Supplemental Information: 11 pages, including title page, 4 Tables, and 2 Figures

Potential toxicity of dissolved metal mixtures (Cd, Cu, Pb, Zn) to early life stage white sturgeon (*Acipenser transmontanus*) in the Upper Columbia River, Washington, United States

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Table S1. Summary of previously published results of metal (Cd, Cu, Pb, Zn) toxicity studies with early life stage sturgeon.									
Metal	Age at start of test	Test Duration	Endpoint	Effective Mortality	Cd	Cu	Pb	Zn	Reference
	days post hatch	days		%	µg/L	µg/L	µg/L	µg/L	
Cd	2	4	Effective mortality	0.00	0.01				Calfee et al. (2014)
Cd	2	4	Effective mortality	0.00	2.10				Calfee et al. (2014)
Cd	2	4	Effective mortality	20.00	4.49				Calfee et al. (2014)
Cd	2	4	Effective mortality	30.00	9.69				Calfee et al. (2014)
Cd	2	4	Effective mortality	55.00	19.50				Calfee et al. (2014)
Cd	2	4	Effective mortality	32.50	47.20				Calfee et al. (2014)
Cu	0	8	Effective mortality	7.50		0.21			Wang et al. (2014)
Cu	0	8	Effective mortality	3.75		0.44			Wang et al. (2014)
Cu	0	8	Effective mortality	3.75		0.92			Wang et al. (2014)
Cu	0	8	Effective mortality	18.75		1.69			Wang et al. (2014)
Cu	0	8	Effective mortality	76.25		3.23			Wang et al. (2014)
Cu	0	8	Effective mortality	98.75		6.91			Wang et al. (2014)
Cu	1	14	Effective mortality	0.00		0.19			Puglis et al. (accepted)
Cu	1	14	Effective mortality	1.67		0.58			Puglis et al. (accepted)
Cu	1	14	Effective mortality	4.17		1.00			Puglis et al. (accepted)
Cu	1	14	Effective mortality	25.83		1.89			Puglis et al. (accepted)
Cu	1	14	Effective mortality	35.83		3.65			Puglis et al. (accepted)
Cu	1	14	Effective mortality	83.33		7.52			Puglis et al. (accepted)

Table S1. (Continued) Summary of previously published results of metal (Cd, Cu, Pb, Zn) toxicity studies with early life stage sturgeon.

Metal	Age at start of test	Test Duration	Endpoint	Effective Mortality	Cd	Cu	Pb	Zn	Reference
	days post hatch	days		%	µg/L	µg/L	µg/L	µg/L	
Cu	1	14	Effective mortality	0.00		0.13			Puglis et al. (accepted)
Cu	1	14	Effective mortality	5.00		0.51			Puglis et al. (accepted)
Cu	1	14	Effective mortality	0.00		0.90			Puglis et al. (accepted)
Cu	1	14	Effective mortality	0.83		1.86			Puglis et al. (accepted)
Cu	1	14	Effective mortality	51.67		3.60			Puglis et al. (accepted)
Cu	1	14	Effective mortality	87.50		7.42			Puglis et al. (accepted)
Cu	1	14	Effective mortality	3.33		0.15			Puglis et al. (accepted)
Cu	1	14	Effective mortality	5.00		0.50			Puglis et al. (accepted)
Cu	1	14	Effective mortality	7.50		0.88			Puglis et al. (accepted)
Cu	1	14	Effective mortality	7.50		1.67			Puglis et al. (accepted)
Cu	1	14	Effective mortality	76.67		3.31			Puglis et al. (accepted)
Cu	1	14	Effective mortality	99.17		7.12			Puglis et al. (accepted)
Cu	2	4	Effective mortality	0.00		0.19			Calfee et al. (2014)
Cu	2	4	Effective mortality	7.50		1.43			Calfee et al. (2014)
Cu	2	4	Effective mortality	55.00		2.66			Calfee et al. (2014)
Cu	2	4	Effective mortality	90.00		5.29			Calfee et al. (2014)
Cu	2	4	Effective mortality	100.00		10.5			Calfee et al. (2014)
Cu	2	4	Effective mortality	100.00		23.55			Calfee et al. (2014)

Table S1. (Continued) Summary of previously published results of metal (Cd, Cu, Pb, Zn) toxicity studies with early life stage sturgeon.

