

SUPPLEMENTAL INFORMATION

**ENVIRONMENTAL CONTAMINATION AND UNUSUAL SNAKE
MORTALITY IN AN URBAN NATIONAL WILDLIFE REFUGE**

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The following material is provided by the authors and was not subjected to peer review or editing by *Herpetological Conservation and Biology*.

TABLE S1. Coordinates of study sites and starting locations for visual searches at National Wildlife Refuges in Louisiana.

Site ID	GPS N	GPS W	Survey Type
<u><i>Bogue Chitto</i></u>			
Lock 3	30.63691	-89.86807	VES
Dumas Wise	30.64236	-89.80199	VES
Honey Island	30.44931	-89.71499	VES
Schmidt	30.44944	-89.70353	VES
<u><i>Big Branch Marsh</i></u>			
Lucille	30.29414	-89.96239	VES
McManus	30.24475	-89.72151	VES
Redeemer	30.31948	-89.93343	VES
Scout	30.27954	-89.89779	VES
<u><i>Bayou Sauvage</i></u>			
Haul Trail North	30.11574	-89.90479	VES, T, CA
Haul Trail South	30.11328	-89.90057	VES, T, CA, EE
Ridge Trail North	30.05691	-89.88036	VES, CA, EE
Ridge Trail South	30.05392	-89.87848	VES, EE
Pipeline Leak	30.11335	-89.89056	CA
Crab Bridge	30.13480	-89.87496	EE
Madere Launch	30.05259	-89.87564	CA
Michoud Road	30.07984	-89.92513	CA, EE
NW Overpass	30.09437	-89.91256	CA

Recovery Road North	30.04838	-89.87717	CA, EE
Recovery Road South	30.03844	-89.87205	CA
SE Overpass	30.10571	-89.89086	CA
SW Overpass	30.08973	-89.90520	CA

*Visual encounter survey (VES), trapping (T), contaminant
analysis (CA; sediment samples), embryo exposure
challenge (EE; water samples).

TABLE S2. Survey effort and snake observations among study sites at National Wildlife Refuges (NWRs) in Louisiana.

Site	Transect		Survey Effort		Snakes (#)		
	Name	Length (km)	Visits (#)	Person- Hours	Live- Normal	Live- Abnormal	Dead
Bayou Sauvage NWR	Haul	1.5	113	250	201	3	49
	Trail						
	Ridge	1.3	19	36	6	0	0
	Trail						
	Site Total	2.8	132	286	207	3	49
Big Branch Marsh NWR	Scout	11.7	52	229	80	0	1
	McManus	1.5	40	80	19	0	1
	Lucille	1.1	26	74	25	0	1
	Redeemer	1.9	11	38	2	0	0
	Site Total	16.2	129	421	126	0	3
Bogue Chitto NWR	Dumas	2.8	25	63	9	0	0
	Wise						
	Honey	2.3	11	65	29	0	0
	Island						
	Lock 3	8.0	8	65	10	0	0
	Schmidt	1.4	22	58	6	0	0

	Site						
		14.5	66	251	54	0	0
	Total						

TABLE S3. Comprehensive list of carcasses detected at Haul Trail in Bayou Sauvage National Wildlife Refuge and relevant findings for specimens subject to postmortem examination

Field Survey Date	Necropsy Conducted ? (USGS Accession #)	Species	Sex^a	Age	Body Condition	Postmortem State	Diagnostic Results^b	Significant Necropsy and Histopathologic Findings^c	Cause of Death^d
5/22/15	No	Glossy Swampsnake (n = 2)	U	Immature	U	Poor	NA	NA	NA
9/25/15	No	Glossy Swampsnake	U	Immature	U	Poor	NA	NA	NA
9/28/15	No	Black-Masked Racer	U	Immature	U	NA	NA	NA	Observed predation

11/3/15	Yes (26922-001)	Glossy Swampsna ke	F	Immature (Hatching year)	Good	Fair	RC liver (-), SC colon (-); GCMS SMB 001-003 (-)	NSF	U
11/5/15	Yes (26922-002)	Glossy Swampsna ke	U	Immature	Good	Fair	RC liver (-), SC colon (-); GCMS SMB 001-003 (-)	Suspect thymic lymphocytic necrosis, Suspect hepatic endothelial sinusoidal necrosis, Renal glomerular and interstitial necrosis	U
11/5/15	Yes (26922-003)	Glossy Swampsna ke	U	Immature	Good	Fair	GCMS SMB 001-003 (-)	Suspect hepatic endothelial sinusoidal necrosis	U
11/5/15	Yes (26922-004)	Glossy Swampsna ke	M	Immature	Good	Fair	NA	NSF	U

