ABSTRACT

SANDERS II, CHARLES WILLIAM. Reproductive Parameters, Heavy Metal Concentrations, and Disease Prevalence in North American River Otters (*Lontra canadensis*) across North Carolina. (Under the direction of Dr. Christopher DePerno).

The North American river otter (*Lontra canadensis*; hereafter otter) is the largest mustelid in North Carolina and was distributed statewide. Populations were decimated by the early 1900s and otter trapping was prohibited in 1938, reopened in 1947, and gradually expanded until 2005. The North Carolina Wildlife Resources Commission (NCWRC) and Great Smoky Mountains National Park combined to release 404 otters to restore populations in western North Carolina. River otters are currently the only harvested otter species worldwide and populations are closely monitored.

Diseases may have an impact on the otter population and other aquatic mammals, through exposure to emerging diseases, contact with domestic animals (e.g., domestic cats), or less robust condition of individuals. Leptospirosis and toxoplasmosis are priority zoonoses and maintained by domestic and wild mammals. Although parvovirus is not zoonotic, it affects pets causing mild to fatal symptoms. Even though biomagnification makes aquatic apex predators particularly vulnerable to environmental contaminants, no prior information exists on the North Carolina otter population.

To determine population dynamics, disease prevalence, and levels of contamination we worked throughout the three Furbearer Management Units (FMUs) and 14 river basins in North Carolina to collect carcasses from trappers during the trapping seasons established by the NCWRC. During 1978-1980 (Period One; Coastal Plain and Piedmont) and the 2009-2013/2014-2016 (Period Two; statewide) trapping seasons, we collected otter carcasses from licensed trappers, fur buyers, and wildlife damage control agents. We conducted necropsies,

analyzed age structure, counted corpora lutea and fetuses for fecundity estimates (Chapter 1), tested brain and kidney tissue for leptospirosis, parvovirus, and toxoplasmosis (Chapter 2), and determined the liver and kidney concentrations of arsenic, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, selenium, thallium, and zinc (Chapter 3).

During Period One, 617 otter carcasses (330 male, 287 female) were collected from the Coastal Plain and Piedmont. During Period Two, we collected 822 (524 male, 298 female) otter carcasses across North Carolina. Age distributions for all otters were skewed toward the younger age classes and did not differ between collection periods. We detected a 45% increase in fecundity overall between Periods One and Two, and reproduction that was absent by juvenile and yearling otters during Period One was present during Period Two. Three otters (1%) tested positive for *Leptospira interrogans*, 41 (19%) for *Parvovirus* spp, and 53 (24%) for *Toxoplasma gondii*. All elements except for cadmium were detected at higher levels in liver samples compared to kidney samples. Most element concentrations remained stable or increased with age. Some river basins and FMUs were significantly higher than the others.

Our results indicate the reproductive distribution has gradually shifted to include younger otters. There are many drivers of reproduction, including food, habitat, environmental contaminants, and population in general. However, otter populations may experience different age structure and fecundity levels depending on harvest pressure and environmental stressors. Although parvovirus and toxoplasmosis are relatively common in North Carolina otters, the otter harvest has remained steady and the population appears to be abundant and self-sustaining. Therefore, parvovirus and toxoplasmosis do not currently appear to be negatively impacting the population. None of the elements we tested 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. Harvest should be closely monitored and regulated, and future studies should assess the effects of disease and environmental stressors on otters and other semi-aquatic mammals, examine transmission parameters between domestic and wild species, and the sublethal effects of infection.

Reproductive Parameters, Heavy Metal Concentrations, and Disease Prevalence in North American River Otters (*Lontra canadensis*) Across North Carolina

by
Charles William Sanders II

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DEDICATION

To my wife, Tiffany, for her love and unwavering support, my children, Amanda and Melody, for their patience and understanding, my mother, Brenda, for the dedication she inspired, and my father, Ronald, for instilling a love of all things wild.

BIOGRAPHY

Charles William Sanders II was born in Wilmington, North Carolina, on June 12, 1980. The youngest of four children, he began following his father through the woods almost as soon as he could walk, and developed a passion and appreciation for nature, wildlife, hunting, fishing, and eventually trapping. He graduated from Topsail High School (Hampstead, NC) in 1998. He spent two years serving as a missionary in Atlantic Canada for the Church of Jesus Christ of Latter-Day Saints, earned an A.A.S. degree in Automotive Systems Technology from Cape Fear Community College (Wilmington, NC) in 2002, and served a short stint as a Satellite Systems Operator/Maintainer in the United States Army. He met his wife, Tiffany, while in the Army and they married in 2003. They have two daughters, Amanda and Melody. He entered North Carolina State University's Fisheries, Wildlife, and Conservation Biology Program in 2009 to pursue his lifelong dream of becoming a wildlife biologist. He completed a Bachelor of Science Degree in that program, with dual concentrations in Wildlife and Fisheries, and a minor in Forest Management in May 2013. He then served as a wildlife technician for the Kentucky Department of Fish and Wildlife and the North Carolina Wildlife Resources Commission. In August 2015 he reentered the Fisheries, Wildlife, and Conservation Biology Program at N.C. State University to begin work on his Master of Science Degree. He earned a Graduate Certificate in Geographic Information Systems in 2018, and his M.S. Degree in Fisheries, Wildlife, and Conservation Biology in 2019. He hopes to transition to a full-time wildlife biologist position working with furbearers and/or in human/wildlife conflict.

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CHAPTER ONE

VARIATIONS IN REPRODUCTION AND AGE STRUCTURE IN THE NORTH AMERICAN RIVER OTTER (*Lontra canadensis*) IN NORTH CAROLINA, USA

ABSTRACT

During colonial times the North American river otter (*Lontra canadensis*) was distributed across North Carolina, but populations were decimated by the early 1900s. Otter trapping was prohibited in 1938, reopened in 1947, and gradually expanded until it was opened statewide in 2005. Between 1986-1992, the North Carolina Wildlife Resources Commission (NCWRC) and Great Smoky Mountains National Park combined to release 404 otters in translocation efforts to restore populations in western North Carolina. River otters are the only harvested otters worldwide, and populations are closely monitored through surveys, necropsies, and tooth collections. We worked throughout the three Furbearer Management Units and 14 river basins in North Carolina to collect carcasses from licensed trappers. We collected otters during the trapping seasons established by the NCWRC. During the 1978-80 (Period One; Coastal Plain) trapping seasons, and in the current study during 2009-2013/2014-2016 (Period Two; statewide) trapping seasons, we collected otter carcasses from licensed trappers, fur buyers, and wildlife damage control agents. We conducted necropsies, used cementum annuli of the lower canine for age-analysis, and counted corpora lutea and fetuses for fecundity estimates. During Period One, we collected 617 otter carcasses (330 male, 287 female) from the Coastal Plain and Piedmont FMUs. During Period Two, we collected 822 (524 male, 298 female) otter carcasses across North Carolina. Age distributions for all otters were skewed toward the younger age classes and

did not differ between collection periods. During Period One, adults in the Coastal Plain had higher corpora lutea counts than during Period Two, while Coastal Plain yearlings and juveniles had higher numbers of corpora lutea during Period Two. During Period Two, corpora lutea counts differed by region, with the Mountain FMU ($\bar{x}$ = 2.6) significantly higher than the Coastal Plain FMU ($\bar{x}$ = 1.6), or the Piedmont FMU ($\bar{x}$ = 1.9). Within the Coastal Plain FMU, total reproduction increased by 45% from Period One to Period Two. Although the adult reproduction in the Coastal Plain FMU dropped 16% from Period One to Period Two, juveniles and yearlings began reproducing regularly between periods. Our results indicate that reproduction has shifted from 1978 to 2018 to include younger otters. Reproduction in wildlife populations is driven by food, habitat, environmental contaminants, and density dependence within the population. However, otter populations across the range may experience different age structure and fecundity levels depending on harvest pressure and environmental stressors. Harvest should be closely monitored and regulated, and future studies should be conducted to further assess the effects of environmental stressors (e.g, contaminants, water quality) on otters and other semi-aquatic mammals including beaver, muskrat, mink, and nutria.

KEYWORDS

age structure, corpora lutea, juvenile, *Lontra canadensis*, reproduction, river otter, variation, yearling

INTRODUCTION

In North Carolina, colonial records indicate a statewide distribution of North American river otters (*Lontra canadensis*; hereafter otter) until the late 19th century. In the early 20th

century, poor farming and logging practices devastated streams, which coupled with unregulated otter harvest, decimated otter populations in the Piedmont and Mountain Furbearer Management Units (FMUs; Figure 1). In the Coastal Plain FMU, large swamps and wetlands provided a refuge that buffered the surviving otter populations (Wilson 1960, Melquist and Dronkert 1987).

North Carolina prohibited otter trapping from 1938-1946 (Wilson 1960). From 1947 - 1983, the newly created North Carolina Wildlife Resources Commission (NCWRC) restricted otter harvest to the east of US highway one, within the Coastal Plain FMU and the eastern edge of the Piedmont FMU (Figure 2). From 1984-2005, the regulated trapping season was expanded to encompass much of the Piedmont FMU, extending to the eastern boundaries of Stokes, Forsyth, Davie, Iredell, and Mecklenburg counties (Figure 2). In fall of 2005, the otter trapping season was opened statewide, including the entire Mountain FMU.

Between 1986-1992, otters (81 male, 56 female) were translocated by the National Park Service from Louisiana, North Carolina, and South Carolina into the Great Smoky Mountains National Park (Griess 1987, Raesly 2001). Between 1988-1996, the NCWRC translocated otters (160 male, 107 female) from the Coastal Plain FMU to the Mountain FMU (Spelman 1998). Today, otters occupy all three physiographic regions of North Carolina (Mountain, Piedmont, Coastal Plain) with a statewide otter trapping season and no bag limits.

The North American river otter is the only species of otter that is legally harvested for the fur trade (Melquist and Dronkert 1987, Serfass et al. 2015). In 1990 the International Union for Conservation of Nature and Natural Resources Species Survival Commission (IUCN/SSC) Otter Specialist Group published a voluntary action plan for the management of river otters in the United States and Canada (Foster-Turley et al. 1990). The plan included recommendations of the "Working Group on Bobcat, Lynx, and River Otter" to monitor population trends, total

harvest, harvest distribution, and habitat evaluation, as well as to analyze harvested animals for reproduction, pollutants, and other factors (Foster-Turley et al. 1990).

