collection* button again to re-start the background checking process.
9. A new background file should be saved each day, and background levels checked between stations. During these between station checks, a new background scatter file should only be saved when

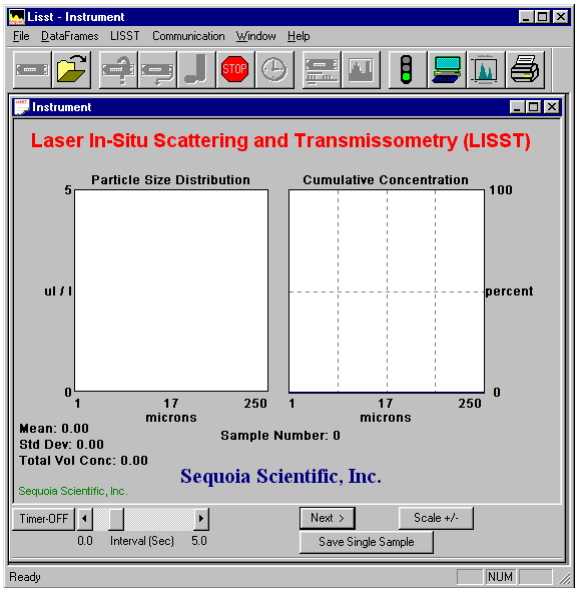
additional deviation from the factory background cannot be remedied by cleaning the mixing chamber and optical lenses with a pre-moistened wipe and rinsing with de-ionized water.

Sample collection

1. Samples are collected from Van-Dorn samplers in 250 mL Nalgene bottles and delivered to LISST operators.
2. The Nalgene sample bottle should be shaken well before sub-sampling.

Analyzing samples

1. Run all Van-Dorn samples collected from the water column at a station before running the surface sample collected using a bucket. This minimizes cross-contamination of the mixing chamber.
2. Connect-to and wake-up the LISST if not already done (as described above) and turn on magnetic stirrer.
3. Choose *File>Open Real-Time Session* (or press the  button to open the real-time session). Choose the most recent background file (saved during the “Checking Background” step) to use and specify output files in which to store the sample information for the run (sample IDs are used as data file names. eg. BM0540199-1.psd and .asc). A display similar to this will appear.



The left hand figure displays a bar chart showing the volume concentration in each of the 32 log spaced size classes. The right hand plot will be the Cumulative Concentration. The slider bar next to the button adjusts the acquisition rate. The slider bar controls the data acquisition time. The

Scale +/- Button adjusts the Particle Size Distribution scale (this can be set to 2.5 for easier real-time viewing).

4. Rinse the mixing chamber (as per instructions above) with de-ionized water.
5. Dilute sample to allow analysis with the LISST instrument and record dilution factor in field notebook.
6. Using sample water, perform two “small rinses” (described above), then close stop-clamp and fill mixing chamber so that optic lenses are completely submerged by the sample.
7. Allow any air bubbles resulting from filling the mixing chamber to escape then click *Start* in the LISST software.
8. Cover the top of the mixing chamber with an opaque object such as a towel in order to mitigate potential interference from sunlight.
9. Allow the LISST to collect 20 samples (scans) then click the *Stop* button. Stopping the LISST does not occur immediately and may take a few seconds to occur – do not proceed until the LISST has received the stop command.
10. Close the Real-Time Session window (data are automatically saved) and drain the sample water from the chamber.
11. Rinse the mixing chamber. You are now ready for the next sample.
12. Once all the samples for a given station are complete, run an analytical blank with DI water as if it were a sample.
13. After finishing with the instrument rinse the mixing chamber and leave filled with de-ionized water. Press the *Put LISST to Sleep* button (looks like a night-sky) or select *LISST>Put LISST to Sleep* to put the LISST back into its low power sleep mode.