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Ecology and Conservation of Semi-aquatic Mammals and their Habitats: Implications for Policy

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Ecology and Conservation of Semi-aquatic Mammals and their Habitats: Implications for Policy

Clayton K. Nielsen

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Recent topics for special issues of the *Journal of Contemporary Water Research & Education* (*JCWRE*) include stormwater management (Hunt 2010); interdisciplinary water resource modeling, research, and education (Link et al. 2013); and watershed management (Edwards 2015). Such topics very clearly fit the aims and scope of *JCWRE*. However, as the title of the present issue indicates, we will deviate somewhat from these more common themes into areas of interest to the wildlife profession (The Wildlife Society 2016). In this brief prologue, I introduce the papers that make up *Ecology and Conservation of Semi-aquatic Mammals and their Habitats: Implications for Policy*.

Prior to introducing the papers in this issue, I will first define the group of wildlife species that are the subject of our theme. A "semi-aquatic mammal" is exactly what it sounds like: a mammal that spends at least some of its time in water. Many semi-aquatic mammal species inhabit the planet, with 10 mammalian Orders of freshwater species alone included in this group. Given their use of both terrestrial and aquatic habitats, semi-aquatic mammals are important to humans and aquatic ecosystems in many ways: as apex carnivores, environmental indicators, and harvestable species, to name a few (Feldhamer et al. 2003). Of course, the habitats used by semi-aquatic mammals are just as important (and probably more so) than the wildlife species themselves, as is well known by this audience. This compilation of papers will broadly address issues regarding wildlife population and habitat status and utility of semi-aquatic mammal species as environmental indicators.

The North American river otter (*Lontra canadensis*) and its habitat is a large focus of this issue. I admit this is in no small part because I have studied river otters for nearly two decades and have an especially strong interest in their ecology and conservation! Two papers address river otters solely in Illinois, USA, where populations have increased considerably following decades of harvest protection and reintroduction efforts in the mid-1990s (Bluett et al. 2004). Holland and van der Merwe utilize otter occupancy data from field surveys to test a prior habitat model; such analyses are relatively rare in the literature. Their work suggests ample caution regarding the appropriate temporal and spatial scale of habitat suitability models for assessing wildlife-habitat relationships. Nielsen presents results from population modeling efforts for river otters, illustrating the rapid population growth possible for this species in highly-suitable habitats with few true competitors. In such situations, wildlife managers should closely monitor population growth, as river otters are occasionally a nuisance species.

Two papers in this issue of *JCWRE* focus on river otters and beavers (*Castor canadensis*). The paper by Nielsen and Kelly measured habitat variables important for both species (as well as several others) as part of their broader assessment of wildlife habitat on mined lands. They found remarkable similarity in habitat conditions on mined lands 30 years following habitat restoration

associated with mining activities, indicating that reclamation activities can indeed be successful. Peterson and Schulte review evidence regarding the toxicological impacts of pollutants to beavers and river otters. They reinforce the notion that these two species can serve as important indicators of environmental health in aquatic systems.

While most of the papers in this issue of *JCWRE* discuss North American systems, I also include a study not of an otter...but of an otter shrew! Decher et al. provide updated distributional information for the Endangered Nimba otter shrew (*Micropotamogale lamottei*) in West Africa. They conclude further surveys are necessary to truly delineate the status of the Nimba otter shrew in this portion of its range and provide further conservation recommendations for the species.

In summary, I hope readers find these topics interesting and gain a further appreciation of wild animal users of aquatic resources. As a wildlife ecologist, I have enjoyed serving as a guest editor for this issue of *JCWRE*, and applaud your efforts for managing and conserving these ecosystems.

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Do River Otters Conform to Habitat Suitability Assessments?

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Abstract: River otters (*Lontra canadensis*) were reintroduced from 1994-1996 into parts of Illinois where the species was extirpated due to over harvest and habitat loss. At the time of reintroduction, managing for the persistence of the population through habitat was very important and research was conducted to determine which watersheds had quality habitat and which needed increased management and protection. In a study conducted in the mid-1990s, biologists used pattern recognition (PATREC) modeling to identify high and low quality habitat for river otters at the subunit level (i.e., divided watershed), based on specific habitat attributes including wooded area, sinuosity, and wetland edge. We compared the habitat quality ratings of subunits with river otter use at 112 bridge sites from 2012-2014 to determine if river otters have distributed themselves according to previously determined habitat quality. We found that the PATREC model was a poor predictor of river otter use when sites were located close to the otter reintroduction points. The PATREC model was most likely a poor predictor of river otter use due to an over-emphasis on the importance of woody vegetation to habitat suitability for river otter in the model. We recommend that future work on the assessment of habitat suitability for river otter use, and accuracy of this assessment, be conducted at a local spatial scale and over a shorter temporal scale. We also recommend that watershed policies and habitat assessments consider changes to land-cover and follow an adaptive management approach to assess habitat suitability for reintroduced species.