The NCWRC regularly monitors the otter harvest through volunteer trapper surveys, fur buyer reporting, and Convention on International Trade in Endangered Species (CITES) tag sales. Voluntary carcass collections are periodically conducted to monitor reproduction, and an annual tooth/skull collection has been initiated for age structure analysis. These processes help fulfill the Otter Specialist Group's first and third recommended conservation priorities (Foster-Turley et al. 1990), which include evaluating the population status of otter populations and analyzing carcasses to increase knowledge of otter reproduction. Therefore, our objective was to determine the age structure and reproductive rates of otters throughout North Carolina and determine if those rates changed by river basin, FMU, and time periods, and varied by age class. We hypothesized that reproduction would vary in the Coastal Plain FMU from 1978 to 2018, would be higher in a reintroduced population (Mountain FMU) compared to a stable population (Coastal Plain FMU), or a population with natural recolonization (Piedmont FMU).

STUDY AREA

We conducted our study across North Carolina. North Carolina is geographically diverse with fourteen different river basins, seventeen terrestrial, and eleven wetland communities (North Carolina Wildlife Resources Commission 2015). For management purposes, the NCWRC established three FMU's (i.e., Mountain, Piedmont, and Coastal Plain) which followed physiographic regions and county boundaries (Figure 1). River otters have been stable within the Coastal Plain FMU since 1978, recolonized the Piedmont FMU naturally by 1984, and were reintroduced into the Mountain FMU between 1986-1992.

METHODS

Data Collection

During 1978-80 (Period One; Coastal Plain and Piedmont FMU) and the 2009-2013/2014-2016 (Period Two; statewide) trapping seasons, we collected otter carcasses from licensed trappers, fur buyers, and wildlife damage control agents. For all otters collected, we recorded the date and location trapped which included specific coordinates, addresses, and/or a general description of the trap site. General descriptions included the county, locality, roads, and any prominent landmarks.

We froze all carcasses until necropsy. During the necropsy, we extracted a lower canine tooth for cementum annuli aging (Stephenson 1977). The samples from Period One were aged at NCSU while the samples from Period Two were sent to Matson's Laboratory (Manhattan, Montana). Otters aged as zero were considered juveniles, otters aged as one-year-old were considered yearlings, and otters aged two years or older were considered adults. We removed female reproductive tracts and preserved them in a 10% formalin solution. We sectioned each ovary in one mm slices similar to Hamilton and Eadie (1964) and counted active corpora lutea. We dissected the uterine horns and counted visible fetuses. During Period One, blastocysts were collected by flushing each uterine horn with sterilized water and examining under a microscope. During Period Two, because blastocysts are quickly degraded (Johnson et al. 2007) we did not collect blastocysts and only report corpora lutea which is consistent with the literature (Docktor et al. 1987, Chilelli et al. 1996, Crimmins et al. 2011)

Data Analysis

We conducted statistical analysis in SAS 9.4 (SAS Institute, Inc, Cary, North Carolina, USA) using Proc TTEST for t-tests, Proc ANOVA for ANOVAs, and Proc GENMOD for

models. We used two-sample t-tests and one-way ANOVA to determine significant differences between Periods One and Two (1978-1980 vs 2009-2013/2014-2016) and between FMUs. We used Tukey's Honestly Significant Difference (HSD) test to examine differences within variables. We used a paired t-test to compare corpora lutea and fetus counts during Period One and Period Two and used a one-way ANOVA to determine difference across age classes. We used Akaike's Information Criterion (AIC) to assess model weights and rank candidate models (Burnham and Anderson 2002). Our generalized linear models contained fixed effects and we limited our candidate model set to two a priori categorical covariates, age and region, to avoid including spurious effects. Due to sample sizes not being distributed across all basins we did not use river basin in our models. We developed relative support for the models by using Akaike weights and then calculated the unconditional variance estimates with their associated 95% confidence intervals (Burnham and Anderson 2002, Anderson 2008).

RESULTS

During Period One, from over 50 trappers and fur dealers, we collected 617 otter carcasses (330 male, 287 female) from the Coastal Plain FMU (315 male, 287 female) and Piedmont FMU (15 males) and determined ages for 330 males and 274 females. No females were collected from the Piedmont FMU during Period One. During Period Two, we collected 822 (524 male, 298 female) otter carcasses across North Carolina from over 50 trappers and fur dealers. We collected 54 from the Mountain FMU (34 male, 20 female), 322 from the Piedmont FMU (204 male, 118 female), and 446 from the Coastal Plain FMU (286 male, 160 female). We obtained ages for all but 4 specimens (2 males, 2 females).

During Period One, the average age of males (n = 330) and females (n = 274) were 1.9 and 1.7, respectively. During Period 2, the average age of males (n = 524) and females (n = 298) were 2.0 and 1.7, respectively. Age distributions for all otters combined across collection periods were skewed toward the younger age classes (Figure 3) and did not differ between collection period ($t = -0.82$, $df = 1213$, $P = 0.4121$). For the Coastal Plain FMU, age distributions that included males and females were similar between collection periods ($t = 0.20$, $df = 417$, $P = 0.84$). During Period Two, male and female age distributions differed within the Piedmont FMU (male = 2.2, female = 1.7; $F = 4.34$, $df = 319$, $P = 0.038$), but were similar in the Mountain FMU (male = 1.9, female = 1.8; $F = 0.16$, $df = 52$, $P = 0.689$) and Coastal Plain FMU (male = 1.6, female = 1.6; $F = 0.95$, $df = 444$, $P = 0.330$).

During Period One, the number of corpora lutea for all Coastal Plain females averaged 1.1. Corpora lutea for juveniles ($\bar{x} = 0.02$), yearlings ($\bar{x} = 0.0$), and adults ($\bar{x} = 2.5$) were significantly different ($F = 248.06$, $df = 270$, $P < 0.0001$) (Table 1), with adults being more likely to have active corpora lutea than yearlings or juveniles ($Q = 3.33$, $df = 270$, $\alpha = 0.05$). During Period Two, the number of corpora lutea for Coastal Plain females across all age classes averaged 1.6. Corpora lutea for juveniles ($\bar{x} = 1.1$), yearlings ($\bar{x} = 1.4$), and adults ($\bar{x} = 2.0$) were significantly different ($F = 12.96$, $df = 143$, $P < 0.0001$) (Table 1); adults were more likely to have corpora lutea than yearlings or juveniles ($Q = 3.35$, $df = 143$, $\alpha = 0.05$). Within the Coastal Plain FMU, corpora lutea counts differed between Period One ($\bar{x} = 1.05$) and Period Two ($\bar{x} = 1.62$; $t = 4.12$, $df = 420$, $P < 0.0001$). Adults during Period One produced higher corpora lutea counts than during Period Two ($t = -2.53$, $df = 166$, $P = 0.0122$), while yearlings ($t = 11.96$, $df = 47$, $P < 0.0001$) and juveniles ($t = 6.92$, $df = 35$, $P < 0.0001$) produced higher counts of corpora lutea during Period Two.

During Period Two, corpora lutea counts differed by FMU ($F = 8.44$, $df = 277$, $P = 0.0003$); the Mountains ($\bar{x} = 2.6$) were significantly higher ($Q = 3.33$, $df = 275$, $\alpha = 0.05$) than the Piedmont ($\bar{x} = 1.9$) and Coastal Plain ($\bar{x} = 1.6$). The top model for corpora lutea incorporated FMU as a classification variable and age as a numeric variable with all effects fixed. This model held 99% of the model weight, and the next closest model was over 13 ΔAIC away, and all covariates were significant via model averaging (Tables 2, 3).

During Period One, the number of fetal counts for all Coastal Plain females averaged 0.8. Adults averaged 2.0 fetuses and were significantly higher ($F = 173.37$, $df = 270$, $P < 0.0001$) than juveniles ($\bar{x} = 0.0$) and yearlings ($\bar{x} = 0.0$; $Q = 3.33$, $df = 270$, $\alpha = 0.05$). During Period Two, the number of fetuses for all Coastal Plain females, regardless of age class, averaged 0.5 (Table 1). Fetus counts were significantly different across age classes ($F = 13.45$, $df = 142$, $P < 0.0001$); adults ($\bar{x} = 1.0$) were greater than yearlings ($\bar{x} = 0.2$) and juveniles ($\bar{x} = 0.0$; $Q = 3.35$, $df = 142$, $\alpha = 0.05$). Fetus counts for all females from the Coastal Plain FMU differed between Period One ($\bar{x} = 0.8$) and Period Two ($\bar{x} = 0.5$; $t = -2.51$, $df = 419$, $P = 0.0126$). Adults during Period One produced higher fetus counts ($\bar{x} = 2.0$) than during Period Two ($\bar{x} = 1.0$; $t = -4.50$, $df = 174$, $P < 0.0001$), while yearling differences were not significant ($t = 1.75$, $df = 47$, $P = 0.0864$).

During Period Two, fetus counts for all females differed by FMU ($F = 3.61$, $df = 274$, $P = 0.0284$); the Mountains ($\bar{x} = 1.2$) were significantly higher ($Q = 3.33$, $df = 274$, $\alpha = 0.05$) than the Piedmont ($\bar{x} = 0.5$) and Coastal Plain ($\bar{x} = 0.5$). The top model for fetus counts incorporated FMU as a classification variable and age as a numeric variable with all effects fixed. This model held 54% of the model weight. The next closest model (Age only) was only 0.4 ΔAIC away and

carried 46% of the model weight. The covariates were significant via model averaging except for the Coastal Plain FMU variable (Tables 2, 3).

We examined litters sizes by eliminating all samples without visually verified fetuses or blastocysts (blastocysts were only collected during Period One). Hence, we had 87 and 57 specimens from Periods One and Two, respectively. Corpora lutea counts ($\bar{x}$ = 3.0, $\bar{x}$ = 2.6) were significantly different from fetus counts during Period One ($\bar{x}$ = 2.6, t = 4.90, df = 86, P <0.0001), but not Period Two ($\bar{x}$ = 2.6, t = -0.11, df = 56, P = 0.9105). During Period One all specimens with visible fetuses were adults, but during Period Two we analyzed 49 adults, seven yearlings, and one juvenile. The one juvenile was aged by a broken tooth and was given a one-year error, making it possible for it to be a yearling. Period Two corpora lutea counts ($\bar{x}$ = 2.7, $\bar{x}$ = 2.1) and fetus counts ($\bar{x}$ = 2.7, $\bar{x}$ = 2.6) were similar between adults and yearlings (F = 0.44, df = 56, P = 0.6487) suggesting that fetus counts supported the corpora lutea counts as accurate estimators of litter size and the difference between the two metrics during Period One could be from the difficulty of isolating and identifying blastocysts.

