Supporting Information for Croteau et al.

Silver bioaccumulation dynamics in a freshwater invertebrate after aqueous and dietary exposures to nanosized and ionic Ag

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13 pages (8 figures; 1 table; 6 equations)

Silver nanoparticles.

cit-Ag NPs were synthesized at 25 °C by adding 0.6 mL of $NaBH_4$ (10 mM) to a mixture of 20 mL of sodium citrate (1.25 mM) and 0.5 mL of $AgNO_3$ (10 mM). The solution was left stirring for 3 hours and thereafter stored in dark for further use. HA-Ag NPs were synthesized at room temperature by adding 10 mL of $NaBH_4$ (10 mM) to a mixture of 10 mL $AgNO_3$ (1 mM) and 10 mL of humic suspension (0.001 wt%). The solution was left stirring for 3 hours and thereafter stored in dark for further use. Humic acid (100%) was purchased from Alfa Aesar, UK (CAS: 1415-93-6).

The size and morphology of the Ag NPs were evaluated using TEM (Hitachi H700, 100 kV). Surface charge was measured using zeta potential module on Malvern Zetasizer Nano (Malvern Instruments) equipped with He-Ne 633 nm laser. The zeta potential of each Ag NPs suspension was measured in MOD water (Table S1) using a Ag NPs concentration of 20ppm.

Samples for imaging were prepared by depositing a portion of sample on an aluminum stub for Scanning Electron Microscopy (SEM) analysis (Phillips XL30). Samples were dried at 40°C prior to imaging and coated with Au-Pd. Images were collected with accelerating voltage of 5 kV and at a working distance of 5mm.

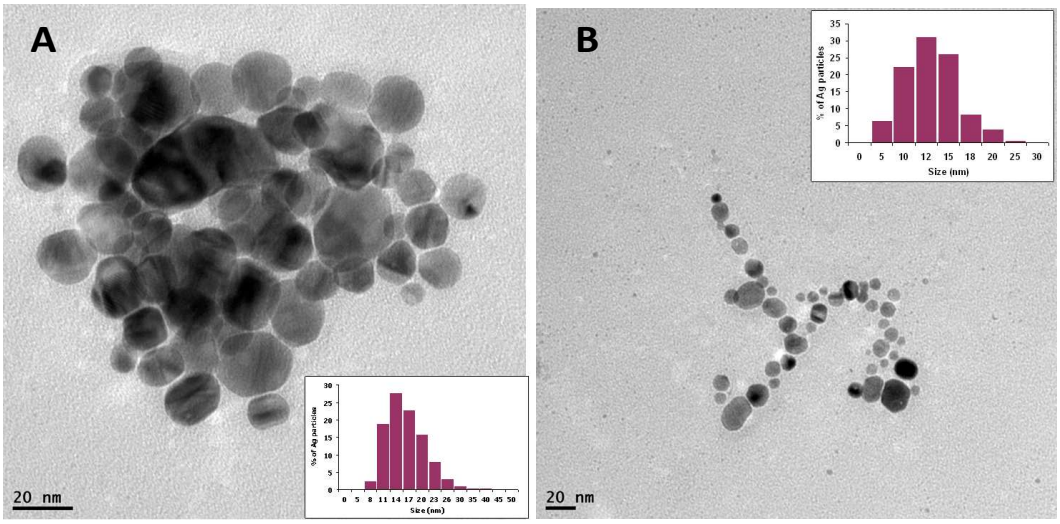

Figure S1. TEM image and size distribution for (a) cit-Ag NPs and (b) humic-Ag NPs (from Misra et al. 9)

Table S1. Ionic composition[1], hardness, pH and ionic strength of moderately hard water[2]

Ca^{2+} (µm)	349
Mg^{2+} (µm)	499
Na^{+} (µm)	1143
K^{+} (µm)	54
Cl^{-} (µm)	54
SO_4^{2-} (µm)	848
CO_3^{2-} (µm)	1143
Hardness (mg $CaCO_3$ l^{-1})	80-100
pH	7.45
Ionic strength	4.74e-03

[1] Nominal concentrations

[2] US EPA 2002 U.S. Environmental Protection Agency: *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms*; U.S. EPA, Washington DC, 2002; EPA-821-R-02-012.