11/5/15	Yes (26922-005)	Glossy Swampsnake	U	Immature	Good	Fair	<i>Ophidiomyces</i> PCR skin (-); <i>Ophidiomyces</i> fungal culture skin (-);	Depigmentation of ventral scales; Epidermitis, necrotizing, mycotic consistent with <i>Ophidiomyces ophiodiicola</i> ; Atrophy of fat	U
11/5/15	Yes (26922-006)	Glossy Swampsnake	U	Immature	Fair	Fair	NA	Suspect dehydration	U
11/6/15	No (saved for future analysis; 26922-007:010)	Glossy Swampsnake (n = 4)	U	Immature	Fair	Fair	NA	NA	NA

12/6/15	Yes (26922-011)	Western Mudsnake	F	Immature	Good	Fair	RC liver (-), SC colon (+); GCMS SMB (-)	Suspect dehydration; Granulomatous colitis and enteritis, verminous enteropathy, suspect hepatic necrosis	Salmonell osis
1/15/16	No	Glossy Swampsna ke	U	Immature	U	Poor	NA	NA	NA
1/17/16	Yes (26922-012)	Western Ribbon Snake	U	Immature	Fair	Fair	RC liver, colon MG	Suspect cutaneous chemical or thermal burn; Protozoal hepatitis (possibly <i>Hepatozoon</i> sp.), nephritis, colonic serositis, ulcerative epidermitis,	Protozoal infection

								verminous pneumonitis	
2/15/16	No	Glossy Swampsna ke	U	Immature	U	Poor	NA	NA	NA
3/3/16	No	Western Mudsnake	U	Adult	U	Poor	NA	NA	NA
3/15/16	No	Glossy Swampsna ke	U	Immature	U	Poor	NA	NA	NA
4/19/16	Yes (27197- 001)	Glossy Swampsna ke	U	Immature (After hatching year)	Emaciat ed	Excellent	VI liver (-), WNV liver (-)	Atrophy of fat, renal pallor; Hepatic necrosis, Interstitial pneumonia, Interstitial nephritis	U (suspect viral infection)
4/23/16	No	Glossy Swampsna ke (n = 4)	U	Immature	U	Fair (n = 1), Poor (n = 3)	NA	NA	NA

5/6/16	No	Glossy Swampsna ke	U	Immature	U	Good	NA	NA	NA
5/15/16	No	Glossy Swampsna ke	U	Immature	U	Poor	NA	NA	NA
5/17/16	No	Glossy Swampsna ke	U	Immature	U	Poor	NA	NA	NA
5/18/16	No	Black- Masked Racer	U	Immature	U	Poor	NA	NA	NA
5/24/16	No	Glossy Swampsna ke	U	Immature	U	Poor	NA	NA	NA
5/30/16	No	Glossy Swampsna ke (n = 2)	U	Immature	U	Poor	NA	NA	NA

6/9/16	No	Glossy Swampsna ke	U	Immature	U	Poor	NA	NA	NA
8/26/16	No	Western Mudsnake	U	Not recorded	U	Poor	NA	NA	NA
10/3/16	Yes (27573- 001)	Black- Masked Racer	M	Adult	Poor	Good	RC liver <i>Salmonella</i> sp., MG; Intestine: <i>Kalicephalus</i> sp., <i>Cryptosporidium</i> sp., nematode Stomach: nematode; VI kidney/liver (-)	Atrophy of fat; Gastric hyperplasia with <i>Cryptosporidium</i> sp. infection, Myositis, lymphogranulocytic	U
10/19/16	No	Glossy Swampsna ke	U	Not recorded	U	Poor	NA	NA	NA

