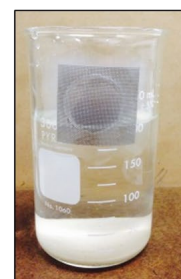


7-day NaCl toxicity test conducted in a water bath



Test beaker
containing 200 mL
of water and 3 mL
of sand substrate

NaCl solutions

Replicate beakers

**Splitter unit used
to renew water for
4 replicates per
concentration**



Manifold: silicone stoppers
with needle inserts

Figure S1. Setup of short-term 7-d toxicity test with juvenile fatmucket (*Lampsilis siliquoidea*).

125 mg/L

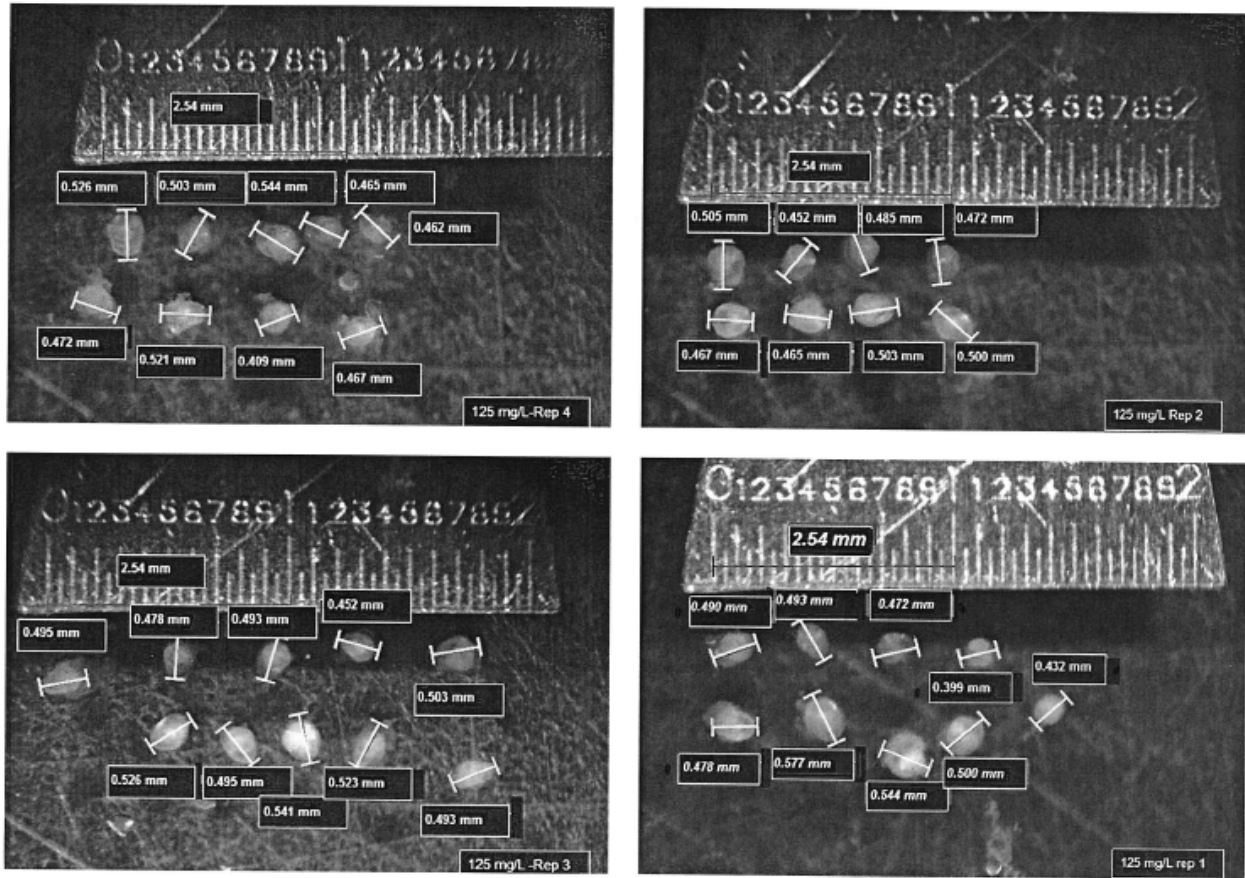


Figure S2. An example of length measurements of surviving mussels from the 4 replicates in the 125 mg NaCl/L treatment using a digitizing system (M Garton, Great Lake Environmental Center, Traverse City, MI, USA).

Table S1. Nominal concentrations of the 6 chemicals in 2 mock effluents for the 7-d test with cladoceran (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), and notched rainbow (*Villosa constricta*) in USEPA moderately hard reconstituted water

Mock effluent 1: The concentration in the 12.5% dilution (except for lead in the 25% dilution due to low solubility) was set at the USEPA chronic water quality criterion for each of the 6 chemicals for the water quality characteristics of the moderately hard reconstituted water (see text)

Conc. (µg/L)	Control	3.125%	6.25%	12.5%	25%	50%	100% ^a
Ammonia	Control	250	500	1000	2000	4000	8000
Cadmium	Control	0.06	0.13	0.25	0.5	1.0	2.0
Copper	Control	0.38	0.75	1.5	3	6.0	12
Nickel	Control	13	26	52	104	208	416
Lead	Control	0.31	0.63	1.25	2.5	5.0	10
Zinc	Control	30	60	120	240	480	960

*Mock effluent 2: The concentration in the 12.5% dilution (except for lead in the 25% dilution due to low solubility) was set at the concentration close to chronic effect concentrations for unionid mussels (*Lampsilis siliquoidea* and *Villosa iris*) in previous 28-d exposures at hardness ~100 mg/L, dissolved organic carbon ~0.5 mg/L, pH ~8.3, and 20 to 22°C (Wang et al. 2010, 2011ab; Besser et al. 2011)*

Conc. (µg/L)	Control	3.125%	6.25%	12.5%	25%	50%	100% ^a
Ammonia	Control	100	200	400	800	1600	3200
Cadmium	Control	2.0	4.0	8.0	16	32	64
Copper	Control	2.0	4.0	8.0	16	32	64
Nickel	Control	11.5	23	46	92	184	368
Lead	Control	5.5	11	22	44	88	176
Zinc	Control	31	62	124	248	496	992

^a *C. dubia* and notched rainbow were not tested in 100% effluent (see text).

