Biogeochemical controls of uranium bioavailability from the dissolved phase in natural freshwaters

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ASSOCIATED CONTENT

Supporting Information: 14 pages, 7 tables, 5 figures, 6 equations and text

Table S1. Snail's averaged size (± SD) for each experiment

Experiment	Dry weight (mg)	*n*	Size range (mg dry weight)
Waterborne U uptake, Experiment set 1			
Very soft	8.5 ± 4.5	58	[2.6-19.3]
Soft	6.5 ± 2.0	59	[2.6-12.0]
MOD	4.9 ± 1.3	59	[2.3-8.6]
Kanab Creek	8.0 ± 2.0	58	[4.4-13.9]
Waterborne U uptake, Experiment set 2			
pH 6	3.1 ± 0.9	30	[2.1-4.8]
pH 6.5	3.9 ± 1.2	50	[1.7-6.7]
pH 7	4.3 ± 0.7	20	[3.4-6.1]
pH 7.5	5.4 ± 1.1	20	[3.8-7.8]
pH 8	3.7 ± 1.0	30	[2.1-6.1]
Waterborne uptake, Experiment 3			
NOM	4.2 ± 1.5	40	[2.1-7.4]
Waterborne uptake, Experiment 4			
^{44}Ca	3.1 ± 0.73	50	[2.1-5.3]
Elimination, Experiment 5			
k_e	8.4 ± 2.9	83	[3.4-14.7]

Table S2. Measured total dissolved U concentrations as well as initial and final pH for the experiments 1-3. Final pH was used for speciation calculations, as noted below; see Table 1 for major ion composition; MDL = method detection limit. Log pCO_2 (atm) of experiments 2 and 3 calculated by PHREEQC for experimental pH and alkalinity (calc) and measured from gas mixer outflow (meas).

Media	$[U]_{water}$ (nM)	pH (initial)	pH (final)	pCO_2 (calc)	pCO_2 (meas)
Experiment set 1					
Very soft	< MDL	7.38	6.80		
	0.68	7.33	6.74		
	6.8	7.36	6.81		
	64	7.33	6.78		
	735	7.34	7.03		
	4570	7.25	7.22		
Soft	< MDL	7.74	7.47		
	0.50	7.74	7.40		
	6.8	7.75	7.36		
	79	7.76	7.38		
	899	7.77	7.32		
	4680	7.84	7.61		
MOD	0.029	8.07	7.61		
	0.86	8.07	7.61		
	9.2	8.12	7.65		
	95	8.12	7.64		
	946	8.12	7.71		
	5260	7.99	7.59		
Kanab Creek	0.070	7.71	7.43		
	0.86	7.65	7.28		
	8.2	7.77	7.27		
	83	7.79	7.26		
	993	7.76	7.27		
	5120	7.73	7.36		
Experiment set 2					
MOD	0.44	6.05	6.06	-1.20	-1.16
	6.8	6.00	6.02	-1.16	-1.16
	19.4	6.05	6.07	-1.21	-1.16
	0.40	6.54	6.53	-1.67	-1.63
	6.3	6.49	6.53	-1.67	-1.63
	7.8	6.56	6.57	-1.71	-1.66
	18.2	6.56	6.55	-1.69	-1.66
	19.4	6.54	6.52	-1.66	-1.63
	9.1	7.05	7.04	-2.18	-2.06
	22.2	7.06	7.05	-2.19	-2.06
	9.3	7.52	7.45	-2.59	-2.51
	23.4	7.50	7.48	-2.62	-2.51
	8.0	8.05	8.02	-3.17	-3.20
	22.3	8.05	8.00	-3.15	-3.20
	45.9	8.04	8.00	-3.15	-3.20
Experiment 3					
MOD	3.3	7.55	7.48	-2.64	-2.68
	12.9	7.52	7.44	-2.60	-2.68
	29.0	7.50	7.43	-2.59	-2.68
	112	7.54	7.51	-2.67	-2.68

Table S3. Chemical properties of DOM HPoA isolate. The sample was obtained from the St. Louis River at river mile 94 on 6/27/2012 according to the methods described in Aiken et al.[28] Elemental composition was measured by Huffmann Laboratories, Inc., and SUVA was calculated as the UV absorption at 254 nm divided by the DOC concentration, according to Weishaar et al. (2003).