Keywords: *Illinois, Lontra canadensis, pattern recognition (PATREC) model, reintroduced populations*

Management strategies often include policies to prioritize and protect certain habitat types to promote existence of species of conservation concern. Occasionally these policies are put into place without an adaptive management plan in mind, and hence no post-policy assessments are conducted (see Sutherland et al. 2004). However, when the successes are evaluated and reported, adaptive management plans can be used by other researchers to improve on their own designs (McLain and Lee 1996), especially where reintroduction efforts are concerned (Salz and Rubenstein 1995). When practiced correctly, adaptive management approaches prove valuable in species management by incorporating knowledge from multiple sources and modeling techniques (Sedon et al. 2007).

Many native species are at increased risk of extirpation due to rapid rises in habitat loss and fragmentation, introductions of non-native species, disease, and exploitation. Reintroductions, translocations, and augmentations have increased dramatically over the past decades as a result of species population declines (Dodd and Seigel 1991; Seigel and Dodd 2002). The stakes associated with reintroduction efforts are usually high from both a monetary and biodiversity point of view (Kleiman 1989). Because reintroduction biology frequently deals with endangered or threatened species, it is often impossible to perform replicated experiments on reintroductions. However, there are ways to deal with the lack of reproducibility, which include replicating the whole study (in a metapopulation fashion) or more recently, using adaptive management approaches (see Sedon et

al. 2007). Many animal reintroductions take place in areas that were identified as suitable or ideal habitat for a specific species, and by following an adaptive management approach one can evaluate whether the species is actually using the previously identified "suitable habitat."

A recent example of a successful species reintroduction involves river otters (*Lontra canadensis*) in Illinois. River otters are mid-sized apex predators in riparian-stream systems (Gittleman and Gompper 2005). As apex predators, river otters may provide top-down control which can affect disease rates, CO_2 production, and biodiversity in the system (Estes et al. 2011). River otters primarily prey on fish and crayfish, but will also consume other aquatic invertebrates, amphibians, and mammals (Toweill 1974; Reid et al. 1994; Williamson 2009). Transfer of nutrients from these prey items to the local terrestrial system allows N to cycle within the local area and decreases the rate of nutrient spiraling (Ben-David et al. 1998; Roemer et al. 2009). River otters were listed as state endangered in Illinois in 1989. Decreased habitat availability and water quality, as well as over-exploitation by fur trappers, caused extirpation of river otters throughout most of midwestern North America (Melquist et al. 2003). Habitat and water quality improvements due to the Clean Water Act (1972), recovery of beaver populations, and reestablishment of wetlands by the early 1990's indicated that a reintroduction program in Illinois may be successful (Bluett 1995). To augment the river otter population, a recovery team was formed by the Illinois Department of Natural Resources (IDNR) to plan and implement a reintroduction program (Bluett et al. 1999). River otter sightings within Illinois identified relict populations in northwestern and extreme southern Illinois (Anderson 1995). The IDNR released 346 river otters from 1994 to 1996 in central Illinois in an effort to reestablish river otter populations throughout the state (Figure 1; Bluett et al. 1999).

Following the first otter-release, a set of indices were designed that ranked habitat suitability for otters (Woolf et al. 1997). Habitat suitability was determined by using pattern recognition (PATREC). This method used landscape-level habitat characteristics based on the locations of otters in southern Illinois and in other states. Their objective was to identify suitable habitat for river otters in southern Illinois, and to develop methods for continuous monitoring of river otter populations in Illinois.

Observations of river otters and the presence of kits increased in Illinois in the years immediately following the initial reintroduction (Bluett et al. 1999). Continued surveys indicated that the river otter population had become established throughout most of the state, and the species was delisted by IDNR in 2004 (Bluett et al. 2004). In 2012 a study was initiated in Illinois, whereby the presence and distribution of river otters in central and southern Illinois was recorded during repeated visits to bridge sites throughout the area. This study also incorporated land-cover data to investigate which habitat types most influenced river otter presence. Understanding how river otters use the landscape and verifying the accuracy of habitat assessments will increase the effectiveness of programs for river otter management. We compared the results from the most recent study of river otters in Illinois with the initial habitat assessment from Woolf et al. (1997) to determine the accuracy of the initial recommendation in promoting species persistence.

