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Differential dissolution and toxicity of surface functionalized silver nanoparticles in small-scale microcosms: impacts of community complexity

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Supporting Information:

15 pages, 5 tables, 14 figures

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Table S1. Metadata associated with zeta potential measurements.

Surface functionalization	PEG; Silanol; Amine terminated Silica	
Shape	Spherical	
Size distribution (minimum - maximum)	PEG-Ag	50 - 220 nm
	Si-Ag	68 - 295 nm
	Ami-Si-Ag	68 - 615 nm
Initial hydrodynamic diameter (mean)	PEG-Ag	96.6
	Si-Ag	136.5
	Ami-Si-Ag	200.2
Particle concentration from the manufacturer	PEG-Ag	5.9E+9 particles/ml
	Si-Ag	5.1E+9 particles/ml
	Ami-Si-Ag	5.9E+9 particles/ml
Electrophoretic mobility (initial: 0 hour - final: 120 hours)	PEG-Ag	-0.287 - -0.199
	Si-Ag	-1.193 - 1.04
	Ami-Si-Ag	0.992 - 2.213
Model used to compute the zeta potential	Smoluchowski equation	
pH	7.2	
Ionic strength	0.01 mol/L	
Ionic composition	Made according to reference 36	
Temperature	25 °C	
Viscosity	0.8872 mPa	
Macromolecules/NOM	None	
Duration of measurement	4 minutes	
Applied voltage	148 V	
Number of instrument measurements made and averaged to determine each ZP	12	
Total number of replicate measurements	3	

Table S2. AgNP digestion recovery rates.

NP type	Recovery rate (%)	Standard deviation (%)
PEG-Ag	105.4	5.1
Si-Ag	104.2	2.6
Ami-Si-Ag	104.1	2.3

Table S3. AgNP hydrodynamic diameters (HDD) and the corresponding polydispersity index (PDI) measured every 24 hours throughout the experimental period (± indicates the standard error of three sample replicates).

	PEG-Ag (MQW)		PEG-Ag (NCM)		Si-Ag (MQW)		Si-Ag (NCM)		Ami-Si-Ag (MQW)		Ami-Si-Ag (NCM)	
Time (hr)	HDD (nm)	PDI	HDD (nm)	PDI	HDD (nm)	PDI	HDD (nm)	PDI	HDD (nm)	PDI	HDD (nm)	PDI
0	97.7 ± 0.17	0.058 ± 0.006	96.6 ± 0.15	0.041 ± 0.007	132.2 ± 0.5	0.014 ± 0.01	136.5 ± 1.1	0.033 ± 0.009	202.5 ± 2	0.152 ± 0.019	200.2 ± 1.7	0.162 ± 0.005
24	98.2 ± 0.65	0.063 ± 0.008	98.8 ± 0.32	0.053 ± 0.002	131 ± 1.7	0.037 ± 0.002	138.8 ± 0.9	0.078 ± 0.008	211.9 ± 1.1	0.128 ± 0.023	214.1 ± 2	0.161 ± 0.017
48	98.3 ± 0.32	0.058 ± 0.011	99.7 ± 0.61	0.058 ± 0.001	124.1 ± 0.67	0.044 ± 0.01	134.4 ± 2.4	0.107 ± 0.014	199.7 ± 1.23	0.171 ± 0.009	222.1 ± 2.1	0.176 ± 0.013
72	98.8 ± 0.56	0.061 ± 0.004	101.1 ± 0.25	0.06 ± 0.004	120 ± 1.41	0.051 ± 0.007	142.7 ± 0.76	0.173 ± 0.012	162 ± 0.33	0.184 ± 0.01	209.5 ± 1.5	0.174 ± 0.011
96	97.1 ± 0.51	0.07 ± 0.005	100.9 ± 0.82	0.045 ± 0.005	119.4 ± 1.02	0.07 ± 0.01	131 ± 0.76	0.106 ± 0.017	135 ± 0.9	0.155 ± 0.011	220 ± 2.6	0.197 ± 0.011
120	97.1 ± 0.35	0.062 ± 0.01	99.5 ± 0.31	0.058 ± 0.016	117.1 ± 0.92	0.045 ± 0.004	138.4 ± 1.02	0.12 ± 0.012	113.3 ± 0.52	0.149 ± 0.013	208.4 ± 2.3	0.19 ± 0.021

Table S4. Theoretical Ag speciation calculated with Visual-MINTEQ assuming equilibrium in NCM. $AgCl_{(s)}$ highlight in red indicates the percentage of precipitates that can be formed at the modeled concentrations.

a) $AgCl_{(s)}$ starts to form when the input Ag^{+} concentration is above 59 µg L^{-1}.

Input Ag+ concentration (59 µg/L)			Input Ag+ concentration (60 µg/L)		
Ag Speciation	µg Ag/L	% Total	Ag Speciation	µg Ag/L	% Total
Ag(OH)2-	1.25E-09	0%	Ag(OH)2-	1.26E-09	0%
Ag+1	5.02E+00	9%	Ag+1	5.07E+00	8%
Ag2MoO4 (aq)	4.27E-14	0%	Ag2MoO4 (aq)	4.36E-14	0%
AgCl (aq)	3.88E+01	66%	AgCl (aq)	3.92E+01	65%
AgCl2-	1.52E+01	26%	AgCl2-	1.54E+01	26%
AgCl3-2	7.23E-02	0%	AgCl3-2	7.31E-02	0%
AgEDTA-3	2.75E-07	0%	AgEDTA-3	2.78E-07	0%
AgH2BO3 (aq)	9.89E-06	0%	AgH2BO3 (aq)	9.99E-06	0%
AgHEDTA-2	5.53E-08	0%	AgHEDTA-2	5.58E-08	0%
AgNO3 (aq)	1.67E-03	0%	AgNO3 (aq)	1.69E-03	0%
AgOH (aq)	7.34E-05	0%	AgOH (aq)	7.42E-05	0%
AgSO4-	6.33E-03	0%	AgSO4-	6.39E-03	0%
AgCl (s)	0.00E+00	0%	AgCl (s)	4.13E-01	1%
Total	59.07	100%	Total	60.07	100%

b) Ag speciation calculated using the highest abiotic dissolved Ag concentration measured in NCM (Figure 2) for each AgNP.