DOM isloate	ash free elemental composition (pre-dialysis wt %)					$SUVA_{254}$ Initial (L mg C^{-1} m^{-1})	$SUVA_{254}$ After dialysis (L mg C^{-1} m^{-1})
	C	H	O	N	S		
IS12-0012MN	51.4	4.21	42.7	1.2	0.52	4.8	5.4

Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R. Evaluation of specific ultra-violet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. **2003,** *Environ. Sci. Technol*. 37, 4702-4708

Table S4. Total dissolved U concentrations, pHs and Ca concentrations measured at the beginning of Experiment 4

$[^{44}Ca]_{water}$ μM	$[U]_{water}$ (nM)	pH (initial)	pH (final)
40.9	21.4	7.22	7.00
79.2	23.0	7.44	7.11
156	29.7	7.79	7.44
233	29.8	7.84	7.56
312	30.4	8.07	7.68

Modeling U elimination

Uranium elimination was modeled by nonlinear regression using a two-compartment model, as described in the equation S1,

$$C = C_1 e^{-k_1 t} + C_2 e^{-k_2 t} \qquad \text{(S1)}$$

where C is the U concentration in the snail's soft tissues at a given time (nmole g^{-1}), C_1 and C_2 are the U concentrations in the fast and slow exchanging compartments (nmole g^{-1}), respectively, k_1 and k_2 are estimated rate constants of loss (d^{-1}) for each compartment, and t is time (day). Because exposures in nature are long, the slow compartment will usually dominate loss (Wang et al. 1996). For modeling purposes then, k_2 equals k_e, the rate constant of loss. Animal weights were uniform over the course of depuration period and therefore, the effect of growth was ignored (Figure S1).

Wang, W. X.; Fisher, N. S.; Luoma, S. N. Kinetic determinations of trace element bioaccumulation in the mussel Mytilus edulis. Mar. Ecol. Prog. Ser. 1996, 140(1-3): 91-113.

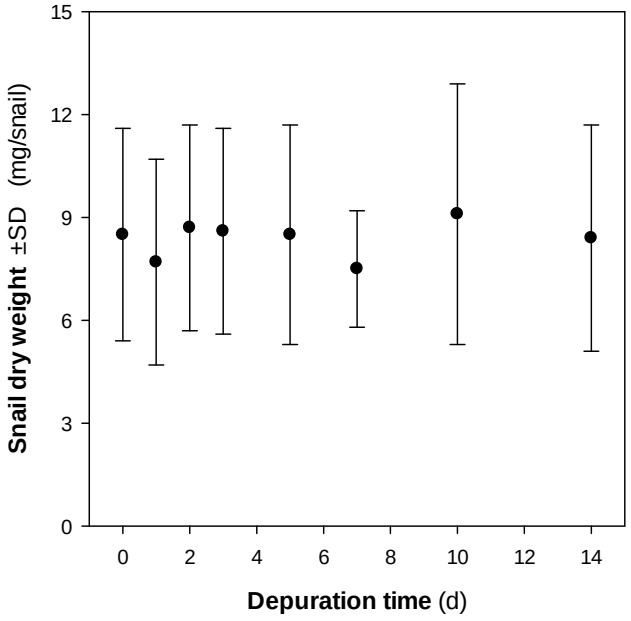

Figure S1. Snail dry weights during depuration (Experiment 5).

Determination of U-DOM conditional binding constants using EDLE methods. The amount of U partitioned into the dialysis bag (Q) was calculated by measuring the U concentrations inside (U_{in}) and outside the bag (U_{out}) with ICP-MS after equilibration. The conditional binding constant (K_{DOMU}) was calculated using:

$$Q = \frac{[U]_{in} - [U]_{out}}{[U]_{out}} \qquad (S2)$$

$$K^{c}_{DOMU} = \frac{Q \times [U_{out}]}{\{UO_2^{+2}\}(DOM)_{in}} \qquad (S3)$$

where the activity of free uranyl cation was calculated with PHREEQC from the outer solution chemistry, assuming that the concentration of that species was the same inside and outside of the dialysis bag (Table S5).

Table S5. Conditional binding constants for U-DOM complexes used in speciation calculations

Initial U (outer solution) (nM)	**Log K_{DOMU}** (L/g)
3.9	8.40
15.3	8.26
34.3	8.21
159	7.91

EDTA was assumed to equilibrate between the inner and outer solutions, and was considered in the free uranyl cation speciation calculation using constants from Hummel et al[21]. Conditional binding constants and DOM concentrations were adjusted to molal units using a molecular weight of 100 g/mol for the speciation calculations, an arbitrary value that mathematically cancels out in the calculation. To verify the speciation calculation, the U speciation under EDLE experimental conditions (with EDTA) was calculated; the calculated Q distribution from the speciation calculation was found to be within 5% of the experimentally measured Q distribution (Table S6). Uranium speciation in MOD water with DOM (experiment 3, no EDTA) was then calculated.