Metal	Age at start of test	Test Duration	Endpoint	Effective Mortality	Cd	Cu	Pb	Zn	Reference
	days post hatch	days		%	µg/L	µg/L	µg/L	µg/L	
Zn	2	4	Effective mortality	2.50				1	Calfee et al. (2014)
Zn	2	4	Effective mortality	0.00				30	Calfee et al. (2014)
Zn	2	4	Effective mortality	0.00				60	Calfee et al. (2014)
Zn	2	4	Effective mortality	10.00				116	Calfee et al. (2014)
Zn	2	4	Effective mortality	100.00				225	Calfee et al. (2014)
Zn	2	4	Effective mortality	100.00				634	Calfee et al. (2014)
Zn	0	8	Effective mortality	1.25				2	Wang et al. (2014)
Zn	0	8	Effective mortality	7.50				22	Wang et al. (2014)
Zn	0	8	Effective mortality	3.75				44	Wang et al. (2014)
Zn	0	8	Effective mortality	15.00				88	Wang et al. (2014)
Zn	0	8	Effective mortality	10.00				165	Wang et al. (2014)
Zn	0	8	Effective mortality	37.50				369	Wang et al. (2014)
Zn	2	4	Effective mortality	2.50				1.29	Calfee et al. (2014)
Zn	2	4	Effective mortality	0.00				29.9	Calfee et al. (2014)
Zn	2	4	Effective mortality	0.00				59.5	Calfee et al. (2014)
Zn	2	4	Effective mortality	10.00				116	Calfee et al. (2014)
Zn	2	4	Effective mortality	100.00				225	Calfee et al. (2014)
Zn	2	4	Effective mortality	100.00				634	Calfee et al. (2014)

Table S1. (Continued) Summary of previously published results of metal (Cd, Cu, Pb, Zn) toxicity studies with early life stage sturgeon.

Table S1. (Continued) Summary of previously published results of metal (Cd, Cu, Pb, Zn) toxicity studies with early life stage sturgeon.

Metal	Age at start of test	Test Duration	Endpoint	Biomass Reduction	Cd	Cu	Pb	Zn	Reference
	days post hatch	days		%	µg/L	µg/L	µg/L	µg/L	
Cd	27	28	Biomass	100.00	0.05				Wang et al. (2014)
Cd	27	28	Biomass	92.44	0.67				Wang et al. (2014)
Cd	27	28	Biomass	91.79	1.31				Wang et al. (2014)
Cd	27	28	Biomass	89.44	2.74				Wang et al. (2014)
Cd	27	28	Biomass	75.23	5.29				Wang et al. (2014)
Cd	27	28	Biomass	4.56	11.68				Wang et al. (2014)
Cu	27	28	Biomass	100.00		0.22			Wang et al. (2014)
Cu	27	28	Biomass	98.37		0.51			Wang et al. (2014)
Cu	27	28	Biomass	95.11		0.95			Wang et al. (2014)
Cu	27	28	Biomass	90.22		1.83			Wang et al. (2014)
Cu	27	28	Biomass	85.87		3.37			Wang et al. (2014)
Cu	27	28	Biomass	1.09		7.34			Wang et al. (2014)
Cu	2	53	Biomass	100.00		0.22			Wang et al. (2014)
Cu	2	53	Biomass	89.95		0.46			Wang et al. (2014)
Cu	2	53	Biomass	89.74		0.93			Wang et al. (2014)
Cu	2	53	Biomass	74.48		1.76			Wang et al. (2014)
Cu	2	53	Biomass	4.23		3.31			Wang et al. (2014)