PEG-AgNP (24 µg/L)			Si-AgNP (53 µg/L)			Ami-Si-AgNP (22 µg/L)		
Ag Speciation	µg Ag/L	% Total	Ag Speciation	µg Ag/L	% Total	Ag Speciation	µg Ag/L	% Total
Ag(OH)2-	5.08E-10	0%	Ag(OH)2-	1.12E-09	0%	Ag(OH)2-	4.66E-10	0%
Ag+1	2.04E+00	9%	Ag+1	4.51E+00	9%	Ag+1	1.87E+00	9%
Ag2MoO4 (aq)	7.07E-15	0%	Ag2MoO4 (aq)	3.45E-14	0%	Ag2MoO4 (aq)	5.94E-15	0%
AgCl (aq)	1.58E+01	66%	AgCl (aq)	3.48E+01	66%	AgCl (aq)	1.45E+01	66%
AgCl2-	6.18E+00	26%	AgCl2-	1.37E+01	26%	AgCl2-	5.67E+00	26%
AgCl3-2	2.94E-02	0%	AgCl3-2	6.50E-02	0%	AgCl3-2	2.70E-02	0%
AgEDTA-3	1.12E-07	0%	AgEDTA-3	2.47E-07	0%	AgEDTA-3	1.02E-07	0%
AgH2BO3 (aq)	4.02E-06	0%	AgH2BO3 (aq)	8.88E-06	0%	AgH2BO3 (aq)	3.69E-06	0%
AgHEDTA-2	2.25E-08	0%	AgHEDTA-2	4.97E-08	0%	AgHEDTA-2	2.06E-08	0%
AgNO3 (aq)	6.80E-04	0%	AgNO3 (aq)	1.50E-03	0%	AgNO3 (aq)	6.24E-04	0%
AgOH (aq)	2.99E-05	0%	AgOH (aq)	6.60E-05	0%	AgOH (aq)	2.74E-05	0%
AgSO4-	2.57E-03	0%	AgSO4-	5.68E-03	0%	AgSO4-	2.36E-03	0%
AgCl (s)	0.00E+00	0%	AgCl (s)	0.00E+00	0%	AgCl (s)	0.00E+00	0%
Total	24.03	100%	Total	53.07	100%	Total	22.03	100%

Table S5. Theoretical Ag speciation calculated with Visual-MINTEQ using measured dissolved silver concentrations in the presence of oprganisms for each type of AgNP in NCM at each exposure concentration (Figure 3). $AgCl_{(s)}$ highlight in red indicates the percentage of precipitates that can be formed at the modeled concentrations.