DISCUSSION

Across North Carolina, the age distribution of harvested otters was stable across the two collection periods (spanned 40 years). During Period Two, the NCWRC estimated ~2,400 otters were harvested annually and based on the age distributions the population appears to be healthy with high reproduction and recruitment. The long-term stable age distribution of harvested otters indicates that habitat is satisfactory and reproduction is stable or increasing (Sulkava et al. 2007, Barrett and Leslie, Jr. 2012, Graser et al. 2012, Rughetti 2016, Marvá and San Segundo 2018, Nadal et al. 2018). Further, an abundance of young otters in the harvest is indicative of high

recruitment and population stability (Rolley 1985, Koons et al. 2006, Flynn and Schumacher 2009, Rughetti 2016).

Within the Coastal Plain FMU, reproduction increased by 45% from Period One to Period Two. Although adult reproduction dropped 16% from Period One to Period Two, juvenile and yearling reproduction began and occurred at a much higher rate than expected during Period Two. Early reproduction has been recorded previously (Liers 1958, Crimmins et al. 2011, Barding and Lacki 2014), but not to the extent that we detected. Our results indicate the reproductive load has shifted to include juvenile and yearling otters. In general, water quality has improved over the years (White 1996), and the expansion and recolonization of beavers has provided more aquatic habitat across the landscape (Naiman et al. 1988, Snodgrass and Meffe 1998, Hood and Larson 2015) which may have contributed to the stability and recovery of the otter population across North Carolina.

The otter reintroduction during the 1990s focused on moving otters from the Coastal Plain FMU, where they were abundant, to the Mountain FMU where they had been extirpated (Spelman 1998). During Period Two, we detected higher reproductive rates in the Mountain FMU compared to the Piedmont or Coastal Plain FMUs. While the sample size in the Mountain FMU was low, the reproductive rate is consistent with other reintroduced populations (Docktor et al. 1987, Crimmins et al. 2011, Barding and Lacki 2014) and is interesting when considering that the Mountain FMU had been extirpated and reintroduced, the Piedmont FMU had been extirpated and recovered naturally, and the Coastal Plain FMU has been stable over time. While the only significant difference in reproduction was in the Mountain FMU, reproduction in the Piedmont FMU was still higher than in the Coastal Plain FMU. Further, adult otters typically average two to three pups per litter, especially in reintroduced and/or recovering populations

(Tabor and Wight 1977, Hill and Lauhachinda 1980, Docktor et al. 1987, Melquist and Dronkert 1987, Johnson et al. 2007, Crimmins et al. 2011, Barding and Lacki 2014). We believe this is the first time that all juveniles from a particular area (Mountain FMU) have been verified as reproductively active.

The number of juveniles and yearlings that we detected as reproductively active is encouraging. Increased fecundity in the presence of abundant resources is an established principle in wildlife management (King et al. 2003, Gamelon et al. 2017), and can explain increased litter size along with yearling and juvenile breeding activity. For example, hard and soft mast fluctuations influence the reproduction of bears, small mammals, and predators (Jensen et al. 2012, Bogdziewicz et al. 2016, Hertel et al. 2018), and food caching birds respond to food abundance (Ruffino et al. 2014). Normally, otters become reproductively active at age two, with delayed implantation causing them to produce their first litter slightly before or around their third birthday (Liers 1958, Hamilton and Eadie 1964, Melquist and Dronkert 1987). Although Liers (1958) documented captive yearling otters giving birth it has always been considered a rare event (Liers 1951, Hamilton and Eadie 1964, Docktor et al. 1987). However, in the last several years, studies of otters in reintroduced populations have observed that reproductive activity in younger individuals has become more common than once thought (Crimmins et al. 2011, Barding and Lacki 2014).

Juvenile and yearling breeding in a species known to not sexually mature until age two may be attributed to environmental pressures (Hamilton and Eadie 1964). A variety of external and internal pressures impact mammal reproduction including endocrine disrupting chemicals (Bergman et al. 2013, Pow et al. 2017), heavy metals (Rzymski et al. 2015), polychlorinated biphenyls (Henson and Chedrese 2004, Sonne et al. 2006, Murphy et al. 2015, Folland et al.

2016), hormones (Petrulis 2013), diet (Ruiz-Olmo et al. 2002, 2011, Ruiz-Olmo and Jiménez 2008), habitat quality (Ruiz-Olmo et al. 2011), and chemical signals (Bieber et al. 2012, Grassel et al. 2016, Coombes et al. 2018). Specifically, endocrine disrupting chemicals impact wildlife (Bergman et al. 2013, Pow et al. 2017), and North Carolina is known to have areas of high concentrations of endocrine disrupting chemicals (Sackett et al. 2015).

While the reproduction levels we observed may be driven by environmental contaminants, there are numerous studies that record breeding in river otters at earlier ages in reintroduced populations (Docktor et al. 1987, Crimmins et al. 2011, Barding and Lacki 2014). We detected breeding in juvenile and yearling otters, in a naturally recovered population (Piedmont FMU) and in a population that has been stable for decades (Coastal Plain FMU). Abundant resources contribute to reproduction, and fish abundance, in general, has improved over the course of our study (Rulifson and Batsavage 2014, Lynch et al. 2016). It is possible that North Carolina follows a similar trend to Minnesota where fisheries were recorded as generally increasing in abundance since 1970, although certain key sport fisheries were declining (Bethke and Staples 2015); but it does not fully explain why we failed to detect juvenile and yearling reproduction during the 1970s. Although the early reproduction we observed in the Mountain FMU my be attributed to the reintroduction, reintroduced populations did not always show the same effects (Chilelli et al. 1996) and we observed the same phenomena in natural regenerated (Piedmont FMU) and stable populations (Coastal Plain FMU), although at lower levels. Hence, we speculate that a combination of complex factors that include contaminants, resources, population density, and other unknown pressures may be contributing to earlier reproduction in Coastal Plain FMU otters. We suggest researchers focus on the effect each covariate has on

reproduction, which will enable us to better understand the environmental influence on otter populations.

MANAGEMENT IMPLICATIONS

During the second half of our study, the otter trapping season was open statewide and during Period Two, the NCWRC estimated the annual harvest at ~2,400 otters, mostly in the Coastal Plain and Piedmont FMUs. Nevertheless, based on the age distributions and fecundity estimates the statewide otter population appears to be stable and healthy with high reproduction and recruitment. However, otter populations across the range may experience different age structure and fecundity levels depending on various stressors. Harvest should be closely monitored and regulated, and future studies should be conducted to further assess the effects of environmental stressors (e.g., contaminants, water quality) on otters and other semi-aquatic mammals including beaver (*Castor canadensis*), muskrat (*Ondatra zibethicus*), mink (*Neovison vison*), and nutria (*Myocastor coypus*).

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Table 1. Corpora lutea and fetus counts in river otters (Lontra canadensis) for Period One (1978-80) and Period Two (2009-16) in North Carolina, USA by Furbearer Management Unit (FMU) and age class. Otters less than a year old were considered juveniles, one-year-old otters are considered yearlings, and all otters age two or older were considered adults.

				Period 1		Period 2	
Age Class	**N_1, N_2**	**FMU**	**Variable**	**Mean**	**SE**	**Mean**	**SE**
Juveniles (0 - 1)	116, 35	Coastal Plain	Corpora Lutea	0.02	0.02	1.11	0.16
			Fetuses	0.00	0.00	0.00	0.00
	0, 31	Piedmont	Corpora Lutea			1.35	0.16
			Fetuses			0.06	0.06
	0, 4	Mountains	Corpora Lutea			1.75	0.25
			Fetuses			0.00	0.00
Yearlings (1 - 2)	43, 48	Coastal Plain	Corpora Lutea	0.00	0.00	1.42	0.12
			Fetuses	0.20	0.08	0.21	0.12
	0, 39	Piedmont	Corpora Lutea			2.00	0.14
			Fetuses			0.15	0.09
	0, 5	Mountains	Corpora Lutea			2.60	0.24
			Fetuses			0.40	0.40
Adults (≥ 2)	115, 65	Coastal Plain	Corpora Lutea	2.49	0.13	2.05	0.12
			Fetuses	2.00	0.13	1.03	0.18
	0, 42	Piedmont	Corpora Lutea			2.24	0.17
			Fetuses			1.07	0.20
	0, 9	Mountains	Corpora Lutea			2.89	0.20
			Fetuses			2.22	0.46

Table 2. Model selection results using Akaike's information criterion (AIC) for the effect of age and Furbearer Management Unit (FMU) on corpora lutea and fetus counts for river otters (*Lontra canadensis*) in North Carolina, USA, during November-February 2009-16. Model weight = $\frac{exp(-0.5*\Delta AIC)}{\Sigma\, exp(-0.5*\Delta AIC)}$, K= number of parameters.

	Corpora lutea					Fetuses				
Model	**AIC**	**ΔAIC**	**Model weight**	**K**	**Log like**	**AIC**	**ΔAIC**	**Model weight**	**K**	**Log like**
Age + FMU	743.9481	0	0.999	5	-366.9740	776.8275	0	0.544	5	-383.4138
Age	757.4973	13.5	0.001	2	-375.7487	777.1839	0.3564	0.456	2	-385.592
FMU	785.0416	41.1	0	4	-388.5208	850.6437	73.8162	0	4	-421.3218
Null	797.6096	53.7	0	1	-396.8048	853.8446	77.0171	0	1	-424.9223

Table 3. Model-averaged coefficients for the effects of age (per year) and Furbearer Management Unit (FMU) on the corpora lutea and fetus counts of river otters (*Lontra canadensis*) in North Carolina during 2009-2016.