Metal	Age at start of test	Test Duration	Endpoint	Biomass Reduction	Cd	Cu	Pb	Zn	Reference
	days post hatch	days		%	µg/L	µg/L	µg/L	µg/L	
Cu	2	53	Biomass	0.00		7.16			Wang et al. (2014)
Cu	1	24	Biomass	100.00		0.15			Wang et al. (2014)
Cu	1	24	Biomass	75.00		0.53			Wang et al. (2014)
Cu	1	24	Biomass	79.07		0.92			Wang et al. (2014)
Cu	1	24	Biomass	63.95		1.90			Wang et al. (2014)
Cu	1	24	Biomass	13.95		3.67			Wang et al. (2014)
Cu	1	24	Biomass	0.87		7.40			Wang et al. (2014)
Pb	25	28	Biomass	100.00			< 0.03		Wang et al. (2014)
Pb	25	28	Biomass	79.49			3.1		Wang et al. (2014)
Pb	25	28	Biomass	92.31			6.2		Wang et al. (2014)
Pb	25	28	Biomass	91.08			13		Wang et al. (2014)
Pb	25	28	Biomass	74.36			28		Wang et al. (2014)
Pb	25	28	Biomass	0.00			60		Wang et al. (2014)
Zn	27	28	Biomass	100.00				1.5	Wang et al. (2014)
Zn	27	28	Biomass	95.45				23	Wang et al. (2014)
Zn	27	28	Biomass	94.89				47	Wang et al. (2014)
Zn	27	28	Biomass	96.02				95	Wang et al. (2014)
Zn	27	28	Biomass	84.09				187	Wang et al. (2014)
Zn	27	28	Biomass	21.59				404	Wang et al. (2014)

Table S2. Summary of equilibrium constants for biotic ligand-cation reactions for 1-site, 2-site, and 4-site metal accumulation models.

	1-site model[a]	2-site model[b]		4-site model[c]				
	Site 1	Site 1	Site 2	Cd Site 1	Cu Site 2	Pb Site 3	Zn Site 4	Reaction
log K (BL-H) =	3.6; 7.5	6.35	6.35	6.7	5.4	4	6.6	$BL^- + H^+ = BL\text{-}H$
log K (BL-Na) =	---	3.89	3.89	3	3	4.2	2.9	$BL^- + Na^+ = BL\text{-}Na$
log K (BL-Mg) =	4.26	3.00	3.00	3.5	3.6	4	3.6	$BL^- + Mg^{+2} = BL\text{-}Mg^+$
log K (BL-Ca) =	3.23	3.88	3.88	5.1	3.6	5.1	3.8	$BL^- + Ca^{+2} = BL\text{-}Ca^+$
log K (BL-Cd) =	7.59	7.61	5.06	5.4	5.4	5.4	5.4	$BL^- + Cd^{+2} = BL\text{-}Cd^+$
log K (BL-CdOH) =	-2.49	---	---	-2.3	-2.3	-2.3	-2.3	$BL^- + Cd^{+2} + H_2O = BL\text{-}CdOH + H^+$
log K (BL-Cu) =	6.92	9.87	7.04	11.4	7.4	7.4	7.4	$BL^- + Cu^{+2} = BL\text{-}Cu^+$
log K (BL-CuOH) =	-1.08	---	---	2.7	-1.3	-1.3	-1.3	$BL^- + Cu^{+2} + H_2O = BL\text{-}CuOH + H^+$
log K (BL-Pb) =	6.22	10.20	7.44	6.65	6.65	6.65	6.65	$BL^- + Pb^{+2} = BL\text{-}Pb^+$
log K (BL-PbOH) =	-1.49	---	---	-1.1	-1.1	-1.1	-1.1	$BL^- + Pb^{+2} + H_2O = BL\text{-}PbOH + H^+$
log K (BL-Zn) =	5.13	7.10	5.43	8.5	5.5	5.5	5.5	$BL^- + Zn^{+2} = BL\text{-}Zn^+$
log K (BL-ZnOH) =	-3.83	---	---	-0.8	-3.8	-3.8	-3.8	$BL^- + Zn^{+2} + H_2O = BL\text{-}ZnOH + H^+$

[a]Farley & Meyer (2015)

[b]Balistrieri & Mebane (2014)

[c]Santore & Ryan (2015)

Table S3. Logistic fits for biotic ligand-metal accumulation (fraction of total sites) versus combined response (Effective Mortality or Reduction in Biomass) (%) for 1-site, 2-site, and 4-site metal accumulation models.