PEG-Ag (Exposure con. 100 µg/L)			PEG-Ag (Exposure con. 1000 µg/L)			PEG-Ag (Exposure con. 5000 µg/L)		
Dissolved Ag measured in NC (2.1 µg/L)			Dissolved Ag measured in NC (132.9 µg/L)			Dissolved Ag measured in NC (906.2 µg/L)		
Ag Speciation	Mass (µg/L)	% Total	Ag Speciation	Mass (µg/L)	% Total	Ag Speciation	Mass (µg/L)	% Total
Ag(OH)2-	4.36E-11	0%	Ag(OH)2-	1.26E-09	0%	Ag(OH)2-	1.26E-09	0%
Ag+1	1.75E-01	9%	Ag+1	5.07E+00	4%	Ag+1	5.08E+00	1%
Ag2MoO4 (aq)	5.21E-17	0%	Ag2MoO4 (aq)	4.36E-14	0%	Ag2MoO4 (aq)	4.38E-14	0%
AgCl (aq)	1.35E+00	66%	AgCl (aq)	3.92E+01	29%	AgCl (aq)	3.92E+01	4%
AgCl2-	5.31E-01	26%	AgCl2-	1.54E+01	12%	AgCl2-	1.53E+01	2%
AgCl3-2	2.53E-03	0%	AgCl3-2	7.30E-02	0%	AgCl3-2	7.28E-02	0%
AgEDTA-3	9.60E-09	0%	AgEDTA-3	2.78E-07	0%	AgEDTA-3	2.78E-07	0%
AgH2BO3 (aq)	3.45E-07	0%	AgH2BO3 (aq)	9.99E-06	0%	AgH2BO3 (aq)	1.00E-05	0%
AgHEDTA-2	1.93E-09	0%	AgHEDTA-2	5.59E-08	4.20E-10	AgHEDTA-2	5.59E-08	0%
AgNO3 (aq)	5.84E-05	0%	AgNO3 (aq)	1.69E-03	0%	AgNO3 (aq)	1.69E-03	0%
AgOH (aq)	2.56E-06	0%	AgOH (aq)	7.42E-05	0%	AgOH (aq)	7.43E-05	0%
AgSO4-	2.21E-04	0%	AgSO4-	6.39E-03	0%	AgSO4-	6.40E-03	0%
AgCl (s)	0.00E+00	0%	AgCl (s)	7.34E+01	55%	AgCl (s)	8.48E+02	93%
Total	2.06	100%	Total	133.03	100%	Total	907.29	100%
Si-Ag (Exposure con. 100 µg/L)			Si-Ag (Exposure con. 1000 µg/L)			Si-Ag (Exposure con. 5000 µg/L)		
Dissolved Ag measured in NC (26.4 µg/L)			Dissolved Ag measured in NC (229.9 µg/L)			Dissolved Ag measured in NC (815.96 µg/L)		
Ag Speciation	Mass (µg/L)	% Total	Ag Speciation	Mass (µg/L)	% Total	Ag Speciation	Mass (µg/L)	% Total
Ag+1	2.25E+00	9%	Ag+1	5.07E+00	2%	Ag+1	5.08E+00	1%
Ag2MoO4 (aq)	8.56E-15	0%	Ag2MoO4 (aq)	4.36E-14	0%	Ag2MoO4 (aq)	4.37E-14	0%
AgCl (aq)	1.74E+01	66%	AgCl (aq)	3.92E+01	17%	AgCl (aq)	3.92E+01	5%
AgCl2-	6.81E+00	26%	AgCl2-	1.53E+01	7%	AgCl2-	1.53E+01	2%
AgCl3-2	3.24E-02	0%	AgCl3-2	7.30E-02	0%	AgCl3-2	7.28E-02	0%
AgEDTA-3	1.23E-07	0%	AgEDTA-3	2.78E-07	0%	AgEDTA-3	2.78E-07	0%
AgH2BO3 (aq)	4.43E-06	0%	AgH2BO3 (aq)	9.99E-06	0%	AgH2BO3 (aq)	1.00E-05	0%
AgHEDTA-2	2.47E-08	0%	AgHEDTA-2	5.59E-08	0%	AgHEDTA-2	5.59E-08	0%
AgNO3 (aq)	7.49E-04	0%	AgNO3 (aq)	1.69E-03	0%	AgNO3 (aq)	1.69E-03	0%
AgOH (aq)	3.29E-05	0%	AgOH (aq)	7.42E-05	0%	AgOH (aq)	7.43E-05	0%
AgSO4-	2.83E-03	0%	AgSO4-	6.39E-03	0%	AgSO4-	6.40E-03	0%
AgCl (s)	0.00E+00	0%	AgCl (s)	1.71E+02	74%	AgCl (s)	7.57E+02	93%
Total	26.44	100%	Total	230.22	100%	Total	816.96	100%
Ami-Si-Ag (Exposure con. 100 µg/L)			Ami-Si-Ag (Exposure con. 1000 µg/L)			Ami-Si-Ag (Exposure con. 5000 µg/L)		
Dissolved Ag measured in NC (7.7 µg/L)			Dissolved Ag measured in NC (84.6 µg/L)			Dissolved Ag measured in NC (309.8 µg/L)		
Ag Speciation	Mass (µg/L)	% Total	Ag Speciation	Mass (µg/L)	% Total	Ag Speciation	Mass (µg/L)	% Total
Ag+1	6.57E-01	9%	Ag+1	5.07E+00	6%	Ag+1	5.08E+00	2%
Ag2MoO4 (aq)	7.32E-16	0%	Ag2MoO4 (aq)	4.36E-14	0%	Ag2MoO4 (aq)	4.36E-14	0%
AgCl (aq)	5.07E+00	66%	AgCl (aq)	3.92E+01	46%	AgCl (aq)	3.92E+01	13%
AgCl2-	1.99E+00	26%	AgCl2-	1.54E+01	18%	AgCl2-	1.53E+01	5%
AgCl3-2	9.47E-03	0%	AgCl3-2	7.30E-02	0%	AgCl3-2	7.30E-02	0%
AgEDTA-3	3.60E-08	0%	AgEDTA-3	2.78E-07	0%	AgEDTA-3	2.78E-07	0%
AgH2BO3 (aq)	1.29E-06	0%	AgH2BO3 (aq)	9.99E-06	0%	AgH2BO3 (aq)	9.99E-06	0%
AgHEDTA-2	7.23E-09	0%	AgHEDTA-2	5.58E-08	0%	AgHEDTA-2	5.59E-08	0%
AgNO3 (aq)	2.19E-04	0%	AgNO3 (aq)	1.69E-03	0%	AgNO3 (aq)	1.69E-03	0%
AgOH (aq)	9.61E-06	0%	AgOH (aq)	7.42E-05	0%	AgOH (aq)	7.42E-05	0%
AgSO4-	8.28E-04	0%	AgSO4-	6.39E-03	0%	AgSO4-	6.39E-03	0%
AgCl (s)	0.00E+00	0%	AgCl (s)	2.50E+01	30%	AgCl (s)	2.51E+02	81%
Total	7.73	100%	Total	84.68	100%	Total	310.20	100%

Figure S1. The potential interactions of the four species in the nanocosm assay. Algae serves as primary producer and produces oxygen for other species in the system. Bacteria are the representative decomposers of the system. Both algae and bacteria are a primary food source of *D. magna*. Zebrafish do not interact with other species directly before they hatch as their chorion serves as a barrier to those interactions. After zebrafish hatching at 80 hours, they start mouth gaping behavior and can potentially ingest some algae and bacteria.