Methods

Study Area

The research conducted in the two comparison studies covered the majority of the southern third of Illinois, but there were minor differences in the study areas. Data used in the present study only included watersheds where the two previous study areas overlapped. Those watersheds included the Embarras, Little Wabash, Lower Wabash, Skillet, Saline, Cache, Big Muddy, Lower Kaskaskia, Middle Kaskaskia, Shoal, Lower Mississippi, and Ohio (42,282 km^2; Figure 1). Natural divisions within the study area were the grand prairie, middle Mississippi riverlands, southern till plain, Wabash border, Ozarks, lower Mississippi river bottomlands, Shawnee hills, and coastal plain (Schwegman 1973). Public areas within the study area included Shawnee National Forest, Crab Orchard National Wildlife Refuge, Middle Mississippi River National Wildlife Refuge, Cypress Creek National Wildlife Refuge, 13 state parks, and additional state-managed natural areas.

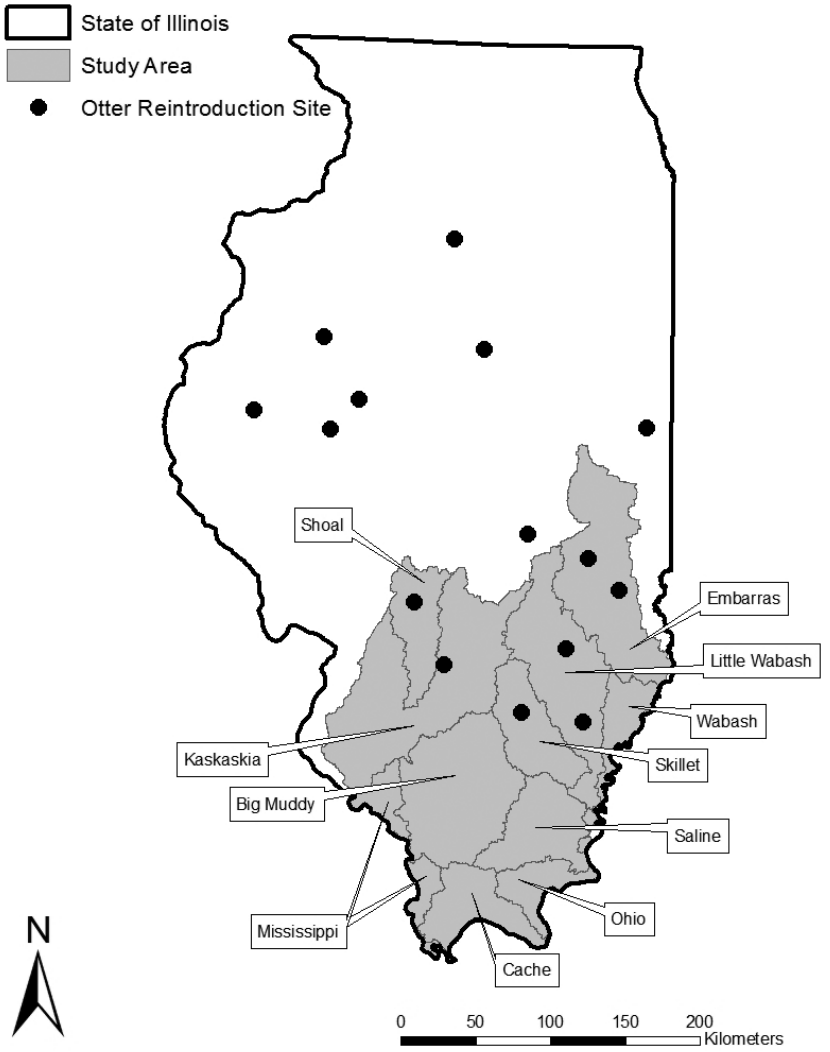


Figure 1. Map depicting reintroduction points and study area for the assessment of using PATREC model values to estimate proportion of visits with river otter use in Illinois, 2012-2014.

Elevation within the study area ranged from 88 to 324 m and the drainage density for the study area was 1.37 km of stream/km^2. Land-cover for the study area comprised primarily crops (48%), forest (23%), and pasture (16%); followed by areas with minor development (8%) and less than 2% each of open water, woody wetlands, grasslands, highly urbanized area, emergent wetlands, and bare land (Homer et al. 2015).

Data Acquisition and Analysis

PATREC is a habitat modeling method by which habitat is classified into different categories (e.g., suitable or unsuitable) based on certain habitat attributes (e.g., vegetation types, distance to urban settlements, etc.; Grubb 1988). The PATREC model output is a score between 0-1, which represents the probability that an area falls into a high suitability category. We used data from Woolf et al. (1997), a study that used the PATREC approach to classify subunits (natural watershed divided further to be of similar size at the landscape level) as suitable or unsuitable for river otters. The habitat attributes they considered for evaluation in their assessment included food availability, bank cover type, and human impacts. Specifically they suggested that food availability would be a function of the presence of water year round. The presence

of woody vegetation on riverbanks would be indicative of suitable habitat for cover and dens for river otters, and urban development would be an indication of human impacts. Model refinements resulted in four habitat attributes: wooded stream length; stream shape index; increase in wooded riparian habitat due to wooded perennial wetlands; and length of intermittent wetland edge (Woolf et al. 1997). These attributes were then used to evaluate suitability of subunits for river otters. Based on these criteria and using National Wetlands Inventory data and other land-cover layers, subunits were classified as high, medium, or low suitability for river otters (Figure 2) and estimated river otter densities were assigned to each subunit based on these classifications.