Biodynamic model equations.

Under steady-state conditions, metal concentrations in an exposed organism ($[M]_{ss}$, µg g^{-1}) can be predicted as

(S1) $$[M]_{ss} = \frac{k_{uf} \times [M]_{food}}{k_e + k_g} + \frac{k_{uw} \times [M]_{water}}{k_e + k_g}$$

k_{uf} integrates the influences of food ingestion rate (IR) and metal assimilation efficiency (AE).

(S2) $$k_{uf} = AE \times IR$$

Food IR (g of ingested food g^{-1} (body tissue) day^{-1}) can be determined during pulse-chase feeding experiment by mass-balance calculations using the accumulated amount of metal in the organism after depuration (M_{org} in ng), the amount of metal egested in the feces during depuration (M_{feces} in ng), the metal concentration in the food ($[M]_{food}$ in ng g^{-1}), the organism's dry weight (wt_{org} in ng) and the exposure duration (T in day),

(S3) $$IR = \frac{\left(M_{org} + M_{feces}\right)}{[M]_{food} \times wt_{org} \times T}$$

Metal AE can be similarly calculated as

(S4) $$AE\ (\%) = \frac{M_{org}}{M_{org} + M_{feces}} \times 100$$

Metal loss can be modelled by non-linear regression using equation S5,

(S5) $$[M]_{snail} = [M]^0_{snail}\ e^{-kt}$$

where $[M]_{snail}$ is metal concentration at a given time (µg g^{-1}), $[M]^0_{snail}$ is the metal concentration (µg g^{-1}) at the beginning of depuration, k is the estimated rate constant of loss (d^{-1}), and t is depuration time (d). If growth is negligible ($k_g <= 0$, see eq S6 below), then k equals k_e. If growth is significant ($k_g > 0$), then k equals $k_e + k_g$. The value of k is determined in depuration

experiments where exposed organisms are transferred in cleaned environment. The slope of the regression between $[M]_{snail}/[M]^0_{snail}$ represents k.

Growth can be determined by fitting the dry weight of the experimental organisms (Wt_{snail} in mg dry wt) to an exponential growth function where Wt^0_{snail} is the weight at the beginning of the experiment (mg dry wt), k_g is the growth rate constant (d^{-1}) and T is the time (d).

(S6) $$Wt_{snail} = Wt^0_{snail}\ e^{\ ggT}$$

EDTA extraction procedure.

Because metal uptake is likely to vary whether the metals are adsorbed onto surfaces or incorporates into food, we determine whether Ag was absorbed or adsorbed onto the diatom surfaces upon waterborne exposures. For this, we exposed diatoms to two dissolved Ag concentrations (1 and 10 µg l^{-1}) in SO water for 24 h. The dissolved Ag concentrations were in the range of Ag concentrations used in the waterborne exposure experiments. Upon exposure, diatoms were harvested onto a 1.2-µm Isopore™ membrane filter (Millipore) and rinsed with SO water. Half of the filter holding the labeled diatoms was dried for 24 h at 40°C prior to metal analysis. The other half was re-suspended in a 5 mM solution of ethylenediaminetetraacetic acid (EDTA) for 1-minute (Hassler et al. *Limnol. Oceanogr. Methods* **2004**, *2*: 237-247). The EDTA-washed diatoms were then harvested onto a 1.2-µm Isopore™ membrane filter (Millipore), rinsed with SO water and dried for 24 h at 40°C prior to metal analysis.

