Environ Monit Assess (2020) 192:146
https://doi.org/10.1007/s10661-020-8106-8

Metal contamination of river otters in North Carolina

Charles W. Sanders II · Krishna Pacifici · George R. Hess · Colleen Olfenbuttel · Christopher S. DePerno

Received: 15 May 2019 / Accepted: 21 January 2020

Abstract Aquatic apex predators are vulnerable to environmental contaminants due to biomagnification. North American river otter (*Lontra canadensis*) populations should be closely monitored across their range due to point and nonpoint pollution sources. Nonetheless, no information exists on environmental contaminants in the North Carolina otter population. Metals and metalloids occur naturally across the landscape, are essential for cellular function, and become toxic when concentrated unnaturally. We conducted our study across the three Furbearer Management Units (FMU) and 14 river basins of North Carolina. We determined the concentrations of arsenic, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, selenium, thallium, and zinc in liver and kidney samples from 317 otters harvested from 2009 to 2016. Arsenic, lead, and thallium samples were tested at levels below the limit of detection. With the exception of cadmium, we detected all other elements at higher levels in the liver compared with the kidney. Specifically, cadmium, cobalt, copper, iron, magnesium, manganese, mercury, molybdenum, and zinc levels differed by tissue type analyzed. Most element concentrations remained stable or increased with otter age. We detected higher levels of mercury and selenium in the Lower Pee Dee and Cape Fear river basins. River basins within the Mountain FMU were higher in cadmium, copper, iron, lead, and zinc, whereas the Coastal Plain FMU was lower in cobalt and manganese. None of the elements occurred at toxic levels. Our research establishes baseline concentration levels for North Carolina, which will benefit future monitoring efforts and provide insight into future changes in the otter population.

Keywords Heavy metals · Kidney · Liver · *Lontra canadensis* · North Carolina · River otter

C. W. Sanders II, (✉) · K. Pacifici · G. R. Hess · C. S. DePerno
Fisheries, Wildlife, & Conservation Biology Program, Department of Forestry and Environmental Resources, College of Natural Resources, North Carolina State University, Raleigh, NC 27695, USA
e-mail: cwsander@ncsu.edu

C. Olfenbuttel
Surveys and Research Program, Wildlife Management Division, North Carolina Wildlife Resources Commission, Pittsboro, NC 27312, USA

Introduction

The North American river otter (*Lontra canadensis*, hereafter "otter") is the largest mustelid known to inhabit North Carolina. Colonial records indicate a statewide distribution until the 19th and early 20th centuries when unsustainable farming and logging practices coupled with unregulated harvest negatively impacted streams, fish stocks, and the otter population (Wilson 1960; Melquist and Dronkert 1987). Subsequently, North Carolina prohibited otter trapping between 1938 and 1946 (Wilson 1960) and translocated otters from the Coastal Plain to the Mountains from 1989 to 1996 (Spelman 1998); the statewide population recovered by 2005. Today, the North Carolina Wildlife Resources

Published online: 28 January 2020

Commission (NCWRC) considers the population to be healthy and robust and manages otters with a regulated annual trapping season across the state.

Aquatic apex predators are particularly vulnerable to environmental contaminants due to trophic level biomagnification, and contaminants in otter populations should be closely monitored (Fairbrother 2001; Mason and Wren 2001). The International Union for the Conservation of Nature (IUCN) acknowledges that metals (e.g., mercury, lead, and cadmium) can play a part in population declines where they occur in unnaturally high concentrations (Foster-Turley et al. 1990). Throughout the otter range, there is a variety of point and nonpoint source pollution, from agriculture and development to industry (Sackett et al. 2009, 2015; Miller and Mackin 2013; Martinez-Finley et al. 2015). However, to our knowledge, no information exists on the environmental contaminants in the North Carolina otter population.

Metals and metalloids occur naturally across the landscape, are essential for cellular function, and only become toxic when concentrated unnaturally (Hoffman et al. 2001). Examples of metals essential to bodily functions include calcium, cobalt, copper, iron, manganese, selenium, and zinc (Adriano 2001), whereas non-essential metals include arsenic, cadmium, lead, and mercury (Martinez-Finley et al. 2015). Mercury, cadmium, and lead are all believed to biomagnify (Hoffman et al. 2001; Evers et al. 2005). While mercury is only toxic as methylmercury (MeHg) because the methyl ion is required to facilitate biological functions, methylation can occur within the body (Rowland et al. 1975). Although the risk appears to be low, it is not negligible and is worthy of investigation (Osowski et al. 1995; Martín-Doimeadios et al. 2017).

Elements and metals including calcium, cadmium, cobalt, copper, mercury, iron, lead, magnesium, manganese, molybdenum, selenium, and zinc have been documented in fur, brain, bone, kidney, and liver tissues of otters across their range (Sheffy and Amant 1982; Anderson-Bledsoe and Scanlon 1983; Wren et al. 1988; Harding et al. 1998; Klenavic et al. 2008). Eisler (2000) included arsenic, cadmium, copper, lead, mercury, molybdenum, selenium, and zinc in the Chemical Risk Assessment Handbook for the United States Geological Survey (USGS).

Arsenic, cadmium, and lead are known carcinogens that bioaccumulate and whose negative effects typically include reproductive issues such as low sperm count, fetal death, malformation, endocrine disruption, and death (Wadi and Ahmad 1999; Eisler 2000; Henson and Chedrese 2004; Burger 2008; Rzymski et al. 2015). Some species such as mallards (*Anas platyrhynchos*), zebrafish (*Danio rerio*), humans (*Homo sapiens*), and great tits (*Parus major*) have shown a sensitivity to low arsenic levels, particularly resulting in stunted growth (Camardese et al. 1990; Boyle et al. 2008; Rahman et al. 2017; Sánchez-Virosta et al. 2018). Although lead is commonly used by humans, it is toxic when ingested and levels tend to be elevated near mining or smelting operations (Eisler 2000).

Calcium, copper, zinc, and molybdenum are all essential nutrients to bodily function (Eisler 2000). Calcium, copper, and zinc are parts of numerous essential molecules and enzymes that regulate processes such as melanin production and the biosynthesis of RNA and DNA (Eisler 2000). Molybdenum is a component of several enzymes required for various stages of metabolism and helps to regulate other metals such as copper and mercury (Eisler 2000). All three have many anthropogenic uses and at high concentrations can be toxic or result in medical issues such as kidney stones (Eisler 2000; Niemuth et al. 2014).

Mercury has no known biological benefit, while selenium is an essential micronutrient that helps fight oxidation (Eisler 2000). Although industrial mercury emissions have been declining for some time, it is still released largely through fossil fuel combustion, waste incineration, cement production, and metals-related industry (Chalmers et al. 2011; Muntean et al. 2014; Weiss-Penzias et al. 2016; Obrist et al. 2018). Both elements occur naturally and mercury biomagnifies through trophic levels (Wolfe et al. 1998; Yang et al. 2008; Tan et al. 2016). Methylated mercury can be absorbed efficiently by the body, which can then show sublethal effects such as impairments on reproduction, growth, behavior, and sensory issues in low levels, and is lethal in high doses (Ullrich et al. 2001). Selenium deficiency can cause anemia, slow growth, and reduced fertility, while excessive selenium over time is lethal (Flueck et al. 2012). Interestingly, selenium in organisms has an inverse relationship with mercury that can serve as protection against mercury toxicity (Yang et al. 2008).

The effects of overexposure to metals and metalloids on otters vary. Wolfe et al. (1998) summarized the toxic effects (i.e., ataxia, anorexia, brain lesions, immune suppression, reduced vision and motor function, impaired fertility, and fetal death) of mercury on wildlife, noting that many effects are sublethal. Unfortunately, the direct effects of many elements other than mercury and lead are less well studied (Rattner and Shore 2001). Therefore, our objective was to establish baseline kidney and liver concentrations of arsenic, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, selenium, thallium, and zinc for otters throughout the state of North Carolina.

Study area

We conducted our study across North Carolina. For management purposes, the North Carolina Wildlife Resources Commission (NCWRC) divided the state into three Furbearer Management Units (FMUs; Mountain, Piedmont, and Coastal Plain), which followed physiographic regions and county boundaries (Fig. 1). However, because otters are semi-aquatic, their territories are linear and tend to correspond with river basin geographic features (Melquist and Hornocker 1983; Melquist and Dronkert 1987; Reid et al. 1994; Sauer et al. 1999; Blundell et al. 2001), we focused our study on the 14 river basins that occur throughout North Carolina (Fig. 1).

Methods

Data collection

We collected otter carcasses from licensed trappers during the regulated 2009–2010 through 2015–2016 trapping seasons for North Carolina. Trapping seasons began 1 November or 1 December and ended the last day of the subsequent February. Trappers provided the carcass, location of the trap, and the date removed from the trap. We kept the carcasses frozen until necropsy. During the necropsy, we collected four grams each of liver and kidney tissue and extracted the lower canine teeth for cementum annuli aging (Stephenson 1977). We sent all teeth to Matson's Laboratory (Manhattan, MT) for aging, and we used 1 April as the birthdate for all otters to standardize age classes.

We sent the liver and kidney samples to the Pennsylvania Animal Diagnostics Laboratory (PADLS, New Bolton, Pennsylvania) for analyses. The PADLS used inductively coupled plasma mass spectrometry (ICP-MS) to determine the concentrations of arsenic, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, selenium, thallium, and zinc in liver and kidney samples for each otter. We recorded results as μg/g wet weight.

Data management and modeling

Spatially, we divided the specimens into FMUs and river basins (Fig. 1) and used five age classes (0–4). We combined ages four and higher because sample sizes for those ages were too low to be statistically meaningful on their own. For values below the limit of detection (LOD), we substituted the value of the LOD divided by the square root of two ($\frac{LOD}{\sqrt{2}}$), and elements with less than 40% of samples testing below the LOD were considered suitable for robust analysis following the guidelines provided by Hornung and Reed (1990).