We used data obtained from river otter sign surveys conducted at 112 bridge sites from 2012 – 2014, collected for a larger dynamic occupancy modeling study (Nielsen et al. 2015). Sites were visited four times from January – April each year by two observers. Each observer surveyed a 400 m stream segment per visit, walking along 800 m of stream bank looking for river otter sign (Lesmeister and Nielsen 2011). Observers surveyed a stream segment located either fully up or downstream of the bridge. A site was considered used by river otters if at least one observer identified river otter tracks or scat during the visit. We calculated proportion of visits with river otter detection for each site each year (site-year) based on the number of visits with river otter observations and the number of visits to the site that year (N = 306 site-years). Not all sites were visited every year due to logistical constraints and landowner permissions (1 year = 5 sites; 2 years = 21 sites). Flooding occasionally affected site visits resulting in only three visits in a year rather than four (23 site-years).

We assessed the accuracy of the habitat suitability models from Woolf et al. (1997) in Program R 3.1.2 (R Core Team 2014). We used a generalized linear model with a binomial distribution of y-values to determine the effect of subunit model scores and distance to nearest otter-reintroduction point, as well as an interaction between these two variables, on the proportion of visits with a river otter detection for each site-year. Distance to nearest otter-reintroduction point was calculated as the Euclidian distance from the bridge site to the nearest otter-reintroduction point. Year and site were included as random effects to account for possible range expansion of river otters or other temporal variation throughout the study, and to eliminate bias due to repeated sampling of the same sites over multiple years.

Results and Discussion

The 112 sites used in this analysis were in 83 of the subunits described by Woolf et al. (1997) with up to four sites being in one subunit. PATREC model scores ranged from 0.05 – 0.91, with an average score of 0.46 for all 112 sites (Table 1). Proportion of visits with otter detections ranged from 0 – 1, with an average of 0.33 and tended to increase over the study period averaging 0.28 in 2012, 0.30 in 2013, and 0.42 in 2014.

The global model indicated that all variables were significant. The PATREC model score (β = -1.982, SE = 0.884, p = 0.025), distance to nearest otter-reintroduction point (β = -0.032, SE = 0.010, p = 0.002), and their interaction (β = 0.043, SE = 0.015, p = 0.005) indicated that as the distance from surveyed sites to otter reintroduction points increased; the ability of PATREC model scores to predict proportion of visits with river otter detections also increased (Figure 3). PATREC model scores were a poor predictor of proportion of visits with river otter detections when sites were close to reintroduction points.

We elucidated the interaction between PATREC model score and distance to nearest otter-reintroduction point by examining the extremes of the relationship. Most of the subunits in the Embarras, Little Wabash, and Skillet watersheds, where five of the reintroduction points occurred, were classified as low quality habitat by the PATREC model (Figure 2). Woolf et al. (1997) stated that river otters were likely to first move into high quality areas near otter reintroduction sites; however after 20 years, river otters are still using subunits categorized as low quality near the original reintroduction sites. This length of time should be sufficient to allow river otter populations to move into higher quality habitat and away from low quality habitat, assuming low quality habitat significantly affects survival and

Table 1. Summary of data used in the analysis of 112 sites and 83 management subunits (natural watersheds divided to be of similar size) to determine the accuracy of PATREC model values in estimating proportion of visits with river otter use in Illinois from 2012-2014.

Watershed	# Sites	---Site Habitat Ranking---			Average PATREC Score	Distance to Otter Reintroduction Point (km)[a]	Proportion of Visits with River Otters Use (km)[a]
		High	Medium	Low			
Big Muddy	18	14	4	0	0.79	79.2	0.24
Cache	11	10	1	0	0.70	135.1	0.31
Embarras	14	0	3	11	0.17	36.7	0.28
Kaskaskia	21	3	3	15	0.31	46.0	0.31
Little Wabash	16	3	2	11	0.33	20.6	0.42
Mississippi	4	2	2	0	0.75	115.7	0.27
Ohio	4	2	2	0	0.60	114.1	0.48
Saline	8	4	1	3	0.54	81.1	0.26
Shoal	8	5	2	1	0.56	18.0	0.38
Skillet	6	1	1	4	0.30	25.6	0.56
Wabash	2	0	0	2	0.10	34.5	0.38