10/19/16	Yes (27595-002)	Black-Masked Racer	F	Adult	Excellent	Good	RC lung, liver MG, SC colon (+) with MG, FC skin <i>Bartalinia pondoensis</i> ; <i>Ophidiomyces</i> PCR skin (+, suspected environmental contamination based on absence of skin abnormalities); Liver, blood smear: <i>Hepatozoon</i> sp. Stomach: <i>Cosmocercoides</i> , <i>Strongyloides</i> , NOS, nematode; VI kidney/liver (-)	Cutaneous scars; Hepatic necrosis with infarctions, Necrohemorrhagic colitis, Splenic atrophy, Granulomatous hepatitis and colitis	Bacterial septicemia
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10/23/16	Yes (27595-003)	Cottonmouth	U	Immature	Emaciated	Fair	RC liver <i>Salmonella</i> with MG, SC colon (-); Stomach: <i>Cryptosporidium</i> sp.; <i>Ophidiomyces</i> PCR skin (+, suspected environmental contamination based on absence of skin abnormalities); VI kidney/liver (-)	Atrophy of fat, Splenic and thymic atrophy; Disseminated protozoal infection (suspect <i>Hepatozoon</i> sp.); Gastric glandular atrophy; possible <i>Salmonella</i> sp. III	U
		h			ed			21:z10:e,n,x,z15 infection of liver	

(may be artifact of decomposition).									
10/23/16		Black-Masked Racer	U	Adult	U	Poor	NA	NA	NA
11/1/16	No	U (possibly Black-Masked Racer)	U	U	Poor	U	NA	NA	NA
12/5/16	No	Mississippi Green Watersnake	U	Adult	U	Excellent (carcass covered in mucus, suspected predation attempt by observed	NA	NA	NA

Northern Cottonmouth)									
12/11/16	Yes (27655-001)	Black-Masked Racer	M	Adult	Excellent	Fair	RC liver, colon <i>Salmonella enterica</i> serovar Infantis with MG, FC skin <i>Diaportha phaseolorum</i> , <i>Ophidiomyces</i> PCR skin (-)	Necrotizing hepatitis, colitis, proctitis and coelomitis, Esophagitis	Salmonellosis
1/14/17	Yes (27695-001)	Mud Snake	F	Adult	Excellent	Not recorded	RC distal colon, stomach MG, RC liver, colon, <i>Salmonella</i> sp. with MG. RC skin bacterial and fungal MG, <i>Ophidiomyces</i>	Hepatic and intestinal necrosis	Salmonellosis

							PCR skin (-); GCMS		
							Liver (-); Intestine:		
							cestode, capillarid,		
							<i>Gnathostoma</i> sp.		
3/15/17	No	U (possibly Black- Masked Racer)	U	U	U	Poor	NA	NA	NA
4/17/17	No	Western Mudsnake	U	Immature (Hatchin g year)	U	Poor	NA	NA	NA
6/29/17	No	Northern Cottonmout h	U	Adult	U	Poor	NA	NA	NA
6/29/17	No	Glossy Swampsna ke	U	Not recorded	U	Poor	NA	NA	NA

11/12/17	No	U (possibly <i>Nerodia</i> sp.)	U	U	U	Poor	NA	NA	NA
11/12/17	No	Eastern Black Kingsnake	U	Immature (Hatchin g year)	U	Poor	NA	NA	NA

^aM=male; F=female; U=undetermined

^bRC=Routine aerobic bacteria culture; SC=Salmonella culture; GCMS=Gas chromatography–mass spectrometry; SMB=pooled skin, muscle and bone; NA=Not applicable; MG=Mixed growth; VI=Virus isolation; WNV=West Nile Virus; NOS=Not otherwise specified

^cNA=Not applicable; NSF=No significant findings

^dNA=Not applicable; U=undetermined

TABLE S4. Lower detection limits of contaminant analyses conducted on Bayou Sauvage National Wildlife Refuge sediment samples.

Compound	Lower Detection Limit (ppb)
Mercury	1
Polychlorinated Biphenyls 138, 153, 180	3.0
Polycyclic Aromatic Hydrocarbons*, Sulfmeturon Methyl	5.0
PCB 28, 52, 101	6.0
PCB 126	12.0
Glyphosate	20.0
Heavy metals**	100
Calcium, Potassium, Sodium	1,000
Nitrate Nitrogen	2,000
Nitrite Nitrogen	3,000
Chloride, Fluoride, Orthophosphate, Sulfates	5,000

* Acenaphthene, anthracene, benzo(ghi)perylene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(K)fluoranthene, chrysene, dibenz(ah)anthracene, fluoranthene, fluorine, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene.