We used SAS TTEST and SAS MULTTEST to perform t tests (pair-wise and 2-sample) with a Bonferroni

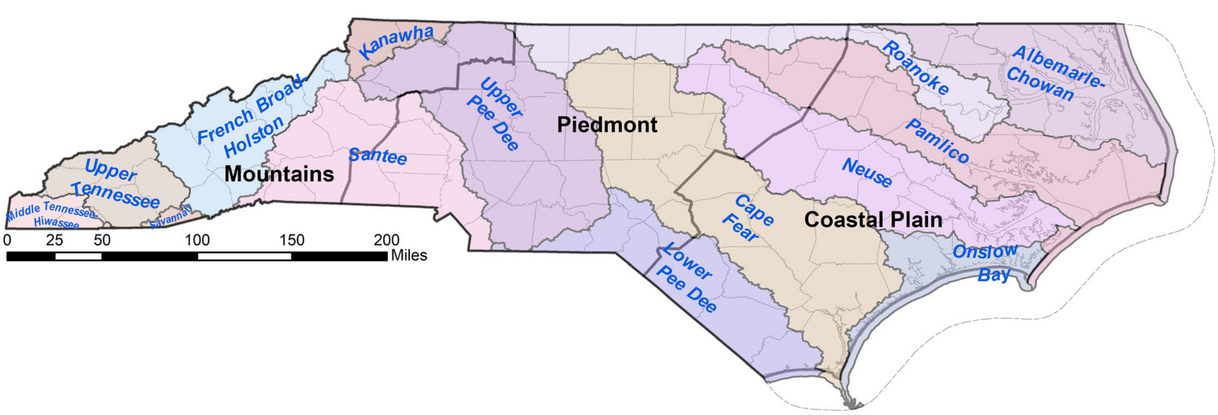

Fig. 1 Furbearer Management Units (FMU) and river basins of North Carolina

Table 1 Heavy and trace element loads (µg/g wet weight) in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016

Element	Liver			Kidney		
	Mean	SE	Median	Mean	SE	Median
Arsenic[a]	0.14	0.0004	0.14	0.14	0.0008	0.14
Cadmium[a]*	0.06	0.0041	0.04	0.2	0.0156	0.11
Calcium	123	4.7878	94	126	5.6311	98
Cobalt[a]*	0.03	0.0010	0.03	0.02	0.0008	0.02
Copper*	8.05	0.2549	6.80	3.69	0.0465	3.60
Iron*	284	6.1591	278	153	2.5066	149
Lead[a]	0.07	0.0275	0.07	0.07	0.0165	.07
Magnesium*	181	1.5499	176	153	1.4821	147
Manganese*	2.74	0.0512	2.57	0.72	0.0197	0.63
Mercury[a]*	2.58	0.1420	1.70	1.68	0.0794	1.24
Molybdenum*	0.80	0.0114	0.76	0.18	0.0022	0.18
Selenium[a]	1.34	0.0340	1.20	1.30	0.0179	1.29
Thallium[a]	0.04	0.0000	0.04	0.035	0.0016	0.04
Zinc*	26.71	0.3037	25.50	21.53	0.2186	20.70

[a] Samples tested below the Limit of Detection. *Significant difference ($P < 0.05$) between kidney and liver sample levels

Table 2 Heavy and trace elements from livers (µg/g wet weight) by sex and Furbearer Management Unit (FMU) in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016

Liver						
Element	µ (SE)	Sex (SE)		FMU (SE)		
		M	F	C	P	M
n	317	167	150	154	125	38
As[a]	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)
Cd[a]⁑	0.06 (0.00)	0.06 (0.01)	0.06 (0.01)	0.05 (0.00) B	0.06 (0.00) B	0.13 (0.02) A
Ca	122.55 (4.79)	117.28 (6.11)	128.42 (7.48)	131.92 (6.71)	112.83 (8.12)	116.57 (11.51)
Co⁑	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)	0.02 (0.00) C	0.04 (0.00) A	0.03 (0.00) B
Cu⁑*	8.05 (0.25)	6.83 (0.28)	9.41 (0.41)	9.10 (0.42) A	6.92 (0.31) B	7.52 (0.59) AB
Fe	283.95 (6.16)	283.38 (6.00)	284.57 (11.20)	289.12 (11.04)	283.83 (6.65)	263.34 (12.63)
Pb[a]	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.08 (0.00)	0.07 (0.00)	0.07 (0.00)
Mg	180.83 (1.55)	181.81 (2.27)	179.73 (2.09)	182.73 (2.26)	177.70 (2.49)	183.39 (3.96)
Mn⁑	2.74 (0.05)	2.73 (0.08)	2.75 (0.07)	2.64 (0.07) B	2.90 (0.08) A	2.64 (0.13) AB
Hg⁑	2.58 (0.14)	2.42 (0.16)	2.76 (0.25)	3.48 (0.25) A	1.79 (0.15) B	1.51 (0.14) B
Mo⁑*	0.80 (0.01)	0.84 (0.02)	0.74 (0.02)	0.77 (0.02) B	0.84 (0.02) A	0.77 (0.03) B
Se⁑	1.34 (0.03)	1.27 (0.04)	1.41 (0.06)	1.46 (0.06) A	1.23 (0.04) B	1.18 (0.05) B
Tl[a]	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)
Zn⁑	26.71 (0.30)	26.52 (0.45)	26.93 (0.40)	27.52 (0.44) A	25.30 (0.46) B	28.07 (0.87) A

[a] Majority of samples tested below the Limit of Detection. *Statistically significant difference between sexes ($P < 0.05$). ⁑Statistically significant difference between FMU's ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

Correction and one-way ANOVA's within SAS ANOVA (SAS Institute, Inc., Cary, NC, USA) to test for differences between individual categories. We used the Brown and Forsythe test to determine the homogeneity of variance (Brown and Forsythe 1974) and Welch's ANOVA to correct for variance heterogeneity (Welch 1947, 1951) when appropriate. We used Tukey's Honestly Significant Difference to determine differences within predictor classes.

Results

From November 2009 through February 2016, we collected 823 otters from over 50 licensed trappers and fur dealers. We processed liver and kidney samples from all 38 viable Mountain FMU specimens and randomly selected 125 Piedmont FMU specimens and 154 Coastal Plain FMU specimens for a total of 317 otters. Over 95% of arsenic (315 liver, 312 kidney), lead (307 liver, 311 kidney), and thallium (317 liver, 316 kidney) samples tested at levels below the LOD (0.2, 0.1, and 0.05 μg/g, respectively). Other elements that returned results below the LOD included cadmium (59 liver, 18 kidney), cobalt (6 liver, 32 kidney), mercury (22 liver, 26 kidney), and selenium (1 kidney) (0.02, 0.01, 0.5, and 0.15 μg/g, respectively; Table 1).

We compared results between livers and kidneys and detected differences for nine elements (i.e., cadmium, cobalt, copper, iron, magnesium, manganese, mercury, molybdenum, and zinc; $P < 0.0001$; Table 1). Also, we detected differences in liver tissue between males and females for copper ($t = 5.16$, $df = 269$, $P < 0.0001$) and molybdenum ($t = -4.66$, $df = 315$, $P = 0.0001$) concentrations (Table 2). No differences were detected in kidney tissue between males and females (Table 3).

Age class was significant for liver tissues for cadmium ($F = 10.82$, $df = 312$, $P < 0.0001$), copper ($F = 2.54$, $df = 312$, $P = 0.0397$), iron ($F = 3.16$, $df = 312$, $P = 0.0144$), magnesium ($F = 3.09$, $df = 312$, $P = 0.0162$), mercury ($F = 2.63$, $df = 312$, $P = 0.0346$), molybdenum ($F = 3.17$, $df = 312$, $P = 0.0141$), and selenium ($F = 4.44$, $df = 312$, $P = 0.0017$). Older age classes typically had higher concentrations of most elements but were only significant ($Q = 3.88$, $df = 312$, $\alpha = 0.05$) for cadmium, magnesium, molybdenum, and selenium in liver

Table 3 Heavy and trace elements from kidneys (μg/g wet weight) by sex and Furbearer Management Unit (FMU) in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016

Kidney						
Element	μ (SE)	Sex (SE)		FMU (SE)		
		M	F	C	P	M
n	317	167	150	154	125	38
As[a]	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)
Cd⁂	0.2 (0.02)	0.20 (0.02)	0.19 (0.02)	0.14 (0.01) B	0.20 (0.02) B	0.44 (0.09) A
Ca	125.78 (5.63)	128.49 (7.77)	122.75 (8.19)	136.44 (8.67)	113.85 (8.05)	121.78 (16.09)
Co⁂	0.02 (0.00)	0.02 (0.01)	0.02 (0.00)	0.02 (0.00) B	0.03 (0.00) A	0.02 (0.00) A
Cu⁂	3.69 (0.05)	3.66 (0.07)	3.72 (0.06)	3.54 (0.06) B	3.78 (0.07) AB	3.97 (0.16) A
Fe⁂	152.75 (2.51)	153.69 (3.31)	151.71 (3.82)	143.86 (3.17) B	156.02 (3.94) B	178.05 (9.00) A
Pb[a]	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.08 (0.01)
Mg⁂	152.55 (1.48)	154.00 (1.90)	150.93 (2.31)	148.95 (1.99)	154.89 (2.33)	159.42 (5.23)
Mn⁂	0.72 (0.02)	0.75 (0.03)	0.69 (0.03)	0.65 (0.02) B	0.79 (0.03) A	0.80 (0.06) A
Hg⁂	1.68 (0.08)	1.76 (0.11)	1.59 (0.12)	2.00 (0.13) A	1.42 (0.10) B	1.26 (0.11) B
Mo⁂	0.18 (0.00)	0.19 (0.00)	0.18 (0.00)	0.18 (0.00) B	0.19 (0.00) A	0.18 (0.01) AB
Se⁂	1.30 (0.02)	1.26 (0.02)	1.35 (0.03)	1.22 (0.03) B	1.38 (0.02) A	1.37 (0.04) A
Tl[a]	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)
Zn⁂	21.53 (0.22)	21.93 (0.32)	21.09 (0.29)	21.25 (0.31) B	21.35 (0.30) B	23.27 (0.7) A

[a] Majority of samples tested below the Limit of Detection. ⁂Statistically significant difference between FMU's ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

samples (Table 4). Age class was significant for kidney tissues for cadmium (F = 11.09, df = 312, $P < 0.0001$), iron (F = 2.96, df = 312, $P = 0.0200$), mercury (F = 4.23, df = 312, $P = 0.0024$), and selenium (F = 6.92, df = 312, $P < 0.0001$). Older age classes were significantly higher for cadmium, iron, mercury, and selenium (Q = 3.88, df = 312, $\alpha = 0.05$) in kidneys (Table 5).