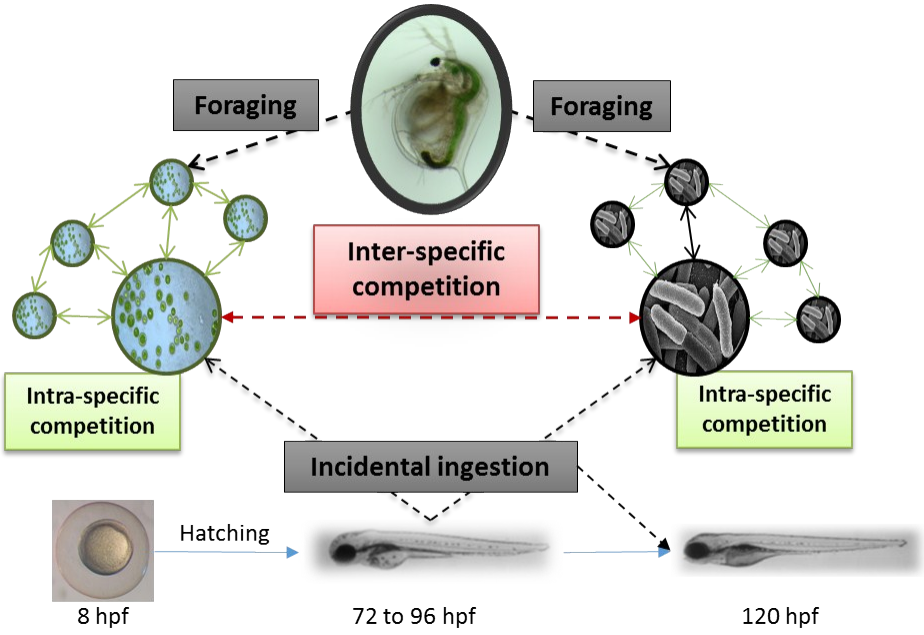

Figure S2. AgNP surface chemistry, TEM images and primary particle sizes as reported by the manufacturer.

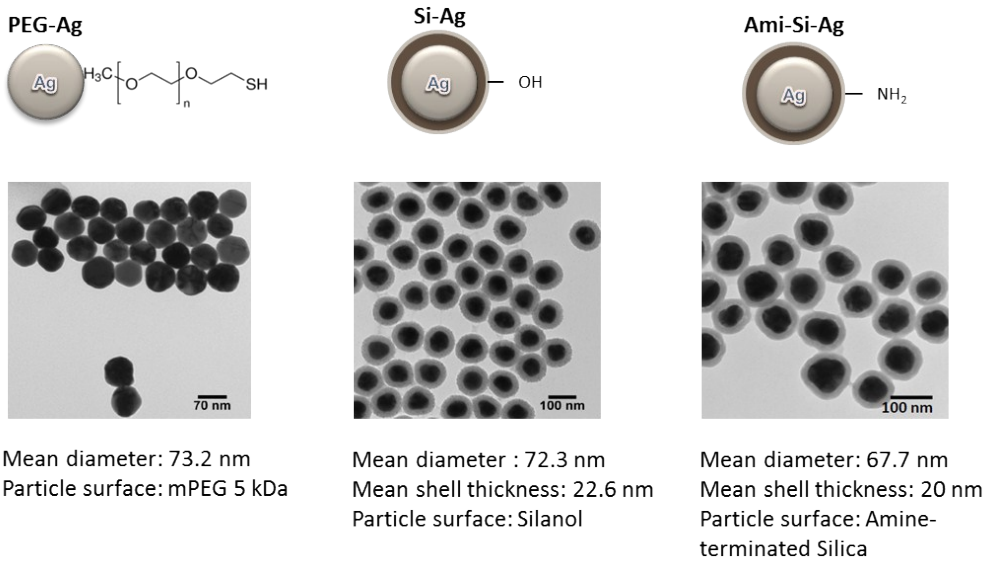

Figure S3. Zeta potential (ZP) of PEG-Ag (orange circle), Si-Ag (green triangle), and Ami-Si-Ag (blue square) in NCM over 120 hours at 10 mg L-1. Standard errors were derived for triplicate measures on each AgNP. Asterisk (*) represents a change in zeta potential from time 0 in Ami-Si-Ag.

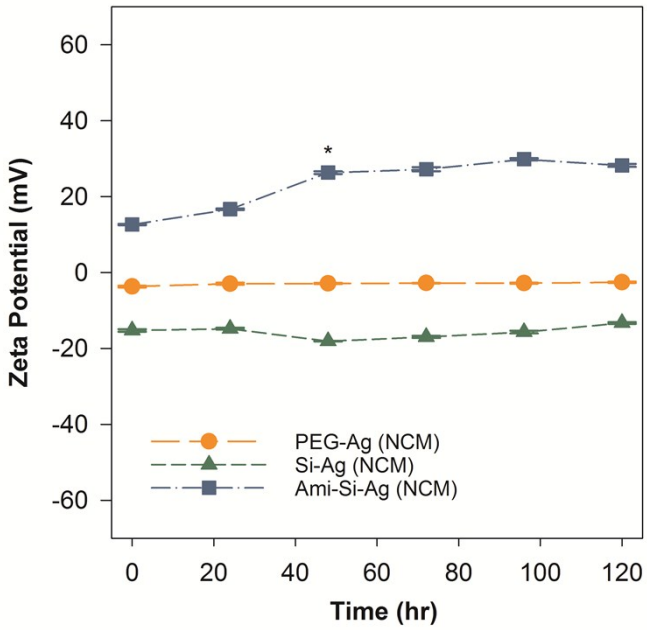

Figure S4. Normalized algal survival in exposure scenarios containing algae and bacteria (A+B), as well as those that added *Daphnia* (A+B+D) and zebrafish (A+B+D+Z) using three different surface functionalized AgNPs. Asterisk (*) indicates significant difference among treatments and the corresponding control.