References:

- Besser JM, Brumbaugh WG, Kemble NE, Ivey CD, Kunz JL, Ingersoll CG, Rudel D. 2011. Toxicity of nickel-spiked freshwater sediments to benthic invertebrates—Spiking methodology, species sensitivity, and nickel bioavailability: U.S. Geological Survey Scientific Investigations Report 2011–5225, 53 p. plus appendixes.
- Wang N, Ingersoll CG, Ivey CD, Hardesty DK, May TW, Augspurger T, Roberts AD, van Genderen E, Barnhart MC. 2010. Sensitivity of early life stages of freshwater mussels (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. *Environ Toxicol Chem* 29:2053-2063.
- Wang N, Mebane CA, Kunz JL, Ingersoll CG, Brumbaugh WG, Santore RC, Gorsuch JW, Arnold WR. 2011a. Influence of dissolved organic carbon on toxicity of copper to a unionid mussel (*Villosa iris*) and a cladoceran (*Ceriodaphnia dubia*) in acute and chronic water exposures. *Environ Toxicol Chem* 30:2115-2125.
- Wang N, Consbrock R, Ingersoll CG, Barnhart MC. 2011b. Evaluation of influence of sediment on the sensitivity of a unionid mussel (*Lampsilis siliquoidea*) to ammonia in 28-day water exposures. *Environ Toxicol Chem* 30:2270-2276.

Table S2. Summary of test conditions for conducting the short-term 7-d toxicity tests with juvenile fatmucket (*Lampsilis siliquoidea*)

Test chemical	NaCl
Test type	Static renewal
Test duration	7 days
Temperature	25±1°C
Light quality	Ambient laboratory light
Light intensity	10-20 $\mu\text{E}/\text{m}^2$ (~500-1000 lux)
Photoperiod	16L:8D
Test chamber size	300 mL (3 mL of silica sand, ~0.2 mm particle size)
Dilution water	Moderately hard reconstituted water (see text)
Test solution volume	200 mL
Renewal of solution	Daily (~80% replacement of water)
Age of test organism	~1 week after transformation
No. Organism/Replicate	10
Replicates/Treatment	4
Feeding	2 mL of algal mixture twice daily. The mixture prepared daily by adding 0.5 mL of Nanno 3600 tm and 1 ml of Shellfish Diet 1800 tm (Reed Mariculture, Campbell, CA) into 900 mL of control water
Chamber cleaning	None
Aeration	None
Dilution factor	0.5
Test concentration	Control, 125, 250, 500, 1000, and 2000 mg NaCl/L
Chemical analysis	Chloride measured in all concentrations on Days 0 and 7. Conductivity measured daily in each of new test solutions and in each old solution after 24-h exposure
Water quality	Temperature, pH, and DO measured in the control, medium, and high concentrations at the beginning and end of each 24-h exposure. Hardness, alkalinity, and ammonia measured in the control, medium, and high concentrations on Days 0 and 7
Endpoint	Survival and growth (shell length)
Test acceptability criterion	≥ 80% survival in controls

Table S3. Daily test checklist for short-term 7-d test with juvenile fatmucket (*Lampsilis siliquoidea*)

Day of Test	-3	-2	-1	0	1	2	3	4	5	6	7	8 and beyond
Calendar Date												
Day of the Week												
Prepare dilution water	X											
Obtain and acclimate mussels	X	X	X									
Feed during acclimation period		X	X									
Temperature of the water upon arrival; general condition of the animals recorded	X											
Prepare test beakers, sand substrate, and other glassware	X	X	X									
Prepare randomization plan for beakers and for adding mussels	X											
Prepare test concentrations				X	X	X	X	X	X	X		
Add organisms				X								
Collect initial organisms				X								
Prepare food		X	X	X	X	X	X	X	X	X	X	
Add food; AM feeding				X	X	X	X	X	X	X	X	
Record time organisms fed (morning)				X	X	X	X	X	X	X		
Add food; PM feeding				X	X	X	X	X	X	X		
Record time organisms fed (afternoon)				X	X	X	X	X	X	X		
Water renewal					X	X	X	X	X	X		
Temperature				X	X	X	X	X	X	X	X	
pH				X	X	X	X	X	X	X	X	
DO				X	X	X	X	X	X	X	X	
Conductivity				X	X	X	X	X	X	X	X	
Hardness, alkalinity, ammonia				X							X	
Chloride Analysis				X							X	
End test; recover, count organisms											X	
Measure shell length												As soon as possible

Table S4. Mean water quality characteristics (\pm standard deviation; a range in parentheses) in 7-d mock effluent toxicity tests with cladoceran (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), and notched rainbow (*Villosa constricta*)^a