** Arsenic, aluminum, barium, beryllium, cadmium, chromium, iron, lead, magnesium, manganese, selenium, silver, and thallium.

TABLE S5. Expected ranges (ppm) of inorganic compounds in Bayou Sauvage National Wildlife Refuge sediment samples.

Inorganics	Expected Range*	Max Detected
Arsenic	0.0–14.7	44.0
Aluminum	0–48,151	47,797
Barium	6.9–286.1	246.0
Beryllium	<0.1	0.4
Cadmium	0.0–1.0	2.7
Calcium	0–19,613	79,866
Chlorides	0–6,018	28,232
Chromium	5.8–26.0	46.8
Fluoride	<5.0	ND
Lead	0.0–83.3	362.8
Magnesium	752–10,241	8,913
Manganese	0.0–412.5	940.6
Mercury	0.00–0.16	0.51
Nitrate Nitrogen	<2.0	273.9
Nitrite Nitrogen	<3.0	11.1
Potassium	0–6,269	8,032
Selenium	1.3–3.6	4.5

Silver	<0.1	ND
Sodium	0–5,778	11,223
Sulfates	0–485.0	33,102
Thallium	<0.1	0.5
Iron	4,431–32,963	26,557

*Based on 95% confidence intervals (CI) across all samples (see methods). Bold text indicates concentrations that exceeded the expected range at one or more sites.

TABLE S6. Dry-weight concentrations* (ppb) of organic contaminants in Bayou Sauvage National Wildlife Refuge sediment.

	Site**											RECAP	EPA
	SWO	NWO	RRN	RRS	MAD	RTN	HRN	HRS	SEO	MIC	PLL		
% Moisture	67.8	81.2	63.0	50.7	71.0	60.5	54.2	86.3	85.6	89.8	63.1		
<u>Compound</u>													
Glyphosate	462.0	973.4	166.3	195.6	ND	ND	ND	ND	ND	ND	NA	NA	6,300
Sulfmeturon Methyl	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
PCB 28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
PCB 52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
PCB 101	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
PCB 126	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
PCB 138	10.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
PCB 153	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
PCB 180	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
Acenaphthene	ND	ND	69.4	ND	ND	ND	ND	ND	ND	ND	234.1	370	3,600
Anthracene	ND	ND	40.2	ND	ND	ND	ND	ND	ND	ND	ND	2,200	18,000
Benzo(ghi)perylene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
Benzo(a)anthracene	56.7	ND	19.0	ND	ND	ND	ND	ND	ND	ND	19.7	0.62	1.1
Benzo(a)pyrene	52.7	ND	14.3	ND	ND	ND	ND	ND	ND	ND	25.1	0.33	0.11
Benzo(b)fluoranthene	91.2	ND	38.1	ND	53.1	ND	16.4	ND	87.0	ND	65.6	0.62	1.1

Benzo(k)fluoranthene	40.0	ND	14.5	ND	17.8	ND	ND	ND	ND	ND	12.4	6.2	11
Chrysene	61.7	ND	27.3	ND	ND	ND	ND	ND	ND	ND	72.4	62	110
Dibenz (a,h) anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA
Fluoranthene	118.1	ND	190.3	ND	46.5	ND	ND	ND	ND	ND	59.6	220	2,400
Fluorene	ND	ND	52.4	ND	ND	ND	ND	ND	ND	ND	118.7	280	2,400
Indeno(1,2,3-cd) pyrene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA
Naphthalene	38.4	ND	40.5	ND	ND	ND	ND	ND	ND	ND	845.5	6.2	3.8
Phenanthrene	45.0	ND	129.6	ND	23.4	ND	ND	ND	ND	ND	64.5	2,100	NA
Pyrene	123.7	ND	134.7	ND	ND	ND	ND	ND	ND	ND	106.2	230	1,800

ND = not detected

NA = not applicable (not tested)

*Shaded values exceeded the corresponding Non-Industrial Soil Screening Standard set by Louisiana Department of Environmental Quality's (LDEQ) Risk Evaluation Corrective Action Program (RECAP) or the Residential Soil Screening Level set by the Environmental Protection Agency (EPA), or both.

