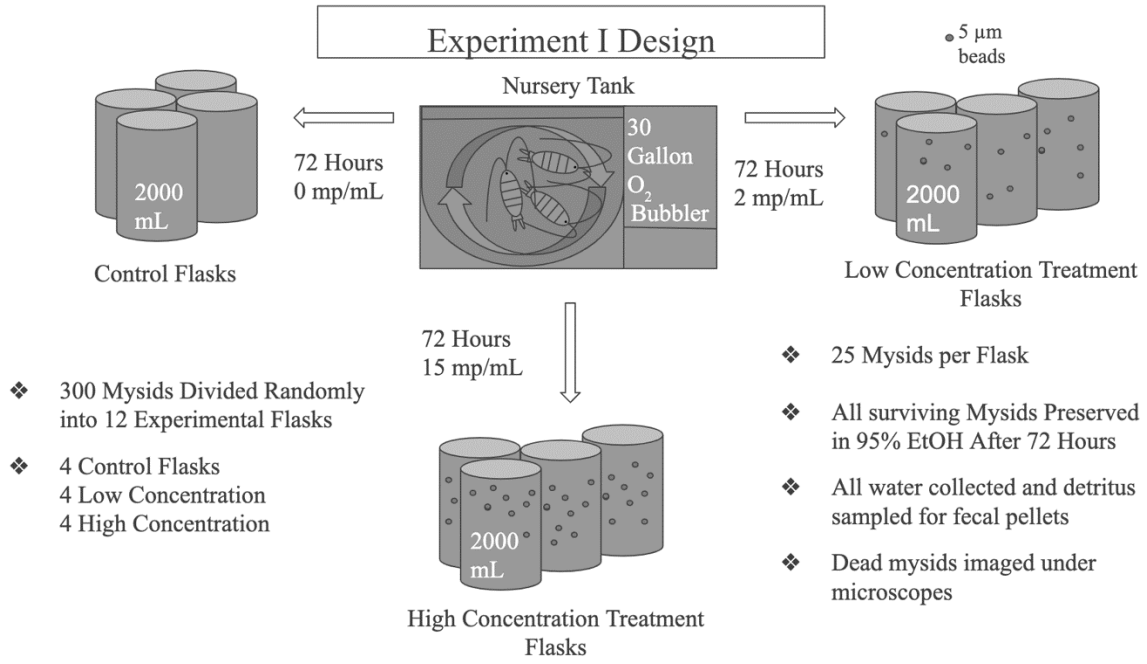
Supplementary Materials

Figures:

S1

Experiment I mysid flasks. (a) 12 mysid dosing flasks, (b) two extra water supply flasks; Marine Facilities Building, University of West Florida, Pensacola, Florida.

S2

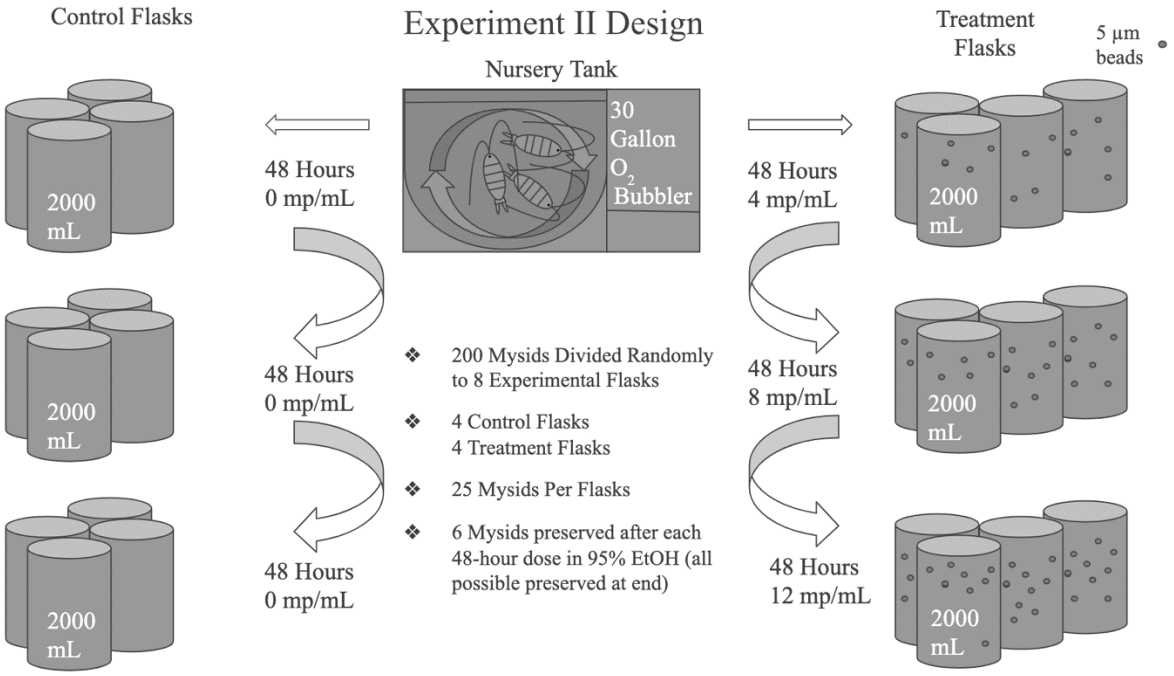


Experiment I design. *Americamysis bahia* exposure to microplastics at constant concentrations of 2 mp/mL or 15 mp/mL, over a three-day span.

S3

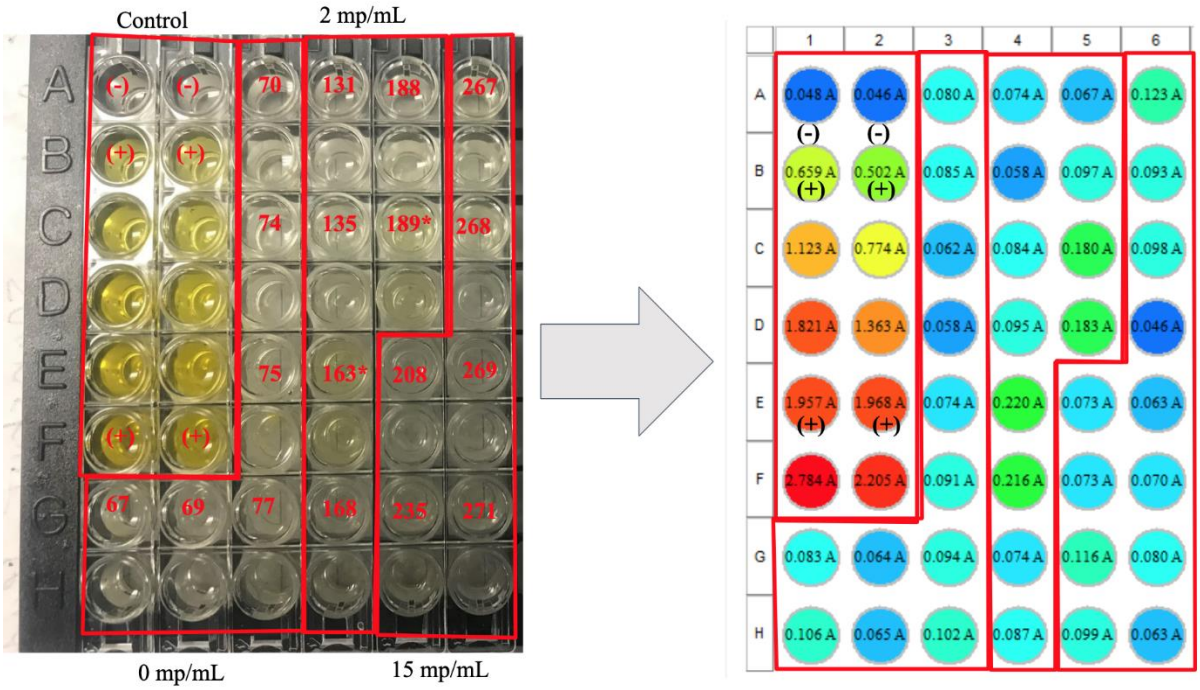
Experiment II mysid flasks. (a) eight mysid dosing flasks, (b) *Artemia* culturing setup, and (c) two extra water supply flasks; Marine Facilities Building, University of West Florida, Pensacola, Florida.

S4



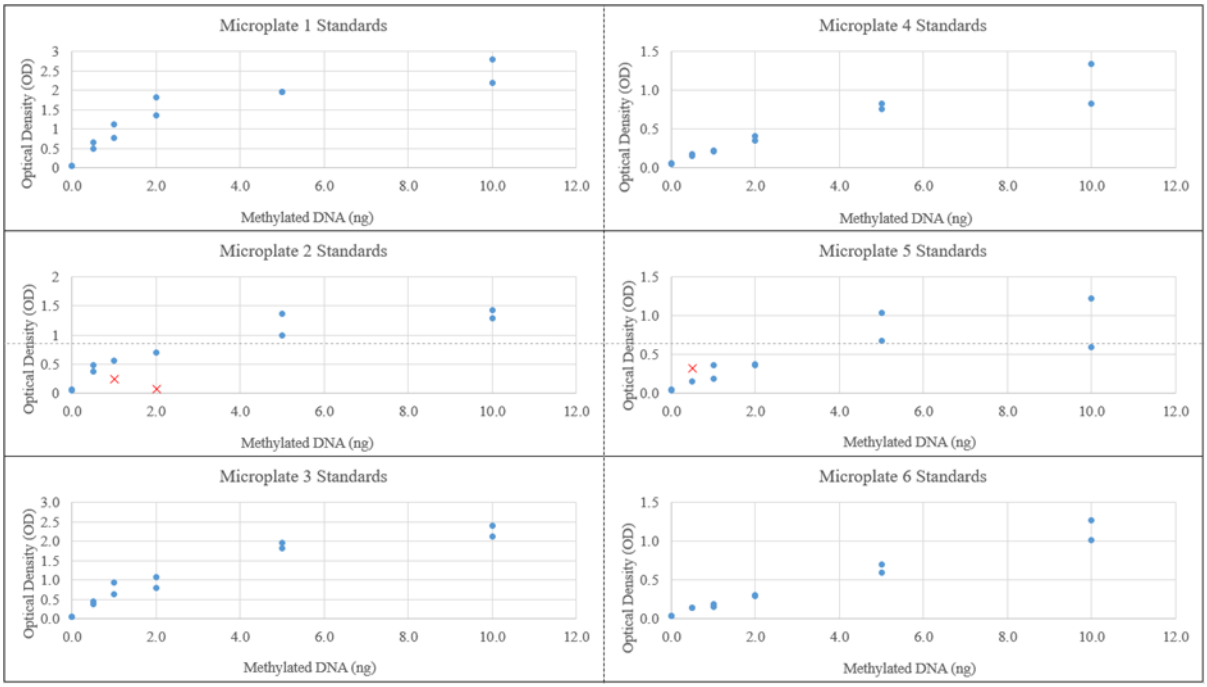
Experiment II design. *Americamysis bahia* exposure to microplastics at increasing concentrations from 4 mp/mL to 8 mp/mL to 12 mp/mL, in three 48-hour increments, over a six-day span.