Table S6. Relative standard deviation of triplicate EDLE experiments, comparison of calculated and experimental Q values, and fraction of the U(VI)-NOM complex over range of experimental U concentrations.

initial U	relative standard deviation in experimental Q, triplicate samples	Q		difference between calculated and experimental Q	U-NOM complex as fraction of total U
(outer solution, nM)	*(%)*	*experimental*	*calculated*	*(%)*	*(%)*
3.9	6%	6.66	6.43	3%	87%
15.3	8%	5.64	5.64	0%	85%
34.3	5%	5.27	5.34	-1%	84%
159	5%	3.49	3.32	5%	78%

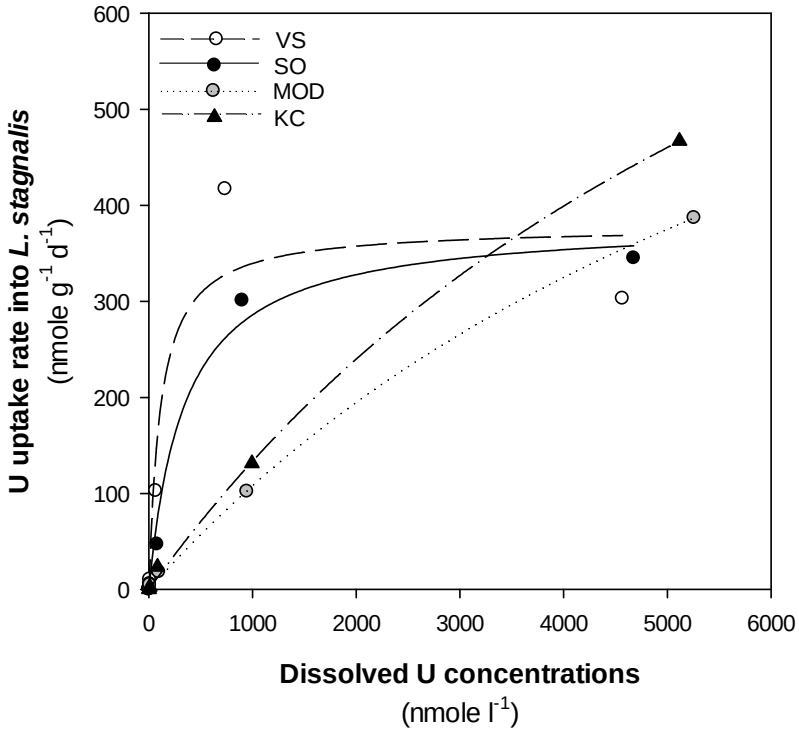

Figure S2. Uranium uptake rate in *L. stagnalis* exposed to aqueous U for 24 h; data taken from Figure 1 and re-plotted on linear scales; error bars omitted for clarity. Lines represent nonlinear regression fits of the mean values to a one site ligand binding saturation model.

Calcium and/or U uptake rates into *L. stagnalis* described by a one site ligand binding saturation model:

$$M\ influx_{\ snail} = \frac{V_{max}\ [M]_{solution}}{(\ K_M + \ [M]_{solution})} \qquad (S4)$$

where $M\ influx_{\ snail}$ is either the ^{44}Ca uptake rate (in µmole g^{-1} d^{-1}) or the U uptake rate (in nmole g^{-1} d^{-1}) into *L. stagnalis* soft tissues, V_{max} is the maximum uptake rate (in either µmole g^{-1} d^{-1} or nmole g^{-1} d^{-1}), $[M]_{solution}$ is the aqueous concentration of either ^{44}Ca (in µmole l^{-1}) or U (in nmole l^{-1}) and K_M is the either the ^{44}Ca or the U concentration at half saturation (in µmole l^{-1} for ^{44}Ca and nmole l^{-1} for U). Because snails were exposed to U for 24 h in experiments 1-3, the U uptake rates are the same as the accumulated U concentrations, and thus Vmax (nmole g^{-1} d^{-1}) equals B_{max} (nmole g^{-1}).