**SW Overpass (SWO), NW Overpass (NWO), Recovery Road North (RRN), Madere Launch (MAD), Ridge Trail North (RTN), Haul Trail North (HTN), Haul Trail South (HTS), SE Overpass (SEO), Michoud Road (MIC), Pipeline Leak (PLL).

TABLE S7. Dry-weight concentrations* (ppm) of inorganic contaminants in Bayou Sauvage National Wildlife Refuge sediment.

	Site**											Louisiana	EPA
	SWO	NWO	RRN	RRS	MAD	RTN	HRN	HRS	SEO	MIC	PLL	RECAP	
% Moisture	67.8	81.2	63.0	50.7	71.0	60.5	54.2	86.3	85.6	89.8	63.1		
<u>Compound</u>													
Arsenic	4.7	6.8	2.5	5.6	14.2	44.0	6.9	3.6	10.9	11.3	4.0	12.0	0.68
Aluminum	15,621	27,728	17,429	23,878	32,310	47,797	36,447	11,883	9,879	6,082	8,401	NA	77,000
Barium	246	164	69	127	141	220	243	52	178	82	89	550	15,000
Beryllium	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.4	16	160
Cadmium	2.3	ND	ND	ND	ND	0.8	0.3	1.0	2.7	ND	0.4	3.9	71
Calcium	21,495	6,606	4,058	79,866	2,444	3,167	4,234	4,479	14,206	9,568	3,902	NA	NA
Chlorides	747	1,489	2,055	1,222	28,232	352	4,361	1,952	1,330	6,582	NA	NA	NA
Chromium	46.8	21.2	9.3	9.9	19.2	39.0	13.4	19.2	23.6	14.2	13.3	NA*	NA*
Fluoride	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	3100
Lead	362.8	78.7	16.5	18.3	34.5	23.8	18.4	31.0	215.2	42.4	71.0	400	400
Magnesium	4,476	8,506	4,461	5,530	7,631	8,913	7,955	3,162	3,562	4,244	2,022	NA	NA
Manganese	518	256	199	941	213	190	121	95	412	270	67	NA	1,800
Mercury	0.5	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	2.3	11
Nitrate Nitrogen	10.0	20.1	ND	ND	118.2	ND	ND	273.9	ND	ND	NA	13,000	130,000

Nitrite Nitrogen	ND	ND	ND	ND	ND	ND	11.1	ND	ND	ND	NA	780	7,800
Ortho Phosphate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA	NA
Potassium	1888	5505	2249	3014	3216	6221	8031	1529	1790	2161	1220	NA	NA
Selenium	2.2	4.5	1.7	2.4	3.3	3.4	2.8	1.9	1.7	2.6	2.4	39	390
Silver	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA	NA
Sodium	1139	2693	1937	2002	11223	1521	5146	2058	1618	5638	453	NA	NA
Sulfates	97	193	1660	379	33102	92	277	969	359	4391	NA	NA	NA
Thallium	ND	ND	ND	ND	ND	0.5	0.5	ND	ND	ND	ND	0.55	0.78
Iron	24,958	24,750	13,618	17,565	21,889	24,202	26,557	8,325	12,375	24,028	7,398	NA	55,000

ND = not detected

NA = Not applicable (not tested)

*Bold values exceeded the expected concentration range, calculated as the mean of all samples + 1.96 standard deviations after removing outliers (see methods).

**SW Overpass (SWO), NW Overpass (NWO), Recovery Road North (RRN), Madere Launch (MAD), Ridge Trail North (RTN), Haul Trail North (HTN), Haul Trail South (HTS), SE Overpass (SEO), Michoud Road (MIC), Pipeline Leak (PLL).



FIGURE S1. Ventral surface of a Black-Masked Racer (*Coluber constrictor latrunculus*) found alive and lethargic on Feb 1, 2016, at Haul Trail (Bayou Sauvage National Wildlife Refuge) with a severe abnormality ~3 cm in length, consistent with a thermal or chemical burn. (Photographed by Kimberly Terrell.)