[a] Averaged across all sites within watershed.

reproduction (Van Horne 1983). The movement into higher quality habitat should reduce the proportion of visits with otter detections in low quality habitat near relocation sites. Additionally we assumed that low quality habitats were used less than high quality habitats (Morrison et al. 2006), but our measurement of river otter use does not reflect how the area is being used. Habitat quality may be more likely to affect denning and forage, but less likely to affect travel. However, the size of subunits classified by the PATREC model and used in this analysis (11,325 – 70,434 ha; Woolf et al. 1997) are larger than the average river otter home range (Female: 1,881.40 ± 665 ha, and Male: 4,495.40 ± 1,422 ha; A.U. Rutter unpublished data) and site use within the subunit is reflective of river otter status in the subunit. Because river otters have not moved away from subunits categorized as low quality by the PATREC model, it can be concluded that these areas are not as low quality as predicted or that habitat in these areas has improved over the last 20 years. These inaccuracies led to a negative relationship between PATREC model score and proportion of visits with river otter detection when sites were close to otter reintroduction points.

Models based on presence-only data and used for the extrapolation of habitat quality are likely to generate results biased towards the original data. The PATREC model heavily relied on subunits in far southern Illinois to establish which attributes were necessary for suitable

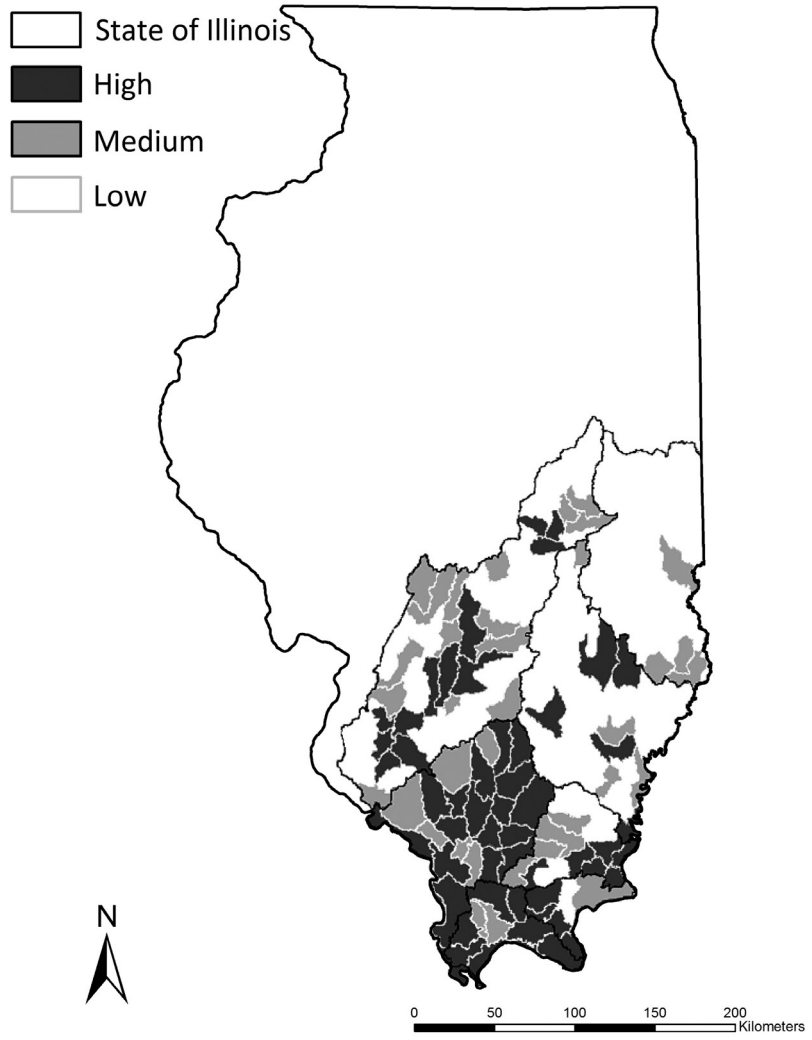


Figure 2. Map adapted from Woolf et al. (1997) depicting the distribution of subunits categorized as High, Medium, or Low quality habitat for river otters.

habitat for river otters. The PATREC modeling process required comparison between present and random subunits, and approximately half of the subunits with river otter present were clustered in the southern Illinois watersheds (Woolf et al. 1997). The land-cover and geology of southern Illinois is very different from central Illinois (i.e., the northern portion of the study area), and this uneven distribution of data resulted in a potential bias of high quality subunits in watersheds in the southern part of the study area. These watersheds were also the farthest away from the otter reintroduction points. The bias of the model towards assigning high and medium quality habitat to subunits in the far southern reaches of the study area resulted in a positive relationship between PATREC model score and proportion of visits with river otter detection when sites were far away from otter reintroduction points. This relationship may also in part be due to the river otter population that was established in the Cache watershed before river otter reintroduction.