	1-site		2-site		4-site	
	value	error	value	error	value	error
Cd						
a	**100**		**100**		**100**	
X_c	**0.33**	0.02	**0.032**	0.002	**0.0016**	0.0002
k	**8**	1	**87**	11	**1522**	260
r^2	**0.894**		**0.895**		**0.814**	
n	**10**		**10**		**10**	
Cu						
a	**100**		**100**		**100**	
X_c	**0.0012**	0.0001	**0.028**	0.001	**0.003**	0.0002
k	**2752**	368	**122**	17	**980**	140
r^2	**0.886**		**0.855**		**0.862**	
n	**48**		**48**		**48**	
Pb[a]						
a	**100**		**100**		**100**	
X_c	**0.001**	0.0001	**0.069**	0.009	**0.00025**	0.00003
k	**3781**	1330	**325**	890	**15295**	5302
r^2	**0.924**		**0.897**		**0.926**	
n	**6**		**6**		**6**	
Zn						
a	**100**		**100**		**100**	
X_c	**0.04**	0.002	**0.129**	0.003	**0.104**	0.004
k	**99**	13	**56**	9	**41**	5
r^2	**0.941**		**0.948**		**0.953**	
n	**17**		**17**		**17**	

[a]Reduction in Biomass only

These logistic fit parameters are from equation 2 in the main text, which is repeated here:

$$Y = \frac{a}{1+exp^{-k*(X-X_c)}}$$

where Y is the fractional biological response, a is the maximum response (i.e., value of 100%), k is the slope of the curve, X is the dose, and X_c is the dose at half the maximum response. The unknown values, k and X_c, are determined by fitting either free metal ion concentrations or calculated accumulation of each metal versus biological response in the single metal toxicity studies. Error is standard error residual to the minimization of the line fit to the observed data.

Table S4. Logistic fits for toxicity function (Tox) versus combined response (Effective Mortality or Reduction in Biomass) (%) for 1-site, 2-site, and 4-site metal accumulation models. See equation 4 in text.

	1-site		2-site		4-site	
	value	error	value	error	value	error
α_{Cd}	**0.1**	0.02	**3.2**	0.5	**53**	10
α_{Cu}	**28**	3	**3.6**	0.3	**28**	3
α_{Pb}	**32**	6	**1.5**	0.2	**348**	76
α_{Zn}	**1**		**1**		**1**	
a	**100**		**100**		**100**	
Tox_c	**0.033**	0.003	**0.1**	0.007	**0.086**	0.009
k	**95**	13	**37**	5	**35**	5
r^2	**0.862**		**0.796**		**0.843**	
n	**87**		**87**		**87**	

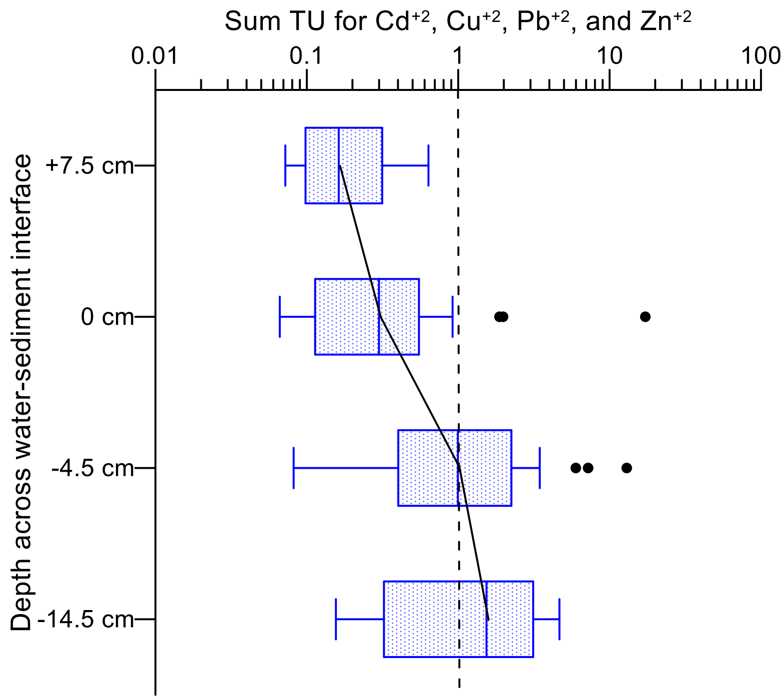

Figure S1. Sum Toxic Unit (TU) for the four free metal ions (Cd^{+2}, Cu^{+2}, Pb^{+2}, and Zn^{+2}) versus depth across the sediment-water interface in the upper reaches of the Columbia River. The whisker plot indicates ranges, 25th percentile, median, 75th percentile, and outliers. The solid line connects the median values and the dashed line indicates a Sum TU of 1.