	Temperature ^a	Dissolved oxygen	pH	Conductivity	Hardness	Alkalinity
	(°)	(mg/L)			(μ S/cm)	(mg/L as CaCO ₃)
<i>Mock effluent-1</i> ^b						
Cladoceran	25 \pm 0.5 (24.5-25.5)	8.9 \pm 0.8 (8.3-9.2)	8.3 \pm 0.1 (8.3-8.4)	291 \pm 29 (264-330)	104 \pm 2.1 (102-104)	101 \pm 2.5 (100-106)
Fathead minnow	25 \pm 0.5 (24.5-25.5)	9.0 \pm 0.8 (8.5-11)	8.3 \pm 0.1 (8.2-8.4)	321 \pm 33 (264-352)	119 \pm 16 (102-134)	107 \pm 4.5 (100-112)
Notched rainbow	25 \pm 0.5 (24.5-25.5)	9.0 \pm 0.6 (8.6-11)	8.3 \pm 0.1 (8.2-8.4)	290 \pm 22 (282-334)	105 \pm 2.3 (104-108)	102 \pm 2.4 (100-104)
<i>Mock effluent-2</i> ^b						
Cladoceran	25 \pm 0.5 (24.5-25.5)	9.0 \pm 0.8 (8.4-9.0)	8.4 \pm 0.1 (8.3-8.4)	278 \pm 8.0 (272-284)	105 \pm 1.6 (104-106)	100 \pm 3.1 (96-100)
Fathead minnow	25 \pm 0.5 (24.5-25.5)	8.9 \pm 0.8 (8.4-11)	8.4 \pm 0.1 (8.3-8.4)	309 \pm 29 (264-314)	119 \pm 16 (102-134)	108 \pm 7.3 (100-116)
Notched rainbow	25 \pm 0.5 (24.5-25.5)	9.1 \pm 0.7 (8.5-11)	8.4 \pm 0.1 (8.3-8.4)	282 \pm 9.6 (276-298)	105 \pm 3.8 (106-112)	101 \pm 2.8 (98-100)

^a Water temperature was measured twice daily during the test and water quality was measured in the control, medium, and high exposure concentrations at the beginning and the end of the tests.

^b See the preparation of the mock effluent in Table S1.

Table S5. Mean measured concentrations (n = 7 for ammonia and 1 to 2 for metals; standard deviation in parentheses) 7-d mock effluent toxicity tests with cladoceran (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), and notched rainbow (*Villosa constricta*)