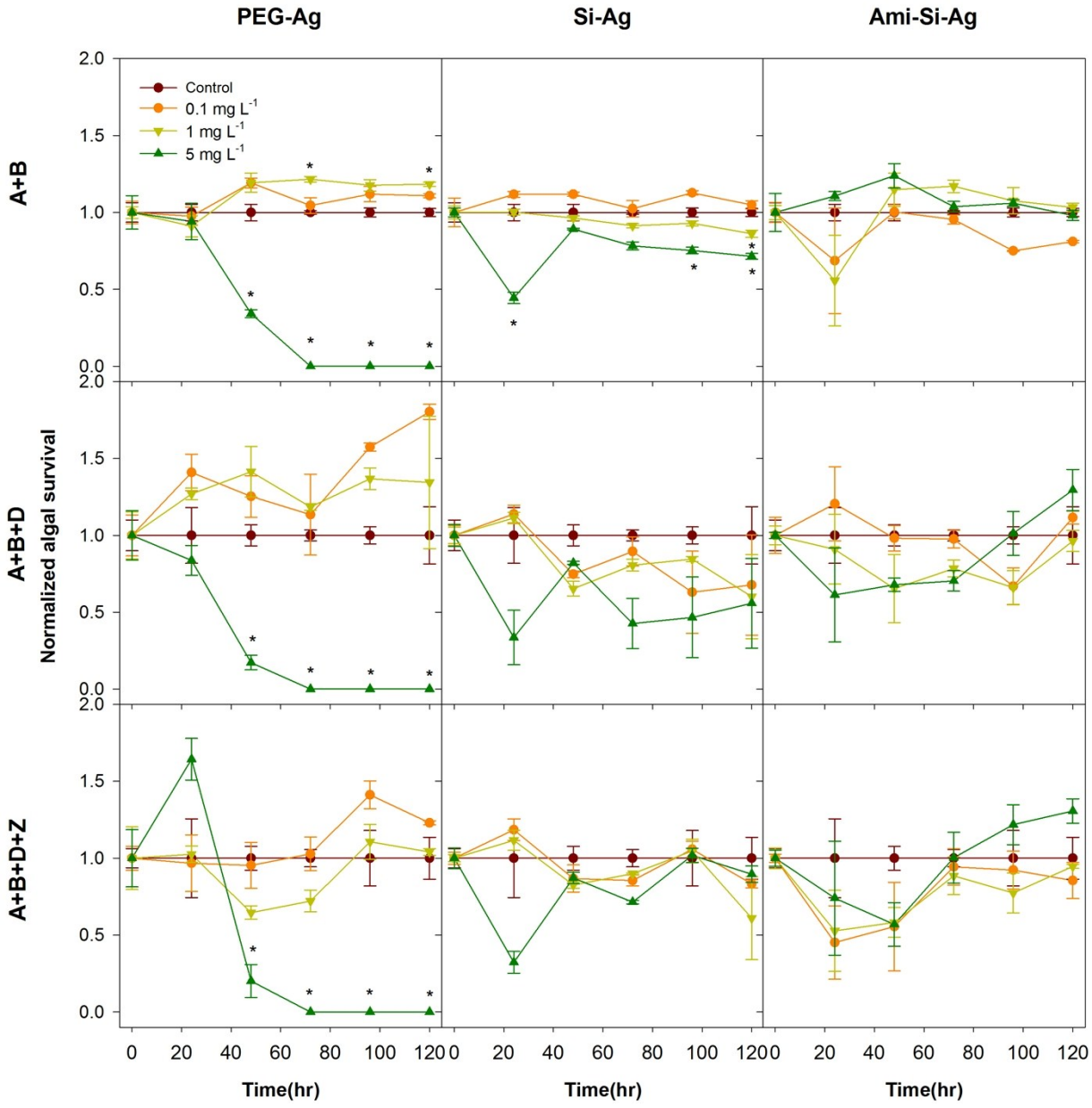

Figure S5. Normalized bacterial survival in three different exposure scenarios with three types of AgNPs. Asterisk (*) indicates significant difference among treatments and the corresponding control.