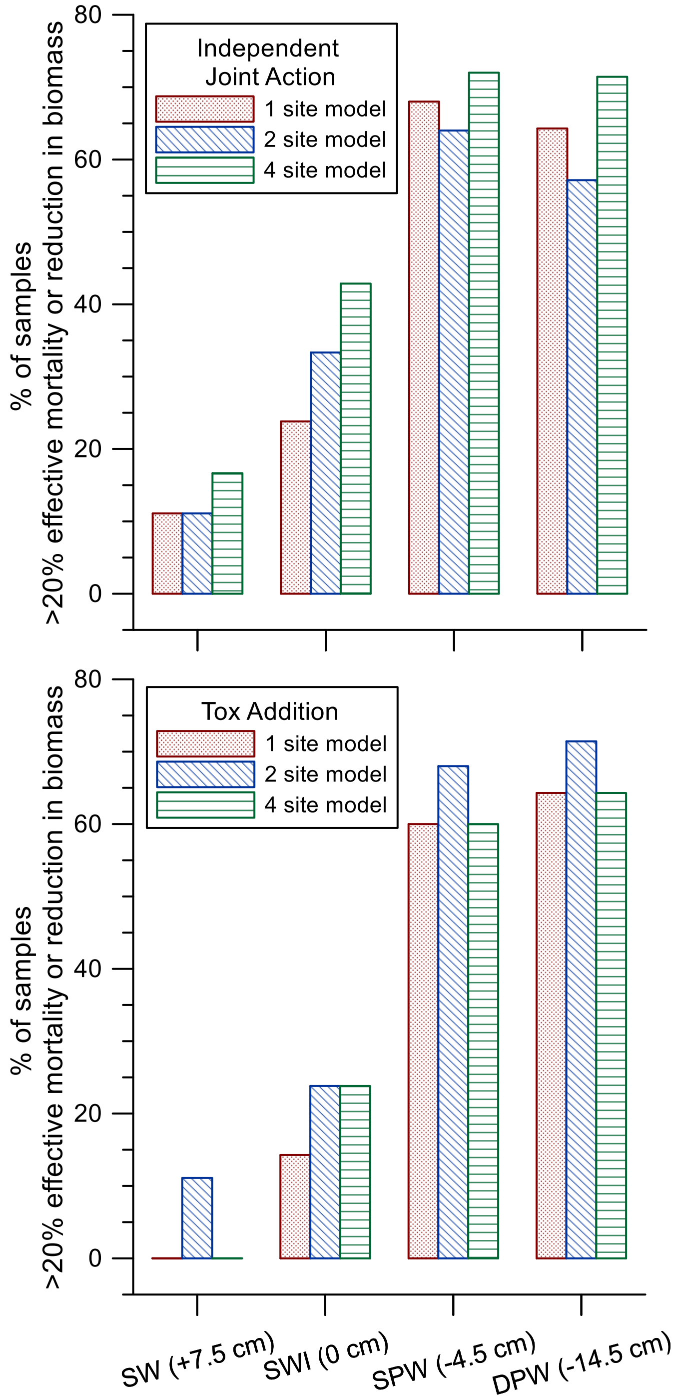

Figure S2. The % of surface (SW), sediment-water interface (SWI), shallow pore water (SPW), and deep pore water (DPW) samples collected in the upper reaches of the Columbia River where >20% shorter-term effective mortality or longer-term reduction in biomass of early life stage sturgeon is predicted using 3 metal accumulation models (1-site, 2-site, 4-site) and 2 response models [independent joint action (upper graph) or Tox addition (lower graph)].