	Corpora lutea			Fetuses		
Variable	**Estimate**	**Unconditional variance SE**	**Unconditional 95% confidence interval**	**Estimate**	**Unconditional variance SE**	**Unconditional 95% confidence interval**
Age	0.180	0.030	(0.121, 0.240)	0.256	0.037	(0.183, 0.329)
FMU (Coastal Plain)	-0.3178	0.116	(-0.545, -0.090)	0.007	0.124	(-0.236, 0.250)
FMU (Mountain)	0.596	0.247	(0.112, 1.079)	0.536	0.263	(0.020, 1.052)

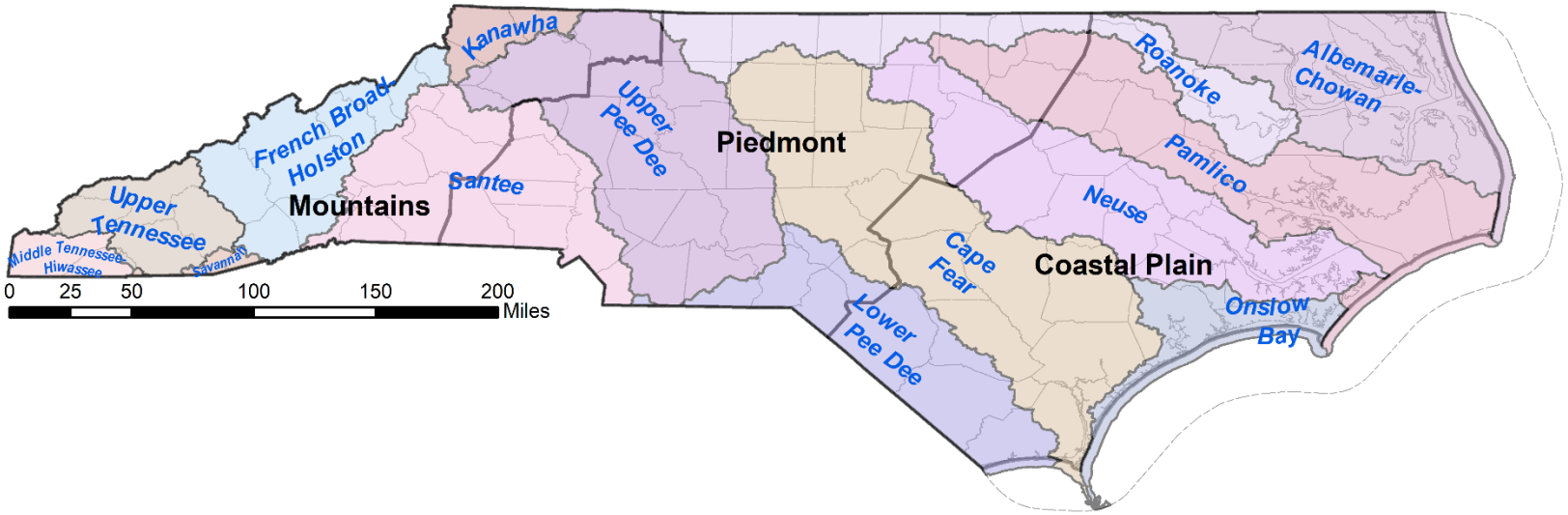

Figure 1. Furbearer Management Units and river basins of North Carolina, 1978-2016.

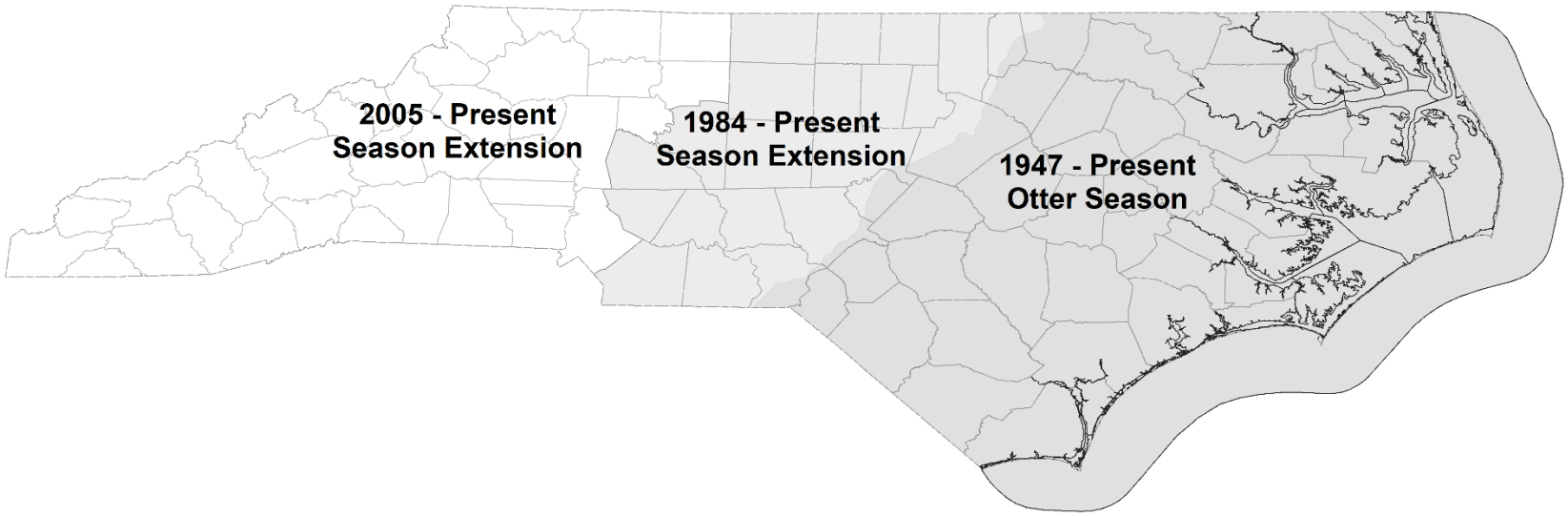

Figure 2. River otter (*Lontra canadensis*) trapping seasons from 1947 - present in North Carolina.

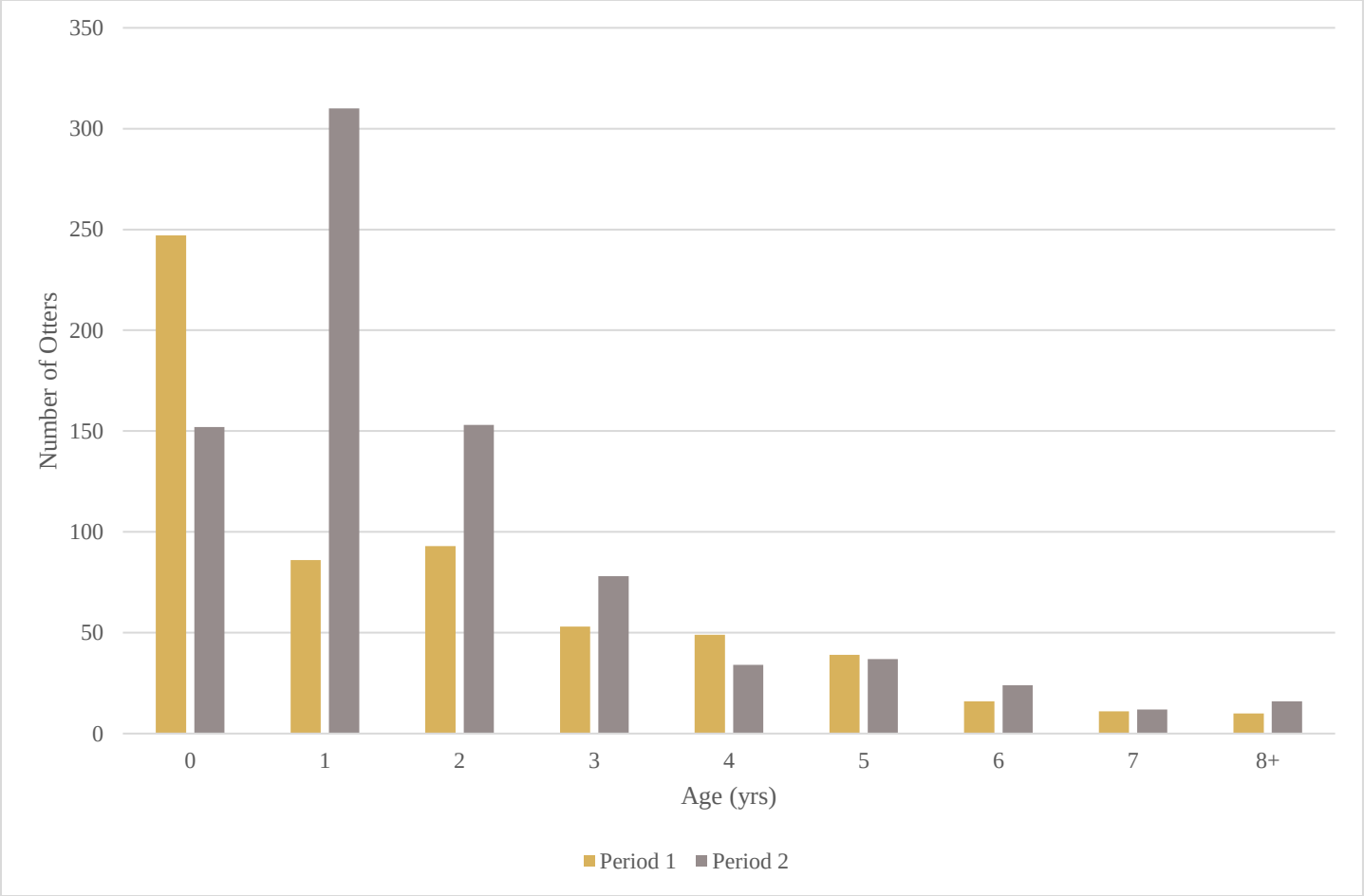

Figure 3. Age distribution of harvested river otters (*Lontra canadensis*) during Period One (1978-1980) and Period Two (2009-2016) in North Carolina.

CHAPTER TWO

LEPTOSPIROSIS, PARVOVIRUS, AND TOXOPLASMOSIS IN THE NORTH AMERICAN RIVER OTTER (*Lontra canadensis*) IN NORTH CAROLINA.

ABSTRACT

The North American river otter (*Lontra canadensis*; hereafter otter) is the largest mustelid in North Carolina and was once extirpated from the central and western portions of the state. Over time and after a successful reintroduction project, otters are abundant and occur throughout North Carolina. However, there is a concern that diseases may have an impact on the otter population, as well as other aquatic mammals, either through exposure to emerging diseases, contact with domestic animals (e.g., domestic cats), or less robust condition of individuals through declines in water quality. Therefore, we tested brain and kidney tissue from harvested otters for leptospirosis, parvovirus, and toxoplasmosis. Leptospirosis and toxoplasmosis are priority zoonoses and are maintained by domestic and wild mammals. Although parvovirus is not zoonotic, it does affect pets causing mild to fatal symptoms. Across the 2014-2015 and 2015-2016 trapping seasons, we tested 220 otters (76 female, 144 male) using real-time polymerase chain reaction (PCR) for leptospirosis, parvovirus, and toxoplasmosis. Of the otters tested, 3 (1%) were positive for *Leptospira interrogans,* 41 (19%) were positive for *Parvovirus* spp, and 53 (24%) were positive for *Toxoplasma gondii*. Although parvovirus and toxoplasmosis are relatively common in North Carolina otters, the otter harvest has remained steady and the population appears to be abundant and self-sustaining. Therefore, parvovirus and toxoplasmosis do not currently appear to be negatively impacting the population. However, subsequent research should examine transmission parameters between domestic and wild species, and the sublethal effects of infection.