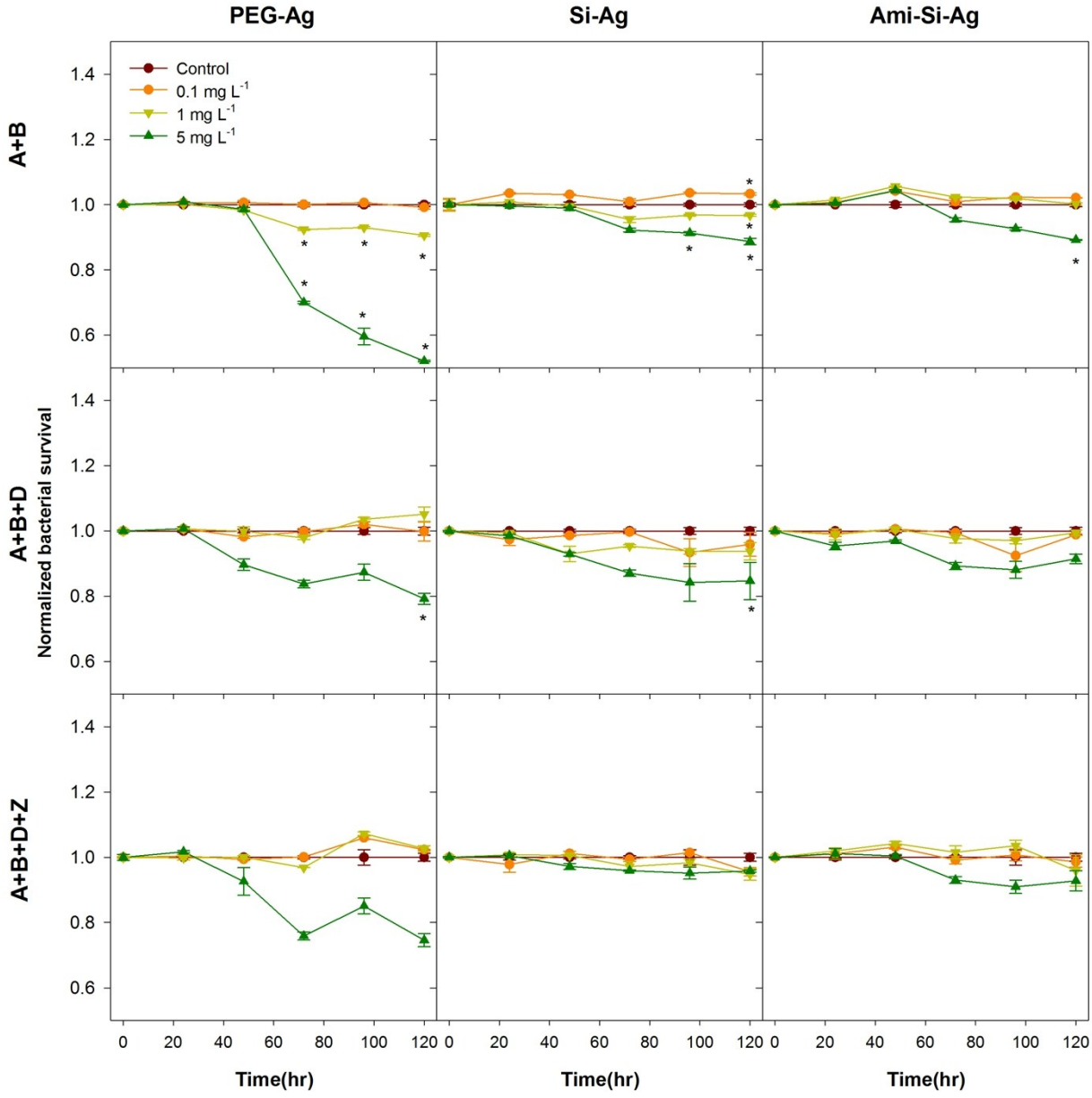

Figure S6. Images of 120-hour *D. magna* after exposure to 5 mg L^{-1} Ami-Si-Ag exposure; A) control; B) *D. magna* in A+B+D exposure scenario; C) *D. magna* in A+B+D+Z exposure scenario.

A) Control B) Ami-Si-Ag (A+B+D) C) Ami-Si-Ag (A+B+D+Z)

Figure S7. Percent of zebrafish mortality and malformation following 120-hours exposure to three different surface functionalized silver nanoparticles. * indicates significant difference from control values. YSE-yolk sac edema; PE-pericardial edema; Circ-circulation.

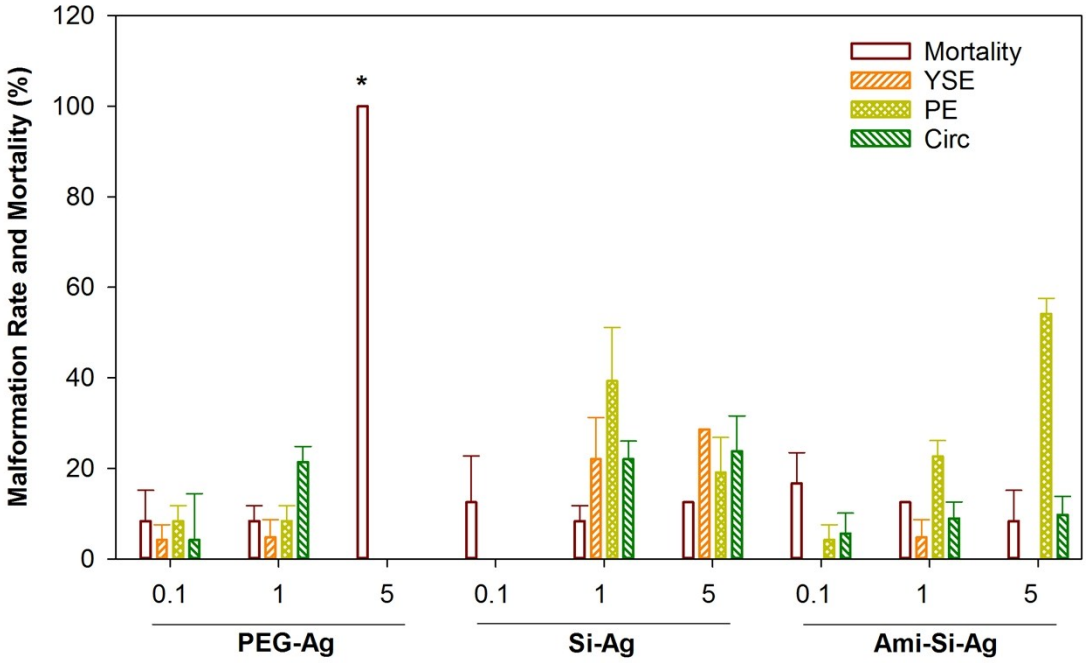

Figure S8. Ag content (%) in unhatched zebrafish chorion (blue) and fish body (green) with 5 mg L^{-1} Si-AgNP exposure after 120 hours under A+B+D+Z exposure scenario.

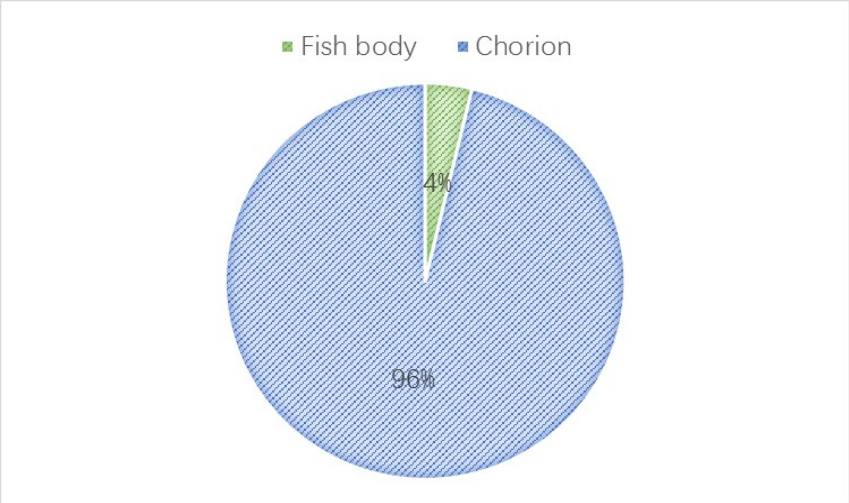

Figure S9. The relationship between the dissolved Ag concentration in the exposure environment and the *Daphnia* (a) and zebrafish (b) silver uptake.