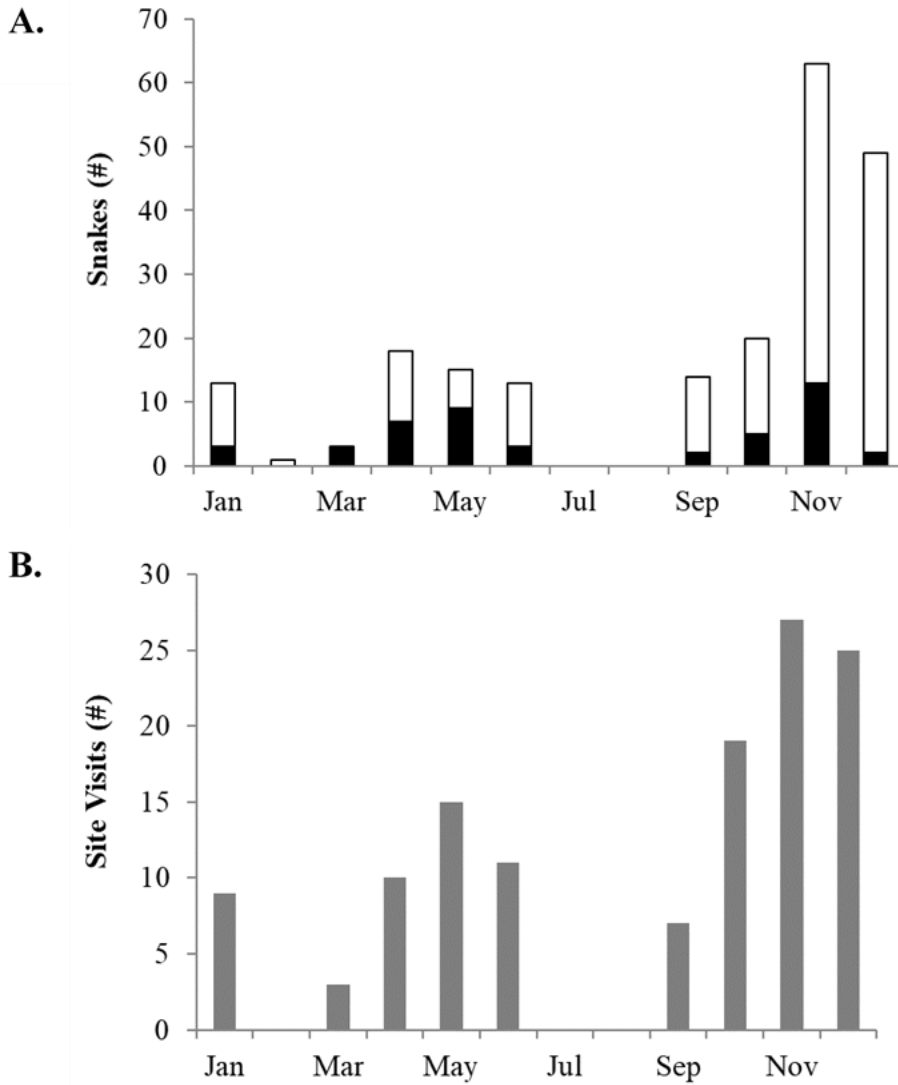


FIGURE S2. Encounters of live (white bars) and dead (black bars) snakes (A) and number of site visits (B) per month at Haul Trail (Bayou Sauvage National Wildlife Refuge).



FIGURE S3. Ventral surface of a Western Ribbonsnake (*Thamnophis proximus*; Accession #26922-012) found dead on Jan 17, 2016 at Haul Trail (Bayou Sauvage National Wildlife Refuge) with a severe abnormality ~3 cm in length, consistent with a thermal or chemical burn. This individual was observed alive with the same abnormality 3 days prior. (Photographed by USGS National Wildlife Health Center staff.)