We detected differences between FMUs within livers for cadmium (F = 22.13, df = 314, $P < 0.0001$), cobalt (F = 66.19, df = 314, $P < 0.0001$), copper (F = 8.64, df = 314, $P = 0.0002$), manganese (F = 3.22, df = 314, $P = 0.0415$), mercury (F = 21.54, df = 314, $P < 0.0001$), molybdenum (F = 4.52, df = 314, $P = 0.0116$), selenium (F = 6.83, df = 314, $P = 0.0012$), and zinc (F = 7.46, df = 314, $P = 0.0007$). Cadmium and cobalt concentrations were higher in the Mountain FMU (Q = 3.33, df = 314, $\alpha = 0.05$) (Table 2). Copper concentrations were higher in Coastal Plain otters than in Piedmont otters, while the opposite was true for manganese (Table 2). Mercury and selenium concentrations were highest in the Coastal Plain. Molybdenum was highest in the Piedmont while zinc was the lowest (Table 2).

Differences between FMUs were detected within kidneys for cadmium (F = 20.41, df = 314, $P < 0.0001$), cobalt (F = 37.72, df = 314, $P < 0.0001$), copper (F = 5.31, df = 314, $P = 0.0054$), iron (F = 10.04, df = 314, $P < 0.0001$), magnesium (F = 3.26, df = 314, $P = 0.0398$), manganese (F = 6.77, df = 314, $P = 0.0013$), mercury (F = 7.90, df = 314, $P = 0.0004$), molybdenum (F = 7.01, df = 314, $P = 0.0011$), selenium (F = 10.77, df = 314, $P < 0.0001$), and zinc (F = 4.43, df = 314, $P = 0.0126$). Concentrations were typically higher in the Mountain FMU, particularly for cadmium, iron, and zinc (Q = 3.33, df = 314, $\alpha = 0.05$) (Table 3). The Coastal Plain FMU had the lowest concentrations of cobalt, manganese, and selenium and the highest concentration of mercury (Table 3). Copper concentrations were different between the Mountain and Coastal Plain FMUs, but neither were different from the Piedmont. Molybdenum was highest in the Piedmont, but the Mountains were not significantly different from the Piedmont or Coastal Plain (Table 3).

Differences were detected in liver tissues between river basins for cadmium (F = 4.16, df = 314, $P < 0.0001$),

Table 4 Heavy and trace elements from livers (μg/g wet weight) by age class in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016

Liver						
Element	μ (SE)	Age class (SE)				
		0	1	2	3	4+
n	317	65	105	53	32	62
As[a]	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.15 (0.00)	0.14 (0.00)
Cd[a]*	0.06 (0.00)	0.04 (0.00) C	0.04 (0.00) C	0.07 (0.01) BC	0.10 (0.01) AB	0.10 (0.11) A
Ca	122.55 (4.79)	120.43 (9.35)	120.71 (8.25)	108.61 (7.31)	123.53 (10.94)	139.30 (15.38)
Co	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)
Cu*	8.05 (0.25)	9.12 (0.68)	7.74 (0.37)	7.78 (0.55)	9.33 (1.18)	7.03 (0.43)
Fe*	283.95 (6.16)	245.49 (8.94) B	304.81 (14.76) A	291.68 (10.04) AB	275.09 (15.90) AB	286.89 (10.45) AB
Pb[a]	0.07 (0.00)	0.07 (0.00)	0.08 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)
Mg*	180.83 (1.55)	184.00 (3.30) AB	178.64 (2.46) AB	172.09 (3.02) B	191.47 (5.58) A	183.18 (4.12) AB
Mn	2.74 (0.05)	2.90 (0.14)	2.66 (0.09)	2.68 (0.12)	2.80 (0.15)	2.73 (0.10)
Hg*	2.58 (0.14)	1.97 (0.20)	2.33 (0.24)	2.80 (0.32)	3.42 (0.49)	3.01 (0.43)
Mo*	0.80 (0.01)	0.75 (0.02) AB	0.77 (0.02) AB	0.81 (0.03) AB	0.85 (0.05) A	0.85 (0.03) A
Se*	1.34 (0.03)	1.12 (0.05) B	1.28 (0.06) AB	1.41 (0.06) AB	1.50 (0.10) A	1.51 (0.10) A
Tl[a]	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)
Zn	26.71 (0.30)	27.09 (0.62)	25.92 (0.51)	25.96 (0.72)	28.66 (1.10)	27.30 (0.71)

[a] Samples tested below the Limit of Detection. *Statistically significant difference between age classes ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

Table 5 Heavy and trace elements from kidneys (μg/g wet weight) by age class in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016

Kidney						
Element	μ (SE)	Age class (SE)				
		0	1	2	3	4+
n	317	65	105	53	32	62
As[a]	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.15 (0.00)	0.14 (0.00)
Cd*	0.2 (0.02)	0.11 (0.01) B	0.11 (0.01) B	0.20 (0.02) B	0.25 (0.04) B	0.41 (0.06) A
Ca	125.78 (5.63)	120.71 (8.50)	131.71 (10.60)	111.44 (8.74)	106.86 (6.39)	143.05 (18.91)
Co	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)
Cu	3.69 (0.05)	3.45 (0.10)	3.68 (0.08)	3.76 (0.11)	3.78 (0.14)	3.83 (0.11)
Fe*	152.75 (2.51)	136.71 (5.80) B	158.77 (4.05) A	152.77 (6.18) AB	153.32 (9.32) AB	159.07 (5.03) A
Pb[a]	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)
Mg	152.55 (1.48)	153.98 (2.76)	154.50 (2.88)	150.10 (3.81)	151.94 (5.03)	150.13 (2.88)
Mn	0.72 (0.02)	0.67 (0.04)	0.73 (0.04)	0.73 (0.05)	0.78 (0.06)	0.72 (0.04)
Hg*	1.68 (0.08)	1.23 (0.14) AB	1.50 (0.12) AB	1.98 (0.20) A	2.06 (0.27) A	2.01 (0.21) A
Mo	0.18 (0.00)	0.18 (0.01)	0.19 (0.00)	0.19 (0.00)	0.18 (0.01)	0.18 (0.00)
Se*	1.30 (0.02)	1.20 (0.04) B	1.24 (0.03) B	1.40 (0.04) A	1.31 (0.05) AB	1.43 (0.04) A
Tl[a]	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.03 (0.00)	0.04 (0.00)	0.04 (0.00)
Zn	21.53 (0.22)	21.20 (0.40)	21.41 (0.36)	21.32 (0.53)	21.87 (0.93)	22.10 (0.53)

[a] Majority of samples tested below the Limit of Detection. *Statistically significant difference between age classes ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

calcium (F = 2.12, df = 314, $P = 0.0225$), cobalt (F = 7.60, df = 314, $P < 0.0001$), copper (F = 3.09, df = 314, $P < 0.0009$), mercury (F = 10.91, df = 314, $P < 0.0001$), and selenium (F = 2.56, df = 314, $P = 0.0055$). Cadmium concentrations were higher in the French Broad and Middle Tennessee/Hiwassee basins, while calcium concentrations were highest in the Neuse and Onslow Bay basins (Q = 4.59, df = 306, α = 0.05) (Tables 6 and 7). Cobalt concentrations were highest in the Upper Pee Dee, while copper concentrations were highest in the Lower Pee Dee and Onslow Bay. The Lower Pee Dee had the highest mercury and selenium concentrations, but the results were mixed for the other basins with the Cape Fear being second highest for mercury and Upper Pee Dee second highest for selenium (Tables 6 and 7).

Differences were detected in kidney tissues between river basins for cadmium (F = 4.14, df = 314, $P < 0.0001$), cobalt (F = 4.54, df = 314, $P < 0.0001$), copper (F = 4.69, df = 314, $P < 0.0001$), iron (F = 2.68, df = 314, $P = 0.0037$), magnesium (F = 2.07, df = 314, $P = 0.0270$), manganese (F = 2.08, df = 314, $P = 0.0261$), mercury (F = 6.88, df = 314, $P < 0.0001$), selenium (F = 2.82, df = 314, $P = 0.0023$), and zinc (F = 3.20, df = 314, $P = 0.0006$). Cadmium concentrations were highest in the Middle Tennessee/Hiwassee, Onslow Bay, and French Broad basins, while calcium was highest in the Cape Fear and Onslow Bay basins (Q = 4.59, df = 306, α = 0.05) (Tables 8 and 9). Cobalt was highest in the Santee and Upper Pee Dee, while copper was highest in Onslow Bay. Iron was highest in the French Broad, while magnesium was highest in Onslow Bay, Middle Tennessee/Hiwassee, and the Santee basins. Mercury was highest in the Lower Pee Dee, and selenium was highest in Onslow Bay, Middle Tennessee/Hiwassee, French Broad, Upper Pee Dee, and the Neuse basins (Tables 8 and 9). Zinc was highest in the Middle Tennessee/Hiwassee and Onslow Bay and lowest in the Cape Fear and Albemarle/Chowan basins.