Species	Mock effluent		Ammonia (mg TAN/L)		Nickel (µg/L)		Copper (µg/L)		Zinc (µg/L)		Cadmium (µg/L)		Lead (µg/L)		
	#	Dilution	Nominal	Measured	Nominal	Measured	Nominal	Measured	Nominal	Measured	Nominal	Measured	Nominal	Measured	
<i>C. dubia</i>	1	0%	0	0.30 (0.32)	0	0.9 (0.2)	0	0.4 (0.2)	0	7.6 (1.6)	0	0.02 (0.01)	0	0.05 (0.04)	
	1	3.12%	0.25	0.32 (0.15)	13	13 (0.8)	0.4	0.7 (0.1)	30	26 (2.9)	0.06	0.06 (0.02)	0.3	0.14 (0.0)	
	1	6.25%	0.5	0.54 (0.14)	26	26 (1.0)	0.8	1.1 (0.1)	60	47 (6.5)	0.13	0.11 (0.04)	0.6	0.25 (0.1)	
	1	12.5%	1.0	0.99 (0.10)	52	55	1.5	1.6	120	96	0.25	0.23	1.3	0.5	
	1	25%	2.0	1.98 (0.14)	104	109	3.0	2.5	240	195	0.5	0.46	2.5	1.0	
	1	50%	4.0	4.01 (0.15)	208	217	6.0	5.4	480	464	1.0	1.03	5	3.2	
	2	0%	0	0.24 (0.22)	0	0.9 (0.2)	0	0.4 (0.2)	0	7.6 (1.6)	0	0.02 (0.01)	0	0.05 (0.04)	
	2	3.12%	0.1	0.16 (0.08)	12	12 (0.6)	2.0	2.0 (0.3)	31	27 (2.3)	2.0	1.7 (0.2)	5.5	2.7 (0.2)	
	2	6.25%	0.2	0.26 (0.13)	23	24 (2.0)	4.0	3.0 (0.5)	62	41 (11)	4.0	3.1 (0.9)	11	4.4 (1.0)	
	2	12.5%	0.4	0.42 (0.04)	46	49	8.0	6.0	124	95	8.0	8.0	22	10	
	2	25%	0.8	0.80 (0.06)	92	97	16	13	248	232	16	16	44	28	
	2	50%	1.6	1.63 (0.17)	184	193	32	26	496	483	32	33	88	62	
	Fathead minnow	1	0%	0	0.14 (0.09)	0	0.9 (0.2)	0	0.3 (0.03)	0	7.0 (0.6)	0	0.01 (0.0)	0	0.05 (0.4)
		1	6.25%	0.5	0.35 (0.48)	26	31 (5.3)	0.8	1.0 (0.02)	60	59 (24)	0.13	0.15 (0.02)	0.6	0.28 (0.1)
1		12.5%	1.0	0.60 (0.91)	52	60 (7.2)	1.5	1.7 (0.1)	120	119 (33)	0.25	0.25 (0.03)	1.3	0.63 (0.2)	
1		25%	2.0	1.88 (0.34)	104	120 (16)	3.0	2.7 (0.4)	240	239 (62)	0.5	0.53 (0.09)	2.5	1.4 (0.6)	
1		50%	4.0	3.81 (0.60)	208	239 (31)	6.0	5.5 (0.2)	480	520 (79)	1.0	1.1 (0.12)	5.0	3.7 (0.7)	
1		100%	8.0	8.29 (0.27)	416	444	12	10	960	951	2.0	2.0	10	8.0	
2		0%	0	0.18 (0.14)	0	0.8 (0)	0	0.2 (0)	0	6.5 (0)	0	0.02 (0.01)	0	0.1 (0)	
2		6.25%	0.2	0.22 (0.04)	23	27 (2.6)	4.0	3.3 (0.7)	62	53 (27)	4.0	4.1 (0.5)	11	5.6 (2.9)	
2		12.5%	0.4	0.42 (0.04)	46	53 (6.5)	8.0	6.9 (1.3)	124	122 (38)	8.0	8.6 (0.9)	22	14 (5.7)	
2		25%	0.8	0.77 (0.09)	92	109 (17)	16	14 (1.3)	248	274 (59)	16	18 (2.6)	44	36 (11)	
2		50%	1.6	1.53 (0.32)	184	214 (30)	32	27 (2.6)	496	547 (90)	32	36 (4.8)	88	76 (20)	
2		100%	3.2	3.26 (0.12)	368	383	64	54	992	1040	64	66	176	158	
Notched rainbow		1	0%	0	0.07 (0.01)	0	0.9 (0.1)	0	0.2 (0.1)	0	6.8 (0.4)	0	0.014 (0)	0	0.05 (0.03)
		1	3.12%	0.25	0.27 (0.04)	13	14 (0.4)	0.4	0.5 (0.2)	30	30 (8.7)	0.06	0.07 (0)	0.3	0.2 (0)
	1	6.25%	0.5	0.48 (0.07)	26	27 (0.0)	0.8	0.8 (0.3)	60	53 (15)	0.13	0.14 (0.01)	0.6	0.3 (0.1)	
	1	12.5%	1.0	0.93 (0.09)	52	55 (0.5)	1.5	1.4 (0.4)	120	111 (21)	0.25	0.25 (0.03)	1.3	0.6 (0.1)	
	1	25%	2.0	1.93 (0.21)	104	110 (1.4)	3.0	2.4 (0.1)	240	223 (38)	0.5	0.49 (0.04)	2.5	1.3 (0.4)	
	1	50%	4.0	3.91 (0.32)	208	219 (2.1)	6.0	4.9 (0.6)	480	478 (20)	1.0	1.0 (0.02)	5.0	3.4 (0.3)	
	2	0%	0	0.07 (0.01)	0	0.88 (0.2)	0	0.2 (0.1)	0	6.8 (0.4)	0	0.01 (0.01)	0	0.05 (0.04)	
	2	3.12%	0.1	0.13 (0.02)	12	13 (0.1)	2.0	1.6 (0.1)	31	30 (6.0)	2.0	1.9 (0.1)	5.5	2.8 (0.1)	
	2	6.25%	0.2	0.21 (0.02)	23	25 (0.1)	4.0	2.8 (0)	62	47 (20)	4.0	3.9 (0.2)	11	4.5 (1.4)	
	2	12.5%	0.4	0.40 (0.06)	46	49 (0.4)	8.0	5.8 (0.2)	124	114 (28)	8.0	8.2 (0.3)	22	13 (4.1)	
	2	25%	0.8	0.77 (0.10)	92	97 (0.1)	16	13 (0.1)	248	250 (25)	16	17 (0.4)	44	33 (7.1)	
	2	50%	1.6	1.57 (0.22)	184	194 (0.7)	32	25 (0.5)	496	508 (35)	32	33 (0.9)	88	69 (10)	

TAN=total ammonia nitrogen

Table S6. Water quality characteristics and mean survival (n = 4; ± standard deviation) in 7-d feeding experiments with the 3 age groups of juvenile fatmucket (*Lampsilis siliquoidea*)

Age group	Feeding rate	Temp. ^a (°)	Dissolved oxygen (mg/L)	pH	Conductivity (µS/cm)	Hardness	Alkalinity	Ammonia (mg TAN/L)	Survival ^c (%)
						(mg/L as CaCO ₃)			
<i>7-d experiment</i>									
1-wk old	2x1 mL	24.7 ± 0.2	8.1 / 7.9 ^b	8.1 / 8.1	242 / 280	102 / 122	90 / 108	0.03 / 0.10	98 ± 5.0
	2x2 mL	24.7 ± 0.2	8.1 / 7.6	8.1 / 8.1	242 / 281	102 / 120	90 / 110	0.03 / 0.13	98 ± 5.0
	2x3 mL	24.7 ± 0.2	8.1 / 7.7	8.1 / 8.1	242 / 287	102 / 120	90 / 108	0.03 / 0.17	93 ± 9.6
	1x4 mL	24.7 ± 0.2	8.1 / 7.6	8.1 / 8.1	242 / 282	102 / 120	90 / 108	0.03 / 0.15	100 ± 0
2-wk old	2x1 mL	24.7 ± 0.2	8.3 / 8.0	8.1 / 8.3	243 / 325	104 / 134	90 / 110	0.03 / 0.09	100 ± 0
	2x2 mL	24.7 ± 0.2	8.3 / 8.1	8.1 / 8.2	243 / 318	104 / 132	90 / 116	0.03 / 0.15	100 ± 0
	2x3 mL	24.7 ± 0.2	8.3 / 8.0	8.1 / 8.3	243 / 314	104 / 134	90 / 114	0.03 / 0.17	100 ± 0
	1x4 mL	24.7 ± 0.2	8.3 / 8.0	8.1 / 8.3	243 / 322	104 / 136	90 / 114	0.03 / 0.17	98 ± 5.0
3-wk old	2x1 mL	24.6 ± 0.2	8.5 / 8.2	8.1 / 8.2	240 / 288	100 / 120	92 / 108	0.02 / 0.12	98 ± 5.0
	2x2 mL	24.6 ± 0.2	8.5 / 8.3	8.1 / 8.2	240 / 280	100 / 120	92 / 106	0.02 / 0.26	100 ± 0
	2x3 mL	24.6 ± 0.2	8.5 / 8.0	8.1 / 8.2	240 / 285	100 / 124	92 / 110	0.02 / 0.33	98 ± 5.0
	1x4 mL	24.6 ± 0.2	8.5 / 7.8	8.1 / 8.2	240 / 284	100 / 120	92 / 108	0.02 / 0.24	100 ± 0

^a Water temperature was measured twice daily during the experiment. Mean and standard deviation (in parentheses) is given.