KEY WORDS

disease, leptospirosis, *Lontra canadensis*, North Carolina, otter, parvovirus, toxoplasmosis

INTRODUCTION

The North American river otter (*Lontra canadensis*; hereafter otter) is the largest mustelid inhabiting North Carolina. Otters were extirpated from the western Mountain Furbearer Management Unit (FMU) and most of the central Piedmont FMU by the early 1900s (Figure 1), with small surviving pockets in some areas (Wilson 1960). Otters were successfully reintroduced to the Mountain FMU from the Coastal Plain FMU during the 1990s (Spelman 1998). After the population recovered, an otter trapping season was opened in the Mountain FMU in November 2005, and bag limits were removed in November 2009. Today, otter populations in all three FMUs are believed to be abundant and self-sustaining. The International Union for Conservation of Nature (IUCN) Red List categorizes five of thirteen otter species as endangered, with only *L. canadensis* listed as "least concern" and "stable" (IUCN 2017). Studies of *L. canadensis* are important because they potentially provide information for vulnerable otter species (Kimber and Kollias 2000).

The IUCN and Natural Resources/Species Survival Commission (IUCN/SSC) Otter Specialist Group does not outline disease as a direct threat to global otter populations (Foster-Turley et al. 1990), however, it is vital to monitor diseases because they may regulate local populations (Kimber and Kollias 2000). Although some diseases can have regulatory or even catastrophic effects on populations (Anderson and May 1978, May and Anderson 1978), they rarely cause extirpations or extinctions. Also, it is possible for a disease to weaken local populations making them vulnerable to stochastic events (Lafferty and Gerber 2002).

Leptospirosis is a bacterial zoonotic disease caused by an aerobic spirochete (*Leptospira interrogans*) and maintained globally by mammals, reptiles, and amphibians (Kimber and Kollias 2000, Plank and Dean 2000, Bengis et al. 2004, Fouts et al. 2016). Infected animals shed leptospires in urine (Plank and Dean 2000) allowing humans and wildlife species to encounter leptospires through contaminated soil, water, animal tissue, or animal bites (Lecour et al. 1989, Everard et al. 1995, Faisal et al. 2012). Because otters are semi-aquatic, infected water sources associated with urban-suburban areas may be detrimental (Gautam et al. 2010). Additionally, leptospirosis has been recorded in many mustelid species (Moinet et al. 2010), black bears associated with urban areas (Sasmal et al. 2019), and is fatal to sea otters (*Enhydra lutris*) (White et al. 2018).

Parvovirus spp. is a highly contagious genus of viruses identified in the 20th century that spreads in felines, raccoons, arctic foxes, mink, and canines through direct contact with an infected animal or by indirect contact with a contaminated object or feces (Parrish 1990, Goddard and Leisewitz 2010). Although parvovirus is not zoonotic, it can cause mild to fatal symptoms in pets and may affect reproduction (Parrish 1990, Kostro et al. 2014). Interestingly, canine parvovirus (CPV) has had devastating effects on gray wolf populations (Mech and Goyal 1995, Mech et al. 2008) and is lethal to Asian small-clawed otters (*Aonyx cinerea*, Gjeltema et al. 2015). All parvoviruses are capable of infecting other species (Allison et al. 2014, Nituch et al. 2015).

Toxoplasma gondii is a single-celled parasite that causes the zoonotic disease toxoplasmosis (Dubey 2008). Toxoplasmosis is globally distributed, but most hosts are asymptomatic. Cats serve as the definitive host, but many species (e.g., mice, pigs, and geese) are intermediate hosts (Dubey 1996, Cenci-Goga et al. 2011, Sandfoss et al. 2011). In humans,

most cases are minor and typically mimic the flu, but toxoplasmosis can be dangerous and even deadly in immunocompromised individuals (Dubey 1996). *Toxoplasma gondii* moves from its feline host to other species most commonly through contact with meat or water contaminated by cat feces (Vanwormer et al. 2013). Sea otter exposure to *T. gondii* may be at least partially influenced by freshwater runoff (Miller et al. 2002, Conrad et al. 2005), and Shapiro et al. (2012) determined that *T. gondii* was the cause of death in 14% of sea otters tested in central California. Additionally, human population density has been connected to *T. gondii* rates in sea otters (Gaydos et al. 2007) and southern river otters (*Lontra provocax*, Barros et al. 2018).

Detection of *L. interrogans*, *Parvovirus* spp., and *T. gondii* in otters may present a possible transmission risk between wildlife, domestic species, and humans, and may be indicative of exposure to aquatic mammals (e.g., muskrats, beaver, mink) and highlight the impacts by humans and domestic species on wild populations. Therefore, our objective was to survey the otter population to determine the prevalence of *L. interrogans*, *Parvovirus* spp., and *T. gondii* across the three FMUs (i.e., Mountains, Piedmont, and Coastal Plain) and 14 river basins of North Carolina. Additionally, we determined if sex or age were important covariates for determining the probability of infection.

STUDY AREA

We conducted our study across the entire state of North Carolina. The North Carolina Wildlife Resources Commission (NCWRC) divided the state into three Furbearer Management Units (i.e., Mountain, Piedmont, and Coastal Plain). The FMUs followed physiographic regions and county boundaries (Figure 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 also focused our study on the 14 river basins that occur throughout North Carolina (Figure 1).

METHODS

Data Collection:

During the 2014-2015 and 2015-2016 regulated trapping seasons, we collected otter carcasses from licensed trappers across North Carolina. Although there were variations in trapping season dates across North Carolina, most of the otters we collected were trapped during January and February. We recorded the date and location trapped including specific coordinates, addresses, and/or a general description of the trap site location. General descriptions included the county, locality, roads, and any prominent landmarks.

We froze all carcasses prior to necropsy. We extracted a lower canine tooth, which was sent to Matson's Laboratory (Manhattan, Montana) for cementum annuli aging (Stephenson 1977). We removed five grams of brain and two grams of kidney tissue which we froze until analysis.

We used IDEXX Laboratories (Columbia, Missouri) for Real-Time Polymerase Chain Reaction (PCR) testing of *L. interrogans, Parvovirus* spp., and *T. gondii*. We extracted total nucleic acids from brains and kidneys with standard protocols using a commercially available platform (One-For-All Vet Kit, Qiagen, Valencia, CA, USA). The canine parvovirus two and *T. gondii* PCR assays were based on the IDEXX BioResearch proprietary service platform (IDEXX Laboratories, Inc., Westbrook, ME, USA) and used a FAM/TAMRA-labeled hydrolysis probe.

Assays passed analytical validation by being tested in triplicate against dilutions of a known positive control and a known positive clinical case sample with the following criteria

being met and reproduced on different run days: amplification efficiency of 95-105%, linearity over five points, calculated coefficient of variation (CV) of crossing points (Cp) equal to or smaller than three percent, r^2 value equal to or larger than 0.993, signal to noise ratio of fluorescent signal ≥10 and analytical sensitivity of ten molecules or less per PCR reaction. Assays passed clinical validation by being tested against well-characterized clinical samples. Sequence analyses were performed on select positive samples during assay validation to confirm amplification of the intended target.

We used a hydrolysis probe-based real-time PCR targeting a housekeeping gene (18S rRNA) to determine the amount of genomic DNA present in the test sample, confirm DNA integrity, and ensure the absence of PCR inhibitors. We performed diagnostic real-time PCR with a standard primer and probe concentrations using a commercially available mastermix (LC480 ProbesMaster, Roche Applied Science, Indianapolis, IN, USA) on a commercially available real-time PCR platform (Roche LightCycler 480). Because brain and kidney tissues are suited for individual diseases and often used in the literature for disease evaluation, we considered specimens positive for a disease if either tissue sample was positive.

Data Management and Modeling:

We used the SAS GENMOD procedure to predict the maximum likelihood of an otter being positive for the disease using logistic regression in a generalized linear model (SAS Institute, Inc, Cary, NC, USA). We treated age (0-13 years old based on cementum annuli) as a numeric variable with sex, river basin, and FMU as classification variables. All ages were assigned based on a date of birth of 1 April annually. Age classifications were assigned by year up to year four, after which all otters age four or greater were combined into a single age class. We limited our candidate model set to four a priori covariates to avoid including spurious effects.

We used Akaike's Information Criterion (AIC) to assess model weights and rank candidate models (Burnham and Anderson 2002). We developed relative support for the models by using Akaike weights and then calculated the unconditional variance estimates with their associated 95% confidence intervals (Burnham and Anderson 2002, Anderson 2008). We ignored non-informative parameters within two ΔAIC units of the top model (Arnold 2010).

We used indicator kriging to predict the probability of testing positive for *Parvovirus* spp. and *T. gondii* throughout North Carolina. We created the kriging models in ArcGIS 10.3 with the Geostatistical Analyst Wizard (Esri, Redlands, CA, USA). We set our threshold value to zero and optimized the semivariogram. We used the standard neighbor type with eight sectors. For parvovirus, we used a maximum of ten neighbors and a minimum of three neighbors, while for *T. gondii* we used a maximum of 5 neighbors and a minimum of two.

RESULTS

We tested 132 (49 female, 83 male) otters from the 2014-2015 season and 88 (27 female, 61 male) from the 2015-2016 season, collected from over 50 trappers and fur dealers. Of those, three (1%) were positive for *L. interrogans*, 41 (19%) were positive for *Parvovirus* spp, and 53 (24%) were positive for *T. gondii* (Table 1). Due to low overall prevalence (1%) we did not model *L. interrogans* further. *Parvovirus* spp. prevalence was highest in yearling otters (age class = 1, 22%, Table 2), highest in the Coastal Plain (24%) and not detected in the Mountains (0%; Table 2). The Lower Pee Dee (35%, Table 3) had the highest prevalence of *Parvovirus* ssp. while the lowest was in the French Broad-Holston, Middle Tennessee-Hiwassee, and Roanoke river basins (0%, Table 3). *Toxoplasma gondii* prevalence ranged from 17% to 43% among FMUs and was highest in females (34%) and individuals four years old or older (33%, Table 2).

The Upper Pee Dee (40%) had the highest prevalence of *T. gondii* among river basins while no positive samples were recorded in the Middle-Tennessee/Hiwassee basin (Table 3).

We documented the significant influence of age and river basin on the occurrence of *Parvovirus* spp. (Tables 4, 5), and age, sex, and FMU for *T. gondii* among the otter population in North Carolina. Of the 15 models we ran for *Parvovirus* spp., four were within two ΔAIC and only explained ~50% of the variation; all models included age, river basin, and/or sex (Table 4). Therefore, we model averaged which indicated the Albemarle, Cape Fear, Neuse, Pamlico, and Upper Pee Dee river basins were significant predictors, whereas age and sex were not significant predictors (Table 5). The best model for *T. gondii* positive otters included FMU, sex, and age and held 79.4% of the model weight.