We reviewed five similar studies from around the USA and Canada (Anderson-Bledsoe and Scanlon 1983; Harding et al. 1998; Klenavic et al. 2008; Sheffy and Amant 1982; Wren et al. 1988) and determined that our values were consistently lower (Table 10). Mercury in particular was much lower than studies in Atlantic Canada and Wisconsin (Klenavic et al. 2008; Sheffy and Amant 1982) in both liver and kidney tissue. Essential

Table 6 Heavy and trace elements from livers (μg/g wet weight) by Mountain/Piedmont river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016. River Basins—*AB/CH* Albemarle/Chowan, *FB* French Broad, *MTH* Middle Tennessee/Hiwassee, *SAN* Santee, *UPD* Upper Pee Dee

Liver					
Mountain/Piedmont river basin					
Element	μ (SE)	FB	MTH	SAN	UPD
n	317	32	1	24	35
As[a]	0.14 (0)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)
Cd*	0.06 (0.00)	0.12 (0.03) A	0.16 (0.00) A	0.09 (0.02) AB	0.07 (0.01) AB
Ca*	123 (4.79)	107.91 (12.25) AB	115.00 (0.00) AB	113.14 (11.02) AB	81.06 (4.72) AB
Co*	0.03 (0.00)	0.03 (0.00) C	0.03 (0.00) C	0.04 (0.00) AB	0.04 (0.00) A
Cu*	8.05 (0.25)	7.74 (0.69) AB	4.16 (0.00) B	7.66 (0.67) AB	6.16 (0.37) B
Fe	284 (6.16)	259.75 (14.22)	274.00 (0.00)	272.08 (14.68)	287.00 (10.14)
Pb[a]	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)
Mg	181 (1.55)	181.63 (4.19)	194.00 (0.00)	189.33 (5.03)	178.74 (5.09)
Mn	2.74 (0.05)	2.70 (0.15)	2.55 (0.00)	3.01 (0.20)	2.95 (0.13)
Hg*	2.58 (0.14)	1.63 (0.15) C	0.35 (0.00) C	1.25 (0.18) C	1.27 (0.25) C
Mo	0.80 (0.01)	0.77 (0.04)	0.66 (0.00)	0.86 (0.05)	0.83 (0.03)
Se*	1.34 (0.03)	1.19 (0.06) B	1.60 (0) AB	1.20 (0.05) B	1.16 (0.07) B
Tl[a]	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)
Zn	26.71 (0.30)	27.64 (0.96)	36.50 (0.00)	28.12 (0.91)	25.52 (0.96)

[a] Majority of samples tested below the Limit of Detection. * Statistically significant difference ($P < 0.05$) between river basins in liver samples. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

nutrients such as iron were also lower (Harding et al. 1998; Wren et al. 1988). We showed considerably lower values in cadmium, copper, lead, and zinc in both liver and kidney tissue than Virginia (Anderson-Bledsoe and Scanlon 1983).

Discussion

Our study was one of the first to conduct a landscape level evaluation of element concentrations in river otters. For nearly every element tested, our concentrations were lower when compared with previous research (Sheffy and Amant 1982; Anderson-Bledsoe and Scanlon 1983; Wren et al. 1988; Harding et al. 1998; Klenavic et al. 2008), likely because our study was conducted statewide without a focus on sources of pollution. For example, otters tested by Harding et al. (1998) and Wren et al. (1988) were selected in part due to their proximity to smelting plants and pulp mills. It is likely the higher concentrations that Harding et al. (1998) detected, when compared with our study, were due to the elevated point-source pollution in the Fraser and Columbia watersheds where they conducted their study. Further, the levels detected in our study were much lower than those observed in Virginia (Anderson-Bledsoe and Scanlon 1983), which may be due to the three decades of time between studies, and the amount of environmental clean-up and regulation that has taken place across the Southeast and the USA (Schmitt and Brumbaugh 1990; Bennear 2007; Stein and Cadien 2009; Anderson and Lockaby 2011; Cristan et al. 2016). To develop benchmark element concentration values, allow for comparisons between studies and comparisons at the population and landscape level, we recommend that otters be sampled across the landscape regardless of point or non-point pollution sources.

Livers and kidneys are filtering organs and typically used to evaluate element concentrations. We detected differences in cadmium, cobalt, copper, iron, magnesium, manganese, mercury, molybdenum, and zinc levels by tissue type, and all (except for cadmium) were detected at higher concentrations in livers compared

Table 7 Heavy and trace elements from livers (μg/g wet weight) by Piedmont/Coastal Plain river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016. River Basins—*AB/CH* Albemarle/Chowan, *CF* Cape Fear, *LPD* Lower Pee Dee, *NE* Neuse, *OB* Onslow Bay, *PAM* Pamlico, *ROA* Roanoke

Liver								
Piedmont/Coastal Plain river basin								
Element	μ (SE)	AB/CH	CF	LPD	NE	OB	PAM	ROA
n	317	39	43	37	42	2	40	18
As[a]	0.14 (0)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)
Cd*	0.06 (0.00)	0.04 (0.01) AB	0.05 (0.01) AB	0.06 (0.01) AB	0.05 (0.01) AB	0.10 (0.02) AB	0.05 (0.01) AB	0.04 (0.01) AB
Ca*	123 (4.79)	107.99 (8.20) AB	130.34 (14.40) AB	140.58 (9.41) AB	150.88 (17.63) A	192.50 (44.50) A	135.10 (19.81) AB	125.54 (22.48) AB
Co*	0.03 (0.00)	0.02 (0.00) C	0.03 (0.00) BC	0.02 (0.00) C	0.03 (0.00) C	0.02 (0.00) C	0.03 (0.00) C	0.04 (0.00) ABC
Cu*	8.05 (0.25)	7.75 (0.78) AB	8.42 (0.69) AB	10.58 (1.15) A	7.68 (0.55) AB	16.60 (3.70) A	8.55 (0.73) AB	6.81 (0.57) AB
Fe	284 (6.16)	284.28 (15.62)	319.86 (33.75)	285.41 (13.35)	298.40 (12.48)	241.00 (88.00)	253.80 (10.84)	285.22 (16.03)
Pb[a]	0.07 (0.00)	0.07 (0.00)	0.09 (0.01)	0.08 (0.00)	0.07 (0.00)	0.07 (0.00)	0.08 (0.01)	0.07 (0.00)
Mg	181 (1.55)	176.33 (3.40)	182.58 (4.74)	185.97 (4.54)	184.74 (4.92)	179.50 (2.50)	175.73 (3.70)	169.17 (6.92)
Mn	2.74 (0.05)	2.48 (0.09)	2.84 (0.16)	2.71 (0.19)	2.61 (0.11)	2.51 (0.06)	2.74 (0.13)	2.75 (0.34)
Hg*	2.58 (0.14)	2.15 (0.21) BC	3.36 (0.48) B	5.63 (0.67) A	2.86 (0.30) BC	1.28 (0.41) C	2.08 (0.22) BC	2.40 (0.44) BC
Mo	0.80 (0.01)	0.74 (0.02)	0.78 (0.03)	0.84 (0.05)	0.80 (0.03)	0.81 (0.26)	0.75 (0.02)	0.84 (0.05)
Se*	1.34 (0.03)	1.22 (0.10) B	1.44 (0.11) AB	1.69 (0.16) A	1.42 (0.08) AB	0.89 (0.13) B	1.27 (0.07) AB	1.44 (0.13) AB
Tl[a]	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)
Zn	26.71 (0.30)	26.90 (0.88)	25.74 (0.78)	26.23 (0.73)	27.25 (0.94)	37.30 (2.40)	26.33 (0.64)	26.56 (1.70)

[a] Majority of samples tested below the Limit of Detection. * Statistically significant difference ($P < 0.05$) between river basins in liver samples. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

Table 8 Heavy and trace elements from kidneys (μg/g wet weight) by Mountain/Piedmont river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016. River Basins—*AB/CH* Albemarle/Chowan, *FB* French Broad, *MTH* Middle Tennessee/Hiwassee, *SAN* Santee, *UPD* Upper Pee Dee

Kidney					
Mountain/Piedmont river basin					
Element	μ (SE)	FB	MTH	SAN	UPD
n	317	32	1	24	35
As[a]	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)
Cd*	0.2 (0.02)	0.40 (0.10) A	0.51 (0.00) A	0.34 (0.10) AB	0.23 (0.04) ABC
Ca	126 (5.63)	123.99 (18.79)	59.90 (0.00)	104.61 (8.91)	84.79 (3.56)
Co*	0.02 (0.00)	0.02 (0.00) AB	0.02 (0.00) AB	0.03 (0.00) A	0.03 (0.00) A
Cu*	3.69 (0.05)	3.97 (0.19) B	4.04 (0.00) AB	3.78 (0.16) B	3.95 (0.14) B
Fe*	153 (2.51)	176.78 (10.10) A	166.00 (0.00) AB	166.33 (9.93) AB	163.51 (7.87) AB
Pb[a]	0.07 (0.00)	0.08 (0.01)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)
Mg*	153 (1.48)	157.41 (5.84) AB	170.00 (0.00) A	165.25 (5.74) A	151.15 (3.64) AB
Mn*	0.72 (0.02)	0.79 (0.07)	0.78 (0.00)	0.85 (0.10)	0.82 (0.05)
Hg*	1.68 (0.08)	1.34 (0.12) BC	0.66 (0.00) C	1.01 (0.09) C	1.13 (0.18) C
Mo	0.18 (0.00)	0.18 (0.01)	0.21 (0.00)	0.19 (0.01)	0.20 (0.01)
Se*	1.30 (0.02)	1.38 (0.06) A	1.54 (0.00) A	1.34 (0.05) AB	1.37 (0.04) A
Tl[a]	0.035 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)
Zn*	21.53 (0.22)	22.78 (0.92) BC	35.50 (0.00) A	22.13 (0.73) BC	21.45 (0.61) BC

[a] Majority of samples tested below the Limit of Detection. * Statistically significant difference ($P < 0.05$) between river basins in kidney samples. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

with kidneys. However, results within the respective organs are often mixed within and across studies, which are further complicated by study site, FMU, and proximity to point and non-point pollution sources making comparisons between studies and FMUs difficult. Therefore, for broad-scale element evaluations in river otters, we recommend continuing to test liver and kidney tissue samples and to evaluate element concentrations across the landscape. Also, more research is needed to evaluate how these elements are filtered and stored within livers and kidneys.