^b Water quality was measured in each treatment at the beginning and the end of the feeding treatment.

^c Mean survival (n=4 replicates; 10 organisms per replicate).

TAN=total ammonia nitrogen.

Table S7. Mean water quality characteristics (\pm standard deviation; a range in parentheses) in 7-d NaCl toxicity tests with the 3 age groups of juvenile fatmucket (*Lampsilis siliquoidea*)^a

Age group	Temperature ^a (°)	Dissolved oxygen (mg/L)	pH	Hardness	Alkalinity	Ammonia
				(mg/L as CaCO ₃)		(mg TAN/L)
1-wk old	24.7 \pm 0.2 (24.4-25.0)	8.1 \pm 0.2 (7.9-8.3)	8.2 \pm 0.1 (8.0-8.4)	112 \pm 6.0 (104-120)	100 \pm 7.3 (90-114)	0.10 \pm 0.08 (0.01-0.21)
2-wk old	24.8 \pm 0.2 (24.4-25.1)	8.2 \pm 0.1 (8.2-8.3)	8.2 \pm 0.1 (8.1-8.2)	112 \pm 7.5 (102-118)	99 \pm 6.5 (90-106)	0.11 \pm 0.08 (0.02-0.20)
3-wk old	24.7 \pm 0.2 (24.4-24.8)	8.0 \pm 0.2 (7.9-8.3)	8.2 \pm 0.1 (8.1-8.3)	113 \pm 6.8 (100-120)	101 \pm 8.7 (90-110)	0.19 \pm 0.14 (0.02-0.34)

^a Water temperature was measured twice daily during the test and water quality was measured at least in the control, medium, and high NaCl concentrations at the beginning and the end of each test. Mean conductivities measured in the control and 5 NaCl concentrations (nominal 0, 125, 250, 500, 1000, and 2000 mg NaCl/L) were 260, 481, 714, 1188, 2169, 3825 μ S/cm for the 1-wk-old mussel test, 253, 513, 746, 1185, 2149, 3891 μ S/cm for the 2-wk-old mussel test, and 266, 509, 742, 1182, 2146, 3900 μ S/cm for the 3-wk-old mussel test.

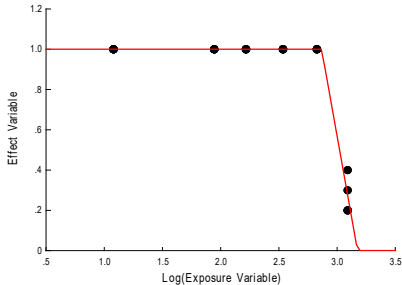
TAN=total ammonia nitrogen.

Table S8. Regressions estimating 20% effect concentrations (EC20s; mg Cl/L) and 95% confidence limits in 7-d NaCl exposures with different age groups of fatmucket (*Lampsilis siliquoides*). Each circle indicates the measured response in each of the 4 replicates, and the line represents the nonlinear regression fit. When insufficient data were available to support adequate analysis by Toxicity Relationship Analysis Program (Erickson 2015), a regression line is highlighted in red and the EC20 is used only for exploratory data analysis

1-wk-old juveniles

Survival

EC20 = 849 (no CL)

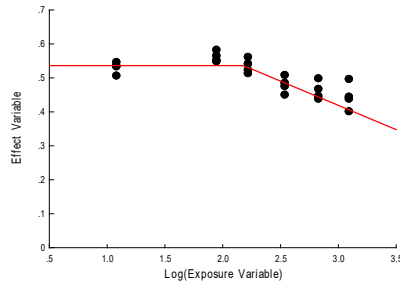


Rectangular distribution

Inadequate partial effects.

Length

EC20 = 854 (535-1364)

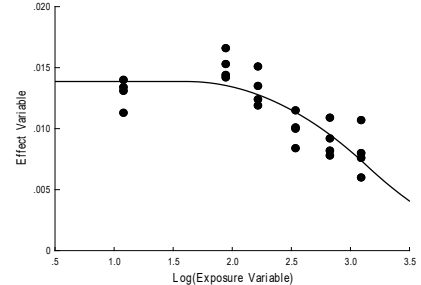


Piecewise Linear

Inadequate partial effects.

Dry weight

EC20 = 381 (180-806)



Threshold Sigmoid

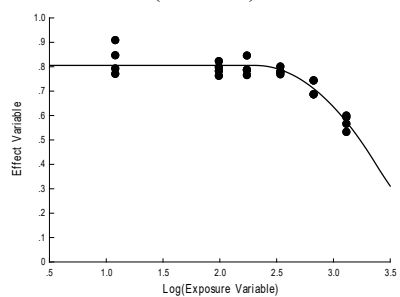
2-wk-old juveniles

Survival

EC20 was not calculated because no concentration caused an effect of >10%.