Two of the four habitat attributes used to quantify suitable habitat in subunits for the PATREC model included aspects of wooded riparian area. Even though the presence of forested riparian buffers increases overall stream quality (Sweeney and Newbold 2014), the probability of

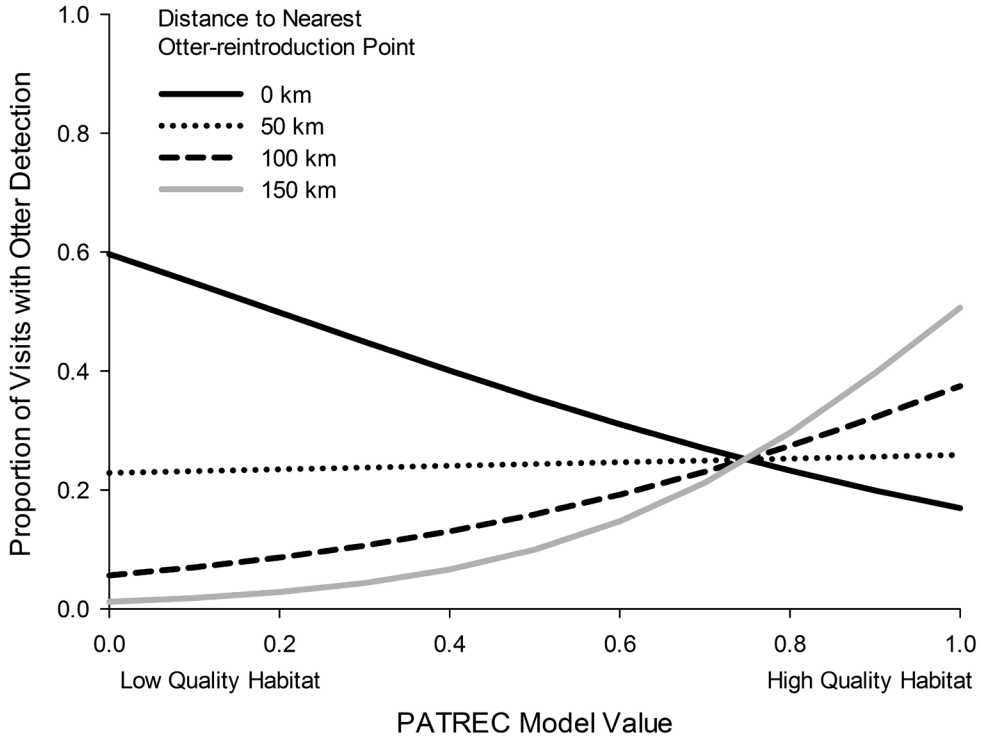


Figure 3. Relationship of PATREC model score and the distance to nearest otter reintroduction point (km) to the estimated proportion of visits used by river otter in Illinois, 2012-2014.

river otter occupancy was not predicted by woody vegetation cover in a recent study, but rather aspects of stream size, urban land-cover, and the presence of mink (*Neovison vison*; Bennett 2014; Nielsen et al. 2015). Additionally, persistence of river otters at sites was correlated with prey availability (Nielsen et al. 2015). Woolf et al. (1997) considered land-cover metrics relating to urban areas and prey availability (assumed to be related to permanent water sources) among the variables evaluated for use in the PATREC model; however these metrics were not used to assess habitat suitability of the subunits. The inclusion of these metrics may have improved the ability of the PATREC model scores to predict the proportion of visits with river otter detection.

Evaluation of the effects of habitat quality on animal movements, behavior, and demographics should be assessed at the scale at which animals perceive differences in quality (Morrison et al. 2006). The PATREC model categorized habitat at the landscape scale for river otters and could not detect variation within subunits at the local scale (Woolf et al. 1997). We assumed that the sites surveyed for river otters were representative of the subunit. In addition to the PATREC model, Woolf et al. (1997) also developed a local scale habitat suitability index (HSI). Since we used local scale data for river otter detection based on relatively short stream segments, an assessment of the accuracy of the HSI would have been more appropriate. Unfortunately, the specific HSI data were not available for use in this analysis. If these data had been available, and river otters perceive habitat quality at the local scale, the HSI may have been more accurately reflected by proportion of visits with river otter detection than the PATREC model.

Our dependent variable, proportion of visits with river otter detection, did not account for the probability of detection. Incorporating probability of detection is important when estimating site occupancy, as sites may be occupied but animals may not be detected (MacKenzie et al. 2002). Therefore our dependent variable may have underestimated river otter use at sites. Probability of river otter detection is affected by observer, substrate, and water level (Jeffress et al. 2011;

Williamson and Clark 2011; Bennett 2014), thus variations in surveys may have affected proportion of visits with river otter detections. We assumed that proportion of visits with detection is related to habitat use, despite known issues of not accounting for probability of detection.