S5



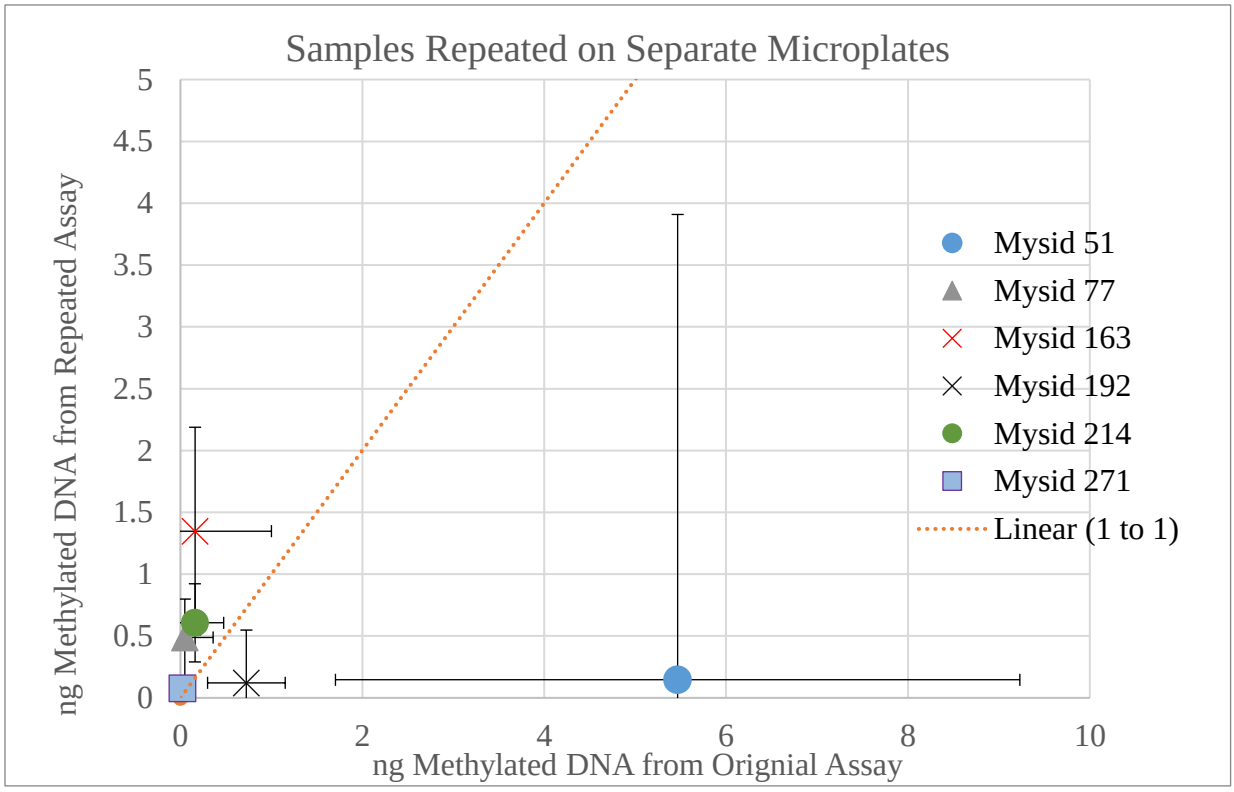
Example of MethylFlash™ microplate and PerkinElmer © readout. A 48-well MethylFlash™ plate and corresponding optical density readout from PerkinElmer microplate spectrophotometer (Model #2030-0030). A standard curve is set up, in duplicate, across 12 wells (wells A1 and A2 are negative control while wells B1, B2, C1, C2, D1, D2, E1, E2, F1, and F2 are positive controls) at 0, 0.5, 1, 2, 5, and 10 ng/µL of polynucleotides containing 50% methylated cytosine. DNA of six individuals from each treatment level of polystyrene microplastic beads (mp/mL = microplastics per milliliter) is compared for methylation signals in duplicate wells (the numbers 67, 69, 70, 74, 75, 77 etc. projected on the physical microplate image are examples of mysid individual ID numbers). Duplicate wells for individuals are always in the same column.

S6



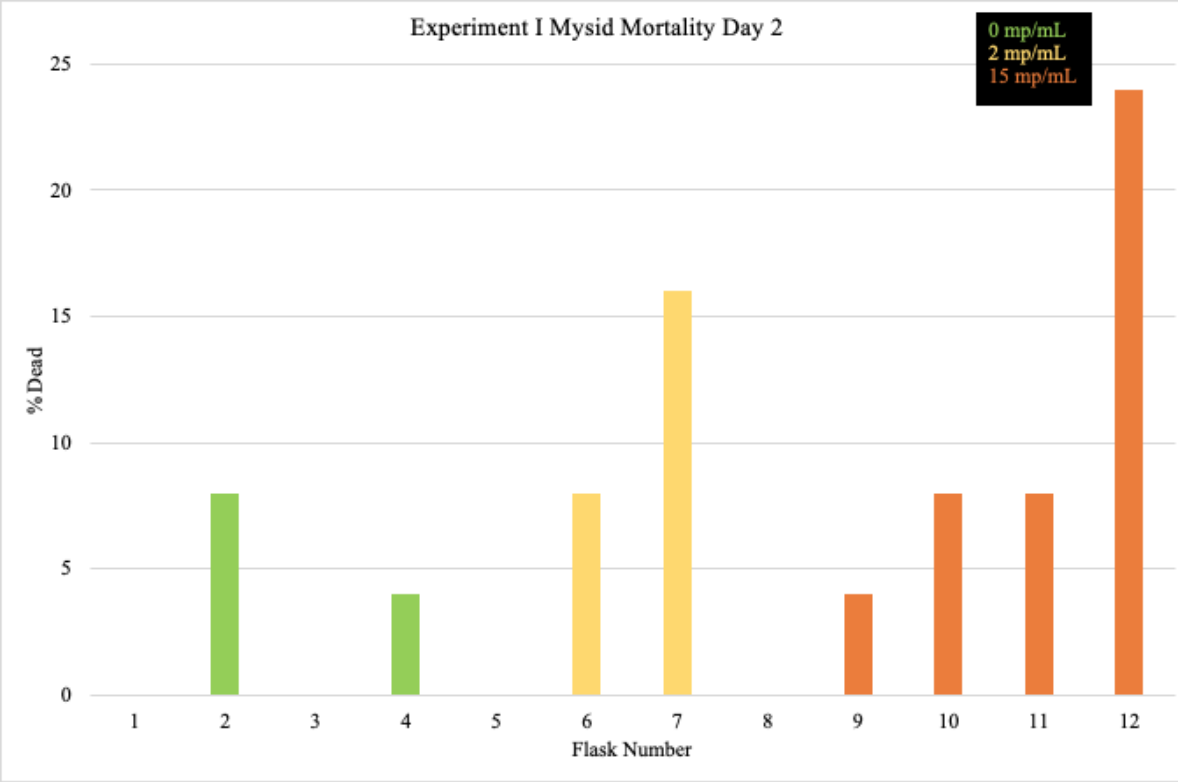
Methylation standard curves; Optical density values for 0, 0.5, 1, 2, 5, and 10 ng of 50% methylated polynucleotide standards on MethylFlash™ microplates; Red "x" denotes optical density values not used in F-factor computations due to evident failure. Microplate 1 (48-well; S23), standard optical density resulted in a non-linear distribution. The standard deviation of duplicate well OD was less than 0.05 in all individuals. All standard optical density readings were used to calculate F-factor values. All sample methylation values on microplate one were calculated using the F-factor corresponding to the standards of 0.5 ng of 50% methylated DNA. In microplate 2 (full, 96-well), one well, each, of the standards at 1 ng and 2 ng methylated DNA were left out of F-factor computation as evident failed wells. The standard deviation of duplicate well OD was greater than 0.05 in four individuals. Forty of forty-two sample methylation values were calculated with the F-factor corresponding to 0.5 ng 50% methylated polynucleotide standards. One sample methylation value was calculated with the F-factor corresponding to 2 ng standard, and the remaining sample was calculated with the F-factor corresponding to 5 ng standard. For microplate 3 (full, 96-well), all standard optical density readings were used to calculate F-factor values. The standard deviation of replicate well OD was greater than 0.05 in four individuals. All sample methylation values on microplate one were calculated using the F-factor corresponding to the standards of 0.5 ng of 50% methylated DNA. For microplate 4 (full, 96-well), all standard optical density readings were used to calculate F-factor values. The standard deviation of replicate well OD was greater than 0.05 in three individuals. Thirty-three of the forty-two sample methylation values were calculated with the f-factor corresponding to 0.5 ng 50% methylated polynucleotide standards. Seven sample methylation values were calculated with the F-factor corresponding to 1 ng standard, and the two remaining samples were calculated with the F-factor corresponding to 2 ng standard. For microplate 5 (full, 96-well), one well of the standards at 0.5 ng methylated DNA was left out of F-factor computation as an evident failed well. The standard deviation of replicate well OD was less than 0.05 in all individuals. All the sample methylation values were calculated with the F-factor corresponding to 0.5 ng 50% methylated polynucleotide standards. For microplate 6 (full, 96-well), standard optical density resulted in a nearly linear distribution, and all standard optical density readings were used to calculate F-factor values. The standard deviation of replicate well OD was greater than 0.05 in three individuals. Thirty-two of forty-two sample methylation values were calculated with the F-factor corresponding to 0.5 ng 50% methylated polynucleotide standards. Six sample methylation values were calculated with the F-factor corresponding to 1 ng standard, and the four remaining samples were calculated with the F-factor corresponding to 2 ng standard.