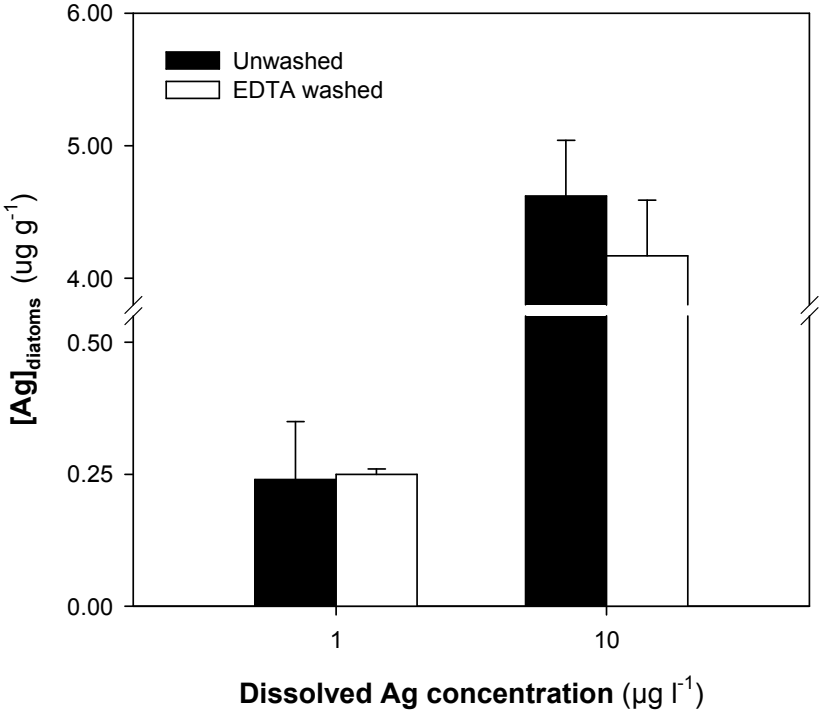

FIGURE S2. Ag concentrations (nmol g^{-1} ± 95% C.I.) in diatoms exposed for 24h to dissolved Ag (as $AgNO_3$). Solid bars are for unwashed algae; open bars are for EDTA-washed algae.

Diatom mats.