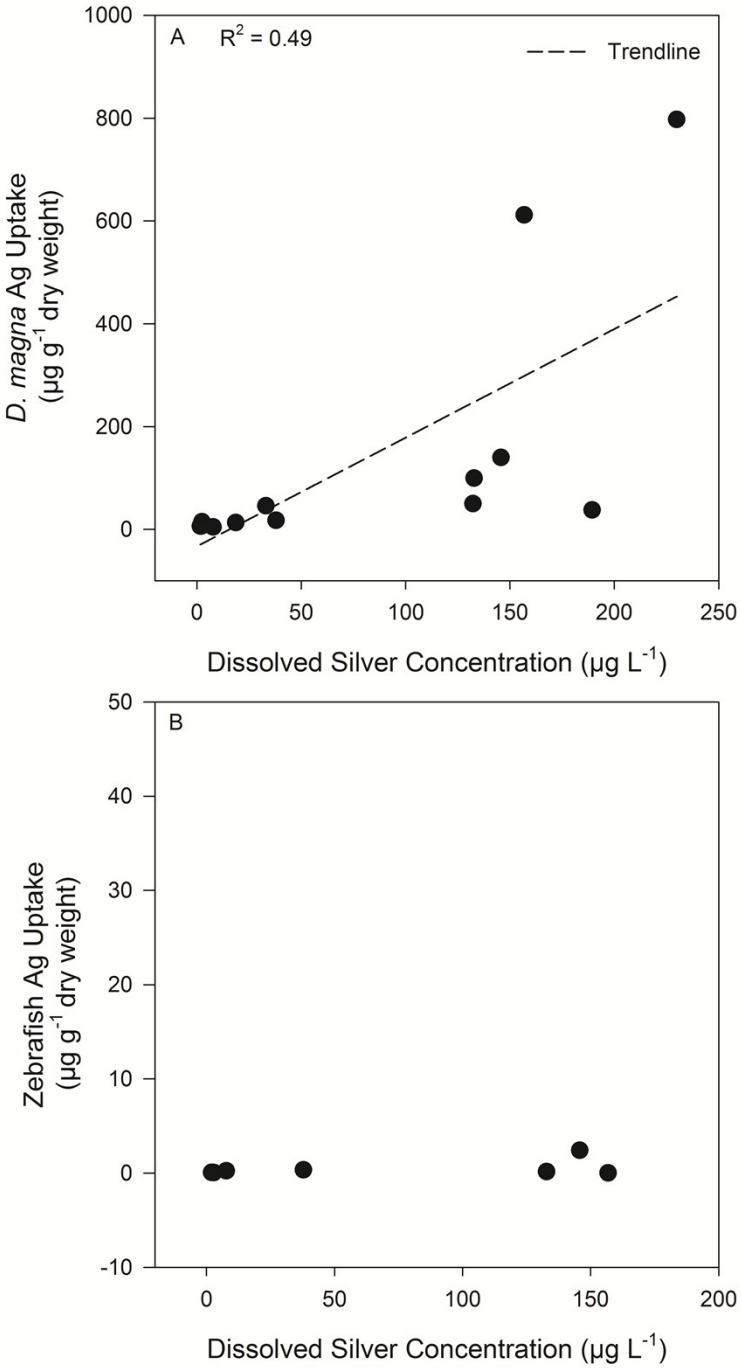

Figure S10. Concentration-response relationship between the dissolved Ag concentration in each exposure nanocosm and algal survival.

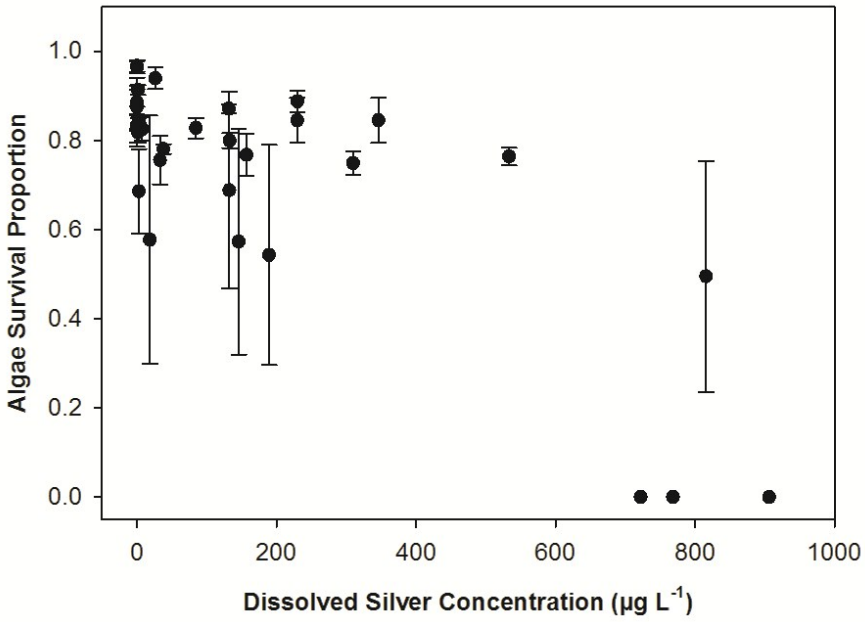

Figure S11. Concentration-response relationship between the dissolved Ag concentration in each exposure nanocosm and bacterial survival.