S7

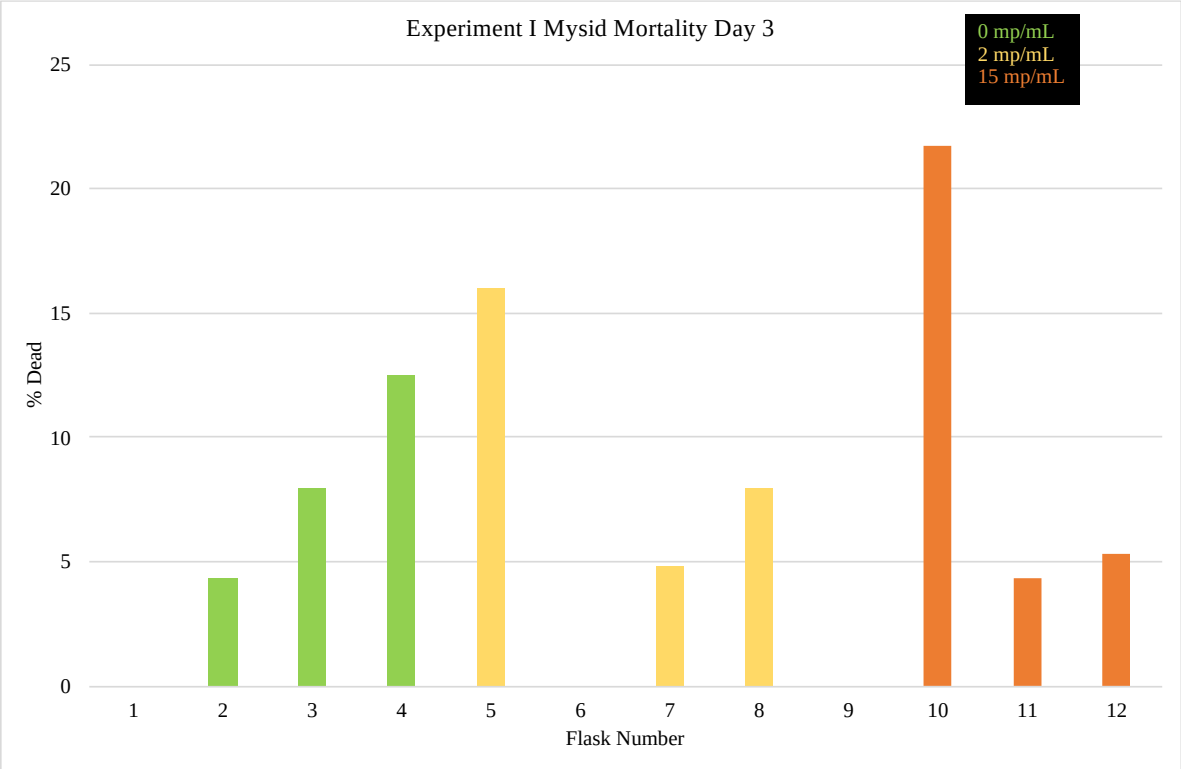


Percent methylation of individuals repeated on separate microplates. The MethylFlash™ assay was repeated for six individuals on separate microplates. *Americamysis bahia* DNA extractions were placed into two replicate wells on both the original assay and the repeated assay. Error bars are standard deviation between average ng of methylated DNA in the original assay and the average ng of methylated DNA in the repeated assay. The linear 1 to 1 line shows the theoretical situation where the amount of methylated DNA calculated in the original assay is equal to the amount of methylated DNA in the repeated assay.