While most element concentrations remained stable across age classes, some increased with age supporting the notion that some elements bioaccumulate during an organism's lifespan (Boening 2000; Martinez-Finley et al. 2015; Julian and Gu 2015). We detected positive accumulation with age in several elements including cadmium, mercury, and selenium, which are all typically associated with manufacturing (Lemly 2004; Burger 2008; Sackett et al. 2009). Selenium, like iron, is an essential nutrient that can be detrimental at excessive levels (Tan et al. 2016). Although yearling and older otters had elevated iron concentrations, they were below the concentrations from other studies (Wren et al. 1988; Harding et al. 1998). Adults have been recorded previously having higher iron levels than juveniles (Grove and Henny 2008), and iron does bioaccumulate over time in animal tissue (Jayaprakash et al. 2015). It is possible that diet could be the main influencer driving the higher iron concentrations in yearlings that we observed (Mylniczenko et al. 2012; Ratnarajah et al. 2016), but, unfortunately, our location data was not precise enough to evaluate specific dietary influences.

Females had higher levels of copper (liver tissue only) and selenium (kidney tissue only) but lower levels of molybdenum than males. Females typically have smaller home ranges than males (Reid et al. 1994; Bowyer et al. 1995; Helon 2013), and the prey base changes throughout the year (Day et al. 2015). Small home ranges may amplify metal concentrations; however, larger home ranges may expose individuals to a wider range of elements. Because exposure levels are often reflective of the environment (Evans et al. 1998; Harding et al. 1998; Elliott et al. 1999; Ramos-Rosas

Table 9 Heavy and trace elements from kidneys (μg/g wet weight) by river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009–2016. River Basins—*AB/CH* Albemarle/Chowan, *CF* Cape Fear, *LPD* Lower Pee Dee, *NE* Neuse, *OB* Onslow Bay, *PAM* Pamlico, *ROA* Roanoke

Kidney								
Piedmont/Coastal Plain river basin								
Element	μ (SE)	AB/CH	CF	LPD	NE	OB	PAM	ROA
n	317	39	43	37	42	2	40	18
As[a]	0.14 (0.00)	0.15 (0.00)	0.14 (0.00)	0.15 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)	0.14 (0.00)
Cd*	0.2 (0.02)	0.11 (0.02) AC	0.18 (0.03) ABC	0.14 (0.02) ABC	0.16 (0.03) ABC	0.50 (0.19) A	0.13 (0.02) ABC	0.14 (0.02) ABC
Ca	126 (5.63)	131.10 (14.02)	157.63 (28.95)	139.00 (10.71)	129.69 (10.18)	248.00 (50.00)	130.96 (16.29)	100.57 (10.44)
Co*	0.02 (0.00)	0.02 (0.00) AB	0.02 (0.00) AB	0.01 (0.00) B	0.02 (0.00) AB	0.02 (0.00) AB	0.02 (0.00) AB	0.02 (0.00) AB
Cu*	3.69 (0.05)	3.16 (0.14) B	3.54 (0.08) B	3.63 (0.10) B	3.64 (0.13) B	5.99 (0.81) A	3.72 (0.10) B	3.86 (0.19) B
Fe*	153 (2.51)	134.06 (6.90) B	145.96 (5.47) AB	146.22 (7.03) AB	157.58 (6.54) AB	165.5 (17.50) AB	142.52 (5.99) B	148.12 (5.93) AB
Pb[a]	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.08 (0.00)	0.07 (0.00)
Mg*	153 (1.48)	141.6 (3.52) AB	146.37 (3.05) AB	152.95 (4.30) AB	157.83 (5.57) AB	178.50 (9.50) A	152.90 (2.93) AB	150.67 (5.03) AB
Mn*	0.72 (0.02)	0.57 (0.04)	0.67 (0.07)	0.67 (0.04)	0.74 (0.04)	0.83 (0.14)	0.76 (0.06)	0.59 (0.03)
Hg*	1.68 (0.08)	1.24 (0.13) BC	2.07 (0.27) B	3.03 (0.29) A	1.97 (0.24) BC	0.89 (0.16) C	1.45 (0.21) BC	1.60 (0.19) BC
Mo	0.18 (0.00)	0.17 (0.01)	0.18 (0.01)	0.18 (0.01)	0.19 (0.01)	0.20 (0.00)	0.19 (0.01)	0.19 (0.00)
Se*	1.30 (0.02)	1.08 (0.06) AB	1.31 (0.04) AB	1.30 (0.07) AB	1.37 (0.05) A	1.63 (0.09) A	1.30 (0.05) AB	1.27 (0.06) AB
Tl[a]	0.035 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.04 (0.00)	0.03 (0.00)	0.04 (0.00)
Zn*	21.53 (0.22)	20.74 (0.78) C	20.66 (0.59) C	21.38 (0.45) BC	21.47 (0.46) BC	29.90 (1.90) AB	21.38 (0.38) BC	21.61 (0.88) BC

[a] Majority of samples tested below the Limit of Detection. * Statistically significant difference ($P < 0.05$) between river basins in kidney samples. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$)

Table 10 Heavy and trace metal loads in river otters (*Lontra canadensis*) from other studies, measured in μg/g (Anderson-Bledsoe and Scanlon 1983[a]; Harding et al. 1998[b]; Klenavic et al. 2008[d]; Sheffy and Amant 1982[e]; Wren et al. 1988[c])

Element	Liver		Kidney	
	Other study means	Our mean	Other study means	Our mean
Arsenic	–	0.14	–	0.14
Cadmium	0.12[a], 0.42[b]	0.06	0.51[a]	0.20
Calcium	220[b], 80.89[c]	123	104[c]	126
Cobalt	0.25[b]	0.03	–	0.02
Copper	12.47[a], 24.87[b], 8.89[c]	8.05	4.89[a], 4.06[c]	3.69
Iron	1121[b], 348[c]	284	169[c]	153
Lead	2.14[a], 0.77[b]	0.07	1.19[a]	0.07
Magnesium	603[b], 185[c]	181	149[c]	153
Manganese	10.79[b], 2.80[c]	2.74	0.72[c]	0.72
Mercury	2.68[b], 7.15[d], 3.34[e]	2.58	8.47[e]	1.68
Molybdenum	1.92[b]	0.80	–	0.18
Selenium	6.72[b]	1.34	–	1.3
Zinc	96.30[a], 79.82[b], 24.48[c]	26.71	121[a], 21.46[c]	21.53

et al. 2013), studies involving wide-ranging and indicator species (e.g., otters and mink) are necessary to understand concentrations of elements at the landscape, river basin, and FMU level (Ben-David et al. 2001; Sutherland et al. 2018; Crowley et al. 2018).

Changes in habitat and sources of pollution play an important role in the distribution of various elements across the river basins and FMUs (Sackett et al. 2009; Vermeulen et al. 2009; Fritsch et al. 2010; Stokeld et al. 2014; Woch et al. 2016; Moskovchenko et al. 2017; Liao et al. 2017; Ren et al. 2019). River basins are units that reflect water movement and drainage across a large landscape. Aquatic animals lend themselves to landscape level evaluations because they occupy and follow landscape level water movement (Ben-David et al. 2001; Carranza et al. 2012; Swinnen et al. 2017; Crowley and Hodder 2019). Further, pollutants from point and non-point sources are concentrated within and downstream of these basins and the home ranges of aquatic animals (Sargaonkar 2006; Leitch et al. 2007). Specifically, we detected higher levels of mercury and selenium in the Lower Pee Dee and Cape Fear basins within the Piedmont and Coastal Plain. It is possible that these higher levels could be from anthropogenic activities in the three major population centers of the Piedmont in North Carolina (Raleigh/Durham/Chapel Hill, Greensboro/Winston-Salem, and Charlotte-Mecklenburg), each with populations greater than 750,000 (United States Census Bureau 2019), from the multiple power plants (coal and nuclear) in the area or from a number of manufacturing facilities (Sackett et al. 2009, 2010). However, the mercury concentrations in these river basins were similar to studies done in Eastern Canada (Klenavic et al. 2008) and Wisconsin (Sheffy and Amant 1982).

River basins within the Mountain FMU were significantly higher in cadmium, copper, iron, lead, and zinc, whereas, the Coastal Plain FMU was lower in cobalt and manganese than the Piedmont and Mountain FMUs. Interestingly, river basins from the Mountain FMU flow west into Tennessee and south into South Carolina with little water movement into the Piedmont and Coastal Plain. River basins within the Piedmont move water south into South Carolina and into the North Carolina Coastal Plain and are responsible for moving pollutants across the landscape (Fig. 1). Importantly, while some of the river basins we studied occur in two FMU's, none inhabit all three, and several (particularly in the Mountains) are either completely contained within a single FMU or flow out of state (Fig. 1).