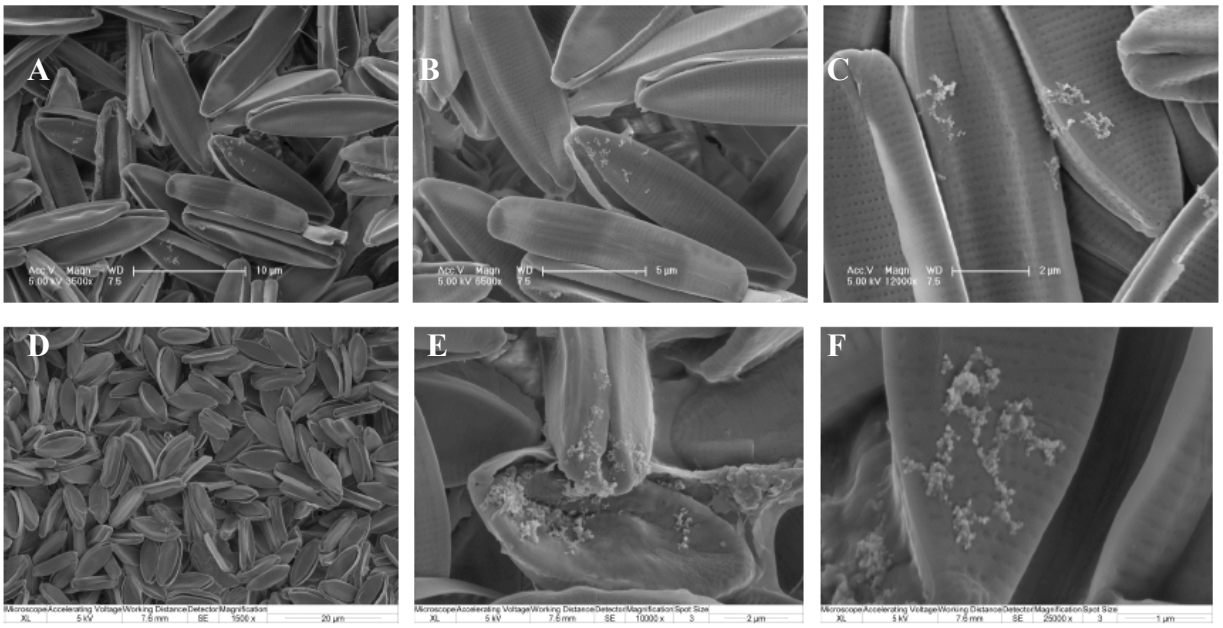

FIGURE S3. Comparative SEM images of diatom mats onto which low concentrations of Ag NPs were filtered through; (a-c) 17 nmol g^{-1} of Ag as HA-Ag NPs (d-f) 7 nmol g^{-1} of Ag as cit-Ag NPs

FIGURE S4. Filter onto which diatoms were harvested, and Ag NPs filtered through (photo taken by J. Garcia-Alonso).

Samples analysis with ICP-MS.

All samples, blanks and standards were introduced by direct injection (peristaltic pump; spray chamber) into the ICP-MS (single-detector; quadrupole). Two analytical replicates were measured for each sample. A replicate consisted of 32 individual measurements that were averaged. External standards, serially diluted from ultra-pure, single-element stock, were used to create calibration curves. To account for instrument drift and change in sensitivity, internal standardization was performed by addition of germanium to all samples and standards, but the calibration blanks. We also reanalyzed one of our standards after every 10 samples. Deviations from standard value were less than 10% for the analyzed Ag isotope at all time.

Snail Ag concentrations measured in the uptake experiments were normalized for the exposure durations to yield Ag influx. Snail Ag concentrations measured in the elimination experiments were standardized for the Ag concentration measured at the beginning of the elimination and expressed in percentage of Ag retained in *L. stagnalis* soft tissues (equation S5). Snail and diatom Ag concentrations are reported on a dry weight basis.