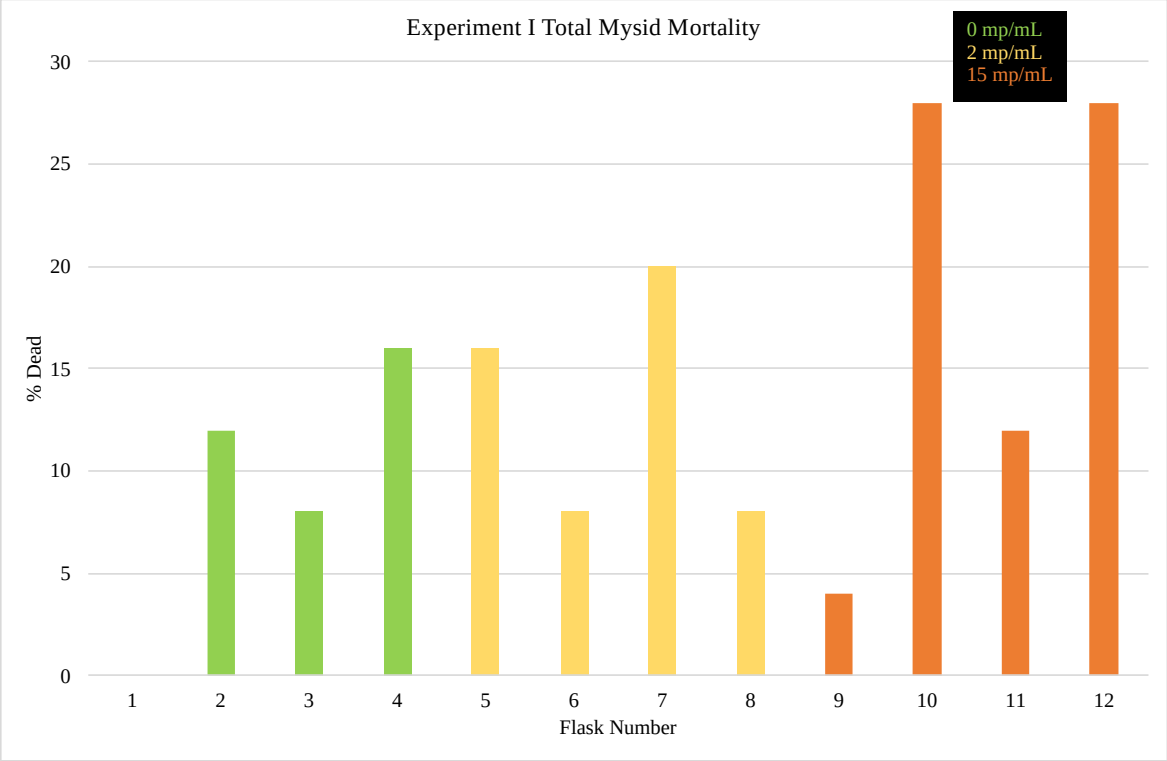
S8 Experiment I Mysid Mortality Day 2



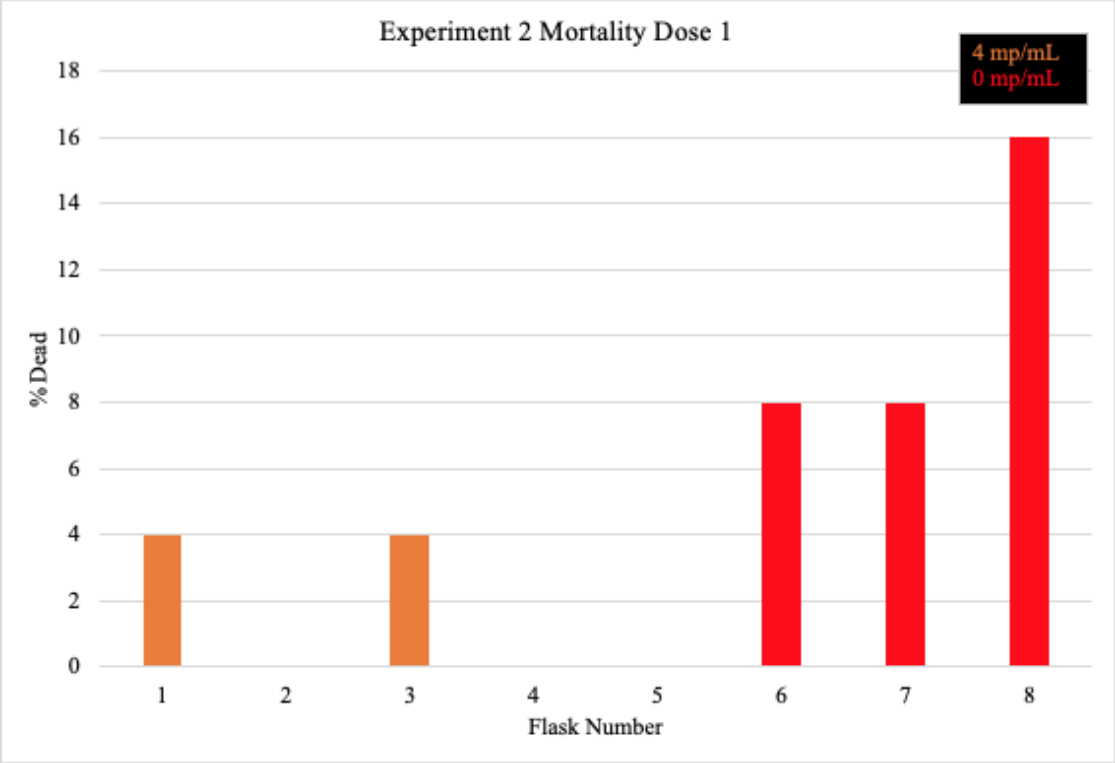
S9 Experiment I Mysid Mortality Day 3



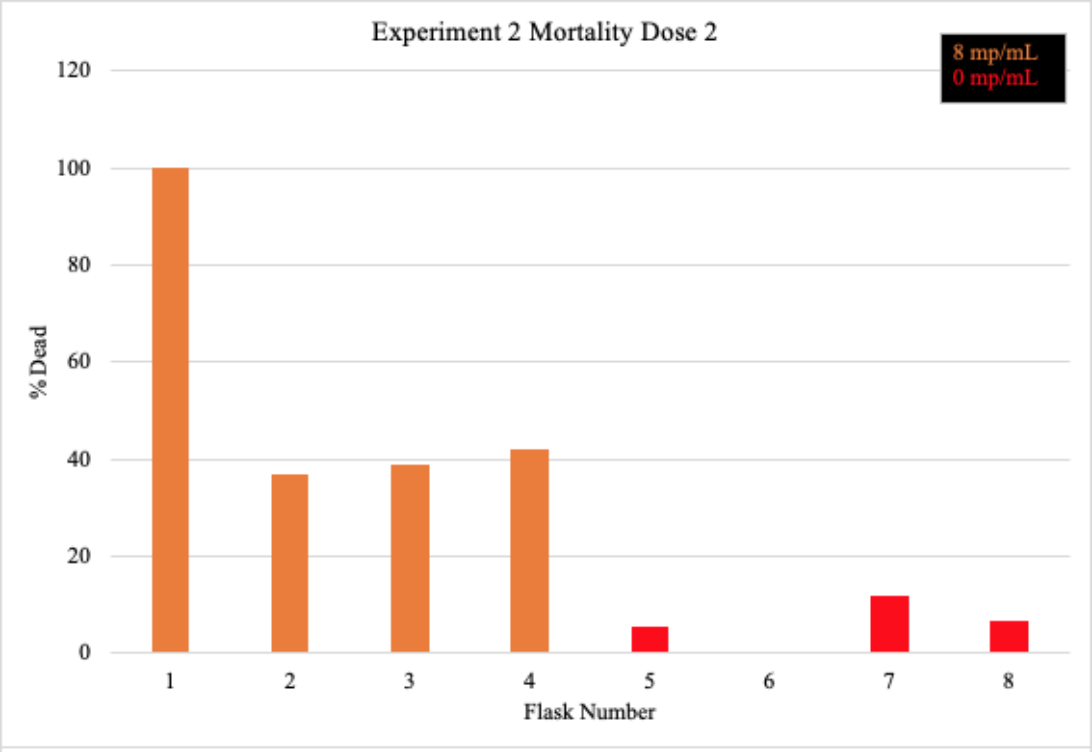
S10 Experiment I Total Mysid Mortality



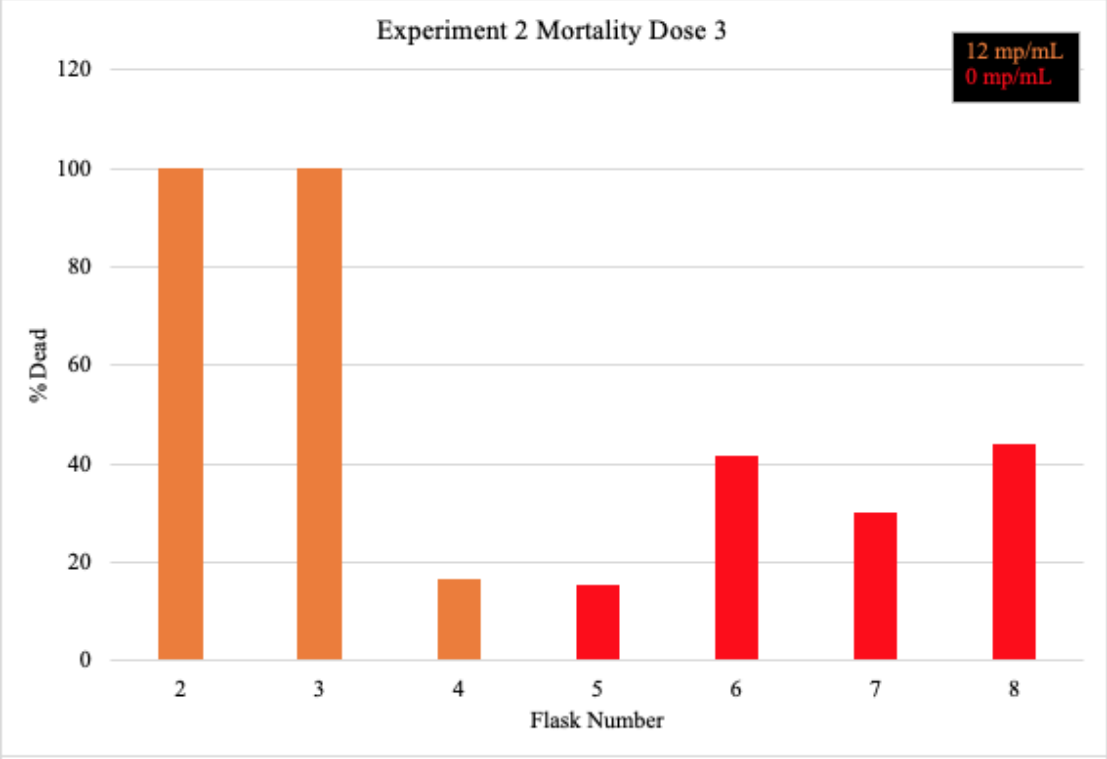
S11 Experiment 2 Mortality Dose 1



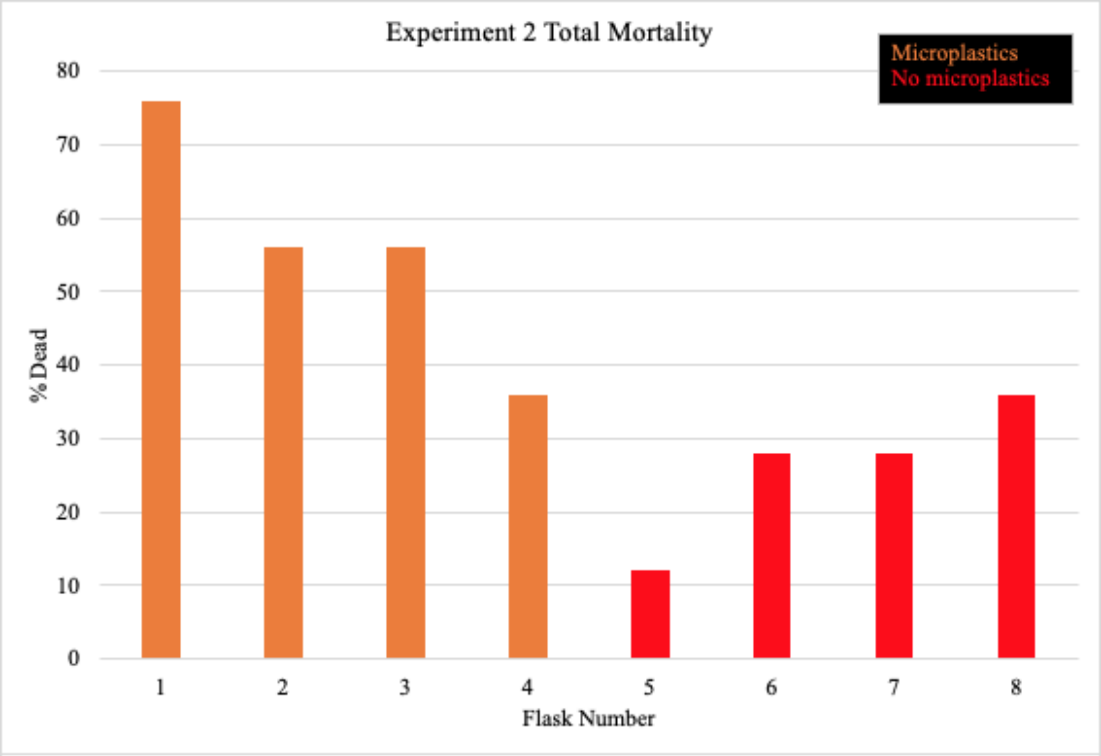
S12 Experiment 2 Mortality Dose 2