To our knowledge, specific thresholds for the elements we evaluated have not been established in river otters except for mercury. Mercury is a known endocrine disruptor and can have many sublethal effects on a variety of systems, including reproduction (Tan et al. 2009). Mink from the Coastal Plain FMU of North

Carolina had liver and kidney concentrations as high as 3.45 μg/g (Osowski et al. 1995). While otters can handle larger concentrations of many toxins in their diet than smaller animals, mercury concentrations over 4 μg/g of MeHg are lethal (Wolfe et al. 1998). Ranched mink have often been used for experimental studies in mustelids, and the lowest observed adverse effect level for mink was a dietary concentration of 1.0 μg/g of MeHg (Wobeser et al. 1976; Wolfe et al. 1998). Similar effects were observed in otters with lethal liver and kidney concentrations beginning at 20 μg/g MeHg (O'Connor and Nielsen 1981). None of our specimens were at lethal levels. We provided statewide baseline levels for all elements including mercury, which will benefit future monitoring efforts and provide insight into future changes in the otter population.

Acknowledgments We thank the North Carolina Wildlife Resources Commission, the Federal Aid to Wildlife Restoration Program, and the Fisheries, Wildlife, and Conservation Biology Program at North Carolina State University for funding. We thank S. Ryan for countless hours collecting tissue samples. We thank the Pennsylvania Animal Diagnostics Laboratory for the professional and relatively quick lab work. Finally, we thank the North Carolina Trappers' Association and its members for providing the otter carcasses and for supporting this project.

Funding information North Carolina Wildlife Resources Commission, the Federal Aid to Wildlife Restoration Program, and the Fisheries, Wildlife, and Conservation Biology Program at North Carolina State University funded this study.

References

Adriano, D. C. (2001). *Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals* (2nd ed.). New York: Springer 867 pp. https://catalog.lib.ncsu.edu/record/NCSU1490714. Accessed.

Anderson, C. J., & Lockaby, B. G. (2011). The effectiveness of forestry best management practices for sediment control in the southeastern United States: A literature review. *Southern Journal of Applied Forestry, 35*, 170–177 http://search.proquest.com/docview/911808661/citation/5C0E43AEF2F240FAPQ/1. Accessed Mar 2019.

Anderson-Bledsoe, K. L., & Scanlon, P. F. (1983). Heavy metal concentrations in tissues of Virginia river otters. *Bulletin of Environmental Contamination and Toxicology, 30*, 442–447.

Ben-David, M., Duffy, L. K., Blundell, G. M., & Bowyer, R. T. (2001). Natural exposure of coastal river otters to mercury: Relation to age, diet, and survival. *Environmental Toxicology and Chemistry, 20*, 1986–1992. https://doi.org/10.1002/etc.5620200917/full Accessed Mar 2017.

Bennear, L. S. (2007). Are management-based regulations effective? Evidence from state pollution prevention programs. *Journal of Policy Analysis and Management, 26*, 327–348. https://doi.org/10.1002/pam.20250 Accessed Mar 2019.

Blundell, G. M., Maier, J. A. K., & Debevec, E. M. (2001). Linear home ranges: Effects of smoothing, sample size, and autocorrelation on kernel estimates. *Ecological Monographs, 71*, 469–489. https://doi.org/10.1890/0012-9615%282001%29071%5B0469%3ALHREOS%5D2.0.CO%3B2 Accessed Jan 2019.

Boening, D. W. (2000). Ecological effects, transport, and fate of mercury: A general review. *Chemosphere, 40*, 1335–1351 http://www.sciencedirect.com/science/article/pii/S0045653599002830. Accessed Oct 2017.

Bowyer, R. T., Testa, J. W., & Faro, J. B. (1995). Habitat selection and home ranges of river otters in a marine environment: Effects of the Exxon Valdez Oil Spill. *Journal of Mammalogy, 76*, 1–11 http://www.jstor.org/stable/1382309. Accessed Jan 2019.

Boyle, D., Brix, K. V., Amlund, H., Lundebye, A.-K., Hogstrand, C., & Bury, N. R. (2008). Natural arsenic contaminated diets perturb reproduction in fish. *Environmental Science & Technology, 42*, 5354–5360. https://doi.org/10.1021/es800230w Accessed Jan 2019.

Brown, M. B., & Forsythe, A. B. (1974). The small sample behavior of some statistics which test the equality of several means. *Technometrics, 16*, 129–132 http://www.jstor.org/stable/1267501. Accessed Mar 2019.

Burger, J. (2008). Assessment and management of risk to wildlife from cadmium. *Sci Total Environ, 389*, 37–45 http://www.sciencedirect.com/science/article/pii/S0048969707008649. Accessed Jan 2019.

Camardese, M. B., Hoffman, D. J., LeCaptain, L. J., & Pendleton, G. W. (1990). Effects of arsenate on growth and physiology in mallard ducklings. *Environmental Toxicology and Chemistry, 9*, 785–795. https://doi.org/10.1002/etc.5620090613 Accessed Jan 2019.

Carranza, M. L., D'Alessandro, E., Saura, S., & Loy, A. (2012). Connectivity providers for semi-aquatic vertebrates: The case of the endangered otter in Italy. *Landscape Ecology, 27*, 281–290. https://doi.org/10.1007/s10980-011-9682-3 Accessed Mar 2019.

Chalmers, A. T., Argue, D. M., Gay, D. A., Brigham, M. E., Schmitt, C. J., & Lorenz, D. L. (2011). Mercury trends in fish from rivers and lakes in the United States, 1969–2005. *Environmental Monitoring and Assessment, 175*, 175–191. https://doi.org/10.1007/s10661-010-1504-6 Accessed Mar 2019.

Cristan, R., Aust, W. M., Bolding, M. C., Barrett, S. M., Munsell, J. F., & Schilling, E. (2016). Effectiveness of forestry best management practices in the United States: Literature review. *Forest Ecology and Management, 360*, 133–151 http://www.sciencedirect.com/science/article/pii/S0378112715005824. Accessed Mar 2019.

Crowley, S. M., & Hodder, D. P. (2019). Factors influencing exposure of North American river otter (*Lontra canadensis*) and American mink (*Neovison vison*) to mercury relative to a large-scale reservoir in northern British Columbia, Canada. *Ecotoxicology, 28*, 343–353. https://doi.org/10.1007/s10646-019-02027-z Accessed Mar 2019.

Crowley, S. M., Hodder, D. P., Johnson, C. J., & Yates, D. (2018). Wildlife health indicators and mercury exposure: A case study of river otters (*Lontra canadensis*) in Central British Columbia, Canada. *Ecological Indicators, 89*, 63–73 http://www.sciencedirect.com/science/article/pii/S1470160X18300682. Accessed Jan 2019.

Day, C. C., Westover, M. D., & McMillan, B. R. (2015). Seasonal diet of the northern river otter (*Lontra canadensis*): What drives prey selection? *Canadian Journal of Zoology, 93*, 197–205. https://doi.org/10.1139/cjz-2014-0218 Accessed Jan 2019.

Eisler, R. (2000). *Handbook of chemical risk assessment*. Boca Raton: Lewis Publishers 1903 pp. https://catalog.lib.ncsu.edu/record/NCSU1384995. Accessed.

Elliott, J. E., Henny, C. J., Harris, M. L., Wilson, L. K., & Norstrom, R. J. (1999). Chlorinated hydrocarbons in livers of American mink (*Mustela vison*) and river otter (*Lutra canadensis*) from the Columbia and Fraser River basins, 1990–1992. *Environmental Monitoring and Assessment, 57*, 229–252 http://www.springerlink.com/index/X482L5QG17R65314.pdf. Accessed Mar 2017.

Evans, R. D., Addison, E. M., Villeneuve, J. Y., MacDonald, K. S., & Joachim, D. G. (1998). An examination of spatial variation in mercury concentrations in otter (*Lutra canadensis*) in south-central Ontario. *Sci Total Environ, 213*, 239–245 http://www.sciencedirect.com/science/article/pii/S0048969798000965. Accessed Mar 2017.

Evers, D. C., Burgess, N. M., Champoux, L., Hoskins, B., Major, A., Goodale, W. M., Taylor, R. J., Poppenga, R., & Daigle, T. (2005). Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. *Ecotoxicology, 14*, 193–221. https://doi.org/10.1007/s10646-004-6269-7 Accessed Mar 2019.

Fairbrother, A. (2001). Putting the impacts of environmental contamination into perspective. In *Ecotoxicology of wild mammals* (pp. 671–690). Chichester: Wiley.

Flueck, W. T., Smith-Flueck, J. M., Mionczynski, J., & Mincher, B. J. (2012). The implications of selenium deficiency for wild herbivore conservation: A review. *European Journal of Wildlife Research, 58*, 761–780. https://doi.org/10.1007/s10344-012-0645-z Accessed Mar 2019.

Foster-Turley, P., Group IOS, Macdonald, S. M., & Mason, C. F. (1990). *Otters: an action plan for their conservation*. Gland: International Union for Conservation of nature and natural resources 126 pp. https://catalog.lib.ncsu.edu/record/NCSU931524. Accessed.

Fritsch, C., Giraudoux, P., Cœurdassier, M., Douay, F., Raoul, F., Pruvot, C., Waterlot, C., de Vaufleury, A., & Scheifler, R. (2010). Spatial distribution of metals in smelter-impacted soils of woody habitats: Influence of landscape and soil properties, and risk for wildlife. *Chemosphere, 81*, 141–155 http://www.sciencedirect.com/science/article/pii/S0045653510007496. Accessed Mar 2019.

Grove, R. A., & Henny, C. J. (2008). Environmental contaminants in male river otters from Oregon and Washington, USA, 1994–1999. *Environmental Monitoring and Assessment, 145*, 49–73. https://doi.org/10.1007/s10661-007-0015-6 .