The increased proportion of visits with detections in 2014 (this study) and increased probability of occupancy in 2014 (Nielsen et al. 2015) indicate that river otters may still be expanding their range throughout Illinois, 20 years after reintroduction efforts. Rates of expansion for recolonizing populations are likely to differ in suitable and unsuitable habitats with slower expansion rates through suitable habitats and higher rates through unsuitable habitats (Lubina and Levin 1988). Additionally, expanding populations, such as those used to create the PATREC model, may use habitats differently than established populations. As the river otter population expands in size and number, habitat preferences may also change (Hobbs and Hanley 1990). If river otter populations are still expanding, the use of low quality habitat is less likely; however, a three year study is not long enough to establish the status of the river otter population or conclude that range expansion is occurring.

Watershed Policy Implications

Woolf et al. (1997) recognized the importance of prey availability and urbanization in determining habitat quality for river otters, but emphasized woody riparian buffers in evaluating habitat suitability. Our results indicate that woody riparian buffers may not be as important for river otter use as originally thought. However, the positive impacts of woody buffers (including woody debris) on streams and rivers are innumerable. The presence of forested buffers (and other woody material) aids in temperature regulation and nutrient absorption, decreases erosion, promotes macro-invertebrate communities, and ultimately leads to greater fish abundances (Erol and Rhandir 2013; Sweeney and Newbold 2014), which may affect river otter use. To the same account, increased urban development impacts streams and watersheds by altering the hydrology of streams during high rain events (increasing flood peak and intensity) and increasing pollutants (Rhandir and Raposa 2014); therefore, managing urban development close to riparian habitats should have impacts on watersheds at more than just a species level (Erol and Rhandir 2013).

Woody riparian vegetation is characteristic of forested upland watersheds, but not all watersheds are naturally forested. Since 1830, Illinois has lost 99.9% of native tallgrass prairie land-cover (Samson and Knopf 1994). The upper reaches of prairie watersheds are naturally open and not forested, resulting in unique systems that differ from forested watersheds (Wiley et al. 1990; Dodds et al. 2004). Although most of the tallgrass prairie has been lost in Illinois, communities in watersheds that were once prairie are most likely still adapted to characteristics of prairie systems, such as open canopy. The historic range of river otters covers most of North America, including the Great Plains region, indicating that river otters are able to utilize a variety of watershed types. The emphasis of Woolf et al. (1997) on riparian woody vegetation gives the impression that watersheds throughout Illinois should be forested.

When management protocols and policies are put in place for overall improvement of the watershed, land-cover changes within the watershed should be considered and will ultimately lead to improvement of habitat for species adapted to recently changed environments. Overall improvement (e.g., decreased urban development near streams and increased prey availability) of stream systems within any watershed type will allow for the persistence of river otters, a species that has generalist habitat preferences within freshwater systems. The habitat variables used here do not seem to predict river otter use; however, other aspects of habitat (i.e., prey availability) are likely important to river otter presence. The identification of variables which relate directly to river otter use is necessary for management of river otter populations. To summarize: there is great value in predictive ecological modeling to assess habitat needs of species as long as previous land-cover types are considered, appropriate habitat quality variables are identified, and adaptive management is used to update assessments. Success of reintroduction programs not only depends on an adaptable species, but also on the accuracy of habitat assessments and maintenance of necessary habitats.

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Modeling Population Growth and Response to Harvest for River Otters in Illinois

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Abstract: Population modeling is an invaluable tool for predicting wildlife population dynamics and the effects of management. I created a state-space population model for unharvested river otters in the Illinois, Kaskaskia, and Wabash river basins in Illinois, and modeled future harvest at 5%, 10%, and 20% levels. River otter populations increased in the absence of harvest during 2000 - 2009 to 8,408 ± 1,934 individuals (λ = 1.11 ± 0.09). Harvest at 0%, 5%, 10%, and 20% levels resulted in continued population growth over time, with annual harvest numbers ranging from 446 - 1,786 in 2010 to 884 - 2,614 in 2014, depending on harvest scenario. I concluded that river otter populations in Illinois were thriving and capable of withstanding a regulated harvest.

Keywords: *harvest, Illinois, population modeling, river otter, trapping*

Population modeling is an invaluable tool for predicting wildlife population dynamics and the effects of management. Wildlife managers often require models that can integrate data from multiple sources that may not be available in successive years (Walters and Hilborn 1978; Nichols et al. 1992; Buckland et al. 2007). Such models also should have the capability to handle the inherent errors in data obtained from hunters and trappers that are regularly implemented into models (Clark and Bjornstad 2004; Buckland et al. 2007).