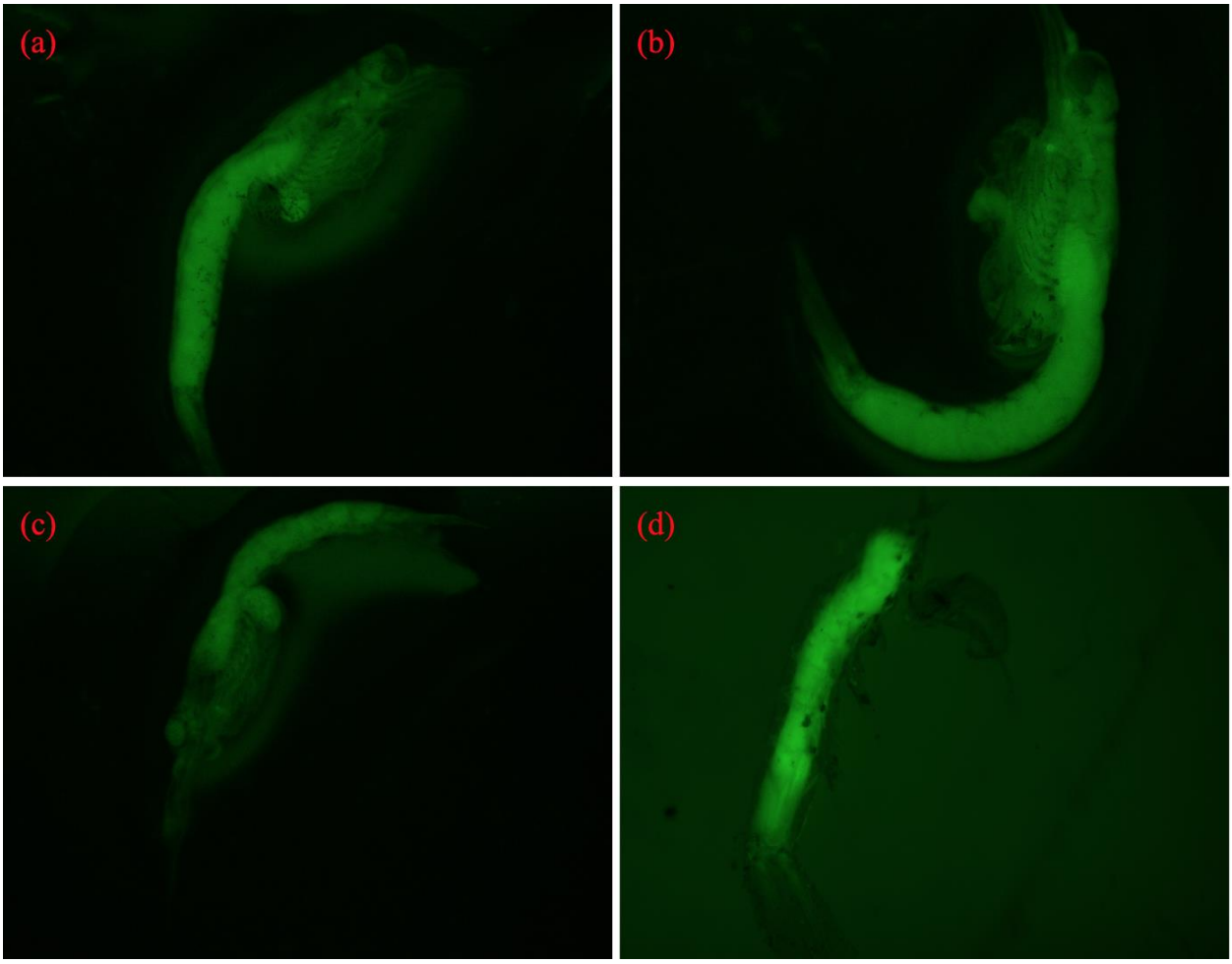
S13 Experiment 2 Mortality Dose 3



S14 Experiment 2 Total Mortality

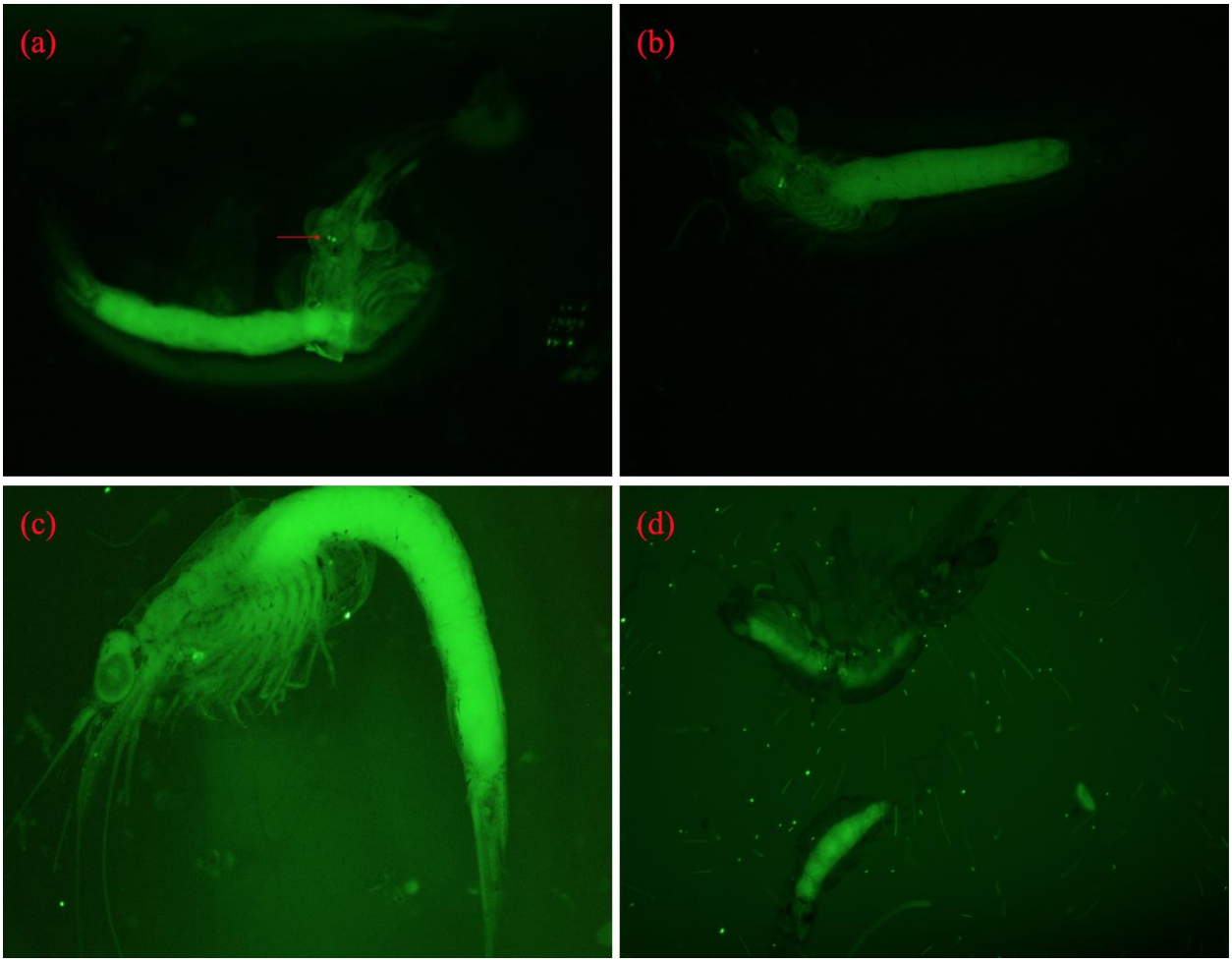


S15 Example Images of Control Group Mysids



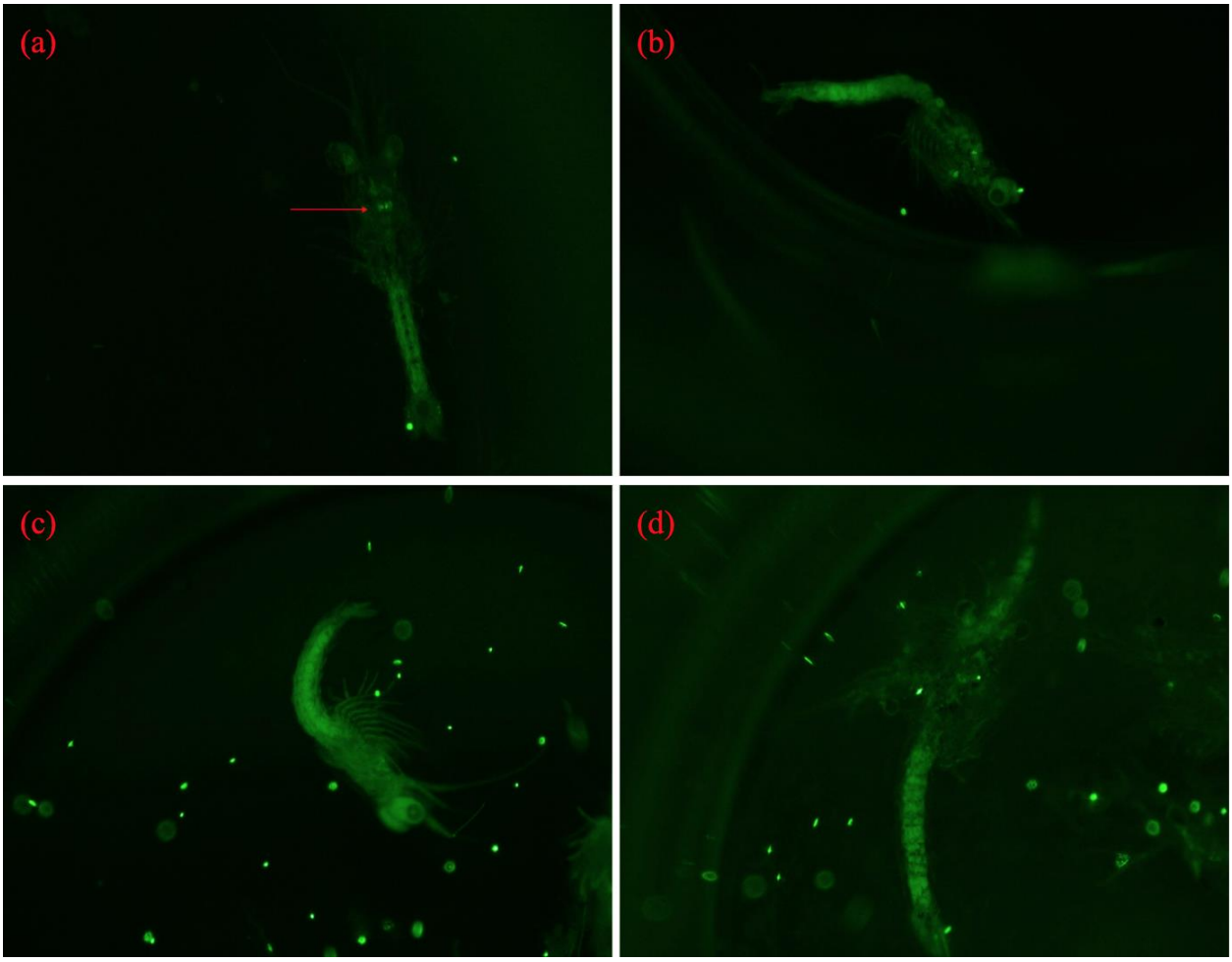
Example microscope images of control group adult. No microplastics were found on any of the control mysids. (a) MyI2 Adult 2 (b) MyI2 Adult 3 (c) MyI3 Adult 1 (d) MyI3 Adult 2 (fragmented).