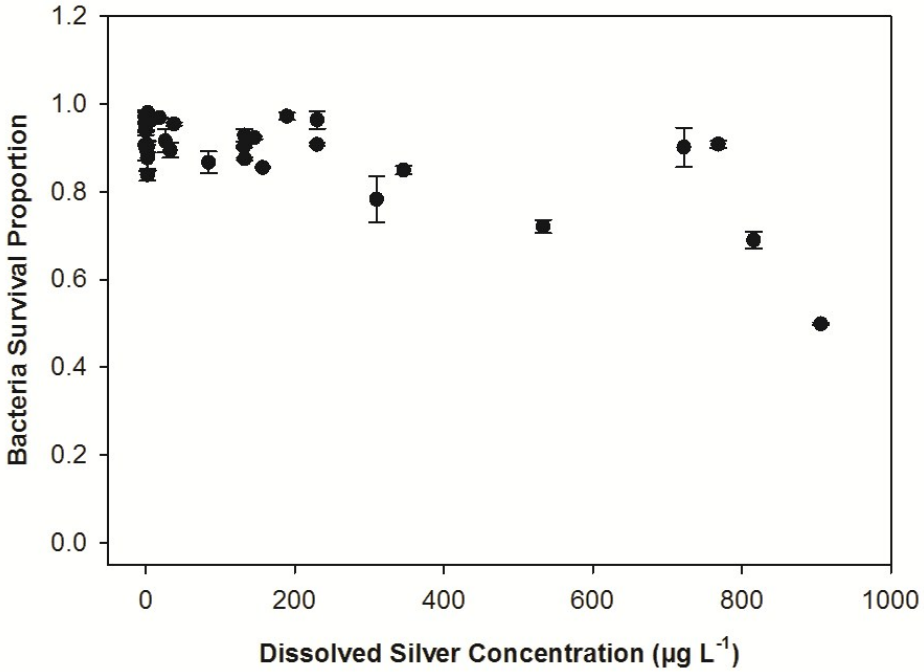

Figure S12. Concentration-response relationship between *D. magna* survival and the dissolved Ag concentration in each exposure nanocosm.

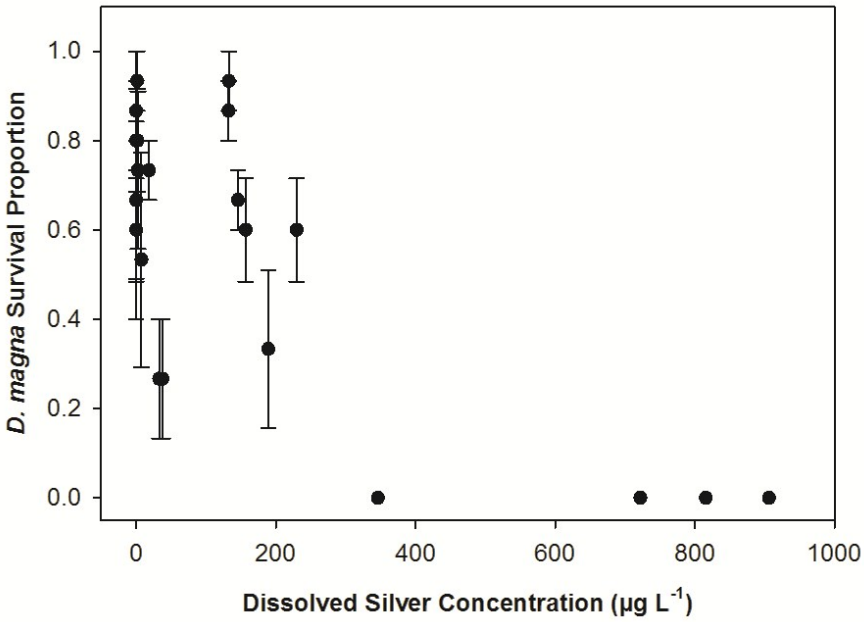

Figure S13. Concentration-response relationship between zebrafish survival and the dissolved Ag concentration in each exposure nanocosm.

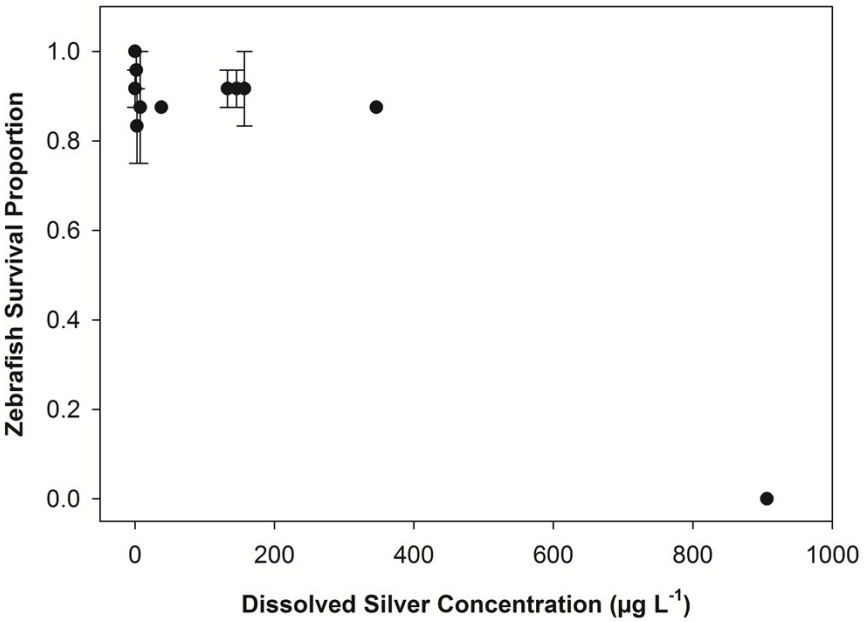

Figure S14. Species sensitivity distributions (SSDs) of organisms in nanocosm using the mean LC_{50} value calculated from Table 2 and the dissolved Ag concentration in the exposure vessels.