Table S7. Binding characteristics (±SE) and rate constant of uptake for the waterborne U uptake by *L. stagnalis.* Also shown are the binding characteristics (±SE) and rate constant of uptake for other metals in MOD water

Water	***Metal***	k_{uw} $l\ g^{-1}\text{-}d^{-1}$	B_{max} nmole g^{-1}	K_U nmole l^{-1}	***log K***	***Reference***
VS	U	1.6 ± 0.01	378 ± 53	112 ± 80		This study
SO	U	0.60 ± 0.01	384 ± 23	343 ± 94		This study
MOD	U	0.55 ± 0.02	977 ± 84	8025 ± 1094	5.1	This study
KC	U	0.28 ± 0.01	1184 ± 117	7854 ± 1228		This study
MOD	Cd	0.82 ± 0.08	171 ± 9		6.6	Croteau and Luoma 2007
MOD	Cu	0.74 ± 0.04	271 ± 21		8.5	Croteau and Luoma 2007
MOD	Ag	1.1 ± 0.1	31 ± 5		7.7	Croteau et al. 2011

Croteau MN; Luoma SN. Characterizing dissolved Cu and Cd uptake in terms of the biotic ligand and biodynamics using enriched stable isotopes. *Environ. Sci. Technol.* **2007,** *41*(9), 3140-3145.

Croteau, M.N., Misra, S.K., Luoma, S.N. Valsami-Jones, E. Silver bioaccumulation dynamics in a freshwater invertebrate after aqueous and dietary exposures to nanosized and ionic Ag. *Environ. Sci. Technol.* **2011**, *45(15)*, 6600-6607.

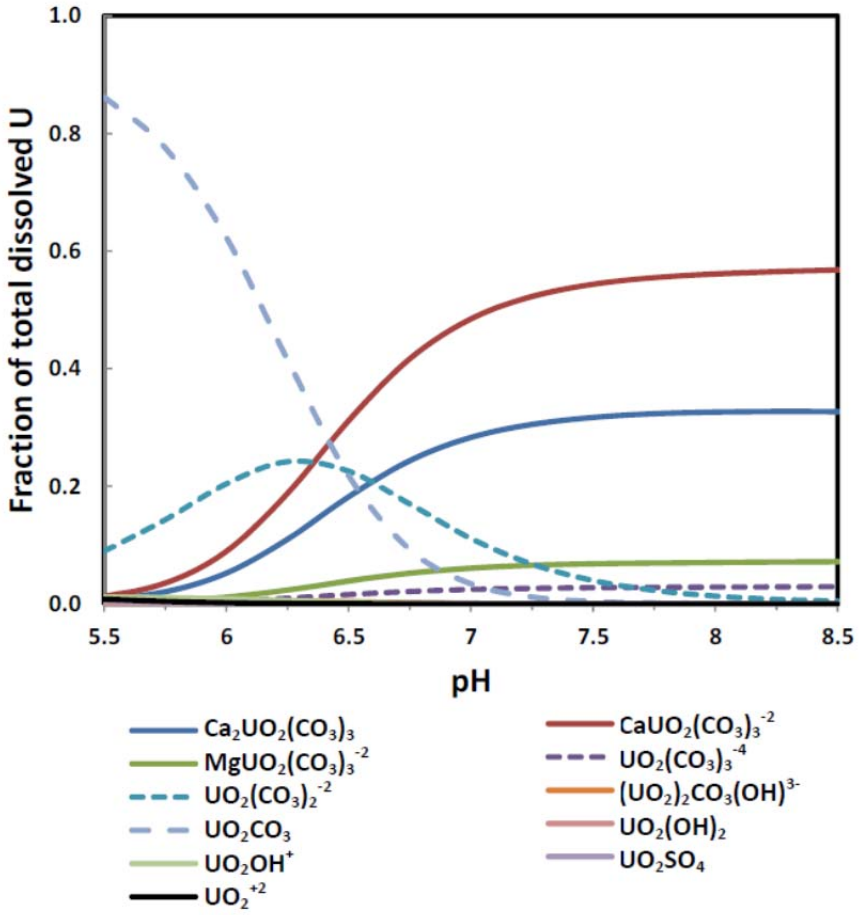

Figure S3. Uranium speciation calculated using PHREEQC for MOD water at pH 7.5 and with 100 nM total U.