S16 Example Images of Dosed Mysids



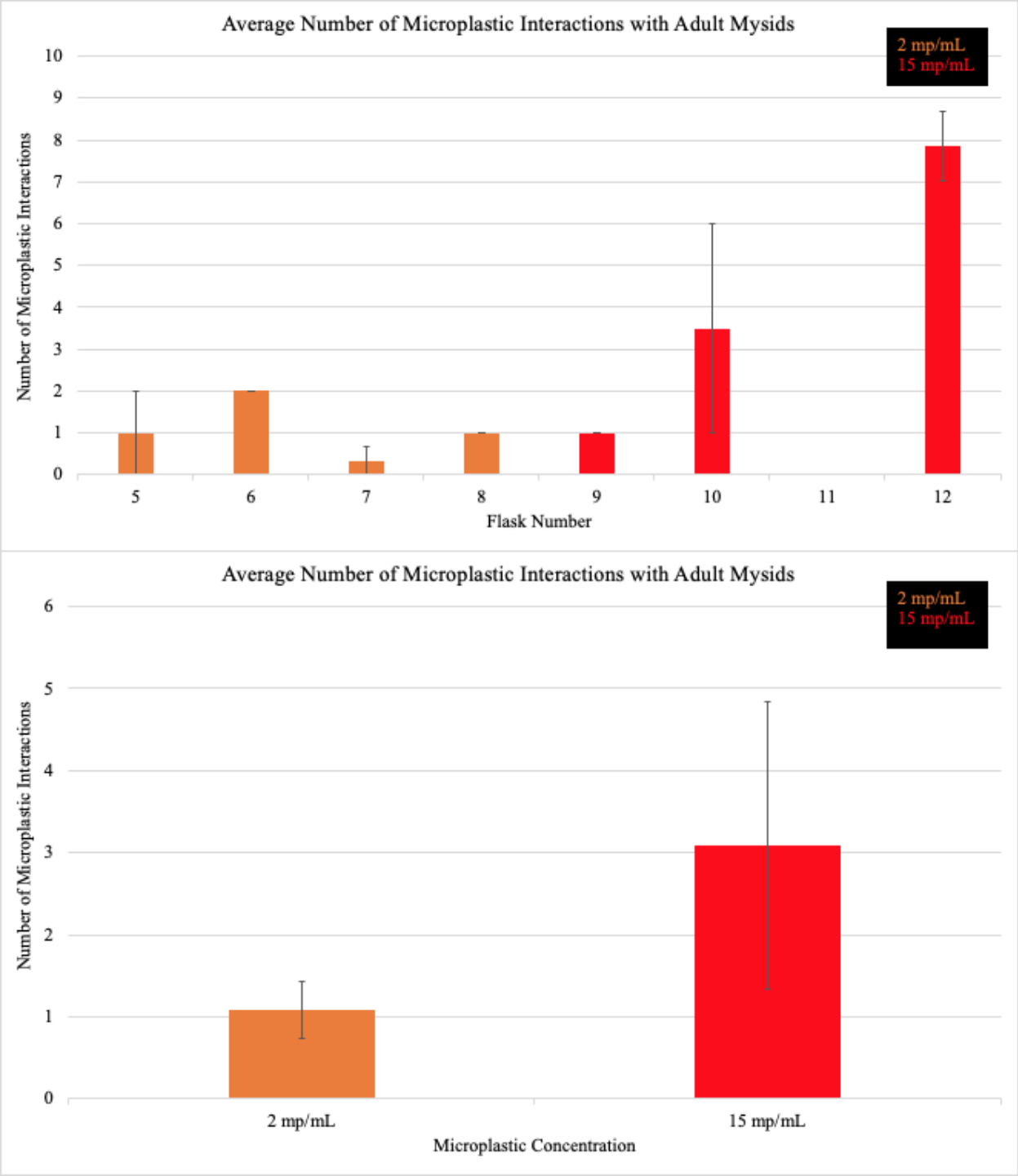
Example microscope images of dosed group adult mysids. All individuals are adult mysids from experiment I treatment groups with microplastics found on them. Microplastics were not seen on every adult, and microplastics were not found on adults in every treatment flask. (a) MyI5 Adult 1 (b) MyI8 Adult 1 (c) MyI10 Adult 1 (d) MyI12 Adult 6.

S17 Example Microscope Images of Dosed Juveniles



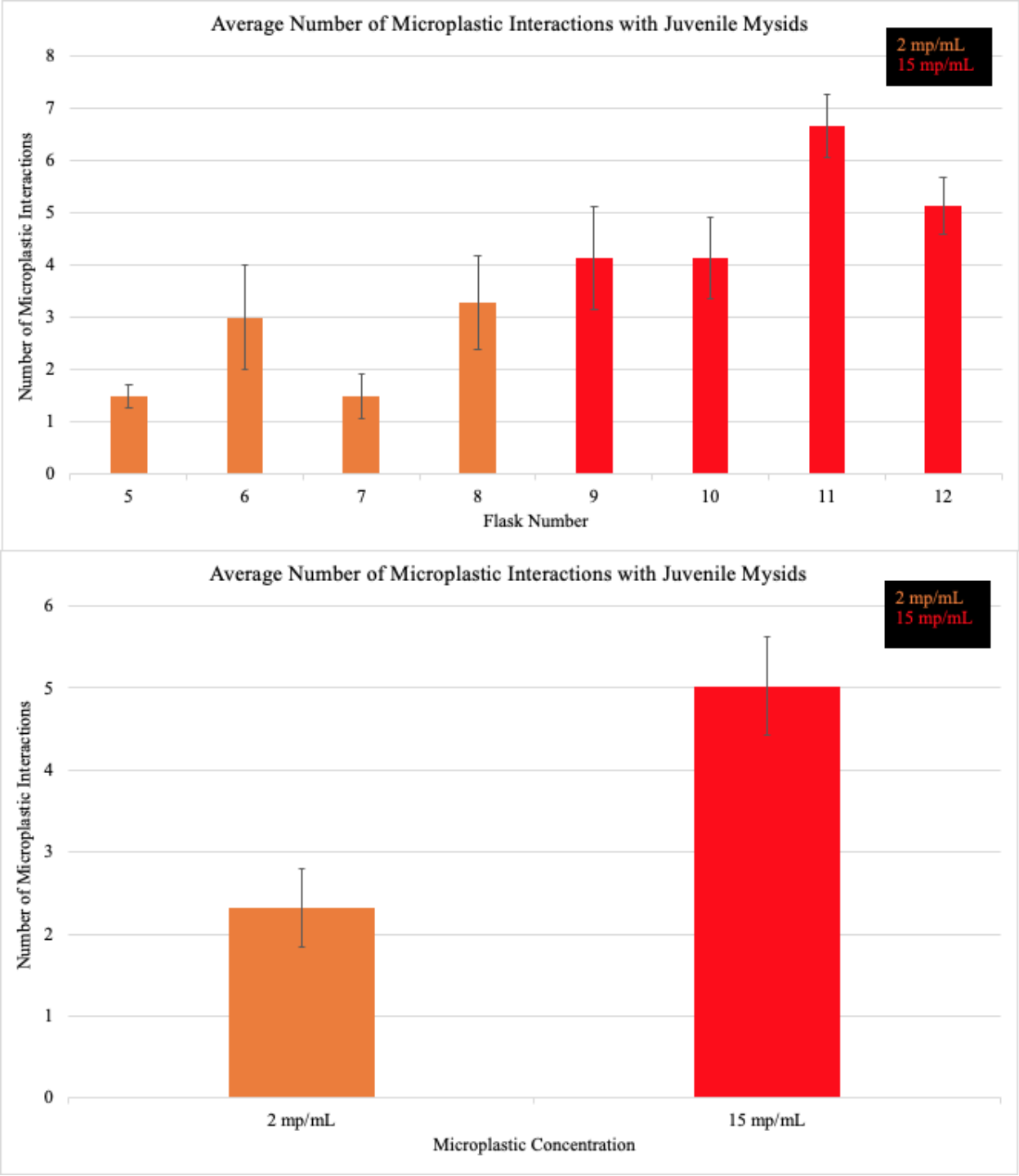
Example microscope images of dosed group juvenile mysids. In experiment I, microplastics were not seen on every juvenile from dosed flasks; however, microplastics were found at least 1 juvenile in all treatment flasks. (a) MyI5 Juvenile 9 (b) MyI6 Juvenile 3 (c) MyI11 Juvenile 1 (d) MyI12 Juvenile 1.