For the indicator kriging analyses, *Parvovirus* spp. and *T. gondii* overlapping points were averaged together, resulting in sample sizes of 97 for each disease analyzed. The standardized mean and the standardized root mean square (RMSS) for *Parvovirus* spp. (0.0018, 1.0373, respectively) and *T. gondii* (-0.0176, 0.0.9880, respectively) demonstrated the indicator kriging had a high degree of model performance. *Parvovirus* spp. appeared to be ubiquitous and at low levels across North Carolina with the lowest prevalence in the Mountain FMU, but with a primary probability of occurrence of 19% across North Carolina (Table 3, Figure 2). *Toxoplasma gondii* was present at relatively high levels throughout North Carolina with high prevalence areas in the Southeast Coastal Plain and eastern part of the Mountain FMU and with a primary probability of occurrence of 24% for all of North Carolina (Table 3, Figure 3).

DISCUSSION

Our study was one of the first to examine diseases in otters in North Carolina. We determined that leptospirosis occurred at low levels throughout North Carolina. Because *L. interrogans* can spread through contaminated soil or water and stays in the soil of an infected area for months or longer (Thibeaux et al. 2017), the potential of zoonotic exposure and impact on aquatic ecosystems is a primary concern. Aquatic and semi-aquatic species such as seals (*Pusa capsica*), mink (*Neovison vison*), and nutria (*Myocastor coypu*) have tested positive on multiple continents (Aviat et al. 2009, Barros et al. 2014, Vein et al. 2014, Namroodi et al. 2018), and leptospirosis is lethal to sea otters (White et al. 2018). Although our low prevalence is encouraging, it may be explained by the difficulty of isolating *L. interrogans*. However, Shearer et al (2014) detected higher prevalence rates using similar methods. We suggest continued monitoring of prevalence rates in aquatic mammal species in North Carolina along with the further study of the transmission routes and effects on various wild aquatic species.

We documented *Parvovirus* spp. in 19% of the samples tested. Although no otter mortality attributed to *Parvovirus* spp. has been documented in North Carolina there have been fatalities for otters (Famini et al. 2013) and Asian small-clawed otters (*Aonyx cinereal*, Gjeltema et al. 2015) recorded. While there are no overarching relationships between anthropomorphic development and disease prevalence (Brearley et al. 2013), the dispersion of some diseases that are spread by direct contact may be aided by the disturbance associated with higher human density, development, agriculture, domestic animals, and pest populations (Gaydos et al. 2007). Specifically, canine parvovirus two (CPV2) is more common in rural areas, often due to the lower likelihood of domestic dogs being vaccinated (Sepúlveda et al. 2014, Zourkas et al. 2015, Curi et al. 2016). The Santee and Lower Pee Dee river basins are largely agricultural, which

may explain the higher prevalence detected in our study and how the model showed other basins significantly lower in prevalence. Interestingly, adult dogs are less affected by parvovirus due to environmental exposure, while weaned puppies less than six months old are usually the most at-risk group (Goddard and Leisewitz 2010). In our study, yearling otters had the highest prevalence, possibly due to greater rates of dispersal, encountering multiple latrine sites, and coming into contact with more otters and other species (Boyle 2006). Because of the 41 specimens that tested positive only ten were positive for in both samples, we suggest continuing to test both kidney and brain tissue for parvovirus.

We documented *T. gondii* in 24% of the samples tested and determined that FMU, sex, and age were significant predictors of *T. gondii* in North Carolina otters. The Mountain FMU had the highest prevalence of *T. gondii* at 43%, possibly due to the small sample size and limited distribution across the FMU. Interestingly, seroprevalence was 45% in Coastal Plain FMU otters during the relocation project in 1996 (Tocidlowski et al. 1997). Those otters formed the base of the Mountain FMU population which may have contributed to the high prevalence we observed. In our study, the prevalence in the heavily populated Piedmont FMU and Upper Pee Dee river basin was significantly higher which was not unexpected due to the established link between anthropomorphic development and toxoplasmosis (Miller et al. 2002, 2008, Conrad et al. 2005, Vanwormer et al. 2013, Barros et al. 2018). Additionally, females and older otters were more likely to test positive which is supported by research indicating that immunocompromised individuals (e.g., pregnant females, older) were at greater risk of contracting *T. gondii* (Dubey 1996, Barros et al. 2018). We observed higher probabilities of female and older otters contracting toxoplasmosis across all three FMUs of North Carolina. While there have been no recorded toxoplasmosis related otter mortalities in North Carolina, sea otter mortalities have

been linked to *T. gondii* in California (Cole et al. 2000, Shapiro et al. 2012, White et al. 2018). While direct mortalities are important there may be sublethal effects of toxoplasmosis, such as litter failure, that are difficult to document (Cenci-Goga et al. 2013, Formenti et al. 2015). We encourage future research to focus on the sub-lethal effects of *T. gondii* on wild otter populations.

Aquatic ecosystems offer a plethora of opportunities for disease to spread and thrive (Johnson and Paull 2011). While river otters seem to be robust to diseases such as leptospirosis, parvovirus, and toxoplasmosis, other species may not be. Other furbearers in particular such as mink, muskrats, and beaver remain at risk (Smith and Frenkel 1995, Forzán and Frasca 2004, Jordan et al. 2005) because drainage focuses exposure towards them (Miller et al. 2002, Shapiro et al. 2012, Ahlers et al. 2015). As an apex predator otters are exposed to diseases not only through the environment, but also through their diet (Krusor et al. 2015, Barros et al. 2018). This makes them an ideal sentinel species and suggests that when otter populations test positive for these diseases other aquatic species in the same areas will also, particularly in areas influenced by the human population.

As human encroachment expands across the landscape, development brings activities, domestic animals, and invasive species that enhance the exposure of wild populations to pathogens (Hess 1994, McCallum and Dobson 2002, Gaydos et al. 2007, Brearley et al. 2013). Our research established baselines that can be used for comparisons to future surveys to monitor the spread of leptospirosis, parvovirus, and toxoplasmosis. While traditional research concludes that zoonoses pass from wild populations to domestic animals and humans (Bengis et al. 2004, Shearer et al. 2014), other studies have determined that wild populations away from human development had lower or no prevalence of the same diseases (Gaydos et al. 2007, Plowright et

al. 2008, Brearley et al. 2013, Becker et al. 2015). As the human-wildlife interface continues to expand, diseases being passed from humans and domestic animals to wild populations and vice-versa are of increasing concern for all three groups.

Disease transmission is often complex and difficult to determine, requiring new methods and approaches (McCallum and Dobson 1995, Plowright et al. 2008, Langwig et al. 2015). While aquatic ecosystems provide a hub for pathogens to be encountered and spread (Gortázar et al. 2007, Johnson and Paull 2011), otters may provide crucial data for the management and conservation of other species. As development and habitat loss increase and force more human-wildlife interactions, subsequent research should examine transmission parameters between domestic and wild species and the sublethal effects of infection. Additionally, future surveys should further elucidate the role of agricultural development and human densities on disease prevalence in river otters across different regions and climes.

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Table 1. Polymerase chain reaction (PCR) results for each disease tested on 220 river otters (*Lontra canadensis*) from North Carolina, 2014-2015 and 2015-2016.

	Total Mean (percent)	**Brain Mean (percent)**	**Kidney Mean (percent)**	**Total Otters Positive**	**Otters Positive (Brain)**	**Otters Positive (Kidney)**	**Otters Positive (Both Tissues)**
L. interrogans	0.014	0	0.014	3	0	3	0
Parvovirus spp.	0.186	0.064	0.168	41	14	37	10
T. gondii	0.241	0.241	0	53	53	0	0

Table 2. Prevalence of *Leptospira interrogans*, *Parvovirus* spp., and *Toxoplasma gondii* in 220 river otters (*Lontra canadensis*) from North Carolina, 2014-2015 and 2015-2016.

	Sex (%)		Age Class (%)					Furbearer Management Unit (%)		
Disease	**Male**	**Female**	**0**	**1**	**2**	**3**	**4**	**Mountain**	**Piedmont**	**Coastal Plain**
n	161	90	57	109	42	17	27	13	99	140
L. interrogans	2.1	0	0	2.1	0	6.3	0	0	1.1	1.6
Parvovirus spp.	18.1	19.7	14.3	22.1	16.7	18.8	16.7	0	12.5	24.0
T. gondii	18.8	34.2	18.4	22.1	27.8	31.3	33.3	42.9	33.0	16.8

Table 3. Prevalence of *Leptospira interrogans*, *Parvovirus* spp., and *Toxoplasma gondii* in 220 river otters (*Lontra canadensis*) from North Carolina, 2014-2015 and 2015-2016. Sixteen specimens were unable to be assigned to a river basin because of a lack of precision in the location data. River Basins- AB/CH: Albemarle/Chowan, CF: Cape Fear, FB: French Broad, LPD: Lower Pee Dee, MTH: Middle Tennessee/Hiwassee, NE: Neuse, OB: Onslow Bay, PAM: Pamlico, ROA: Roanoke, SAN: Santee, UPD: Upper Pee Dee.

Disease	**AB/CH (%)**	**CF (%)**	**FB (%)**	**LPD (%)**	**MTH (%)**	**NE (%)**	**PAM (%)**	**ROA (%)**	**SAN (%)**	**UPD (%)**
n	44	66	11	24	1	21	14	9	10	36
L. interrogans	0	1.9	0	0	0	10.0	0	0	0	0
Parvovirus spp.	11.8	18.5	0	34.8	0	15.0	14.3	0	30.0	8.6
T. gondii	11.8	22.2	33.3	21.7	0	30.0	14.3	11.1	30.0	40.0

Table 4. Model selection results using Akaike's Information Criterion (AIC) for the effect of sex, age, river basin, and region on whether river otters (*Lontra canadensis*) tested positive for *Parvovirus* spp. by PCR in North Carolina, USA, in November-February 2014-16. Model weight = $\frac{exp(-0.5*\Delta AIC)}{\Sigma\, exp(-0.5*\Delta AIC)}$, K= number of parameters.

Model	AIC	ΔAIC	Model weight	K	Log like
River Basin + Age	206.09	0.00	0.247	11	184.09
FMU+ River Basin + Age	206.96	0.87	0.160	13	180.96
River Basin	207.91	1.82	0.100	10	187.91
River Basin + Sex + Age	208.06	1.97	0.092	12	184.06
FMU + River Basin	208.37	2.28	0.079	12	184.37
FMU + Age	208.39	2.30	0.078	4	200.39
FMU + River Basin + Sex + Age	208.80	2.71	0.064	14	180.80
River Basin + Sex	209.90	3.81	0.037	11	187.90
FMU	210.08	3.99	0.034	3	204.08
FMU + River Basin + Sex	210.33	4.24	0.030	13	184.33
FMU + Sex + Age	210.38	4.29	0.029	5	200.38
Age	210.96	4.87	0.022	2	206.96
FMU + Sex	212.02	5.93	0.013	4	204.02
Sex + Age	212.95	6.86	0.008	3	206.95
Null	213.60	7.51	0.006	1	211.60
Sex	215.51	9.42	0.002	2	211.51

Table 5. Model-averaged coefficients for the effects of age (per year), sex, FMU, and river basin on whether a river otter tested positive for *Parvovirus* spp. by PCR in North Carolina during 2014-2016.