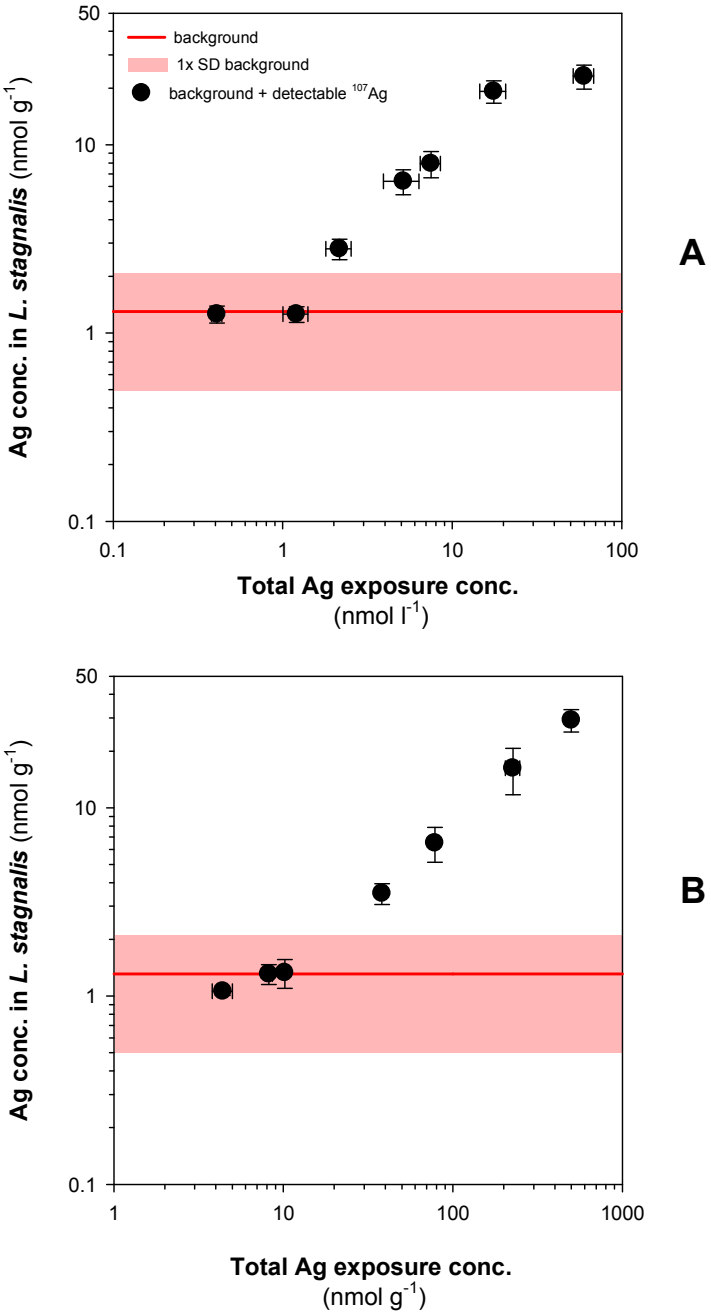

FIGURE S5. Ag concentrations in snails (± 95% C.I.) exposed to (a) waterborne or (b) dietborne Ag. The red line across the exposure concentrations displays the mean background Ag concentrations (n=70). The shaded areas represent 1x the SD of the mean background Ag concentration.