S18 Average Number of Microplastic Interactions with Adult Mysids



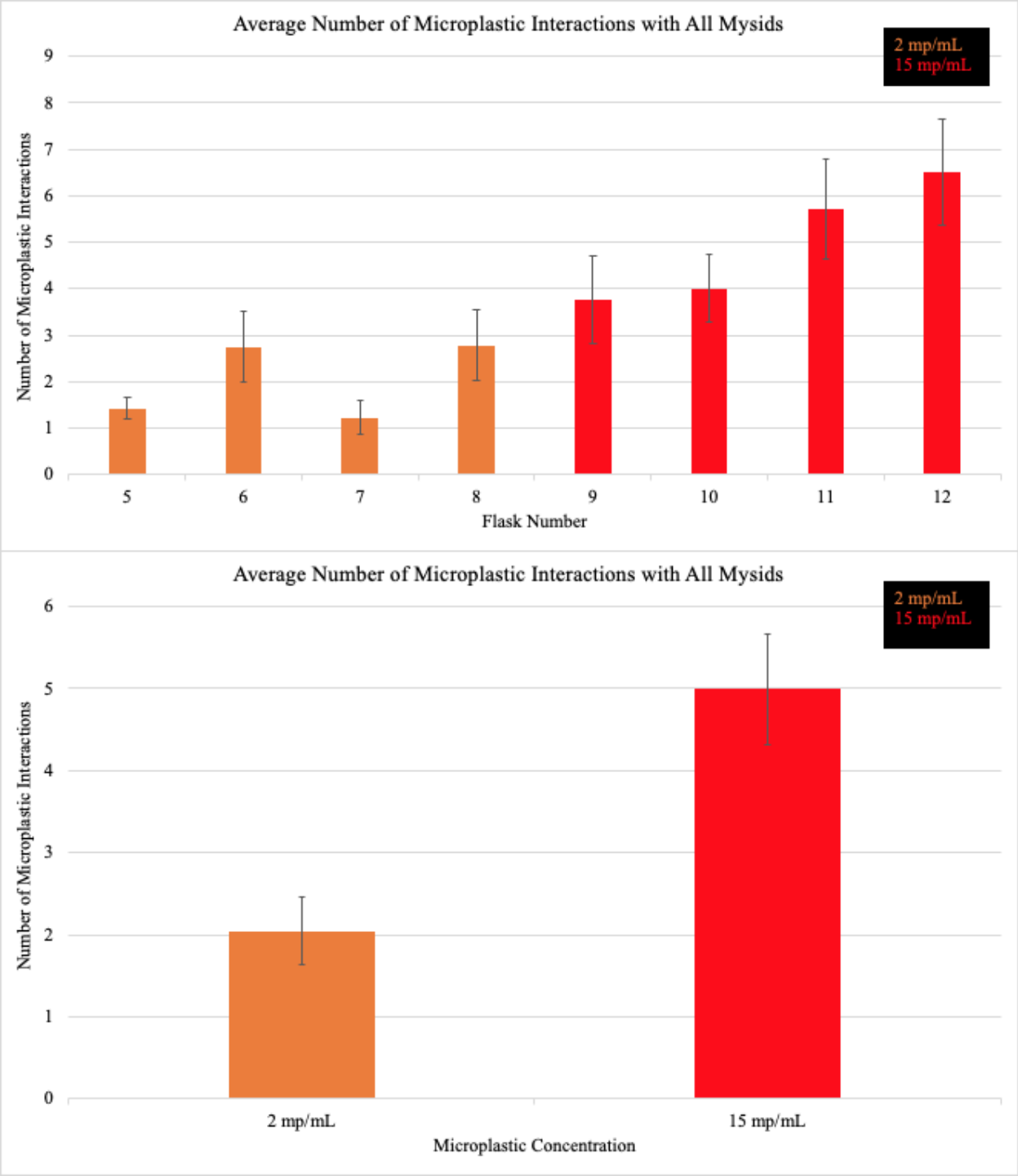
Experiment I microplastic interactions with adult mysids. Number of microplastic interactions per flask +/- standard error (top) and mean number of microplastic interactions per treatment level +/- standard error (bottom). ANOVA results show no difference between the average number of polystyrene microbead interactions with adult mysids per flask treated with either 2 or 15 microplastics per milliliter (mp/mL). p-value 0.304, 1 df, F-value 1.264.

S19 Average Number of Microplastic Interactions with Juvenile Mysids



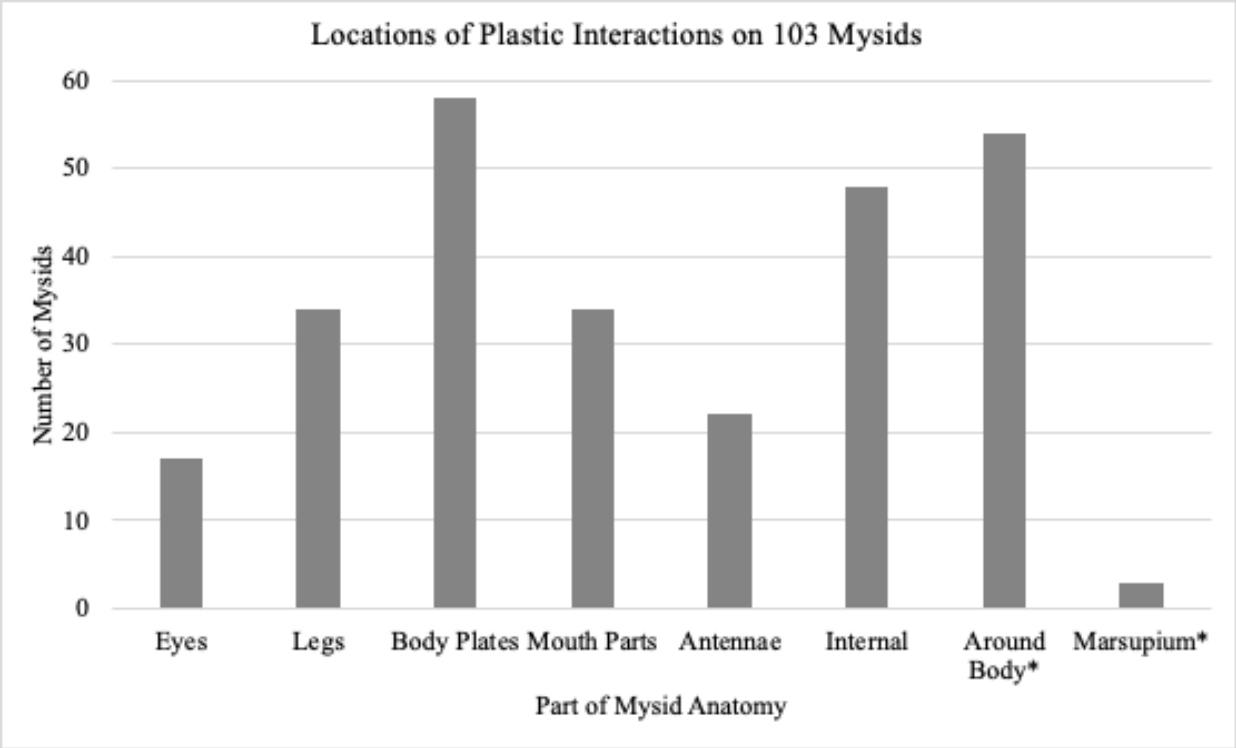
Experiment I microplastic interactions with juvenile mysids. Number of microplastic interactions per flask +/- standard error (top) and mean number of microplastic interactions per treatment level +/- standard error (bottom). ANOVA results show a significant difference between the average number of polystyrene microbead interactions with juvenile mysids per flask treated with either 2 or 15 microplastics per milliliter (mp/mL). p-value 0.012, 1 df, F-value 12.437.

S20 Average Number of Microplastic Interactions with All Mysids



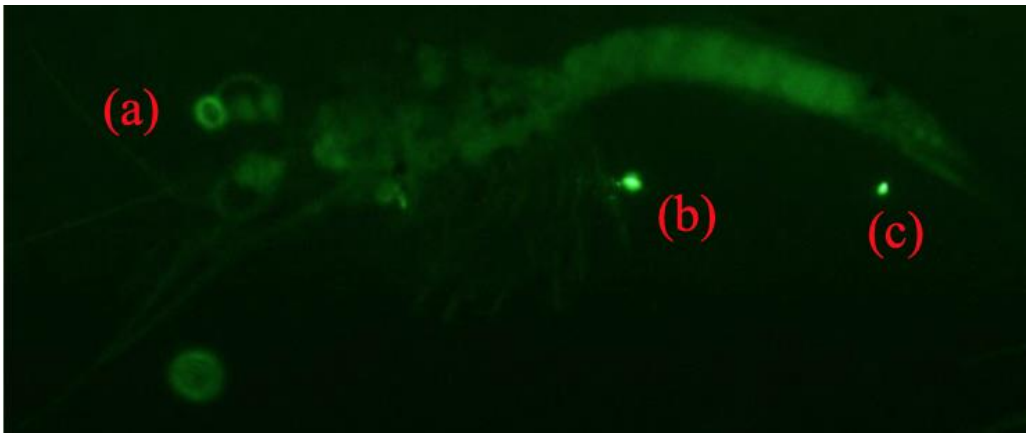
Experiment I microplastic interactions with all mysids. Number of microplastic interactions per flask +/- standard error (top) and mean number of microplastic interactions per treatment level +/- standard error (bottom). ANOVA results show a significant difference between the average number of polystyrene microbead interactions with all mysids per flask treated with either 2 or 15 microplastics per milliliter (mp/mL). p-value 0.010, 1 df, F-value 14.049.

S21



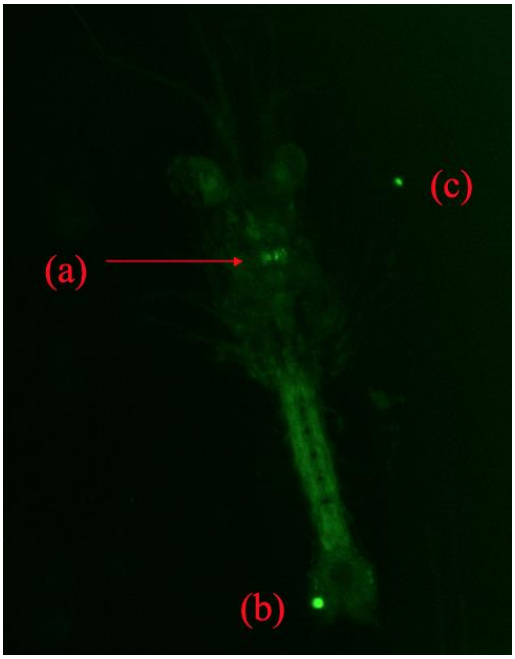
Number of *A. bahia* individuals (n=103) affected per anatomical structure that interacted with polystyrene microbeads. Microbeads interacted with more than one part of the body on 62 individuals.