Length

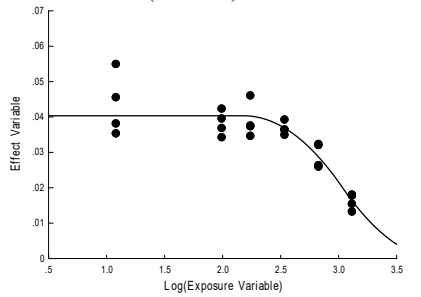
EC20 = 959 (820-1121)



Threshold Sigmoid

Dry weight

EC20 = 523 (363-754)



Threshold Sigmoid

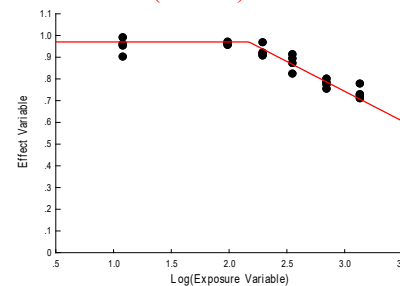
3-wk-old juveniles

Survival

EC20 was not calculated because no concentration caused an effect of >10%.

Length

EC20 = 757 (609-943)

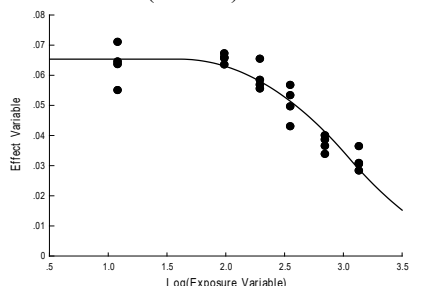


Piecewise Linear

Inadequate partial effects.

Dry weight

EC20 = 330 (216-503)



Threshold Sigmoid

References:

Erickson RJ. 2015. Toxicity Relationship Analysis Program (TRAP), Ver 1.30a. EPA/600/C-11/002. U.S. Environmental Protection Agency, Washington, DC.

Table S9. Mean water quality characteristics (\pm standard deviation; a range in parentheses) in 7-d NaCl toxicity tests with juvenile fatmucket (*Lampsilis siliquoidea*) from the 13 participating laboratories of the interlaboratory study^a

Lab code	Temp. (°C)	Dissolved oxygen (mg/L)	pH	Hardness	Alkalinity	Ammonia
				(mg/L as CaCO ₃)		(mg TAN/L)
1	24.8 \pm 0.7 (24.0-26.0)	8.0 \pm 0.4 (7.3-8.6)	8.0 \pm 0.1 (7.7-8.2)	103 \pm 0 (103-103)	68 \pm 0 (68-68)	0.23 \pm 0.25 (0-0.50)
2	24.9 \pm 0.4 (24.5-26.0)	8.0 \pm 0.4 (7.4-8.6)	7.8 \pm 0.3 (6.5-8.0)	90 \pm 8.8 (82-98)	67 \pm 3.3 (64-70)	<0.20 ^b
3	25.2 \pm 0.3 (25.0-26.0)	8.2 \pm 0.3 (7.7-8.7)	7.7 \pm 0.3 (7.2-8.2)	69 \pm 2.5 (66-72)	48 \pm 1.8 (46-50)	<0.25 ^b
4	24.4 \pm 0.2 (24.1-24.8)	8.4 \pm 0.2 (8.0-8.6)	7.7 \pm 0.1 (7.4-7.9)	85 \pm 1.8 (84-88)	61 \pm 1.5 (59-63)	0.12 \pm 0.03 (0.09-0.15)
5	24.6 \pm 0.2 (24.3-25.0)	8.2 \pm 0.2 (7.8-8.6)	8.0 \pm 0.1 (7.2-8.2)	83 \pm 2.8 (78-86)	57 \pm 2.8 (54-62)	0.15 \pm 0.13 (0.01-0.32)
6	24.4 \pm 0.6 (23.4-25.5)	6.4 \pm 0.7 (6.0-9.1)	7.5 \pm 0.2 (7.2-7.9)	94 \pm 5.6 (87-100)	62 \pm 1.0 (61-64)	0.11 \pm 0.10 (0-0.23)
7	24.9 \pm 0.1 (24.8-24.9)	7.7 \pm 0.4 (7.2-8.7)	7.9 \pm 0.1 (7.9-8.0)	98 \pm 8.5 (90-108)	66 \pm 3.2 (64-72)	0.31 \pm 0.31 (0.02-0.69)
8	24.5 \pm 0.5 (24.1-25.6)	7.8 \pm 0.3 (7.2-8.4)	8.1 \pm 0.3 (7.5-8.5)	76 \pm 2.1 (73-78)	56 \pm 7.1 (44-64)	0.08 \pm 0.02 (0-0.11)
9	24.8 \pm 0.2 (24.5-25.1)	7.0 \pm 1.1 (5.3-8.9)	7.6 \pm 0.1 (7.4-7.8)	86 \pm 2.2 (84-88)	59 \pm 3.3 (56-64)	0.02 \pm 0.01 (0.01-0.03)
10	24.9 \pm 0.3 (24.4-25.8)	7.8 \pm 0.5 (6.4-8.6)	7.8 \pm 0.1 (7.6-8.0)	88 \pm 2.7 (84-92)	63 \pm 3.1 (60-68)	0.07 \pm 0.01 (0.04-0.09)
11	25.1 \pm 0.5 (24.5-26.6)	8.9 \pm 0.6 (7.9-10.1)	7.8 \pm 0.1 (7.6-8.0)	96 \pm 3.7 (90-100)	68 \pm 2.2 (66-70)	0.06 \pm 0.08 (0-0.17)
12	24.4 \pm 0.2 (24.0-24.7)	7.7 \pm 0.3 (7.3-8.3)	7.8 \pm 0.1 (7.7-7.9)	81 \pm 2.8 (78-86)	74 \pm 5.1 (68-80)	<0.05 ^b
13	24.8 \pm 0.2 (24.4-25.1)	7.0 \pm 0.2 (6.4-7.4)	7.5 \pm 0.1 (7.3-7.6)	NM	NM	NM

^a The water quality were measured at least in the control, medium, and high treatments at the beginning and the end of the tests. The temperature, dissolved oxygen, and pH were also measured during the tests before and after daily water renewal.