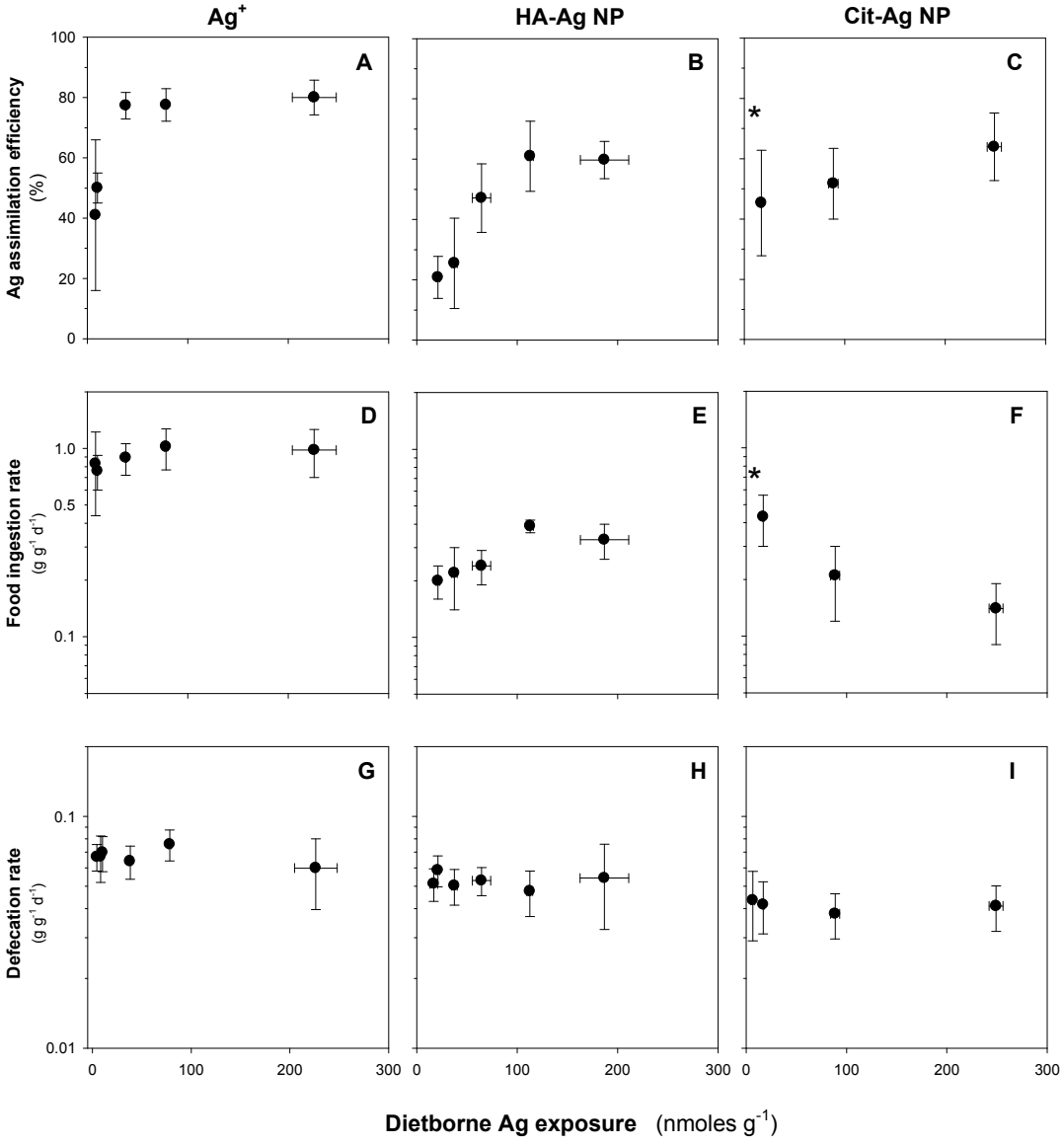

FIGURE S6. Ag AE (%), food IR (g g^{-1} d^{-1}) and defecation rates (g g^{-1} d^{-1}) in snails exposed to diatoms pre-exposed to dissolved Ag (as $AgNO_3$), or to diatoms mixed with HA-AgNPs or cit-AgNPs

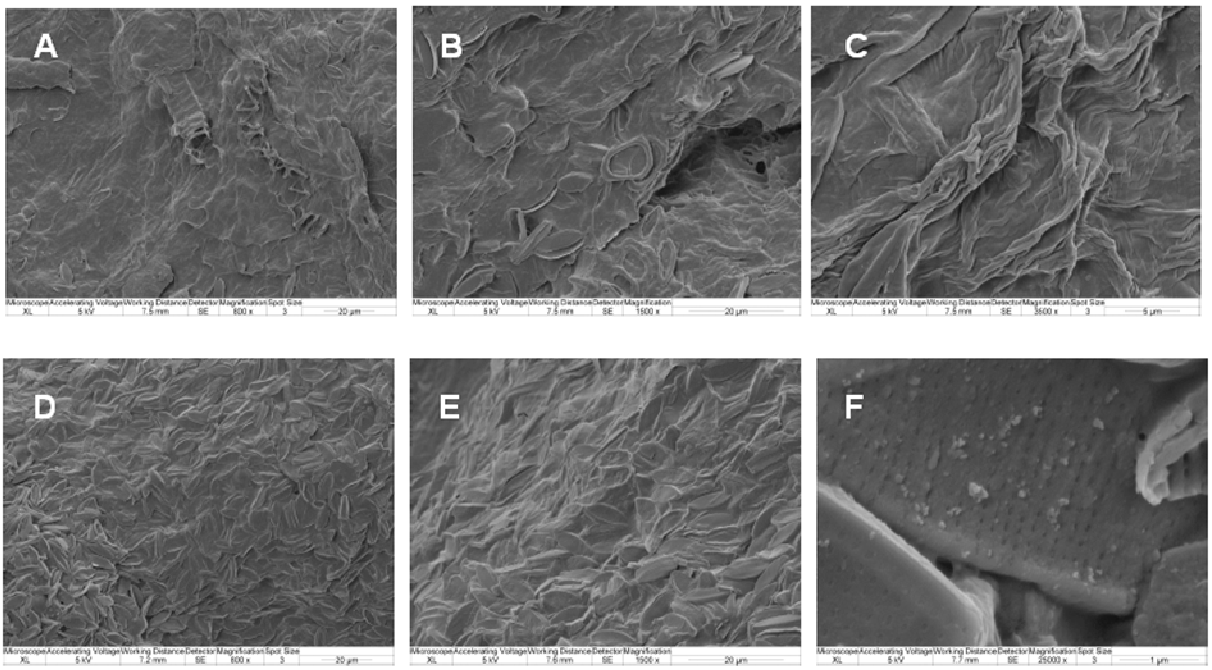

FIGURE S7. Comparative SEM images of feces collected after 48h of depuration for the lowest (a-c) and highest (d-f) dietborne exposures to cit-AgNPs