Variable	Estimate	Unconditional variance SE	Unconditional 95% confidence interval
Age	0.007	0.134	(-0.256, 0.270)
Sex (female)	-0.056	0.398	(-0.836, 0.724)
FMU (Coastal Plain)	0.845	0.529	(-0.192, 1.883)
FMU (Mountain)	-23.307	167431.461	(-328188.971, 328142.357)
River Basin (Albemarle)	-2.245	0.763	(-3.740, -0.750)
River Basin (Cape Fear)	-1.592	0.630	(-2.827, -0.356)
River Basin (French Broad)	-15.063	174071.435	(-341195.075, 341164.949)
River Basin (Lower Pee Dee)	-0.827	0.709	(-2.217, 0.563)
River Basin (Neuse)	-1.844	0.817	(-3.446, -0.242)
River Basin (Pamlico)	-1.976	0.940	(-3.819, -0.133)
River Basin (Roanoke)	-24.928	92123.913	(-180587.798, 180537.941)
River Basin (Santee)	-0.644	1.011	(-2.626, 1.338)
River Basin (Upper Pee Dee)	-2.236	0.902	(-4.003, -0.469)

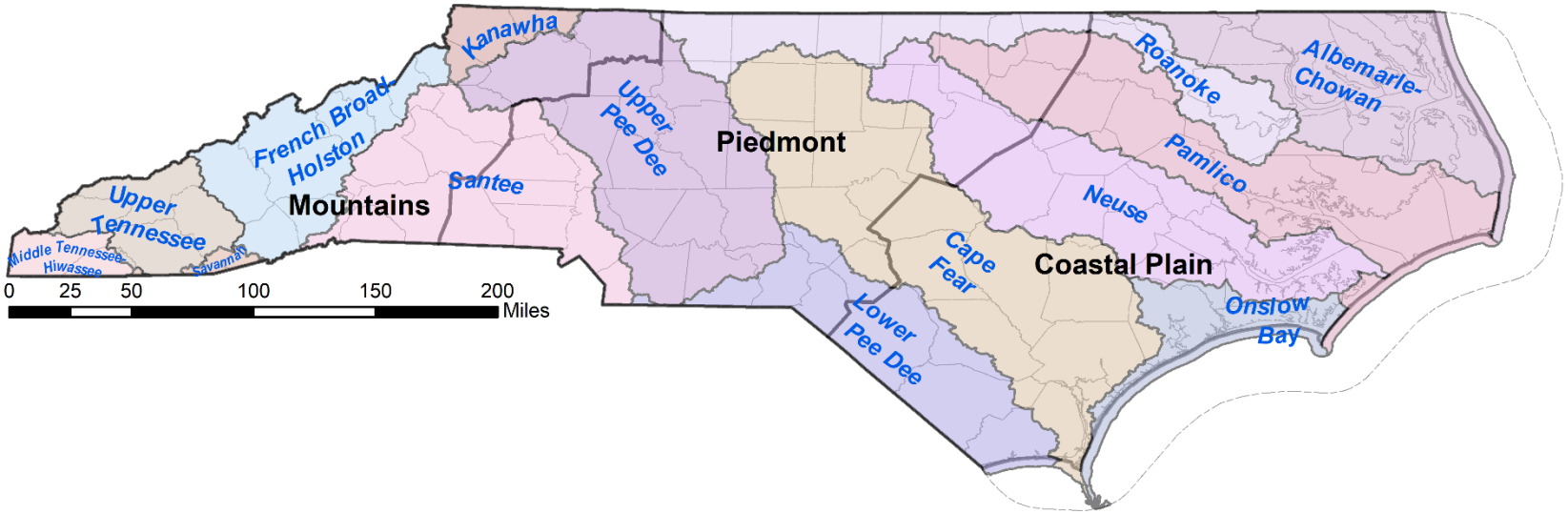

Figure 1. River Basins and Furbearer Management Units in North Carolina, 2014-2015 and 2015-2016.

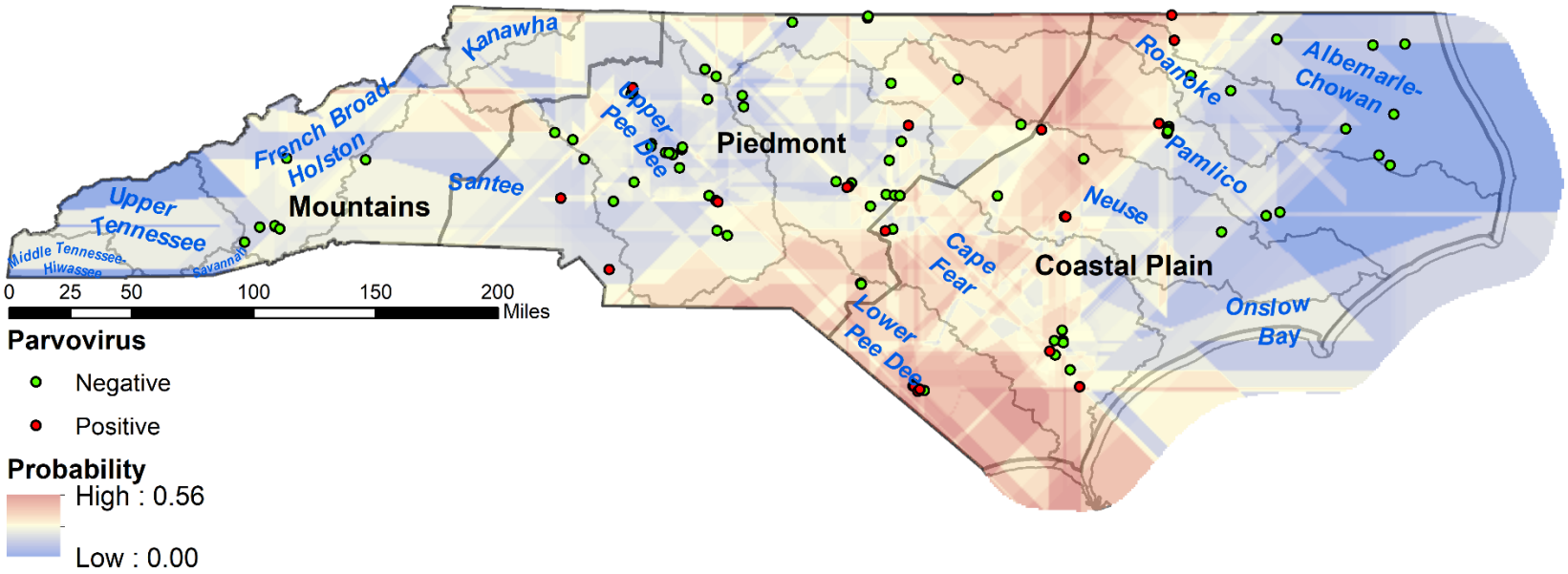

Figure 2. Probabilities of *Parvovirus* spp. infection in harvested North American river otters (*Lontra canadensis*) in North Carolina, 2014-2015 and 2015-2016.

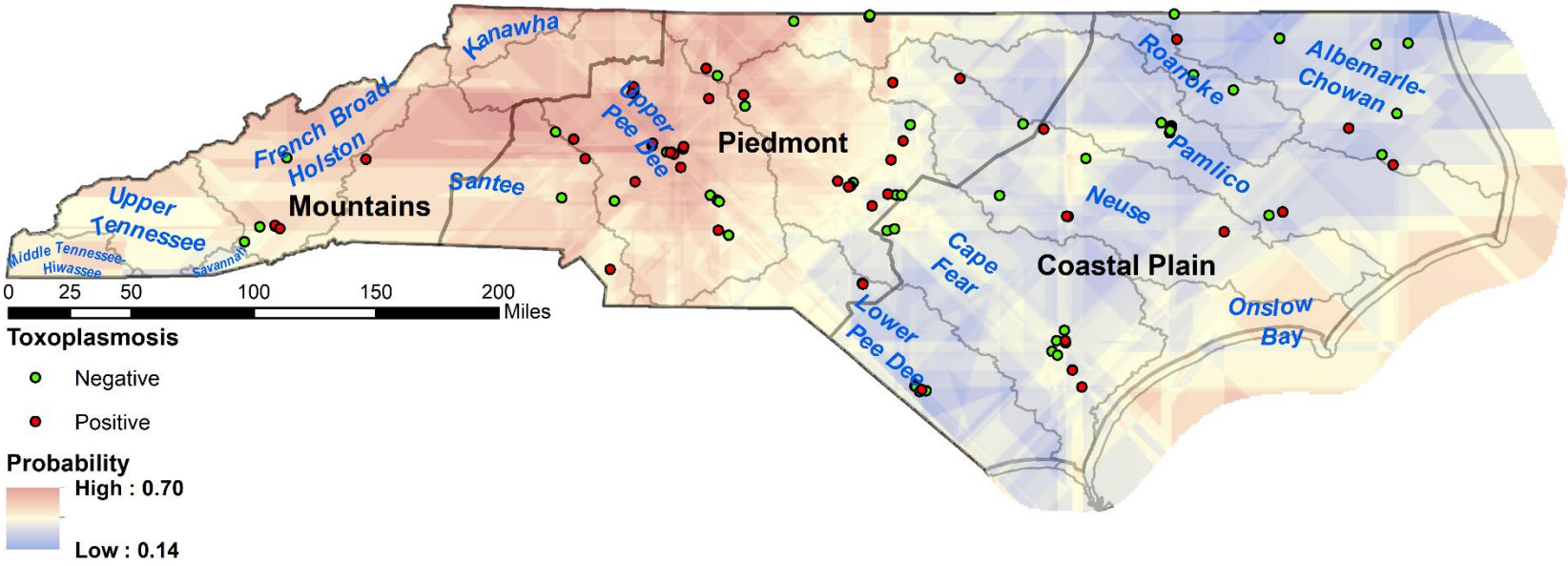

Figure 3. Probabilities of *T. gondii* infection in harvested North American river otters (*Lontra canadensis*) in North Carolina, 2014-2015 and 2015-2016.