Harding, L. E., Harris, M. L., & Elliott, J. E. (1998). Heavy and trace metals in wild mink (*Mustela vison*) and river otter (*Lontra canadensis*) captured on rivers receiving metals discharges. *Bulletin of Environmental Contamination and Toxicology, 61*, 600–607. https://doi.org/10.1007/s001289900803.pdf Accessed Mar 2019.

Helon, D. A. (2013). Summer movements and activity patterns of river otters in Northeastern Ohio, USA. *Proceedings of the International Academy of Ecology and Environmental Sciences Hong Kong, 3*, 181 http://search.proquest.com/docview/1430635475/abstract/F52C6DB553624243PQ/1. Accessed Feb 2019.

Henson, M. C., & Chedrese, P. J. (2004). Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. *Experimental Biology and Medicine, 229*, 383–392. https://doi.org/10.1177/153537020422900506 Accessed Jan 2019.

Hoffman, D. J., Rattner, B. A., Scheunert, I., & Korte, F. (2001). Environmental contaminants. In *Ecotoxicology of wild mammals* (pp. 1–48). West Sussex: Wiley.

Hornung, R. W., & Reed, L. D. (1990). Estimation of average concentration in the presence of nondetectable values. *Applied Occupational and Environmental Hygiene, 5*, 46–51 http://tripsaver.lib.ncsu.edu/pdf/1041632.pdf. Accessed Oct 2018.

Jayaprakash, M., Kumar, R. S., Giridharan, L., Sujitha, S. B., Sarkar, S. K., & Jonathan, M. P. (2015). Bioaccumulation of metals in fish species from water and sediments in macrotidal Ennore creek, Chennai, SE coast of India: A metropolitan city effect. *Ecotoxicology and Environmental Safety, 120*, 243–255 http://www.sciencedirect.com/science/article/pii/S0147651315002651. Accessed Mar 2019.

Julian, P., & Gu, B. (2015). Mercury accumulation in largemouth bass (*Micropterus salmoides* Lacépède) within marsh ecosystems of the Florida Everglades, USA. *Ecotoxicology, 24*, 202–214. https://doi.org/10.1007/s10646-014-1373-9 Accessed Oct 2017.

Klenavic, K., Champoux, L., Mike, O., Daoust, P.-Y., Evans, R. D., & Evans, H. E. (2008). Mercury concentrations in wild mink (*Mustela vison*) and river otters (*Lontra canadensis*) collected from eastern and Atlantic Canada: Relationship to age and parasitism. *Environmental Pollution, 156*, 359–366 http://linkinghub.elsevier.com/retrieve/pii/S0269749108000948. Accessed Feb 2017.

Leitch, D. R., Carrie, J., Lean, D., Macdonald, R. W., Stern, G. A., & Wang, F. (2007). The delivery of mercury to the Beaufort Sea of the Arctic Ocean by the Mackenzie River. *Sci Total Environ, 373*, 178–195 http://www.sciencedirect.com/science/article/pii/S0048969706008230. Accessed Mar 2019.

Lemly, A. D. (2004). Aquatic selenium pollution is a global environmental safety issue. *Ecotoxicology and Environmental Safety, 59*, 44–56 http://www.sciencedirect.com/science/article/pii/S0147651303000952. Accessed Mar 2019.

Liao, J., Chen, J., Ru, X., Chen, J., Wu, H., & Wei, C. (2017). Heavy metals in river surface sediments affected with multiple pollution sources, South China: Distribution, enrichment and source apportionment. *Journal of Geochemical Exploration, 176*, 9–19 http://www.sciencedirect.com/science/article/pii/S0375674216301935. Accessed Mar 2019.

Martín-Doimeadios, R. C. R., Mateo, R., & Jiménez-Moreno, M. (2017). Is gastrointestinal microbiota relevant for endogenous mercury methylation in terrestrial animals? *Environmental Research, 152*, 454–461 http://www.sciencedirect.com/science/article/pii/S0013935116302535. Accessed Jan 2018.

Martinez-Finley, E. J., Caito, S., Fretham, S., Chen, P., & Aschner, M. (2015). Metal toxicology. In A.-D. M. B (Ed.), *Mammalian toxicology* (pp. 171–185). Hoboken: Wiley. https://doi.org/10.1002/9781118683484.ch8/summary Accessed Oct 2017.

Mason, C. F., & Wren, C. D. (2001). Carnivora. In *Ecotoxicology of wild mammals* (pp. 315–370). Chichester: Wiley.

Melquist, W. E., & Dronkert, A. E. (1987). River otter. In J. Bedford & G. Thompson (Eds.), *Wild furbearer management and conservation in North America section IV: Species biology, management, and conservation* (pp. 626–641). Concord: Ashton-Potter Limited.

Melquist, W. E., & Hornocker, M. G. (1983). Ecology of river otters in west Central Idaho. *Wildlife Monographs, 83*, 3–60.

Miller, J. R., & Mackin, G. (2013). Concentrations, sources, and potential ecological impacts of selected trace metals on aquatic biota within the Little Tennessee River Basin, North Carolina. *Water, Air, and Soil Pollution, 224*, 1613–1624. https://doi.org/10.1007/s11270-013-1613-2. Accessed Aug 2018.

Moskovchenko, D. V., Kurchatova, A. N., Fefilov, N. N., & Yurtaev, A. A. (2017). Concentrations of trace elements and iron in the Arctic soils of Belyi Island (the Kara Sea, Russia): Patterns of variation across landscapes. *Environmental Monitoring and Assessment, 189*, 210–213. https://doi.org/10.1007/s10661-017-5928-0. Accessed Mar 2019.

Muntean, M., Janssens-Maenhout, G., Song, S., Selin, N. E., Olivier, J. G. J., Guizzardi, D., Maas, R., & Dentener, F. (2014). Trend analysis from 1970 to 2008 and model evaluation of EDGARv4 global gridded anthropogenic mercury emissions. *Sci Total Environ, 494–495*, 337–350 http://www.sciencedirect.com/science/article/pii/S0048969714008572. Accessed Mar 2019.

Mylniczenko, N. D., Sullivan, K. E., Corcoran, M. E., Fleming, G. J., & Valdes, E. V. (2012). Management strategies of iron accumulation in a captive population of black rhinoceroses (*Diceros bicornis minor*). *Journal of Zoo and Wildlife Medicine, 43* https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine/volume-43/issue-3s/2011-0168.1/MANAGEMENT-STRATEGIES-OF-IRON-ACCUMULATION-IN-A-CAPTIVE-POPULATION-OF/10.1638/2011-0168.1.full. Accessed Mar 2019.

Niemuth, J. N., Sanders, C. W., Mooney, C. B., Olfenbuttel, C., DePerno, C. S., & Stoskopf, M. K. (2014). Nephrolithiasis in free-ranging North American river otter (*Lontra canadensis*) in North Carolina, USA. *Journal of Zoo and Wildlife Medicine, 45*, 110–117 https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine/volume-45/issue-1/2013-0135R2.1/NEPHROLITHIASIS-IN-FREE-RANGING-NORTH-AMERICAN-RIVER-OTTER-iLONTRA-CANADENSIS/10.1638/2013-0135R2.1.full. Accessed Feb 2019.

O'Connor, D. J., & Nielsen, S. W. (1981). Environmental survey of methylmercury levels in wild mink (*Mustela vison*) and otter (*Lutra canadensis*) from the northeastern United States and experimental pathology of methylmercurialism in the otter. In: *Proceedings, Worldwide Furbearer Conference*. Frostburg, MD. pp. 1728–1745.

Obrist, D., Kirk, J. L., Zhang, L., Sunderland, E. M., Jiskra, M., & Selin, N. E. (2018). A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. *Ambio, 47*, 116–140. https://doi.org/10.1007/s13280-017-1004-9 Accessed Mar 2019.

Osowski, S. L., Brewer, L. W., Baker, O. E., & Cobb, G. P. (1995). The decline of mink in Georgia, North Carolina, and South Carolina: The role of contaminants. *Archives of Environmental Contamination and Toxicology, 29*, 418–423. https://doi.org/10.1007/BF00212510 Accessed May 2019.

Rahman, A., Granberg, C., & Persson, L.-Å. (2017). Early life arsenic exposure, infant and child growth, and morbidity: A systematic review. *Archives of Toxicology, 91*, 3459–3467. https://doi.org/10.1007/s00204-017-2061-3 Accessed Jan 2019.

Ramos-Rosas, N. N., Valdespino, C., García-Hernández, J., Gallo-Reynoso, J. P., & Olguín, E. J. (2013). Heavy metals in the habitat and throughout the food chain of the Neotropical otter, *Lontra longicaudis*, in protected Mexican wetlands. *Environmental Monitoring and Assessment, 185*, 1163–1173. https://doi.org/10.1007/s10661-012-2623-z Accessed Feb 2017.

Ratnarajah, L., Nicol, S., Kawaguchi, S., Townsend, A. T., Lannuzel, D., Meiners, K. M., & Bowie, A. R. (2016). Understanding the variability in the iron concentration of Antarctic krill. *Limnology and Oceanography, 61*, 1651–1660. https://doi.org/10.1002/lno.10322 Accessed Mar 2019.

Rattner, B. A., & Shore, R. F. (2001). *Ecotoxicology of wild mammals*. Chichester: Wiley 730 pp. https://catalog.lib.ncsu.edu/record/NCSU1474159. Accessed.

Reid, D. G., Code, T. E., Reid, A. C. H., & Herrero, S. M. (1994). Spacing, movements, and habitat selection of the river otter in boreal Alberta. *Canadian Journal of Zoology, 72*, 1314–1324. https://doi.org/10.1139/z94-175 Accessed Jan 2019.

Ren, H., Zhou, Q., He, J., Hou, Y., Jiang, Y., Rodrigues, J. L. M., Cobb, A. B., Wilson, G. W. T., Hu, J., & Zhang, Y. (2019). Determining landscape-level drivers of variability for over fifty soil chemical elements. *Science of the Total Environment, 657*, 279–286 http://www.sciencedirect.com/science/article/pii/S0048969718348642. Accessed Marh 2019.