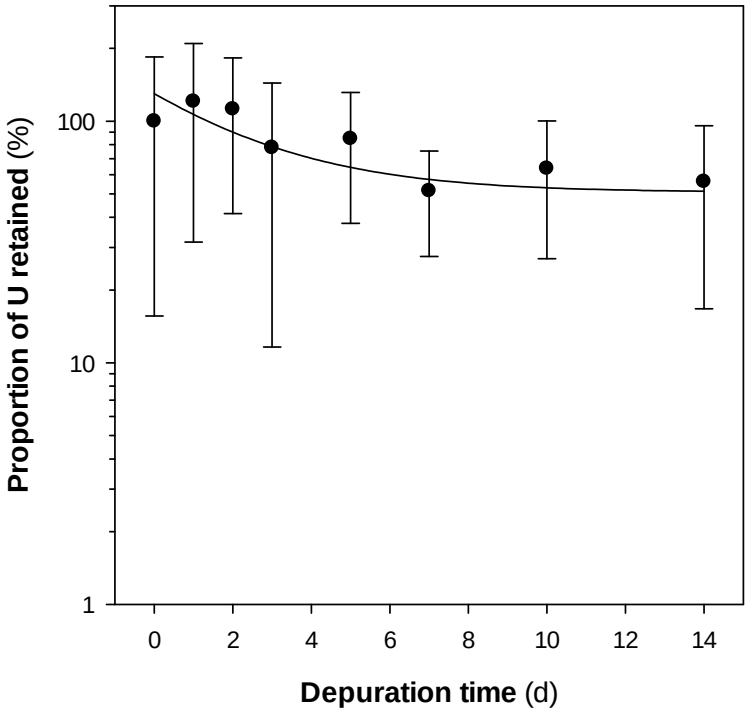

Figure S4. Loss of U over time in *L. stagnalis* after 2 days of exposure to waterborne U. Each symbol represents the averaged percentage of U retained in 10-11 individuals over time (C_t/C_0) where C_t is the concentration of U in tissues at any given time and C_0 is the concentration when unidirectional loss began. The error bars represent ±SD relative to C_0. Solid line represents net loss of U calculated using equation S1.

Uranium bioaccumulation in *L. stagnalis* described according to the free-ion activity model when competition by either H^+ (equation S5) or Ca^{2+} (equation S6) reduces U uptake:

$$[U]_{snail} = F \frac{[UO_2^{+2}]}{([H^+] + K_a)} \qquad (S5)$$

$$[U]_{snail} = F \frac{[UO_2^{+2}]}{(1 + K_{Ca}[Ca^{+2}])} \qquad (S6)$$

where $[U]_{snail}$ is the U concentration in the snail (nmole g^{-1}), $[UO_2^{+2}]$ is the concentration of the free uranyl ion (moles l^{-1}), $[H^+]$ is the concentration of hydrogen ions (mole L^{-1}), $[Ca^{2+}]$ is the concentration of Ca^{+2} (mole L^{-1}), *F* is the product of proportionality constants and K_a and K_{Ca} are proportionality constants for the (pseudo)equilibrium reactions between either H^+ or Ca^{2+} and the binding sites. To account for the competing influence of Mg^{2+}, K_{Ca} should be replaced by K_{Mg} and $[Ca^{2+}]$ by $[Mg^{2+}]$.

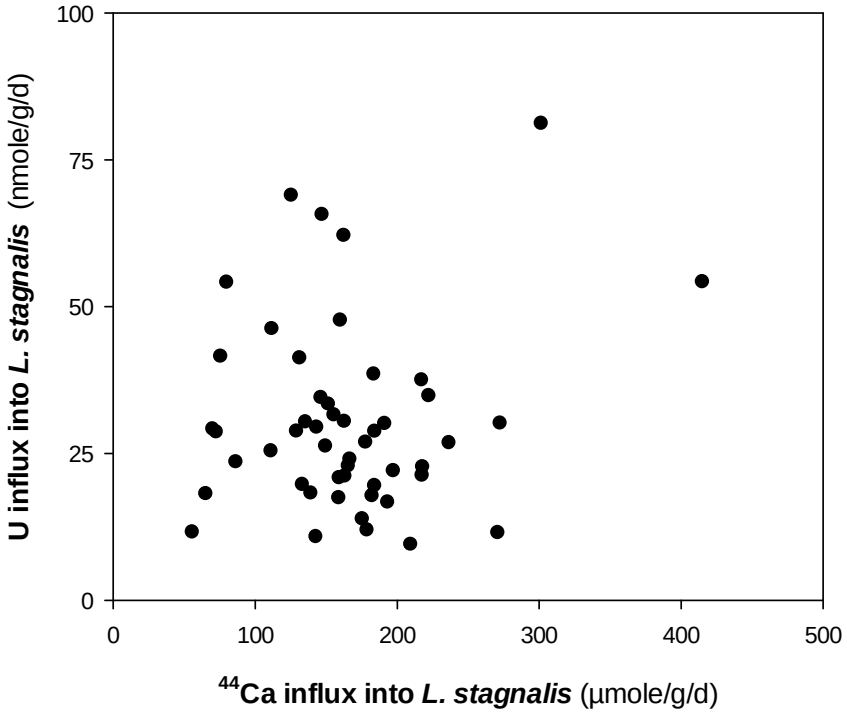

Figure S5. Uranium uptake rates into *L. stagnalis* (nmole g^{-1} d^{-1}) as a function of ^{44}Ca uptake rates (µmole g^{-1} d^{-1}). Each symbol represents an individual.