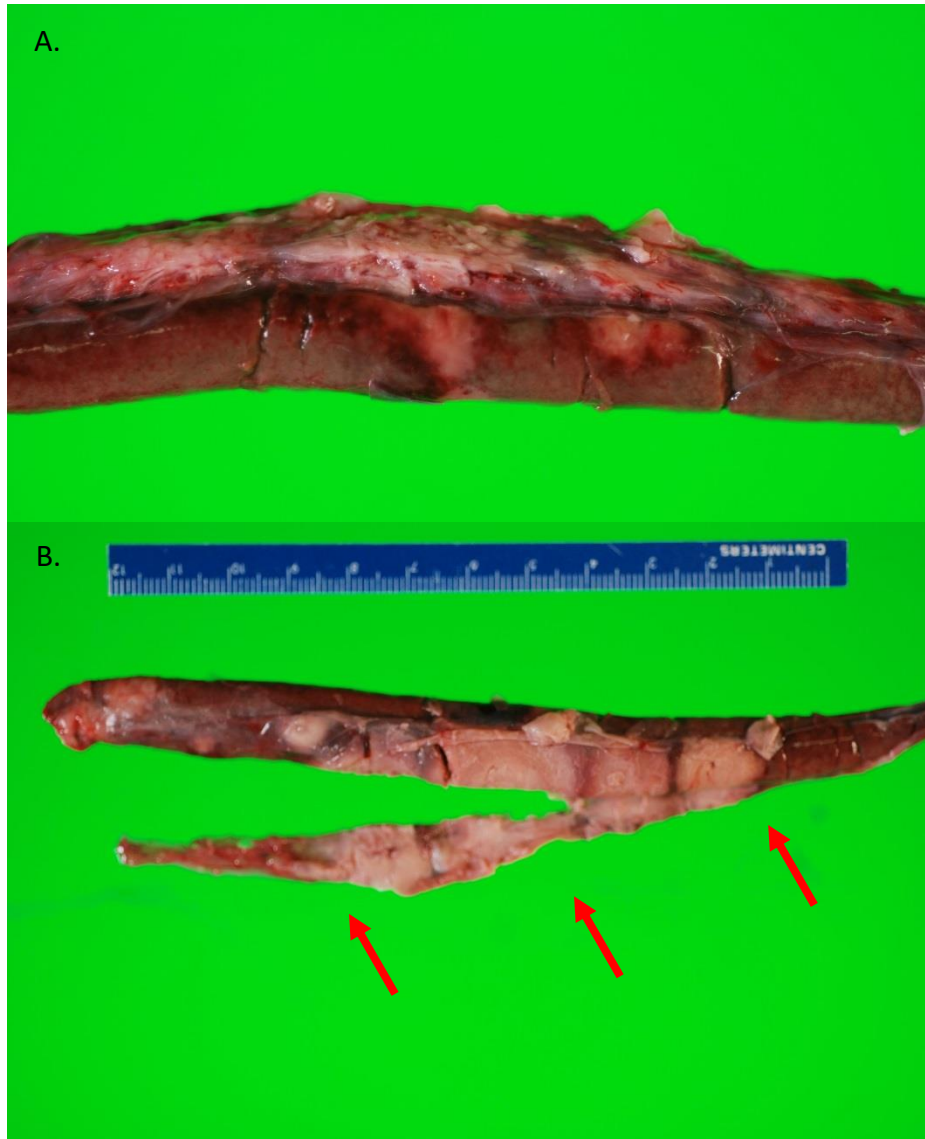


FIGURE S4. Severely (40%) necrotic liver (A) sectioned to reveal an infarction (B; arrows) from an adult female Black-Masked Racer (*Coluber constrictor latrunculus*; Accession #27595-002) found dead on Oct 19, 2016, at Haul Trail (Bayou Sauvage National Wildlife Refuge) infected with *Edwardsiella tarda* (liver), *Salmonella* sp. (colon), *Hepatozoon* sp. (widespread) and lung/intestinal parasites in the genera *Cestoda*, *Trematoda*, *Nematoda*, *Acanthocephala*. (Photographed by USGS National Wildlife Health Center staff.)



FIGURE S5. Images of a Western Mudsnake (*Farancia abacura reinwardtii*; Accession #27695-001) found dead on Jan 14, 2017 at Haul Trail (Bayou Sauvage National Wildlife Refuge). The carcass appeared perfectly intact, and rigor mortis occurred subsequent to collection. Necropsy determined that the snake died of Salmonellosis. (Photographed by Kimberly Terrell.)