S22



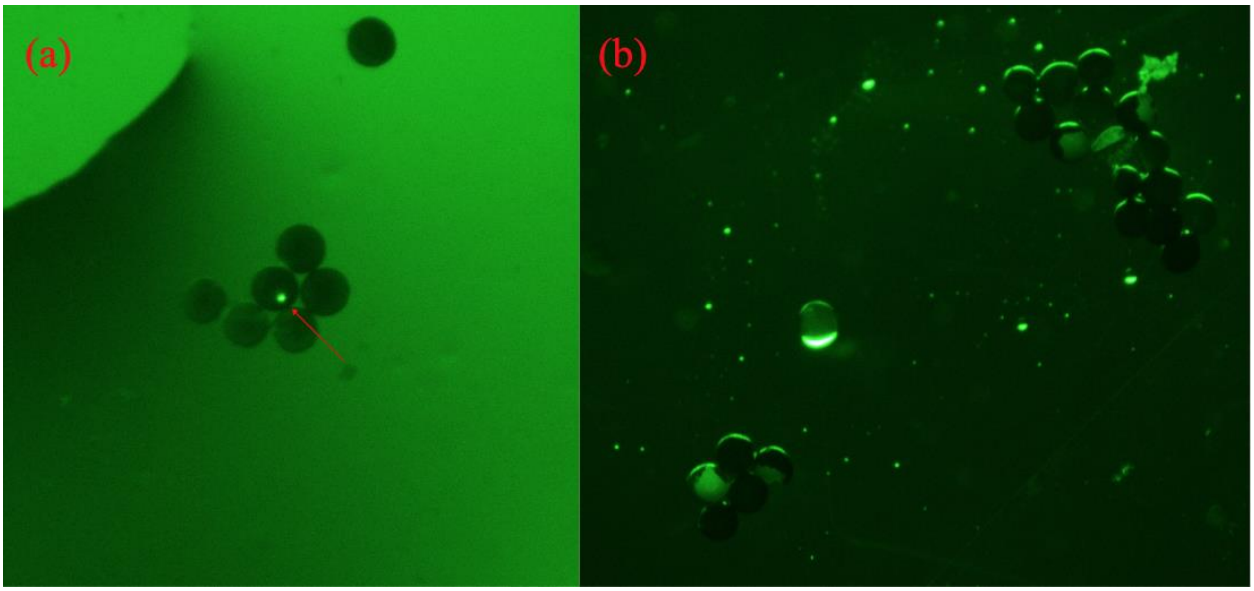
Examples of microbead interactions on a juvenile from flask 10 of experiment I. Microbeads are seen on (a) the right eye (b) the last pereiopod, and (c) around the body. (Nikon SMZ1270 microscope; Nikon DS-Fi3 camera)

S23



Examples of microbead interactions on a mysid from experiment I. Microplastics are seen (a) internally from the dorsal view of the individual, (b) as well as on the uropod, and (c) nearby, but not on the body. (Nikon SMZ1270 microscope; Nikon DS-Fi3 camera)

S24



Images of microplastics among particulate matter. Microscopy of particulate matter from microplastic dosage flasks shows microplastics (a) stuck to brine shrimp cysts and (b) dispersing into small aggregates. (Nikon SMZ1270 microscope; Nikon DS-Fi3 camera)

Tables:

Table S1: Mortality Results – Experiment I

Flask Number	Dead Day 1	Dead Day 2, % Dead	Alive for Day 3	Dead Day 3, % Dead (from alive for day 3)	Total Dead, % Dead	Total Alive (Preserved)
1	0	0, 0%	25	0, 0%	0, 0%	25
2	0	2, 8.0%	23	1, 4.3%	3, 12.0%	22
3	0	0, 0%	25	2, 8.0%	2, 8.0%	23
4	0	1, 4.0%	24	3, 12.5%	4, 16.0%	21
5	0	0, 0%	25	4, 16.0%	4, 16.0%	21
6	0	2, 8.0%	23	0, 0%	2, 8.0%	23
7	0	4, 16.0%	21	1, 4.8%	5, 20.0%	20
8	0	0, 0%	25	2, 8.0%	2, 8.0%	23
9	0	1, 4.0%	24	0, 0%	1, 4.0%	24
10	0	2, 8.0%	23	5, 21.7%	7, 28.0%	18
11	0	2, 8.0%	23	1, 4.3%	3, 12.0%	22
12	0	6, 24.0%	19	1, 5.3%	7, 28.0%	18
0 mp/mL			2 mp/mL		15 mp/mL	

Mysid mortality over the three-day duration of experiment I. Comparisons between control and treatment group % dead were made after each day and at the end of the experiment. Flasks 1-4 (grey) were treated with approximately 0 mp/mL (microplastics per milliliter). Flasks 5-8 (yellow) were treated with approximately 2 mp/mL. Flasks 9-12 (orange) were treated with approximately 15 mp/mL.

Table S2: Mortality Results – Experiment II

Flask Number	Dead Day 1	Dead Day 2	Dose 1 Dead, % Dead	Dose 2 Mysids	Dead Day 3	Dead Day 4	Dose 2 Dead, % Dead	Dose 3 Mysids	Dead Day 5	Dead Day 6	Dose 3 Dead, % Dead	Living Mysids	Total Dead, % Dead	Total Preserved
1	0	1	1, 4.0%	18	9	9	18, 100%	X	X	X	X	0	19, 76.0%	6
2	0	0	0, 0%	19	1	6	7, 36.8%	7	7	X	7, 100%	0	14, 56.0%	11
3	0	1	1, 4.0%	18	2	5	7, 38.9%	6	6	X	6, 100%	0	14, 56.0%	11
4	0	0	0, 0%	19	2	6	8, 42.1%	6	1	0	1, 16.7%	5	9, 36.0%	16
5	0	0	0, 0%	19	1	0	1, 5.3%	13	0	2	2, 15.4%	11	3, 12.0%	22
6	0	2	2, 8.0%	17	0	0	0, 0%	12	3	2	5, 41.7%	7	7, 28.0%	18
7	0	2	2, 8.0%	17	1	1	2, 11.8%	10	0	3	3, 30%	7	7, 28.0%	18
8	0	4	4, 16.0%	15	0	1	1, 6.7%	9	2	2	4, 44%	5	9, 36.0%	16
25 Mysids per Beaker	6 Living Preserved After Dose 1		% of 25	Start of Dose 2	5 Living Preserved After Dose 2		% of Dose 2 Mysids	Start of Dose 3	X Marks 0 Remaining in Flask		% of Dose 3 Mysids	Any Remaining Alive Preserved After Final Dose		
0 mp/mL				4 mp/mL				8 mp/mL				12 mp/mL		

Mysid mortality over the six-day duration of experiment II (0 mp/mL = 0 microplastics per milliliter). Concentrations of microplastics were increased by 4 mp/mL with each dose interval. Comparisons between control and treatment groups % dead were made after each 48-hour dosing interval and at the end of the experiment. The second to last row of the table provides descriptions of the mysid sampling methods during the experiment. The bottom row is a color legend for the table.

Table S3: Frequency of Methylation Detections

Detections of Methylation per Flask

Flask	Number of Methylation Detections	Frequency of Detection	Average Frequency of Detections per Treatment (+/- SE)
I1 (n=18)	16	0.89	
I2 (n=17)	17	1.00	
I3 (n=17)	14	0.82	
I4 (n=18)	17	0.94	0.914 +/- 0.038
I5 (n=18)	15	0.83	
I6 (n=17)	13	0.76	
I7 (n=18)	17	0.94	
I8 (n=18)	16	0.89	0.858 +/- 0.038
I9 (n=17)	13	0.76	
I10 (n=18)	14	0.78	
I11 (n=17)	13	0.76	
I12 (n=17)	15	0.88	0.797 +/- 0.028
Shipped (n=12)	12	1.00	

Frequency of DNA methylation detections in *Americamysis bahia* to a limit of 0.05 ng. Ranked ANOVA results show no difference between treatment groups (Top: p-0.117, 2 df, F-2.754)

Table S4: Number of Microplastic Interactions with Adult Mysids

ADULT MYSIDS - Experiment I						
Flask Number	Number of Mysids	Total Interactions	Average # Interactions / Mysid	SD of Flask	SE of Flask	Average of Treatment (+/- SE of Treatment)
I1	0	0	0	0	0	

I2	3	0	0	0	0	
I3	2	0	0	0	0	
I4	1	0	0	0	0	0 +/- 0
I5	2	2	1.00	1.41	1.00	
I6	1	2	2.00	0	0	
I7	3	1	0.33	0.58	0.33	
I8	2	2	1.00	0	0	1.09 +/- 0.34
I9	1	1	1.00	0	0	
I10	2	7	3.50	3.54	2.50	
I11	1	0	0	0	0	
I12	7	55	7.86	5.79	0.83	3.09 +/- 1.75
Total	25	70				

Table S5: Number of Microplastic Interactions with Juvenile Mysids

JUVENILE MYSIDS - Experiment I

Flask Number	Number of Mysids	Total Interactions	Average # Interactions / Mysid	SD of Flask	SE of Flask	Average of Treatment (+/- SE of Treatment)
I1	3	0	0	0	0	

I2	7	0	0	0	0	
I3	2	0	0	0	0	
I4	6	0	0	0	0	0 +/- 0
I5	10	15	1.50	0.77	0.22	
I6	3	9	3.00	1.73	1.00	
I7	10	15	1.50	1.35	0.42	
I8	7	23	3.29	2.36	0.89	2.32 +/- 0.48
I9	7	29	4.14	2.61	0.99	
I10	7	29	4.14	2.04	0.77	
I11	6	40	6.67	1.51	0.61	
I12	7	36	5.14	1.46	0.55	5.02 +/- 0.60
Total	75	196				

Table S6: Number of Microplastic Interactions with All Mysids

ALL MYSIDS - Experiment I

Flask Number	Number of Mysids	Total Interactions	Average # Interactions / Mysid	SD of Flask	SE of Flask	Average of Treatment (+/- SE of Treatment)
I1	3	0	0	0	0	
I2	10	0	0	0	0	
I3	4	0	0	0	0	
I4	7	0	0	0	0	0 +/- 0

I5	12	17	1.42	0.79	0.23	
I6	4	11	2.75	1.50	0.75	
I7	13	16	1.23	1.30	0.36	
I8	9	25	2.78	2.28	0.76	2.05 +/- 0.42
I9	8	30	3.75	2.66	0.94	
I10	9	36	4.00	2.18	0.73	
I11	7	40	5.71	2.87	1.08	
I12	14	91	6.50	4.29	1.15	4.99 +/- 0.67
Total	100	266				