CHAPTER THREE

METALS CONTAMINATION OF RIVER OTTERS IN NORTH CAROLINA

ABSTRACT

Aquatic apex predators are particularly vulnerable to environmental contaminants due to biomagnification. Contaminants in North American river otter (*Lontra canadensis*) populations should be closely monitored because across their range there is a variety of point and nonpoint source pollution, from agriculture and development to industry. 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 only become toxic when concentrated unnaturally. We conducted our study across the three Furbearer Management Units (FMU) and 14 river basins of North Carolina. We collected otter carcasses from licensed trappers during the regulated 2009-10 through 2015-16 trapping seasons in North Carolina. We used inductively coupled plasma mass spectrometry to determine the concentrations of arsenic, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, selenium, thallium, and zinc in each sample. We conducted analyses on liver and kidney samples from 317 otters harvested between November 2009 through February 2016. Arsenic (315 liver, 312 kidney), lead (307 liver, 311 kidney), and thallium (317 liver, 316 kidney) samples tested at levels below the limit of detection (0.2, 0.1, 0.05, µg/g, respectively). All other elements were detected at higher levels in the liver samples compared to the kidney samples with the exception of cadmium. 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 age of the otters suggesting that

bioaccumulation occurred. We detected higher levels of mercury and selenium in the Lower Pee Dee and Cape Fear river basins within the Piedmont and Coastal Plain FMUs. 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 Mountain or the Piedmont FMUs. 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.

KEY WORDS

heavy metals, kidney, liver, *Lontra canadensis*, North Carolina, river otter, trace elements

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 1938-1946 (Wilson 1960) and translocated otters from the Coastal Plain to the Mountains from 1989-1996 (Spelman 1998); the statewide population recovered by 2005. Today, the North Carolina Wildlife Resources 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 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, 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 nonessential 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 have been documented in fur, brain, bone, kidney, and liver tissues of otters across their range including calcium, cadmium, cobalt, copper, mercury, iron, lead, magnesium, manganese, molybdenum, selenium, and zinc (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 bioaccumulates 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 varies. 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 (Figure 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 also focused our study on the 14 river basins that occur throughout North Carolina (Figure 1).

METHODS

Data Collection:

We collected otter carcasses from licensed trappers during the regulated 2009-10 through 2015-16 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 ageing, 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 (Figure 1) and used age classes from zero to four. 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 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 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, 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, 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 2a). No differences were detected in kidney tissue between males and females (Table 2b).

Age class was significant in 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 samples (Table 3a). Age class was significant in 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 3b).

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 2a). Copper concentrations were higher in Coastal Plain otters than in Piedmont otters, while the opposite was true for manganese (Table 2a). Mercury and selenium concentrations were highest in the Coastal Plain. Molybdenum was highest in the Piedmont while zinc was the lowest (Table 2a).

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 2b). The Coastal Plain FMU had the lowest concentrations of cobalt, manganese, and selenium, and the highest concentration of mercury (Table 2b). 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 2b).

Differences were detected in liver tissues between river basins for cadmium (F = 4.16, df = 314, P < 0.0001), 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, $\alpha = 0.05$) (Table 4a/b). 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 (Table 4a/b).

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 ($Q = 4.59$, $df = 306$, $\alpha = 0.05$) (Table 5a/b). 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 (Table 5a/b). 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 United States and Canada (Anderson-Bledsoe and Scanlon 1983, Harding et al 1998, Klenavic et al 2008, Sheffy and Amant 1982, Wren et al 1988), and found that our values were consistently lower (Table 6). 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 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 our northern neighbors in 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 to 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 to 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 of our Virginia neighbor (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 United States (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 pollutions 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 to 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 (Boening 2000, Julian and Gu 2015, Martinez-Finley et al. 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 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. 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 et al. 2013) studies involving wide-ranging and indicator species (e.g., otters, mink) are

necessary to understand concentrations of elements at the landscape, river basin, and FMU level (Ben-David et al. 2001, Crowley et al. 2018, Sutherland 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, Liao et al. 2017, Moskovchenko 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). 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 (Figure 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 (Figure 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). 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, Osowski et al 1995). 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.

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Table 1. Heavy and trace element loads in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units were measured in µg/g. [a]Samples tested below the Limit of Detection. [b]Significant difference ($p<0.05$) between kidney and liver sample levels.

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

Table 2a. Heavy and trace elements from livers by sex and Furbearer Management Unit (FMU) in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Majority of samples tested below the Limit of Detection. [c]Statistically significant difference between sexes ($P < 0.05$). [d]Statistically significant difference between FMU's ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

Liver

		Sex (SE)		**FMU (SE)**		
Element	**µ (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[ad]	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[d]	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[cd]	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[d]	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[d]	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[cd]	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[d]	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[d]	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

Table 2b. Heavy and trace elements from kidneys by sex and Furbearer Management Unit (FMU) in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Majority of samples tested below the Limit of Detection. [c]Statistically significant difference between sexes ($P < 0.05$). [d]Statistically significant difference between FMU's ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

Kidney

		Sex (SE)		**FMU (SE)**		
Element	**µ (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[d]	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[d]	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[d]	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[d]	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[d]	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[d]	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[d]	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[d]	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[d]	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[d]	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

Table 3a. Heavy and trace elements from livers by age class in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Samples tested below the Limit of Detection. [b]Statistically significant difference between age classes ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

Liver

		Age Class (SE)				
Element	**µ (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[ab]	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[b]	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[b]	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[b]	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[b]	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[b]	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[b]	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)

Table 3b. Heavy and trace elements from kidneys by age class in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Majority of samples tested below the Limit of Detection. [b]Statistically significant difference between age classes ($P < 0.05$). Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

Kidney

		Age Class (SE)				
Element	**µ (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[b]	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[b]	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[b]	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[b]	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)

Table 4a. Heavy and trace elements from livers by Mountain/Piedmont river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Majority of samples tested below the Limit of Detection. [b]Basin showed a significant difference ($P < 0.05$) from the mean in liver samples. River Basins- AB/CH: FB: French Broad, MTH: Middle Tennessee/Hiwassee, SAN: Santee, UPD: Upper Pee Dee. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

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[b]	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[b]	123 (4.79)	107.91 (12.25) AB	115.00 (0.00) AB	113.14 (11.02) AB	81.06 (4.72) AB
Co[b]	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[b]	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[b]	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[b]	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)

Table 4b. Heavy and trace elements from livers by Piedmont/Coastal Plain river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Majority of samples tested below the Limit of Detection. [b]Basin showed a significant difference ($P < 0.05$) from the mean in liver samples. River Basins- AB/CH: Albemarle/Chowan, CF: Cape Fear, LPD: Lower Pee Dee, NE: Neuse, OB: Onslow Bay, PAM: Pamlico, ROA: Roanoke. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

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[b]	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[b]	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[b]	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[b]	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[b]	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[b]	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)

Table 5a. Heavy and trace elements from kidneys by Mountain/Piedmont river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Majority of samples tested below the Limit of Detection. [b]Basin showed a significant difference ($P < 0.05$) from the mean in kidney samples. River Basins- AB/CH: FB: French Broad, MTH: Middle Tennessee/Hiwassee, SAN: Santee, UPD: Upper Pee Dee. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

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[b]	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[b]	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[b]	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[b]	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[b]	153 (1.48)	157.41 (5.84) AB	170.00 (0.00) A	165.25 (5.74) A	151.15 (3.64) AB
Mn[b]	0.72 (0.02)	0.79 (0.07)	0.78 (0.00)	0.85 (0.10)	0.82 (0.05)
Hg[b]	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[b]	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[b]	21.53 (0.22)	22.78 (0.92) BC	35.50 (0.00) A	22.13 (0.73) BC	21.45 (0.61) BC

Table 5b. Heavy and trace elements from kidneys by river basin in 317 North Carolina river otters (*Lontra canadensis*), 2009-2016. Units measured in µg/g. [a]Majority of samples tested below the Limit of Detection. [b]Basin showed a significant difference ($P < 0.05$) from the mean in kidney samples. River Basins- AB/CH: Albemarle/Chowan, CF: Cape Fear, LPD: Lower Pee Dee, NE: Neuse, OB: Onslow Bay, PAM: Pamlico, ROA: Roanoke. Capital letters indicate significance grouping according to Tukey's test ($P < 0.05$).

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[b]	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[b]	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[b]	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[b]	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[b]	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[b]	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[b]	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[b]	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[b]	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

Table 6. 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]).

	Liver		Kidney	
Element	**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

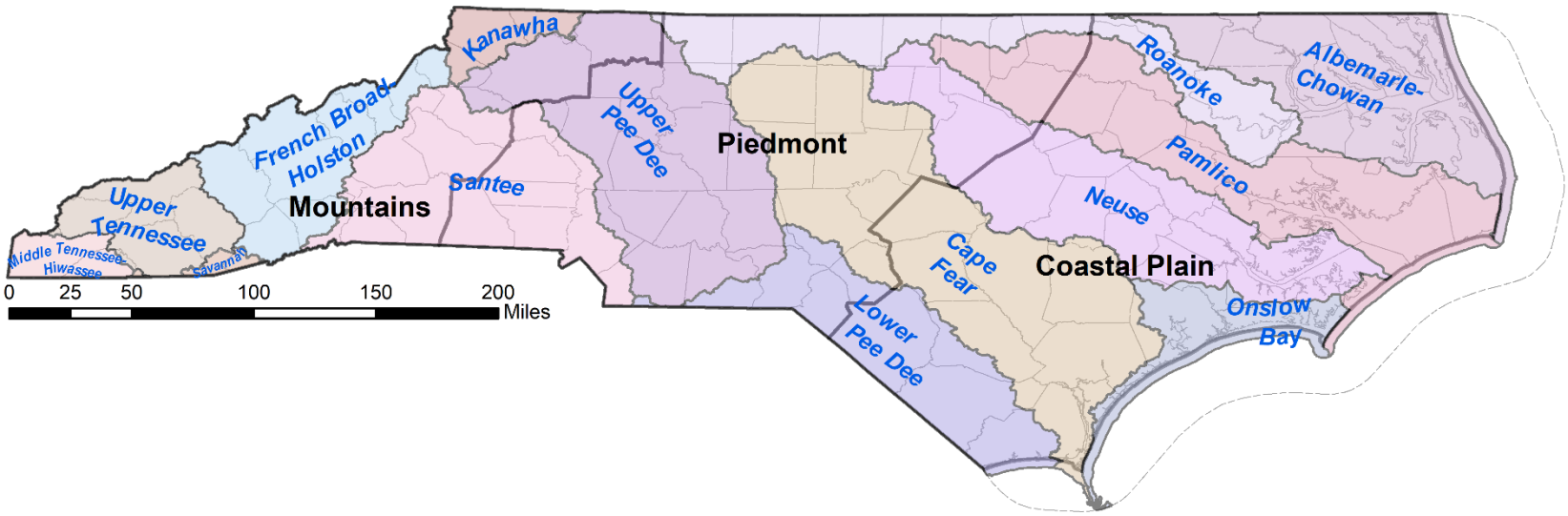

Figure 1. Furbearer Management Units (FMU) and river basins of North Carolina.