Rowland, I. R., Grasso, P., & Davies, M. J. (1975). The methylation of mercuric chloride by human intestinal bacteria. *Experientia, 31*, 1064–1065. https://doi.org/10.1007/BF02326961 Accessed Jan 2018.

Rzymski, P., Tomczyk, K., Rzymski, P., Poniedziałek, B., Opala, T., & Wilczak, M. (2015). Impact of heavy metals on the female reproductive system. *Annals of Agricultural and Environmental Medicine, 22*, 259–264 http://www.aaem.pl/Impact-of-heavy-metals-on-the-female-reproductive-system,72271,0,2.html. Accessed Mar 2019.

Sackett, D. K., Aday, D. D., Rice, J. A., & Cope, W. G. (2009). A statewide assessment of mercury dynamics in North Carolina water bodies and fish. *Transactions of the American Fisheries Society, 138*, 1328–1341. https://doi.org/10.1577/T08-178.1 Accessed Feb 2017.

Sackett, D. K., Aday, D. D., Rice, J. A., Cope, W. G., & Buchwalter, D. (2010). Does proximity to coal-fired power plants influence fish tissue mercury? *Ecotoxicology, 19*, 1601–1611. https://doi.org/10.1007/s10646-010-0545-5 Accessed Oct 2017.

Sackett, D. K., Pow, C. L., Rubino, M. J., Aday, D. D., Cope, W. G., Kullman, S., Rice, J. A., Kwak, T. J., & Law, M. (2015). Sources of endocrine-disrupting compounds in North Carolina waterways: A geographic information systems approach. *Environmental Toxicology and Chemistry, 34*, 437–445. https://doi.org/10.1002/etc.2797 Accessed Apr 2018.

Sánchez-Virosta, P., Espín, S., Ruiz, S., Salminen, J.-P., García-Fernández, A. J., & Eeva, T. (2018). Experimental manipulation of dietary arsenic levels in great tit nestlings: Accumulation pattern and effects on growth, survival and plasma biochemistry. *Environmental Pollution, 233*, 764–773 http://www.sciencedirect.com/science/article/pii/S0269749117331214. Accessed Jan 2019.

Sargaonkar, A. (2006). Estimation of land use specific runoff and pollutant concentration for Tapi river basin in India. *Environmental Monitoring and Assessment, 117*, 491–503. https://doi.org/10.1007/s10661-006-0769-2 Accessed Mar 2019.

Sauer, T. M., Ben-David, M., & Bowyer, R. T. (1999). A new application of the adaptive-kernel method: Estimating linear home ranges of river otters, *Lutra canadensis*. *Canadian Field-Naturalist, 113*, 419–424.

Schmitt, C. J., & Brumbaugh, W. G. (1990). National contaminant biomonitoring program: Concentrations of arsenic, cadmium, copper, lead, mercury, selenium, and zinc in U.S. freshwater fish, 1976–1984. *Archives of Environmental Contamination and Toxicology, 19*, 731–747. https://doi.org/10.1007/BF01183991 Accessed Mar 2019.

Sheffy, T. B., & Amant, J. R. S. (1982). Mercury burdens in furbearers in Wisconsin. *Journal of Wildlife Management, 46*, 1117 http://www.jstor.org/stable/3808255?origin=crossref. Accessed Feb 2017.

Spelman, L. H. (1998). *North American river otter (Lutra canadensis) translocation in North Carolina 1989–1996*. Chester: European Association of Zoo and Wildlife Veterinarians https://repository.si.edu/bitstream/handle/10088/11671/Spelman1998.pdf?sequence=1&isAllowed=y. Accessed Mar 2017.

Stein, E. D., & Cadien, D. B. (2009). Ecosystem response to regulatory and management actions: The southern California experience in long-term monitoring. *Marine Pollution Bulletin, 59*, 91–100 http://www.sciencedirect.com/science/article/pii/S0025326X09000976. Accessed Mar 2019.

Stephenson, A. B. (1977). Age determination and morphological variation of Ontario otters. *Canadian Journal of Zoology, 55*, 1577–1583. https://doi.org/10.1139/z77-206 Accessed Feb 2017.

Stokeld, D., Hamer, A. J., van der Ree, R., Pettigrove, V., & Gillespie, G. (2014). Factors influencing occurrence of a freshwater turtle in an urban landscape: A resilient species? *Wildlife Research, 41*, 163–171 https://bioone.org/journals/Wildlife-Research/volume-41/issue-2/WR13205/Factors-influencing-occurrence-of-a-freshwater-turtle-in-an-urban/10.1071/WR13205.full. Accessed Mar 2019.

Sutherland, C., Fuller, A. K., Royle, J. A., & Madden, S. (2018). Large-scale variation in density of an aquatic ecosystem indicator species. *Scientific Reports, 8*, 1–10 http://search.proquest.com/docview/2054061294/abstract/FD93CBF92CD0422DPQ/1. Accessed Mar 2019.

Swinnen, K. R. R., Strubbe, D., Matthysen, E., & Leirs, H. (2017). Reintroduced Eurasian beavers (*Castor fiber*): Colonization and range expansion across human-dominated landscapes. *Biodiversity and Conservation, 26*, 1863–1876 http://search.proquest.com/docview/1912090531/abstract/38D11933558B4F8BPQ/1. Accessed Mar 2019.

Tan, S. W., Meiller, J. C., & Mahaffey, K. R. (2009). The endocrine effects of mercury in humans and wildlife. *Critical Reviews in Toxicology, 39*, 228–269 https://proxying.lib.ncsu.edu/index.php?url=http://search.ebscohost.com/login.aspx?direct=true&db=fzh&AN=37140623&site=ehost-live&scope=site. Accessed Jan 2018.

Tan, L. C., Nancharaiah, Y. V., van Hullebusch, E. D., & Lens, P. N. L. (2016). Selenium: Environmental significance, pollution, and biological treatment technologies. *Biotechnology Advances, 34*, 886–907 http://www.sciencedirect.com/science/article/pii/S0734975016300623. Accessed Mar 2019.

Ullrich, S. M., Tanton, T. W., & Abdrashitova, S. A. (2001). Mercury in the aquatic environment: A review of factors affecting methylation. *Critical Reviews in Environmental Science and Technology, 31*, 241 https://search.proquest.com/docview/219164226/citation/A0DD7F0087FA4D7FPQ/1. Accessed Oct 2017.

United States Census Bureau. (2019). Annual estimates of the resident population: April 1, 2010 to July 1, 2017. Database record, . https://factfinder.census.gov/faces/tableservices/jsf/pages/productview.xhtml?pid=PEP_2017_PEPANNRES&prodType=table. Accessed Mar 2019.

Vermeulen, F., Van den Brink, N. W., D'Havé, H., Mubiana, V. K., Blust, R., Bervoets, L., & De Coen, W. (2009). Habitat type-based bioaccumulation and risk assessment of metal and As contamination in earthworms, beetles and woodlice. *Environmental Pollution, 157*, 3098–3105 http://www.sciencedirect.com/science/article/pii/S0269749109002486. Accessed Mar 2019.

Wadi, S. A., & Ahmad, G. (1999). Effects of lead on the male reproductive system in mice. *Journal of Toxicology and Environmental Health. Part A, 56*, 513–521. https://doi.org/10.1080/009841099157953 Accessed Mar 2019.

Weiss-Penzias, P. S., Gay, D. A., Brigham, M. E., Parsons, M. T., Gustin, M. S., & ter Schure, A. (2016). Trends in mercury wet deposition and mercury air concentrations across the U.S. and Canada. *Sci Total Environ, 568*, 546–556 http://www.sciencedirect.com/science/article/pii/S0048969716300614. Accessed Mar 2019.

Welch, B. L. (1947). The generalization of 'student's' problem when several different population variances are involved. *Biometrika, 34*, 28–35 http://www.jstor.org/stable/2332510. Accessed Mar 2019.

Welch, B. L. (1951). On the comparison of several mean values: An alternative approach. *Biometrika, 38*, 330–336 https://academic.oup.com/biomet/article/38/3-4/330/257875. Accessed Mar 2019.

Wilson, K. A. (1960). Management of the otter in North Carolina. Memo, North Carolina Wildlife Resources Commission. p. 4.

Wobeser, G., Nielsen, N. O., & Schiefer. (1976). Mercury and mink. I. The use of mercury contaminated fish as a food for ranch mink. *Canadian Journal of Comparative Medicine, 40*, 30–33 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1277515/. Accessed Jan 2018.

Woch, M. W., Kapusta, P., & Stefanowicz, A. M. (2016). Variation in dry grassland communities along a heavy metals gradient. *Ecotoxicology, 25*, 80–90. https://doi.org/10.1007/s10646-015-1569-7 Accessed Mar 2019.

Wolfe, M. F., Schwarzbach, S., & Sulaiman, R. A. (1998). Effects of mercury on wildlife: A comprehensive review. *Environmental Toxicology and Chemistry, 17*, 146–160. https://doi.org/10.1002/etc.5620170203/abstract Accessed Jan 2018.

Wren, C. D., Fischer, K. L., & Stokes, P. M. (1988). Levels of lead, cadmium and other elements in mink and otter from Ontario, Canada. *Environmental Pollution, 52*, 193–202 http://www.sciencedirect.com/science/article/pii/0269749188900036. Accessed Feb 2017.

Yang, D.-Y., Chen, Y.-W., Gunn, J. M., & Belzile, N. (2008). Selenium and mercury in organisms: Interactions and mechanisms. *Environmental Reviews, 16*, 71–92 https://proxying.lib.ncsu.edu/index.php?url=http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=36555298&site=ehost-live&scope=site. Accessed Jan 2019.