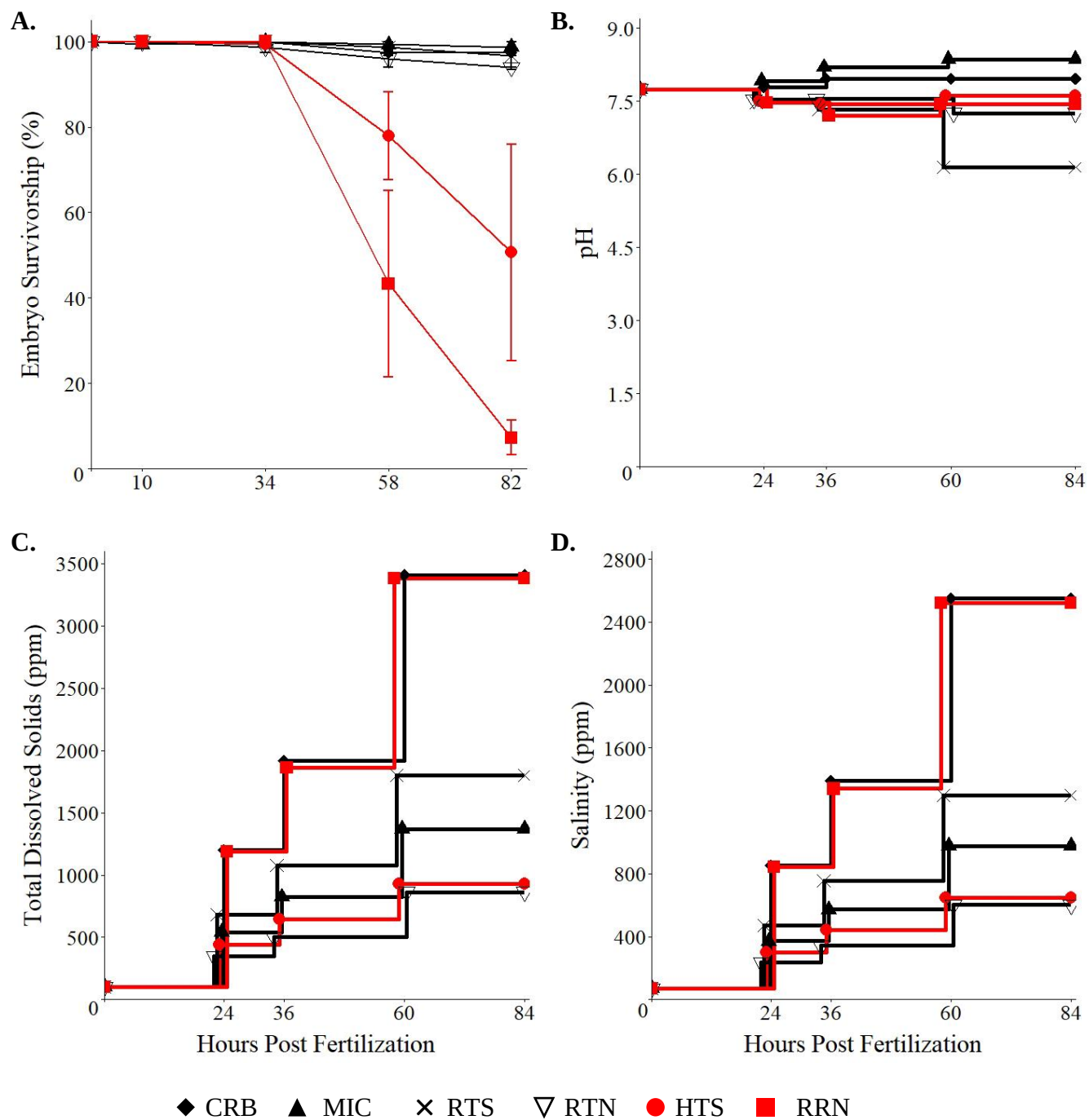


FIGURE S6. Survival of captive-bred Fowler's Toad (*Anaxyrus fowleri*) embryos (A) experimentally exposed to water from six locations in Bayou Sauvage National Wildlife Refuge, with chemical parameters of water treatments (B-D) monitored over 84 hours. Stepwise changes

in water chemistry correspond to incremental additions of treatment water to the embryos (i.e., 25%, 25%, and 50% water changes at 24, 36, and 60 h post-fertilization). Sites of suspected contamination (see methods) are illustrated in red. Site codes: Crabbing Bridge (CRB), Michoud Road (MIC), Ridge Trail South (RTS), Ridge Trail North (RTN), Haul Trail South (HTS), Recovery Road North (RRN). Error bars represent one standard error of the mean.