^b A less-than value is presented when ammonia concentration was below the detection limit.

TAN=total ammonia nitrogen; NM = not measured.

Table S10. Interlaboratory study Lab 1: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity ($\mu\text{S}/\text{cm}$; n=14)		Survival (%)		Length (mm) ^a		Dry weight (μg) ^a	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	7.5	304	21	100	0	NR		NR	
125	76	88	550	30	98	5.0	NR		NR	
250	152	176	787	38	100	0	NR		NR	
500	303	323	1266	55	95	5.9	NR		NR	
1000	607	628	2191	90	98	5.0	NR		NR	
2000	1214	1264	3992	153	100	0	NR		NR	
NOEC					NA		NC		NC	
LOEC					>1264		NC		NC	
IC25 (95% CL)					>1264		NC		NC	

^a The growth data are not reported because the shells of 2 or more surviving mussels in most replicates were crushed and could not be measured (see text).

NR=not reported; NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NC=not calculated; NA=not applicable.

Table S11. Interlaboratory study Lab 2: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (μS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (μg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	2.6	306	22	100	0	0.370	0.016	4.84	0.55
125	76	83	562	23	100	0	0.364	0.005	4.63	0.16
250	152	160	809	26	100	0	0.361	0.005	4.55	0.18
500	303	317	1295	35	100	0	0.349	0.009	4.15	0.29
1000	607	639	2236	43	95	6.7	0.331	0.008	3.57	0.21
2000	1214	1268	4041	89	82	22	0.312	0.005	3.07	0.12
NOEC					NA		317		317	
LOEC					>1268		639		639	
IC25 (95% CL)					>1268		>1268		597 (403-886)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S12. Interlaboratory study Lab 3: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (μS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (μg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	2.3	302	10	84	10	0.368	0.007	4.73	0.23
125	76	82	548	6.2	100	0	0.358	0.010	4.45	0.33
250	152	150	798	6.5	88	10	0.355	0.009	4.34	0.27
500	303	295	1289	8.5	95	10	0.348	0.005	4.10	0.17
1000	607	599	2247	10	95	10	0.341	0.001	3.88	0.04
2000	1214	1151	4103	39	70	18	0.320	0.008	3.25	0.22
NOEC					NA		150		150	
LOEC					>1151		295		295	
IC25 (95% CL)					>1151		>1151		846 (718-1014)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S13. Interlaboratory study Lab 4: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (µS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (µg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	2.5	310	28	100	0	0.396	0.003	5.79	0.11
125	76	73	545	36	100	0	0.403	0.007	6.07	0.28
250	152	137	767	31	100	0	0.387	0.004	5.46	0.15
500	303	281	1230	27	100	0	0.366	0.007	4.70	0.25
1000	607	587	2174	34	74	29	0.349	0.013	4.14	0.44
2000	1214	1175	3988	102	90	0	0.332	0.008	3.74	0.20
NOEC					NA		137		137	
LOEC					>1175		281		281	
IC25 (95% CL)					>1175		>1175		392 (324-620)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S14. Interlaboratory study Lab 5: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (μS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (μg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	2.4	311	19	100	0	0.473	0.027	9.52	1.35
125	76	79	556	5.4	100	0	0.442	0.024	7.91	1.26
250	152	156	811	6.0	100	0	0.454	0.012	8.30	0.76
500	303	315	1301	82	98	5.0	0.455	0.028	8.56	1.41
1000	607	633	2279	12	90	12	0.436	0.029	7.57	1.27
2000	1214	1261	4231	41	90	8.2	0.385	0.027	5.40	1.03
NOEC					NA		633		633	
LOEC					>1261		1261		1261	
IC25 (95% CL)					>1261		>1261		724 (398-1080)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S15. Interlaboratory study Lab 6: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (µS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (µg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	2.1	279	24	95	5.8	0.306	0.008	2.90	0.21
125	76	78	533	23	96	7.1	0.327	0.010	3.48	0.29
250	152	158	787	25	90	12	0.295	0.010	2.66	0.23
500	303	302	1281	25	94	6.4	0.316	0.024	3.22	0.66
1000	607	598	2236	62	97	6.3	0.311	0.006	3.02	0.17
2000	1214	1177	4111	87	52	18	0.275	0.008	2.17	0.19
NOEC					598		598		598	
LOEC					1177		1177		1177	
IC25 (95% CL)					869 (761-1081)		>1177		1060 (831-1157)	

^a The value should be used with caution due to inadequate partial effect for the EC20 estimation.

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits.