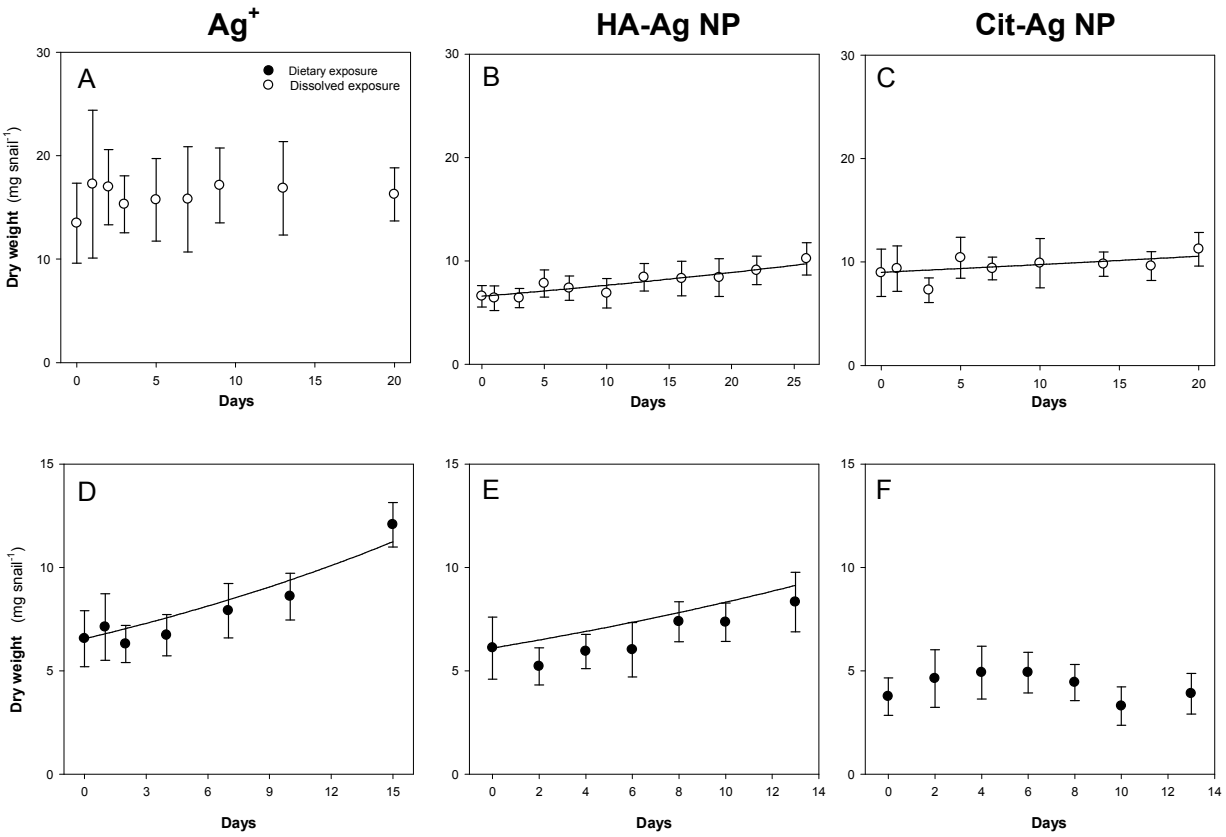

FIGURE S8. Snail's dry weight during depuration. The solid line represents growth as predicted by equation S6