Table S16. Interlaboratory study Lab 7: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (μS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (μg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	3.7	313	23	100	0	0.404	0.013	6.18	0.48
125	76	101	540	22	100	0	0.385	0.032	5.58	1.41
250	152	184	775	33	100	0	0.382	0.005	5.33	0.16
500	303	362	1237	64	100	0	0.386	0.015	5.47	0.57
1000	607	675	2116	80	100	0	0.378	0.016	5.17	0.64
2000	1214	1313	3849	150	100	0	0.330	0.017	3.56	0.50
NOEC					NA		675		675	
LOEC					>1313		1313		1313	
IC25 (95% CL)					>1313		>1313		842 (683-978)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S17. Interlaboratory study Lab 8: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (µS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (µg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	4.3	320	16	100	0	0.421	0.035	7.01	1.41
125	76	78	566	12	95	6.1	0.405	0.021	6.25	0.83
250	152	151	817	20	100	0	0.399	0.015	5.99	0.63
500	303	301	1306	38	94	6.4	0.379	0.009	5.24	0.36
1000	607	556	2281	76	100	0	0.378	0.015	5.17	0.49
2000	1214	1160	4005	161	97	5.6	0.340	0.014	3.89	0.43
NOEC					NA		151		151	
LOEC					>1160		301		301	
IC25 (95% CL)					>1160		>1160		296 (151-787)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S18. Interlaboratory study Lab 9: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (μS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (μg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	1.5	308	5.8	96	7.1	0.354	0.013	4.32	0.41
125	76	73	547	17	98	5.0	0.353	0.016	4.34	0.56
250	152	145	778	19	95	6.1	0.369	0.018	4.84	0.62
500	303	300	1241	27	95	6.1	0.380	0.029	5.35	0.98
1000	607	608	2177	42	93	15	0.350	0.025	4.20	0.79
2000	1214	1136	3944	167	87	10	0.326	0.016	3.47	0.52
NOEC					NA		NA		NA	
LOEC					>1136		>1136		>1136	
IC25 (95% CL)					>1136		>1136		1076 (588-1093)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S19. Interlaboratory study Lab 10: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (µS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (µg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	3.1	327	11	95	10.0	0.459	0.018	8.70	0.87
125	76	98	570	8.6	98	5.0	0.488	0.008	10.28	0.46
250	152	156	817	11	100	0	0.462	0.006	8.83	0.34
500	303	301	1297	26	98	5.0	0.446	0.008	8.03	0.40
1000	607	577	2254	28	92	11	0.428	0.010	7.23	0.53
2000	1214	1219	4097	43	94	6.6	0.374	0.015	4.98	0.56
NOEC					NA		301		301	
LOEC					>1219		577		577	
IC25 (95% CL)					>1219		>1219		598 (469-693)	

^a The value should be used with caution due to inadequate partial effect for the EC20 estimation.

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NA=not applicable.

Table S20. Interlaboratory study Lab 11: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (µS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (µg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	3.1	350	19	100	0	0.381	0.015	5.34	0.60
125	76	98	600	15	100	0	0.377	0.008	5.15	0.32
250	152	156	852	23	Nra		0.359	0.021	4.53	0.73
500	303	285	1328	32	100	0	0.360	0.007	4.48	0.23
1000	607	569	2262	83	NR ^a		0.354	0.003	4.28	0.09
2000	1214	1123	4041	152	NR ^a		0.342	0.016	3.92	0.48
NOEC					NC		285		285	
LOEC					NC		569		569	
IC25 (95% CL)					NC		>1123		957 (251-1068)	

^a One or more replicates had a low recovery of mussels (≤50%) and survival was not reported.

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NC=not calculated; NR=not reported.

Table S21. Interlaboratory study Lab 12: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test. Shaded cell indicates significant reduction relative to the control within a column (Dunnett's test or Steel's many-one rank test, p<0.05)

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)	Conductivity (µS/cm; n=14)		Survival (%)		Length (mm)		Dry weight (µg)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	4.6	304	11	95	5.8	0.405	0.006	6.23	0.19
125	76	77	558	20	80	12	0.412	0.002	6.48	0.12
250	152	164	806	23	88	13	0.403	0.011	6.08	0.41
500	303	303	1299	35	93	15	0.406	0.019	6.23	0.78
1000	607	603	2259	62	80	20	0.382	0.022	5.32	0.80
2000	1214	1205	4096	48	73	10	0.332	0.008	3.64	0.17
NOEC					603		603		603	
LOEC					1205		1205		1205	
IC25 (95% CL)					>1205		>1205		758 (534-850)	

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits.

Table S22. Interlaboratory study Lab 13: Mean survival, shell length, and estimated dry weight (n=4) of fatmucket (*Lampsilis siliquoidea*) in the 7-d NaCl test

Nominal NaCl (mg/L)	Nominal Cl (mg/L)	Measured Cl (mg/L; n=2)		Conductivity (µS/cm; n=7)		Survival (%) ^a		Length (mm) ^a		Dry weight (µg)	
		Day 0	Day 6	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	Control	16	16	186	5.0	100	0	0.483	0.004	10.07	0.35
125	76	18	66	308	71	98	5.0	0.488	0.015	10.26	0.77
250	152	20	116	448	143	100	0	0.488	0.004	10.31	0.15
500	303	20	198	670	255	95	9.1	0.481	0.010	9.82	0.55
1000	607	24	374	1177	513	83	5.8	0.490	0.013	10.36	0.73
2000	1214	20	759	2133	995	87	10	0.474	0.024	9.54	1.26
NOEC						NC ^b		NC		NC	
LOEC						NC ^b		NC		NC	
IC25 (95% CL)						NC		NC		NC	

^a The 7-d test ended on test day 6 by mistake. Survival and growth data were based on the 6-d exposure.

^b Effect concentrations were not calculated because the exposure concentrations were not consistent over the test period and far below the nominal concentrations in most cases.

NOEC=no observed effect concentration; LOEC=lowest-observed-effect concentrations; IC25=25% inhibition concentration; CL=confidence limits; NC=not calculated.