State-space modeling is a relatively novel modeling technique that allows for integration of multiple forms of models, errors, and data sets, which shows great promise for modeling wildlife populations (Buckland et al. 2004). Kalman (1960) originated the idea for the state-space model for use in engineering systems. State-space models have since been used in many applications including population modeling, survival analysis, and disease modeling (Tan and Xiang 1998; Buckland et al. 2004; Tan and Ke 2004).

State-space models are composed of two elements, the state process and the observation process (Borchers et al. 2002). These components allow for a fairly robust estimation of population parameters and an effective way to test for uncertainty. The state process is referred to as the hidden or "true" state model (Buckland et al. 2007). This model generally refers to population processes which can be represented by a population projection matrix and are not explicitly known (e.g., population abundance). This in turn can include sub-processes which may also be modeled as separate Leslie matrices which may include metapopulations or processes such as births and deaths. Habitat modeling and animal movements also can be included in the state process (Thomas et al. 2005).

Two primary methods used in state-space modeling are probability equations and matrix models (Buckland et al. 2004). Probability equations are more commonly used when there is a lack of substantial data for setting the prior distribution of the state variables (Tan and Ke 2004). Matrix models are used commonly when sufficient data to develop a prior distribution are present (Thomas et al. 2005). These methods also are used in combination with the observational process to infer state variables. One of the more common methods for this technique involves a simple time series analysis coupled with recorded observational data to establish a projected Leslie

matrix (Buckland et al. 2004). Within the Leslie matrix, projection state variables are allowed to possess intermediate states. This allows for a variable such as population abundance to have matrix intermediaries that may include births, aging, and survival.

The observation process (i.e., the second portion of the state-space model) seeks to model measurements or observations that can be projected onto the state process; these are generally known quantities obtained from surveys. The observation process can include many of the same aspects of the state process which can then affect the hidden matrices to provide an estimation of state values. The observation process also can include parameters for stochasticity, observational error, and maximum likelihood estimates (Wang 2007). The observational process can be used to determine posterior distributions which can be linearized, and through the use of Bayesian statistics or appropriate Kalman filters, differentiated through the state process (Wang 2007). These in turn can then be assessed through Monte Carlo Markov chain (MCMC) methods to estimate unknown parameters that may be lacking in the state process (Schaub et al. 2007).

Wildlife managers required updated models for river otter (*Lontra canadensis*) populations in Illinois. River otters were listed as a state endangered species in Illinois in 1989 (Bluett et al. 1999). As part of a comprehensive recovery plan developed by the Illinois Department of Natural Resources (IDNR), 346 wild otters were obtained from Louisiana and released in Illinois during January 1994 - March 1997. River otters were downlisted to state-threatened in 1999 following the initial success of the reintroduction program (Bluett et al. 1999). Research then indicated rapid population growth, reproduction, and excellent condition of Illinois river otters (Woolf and Nielsen 2001; Bluett et al. 2005). This research, in conjunction with data collected by the IDNR, resulted in river otters being delisted to non-game status in 2004 (Bluett et al. 2005). Growth in river otter populations in Illinois (similar to that observed in Missouri; Hamilton 1998) led to increased concern regarding potential negative impacts of river otters on native fish and wildlife resources, fish hatcheries, and private ponds (Serfass et al. 1990) as occurred in Missouri (Hamilton 1998).

Research was necessary to monitor population growth for river otters given that population models currently in place for the assessment and management of river otter populations in Illinois were outdated and require incorporation of new data (Woolf and Nielsen 2001). Furthermore, there was potential opportunity for a well-regulated river otter harvest in Illinois (Woolf and Nielsen 2001), and updated information on the biology of this species was necessary to support harvest management while providing a method of monitoring population status. My objective in this study was to use state-space models to forecast population growth in river otters and determine response to varying harvest levels.

Methods

I used state-space modeling to develop a population model for river otters in the Illinois, Kaskaskia, and Wabash river basins in Illinois, where river otters were reintroduced during 1994-97 (Buckland et al. 2004; Moore and Read 2008; Yamamura et al. 2008). My goal was to provide a population estimate for 2009 based on collected data and then project population growth into the future given selected harvest scenarios. I used a Bayesian uncertainty analysis to estimate survival and growth rate from age-at-death data (Moore and Read 2008) following animal necropsy. Population growth was modeled starting with estimates provided in 2001 (Woolf and Nielsen 2001) to the present time (i.e., 2009); I then modeled population growth and response to harvest five years into the future (i.e., until 2014). All models were density-independent.

Empirical Data

To inform model construction, I used empirical data collected from river otter necropsies and previous river otter modeling efforts (Hamilton 1998; Woolf and Nielsen 2001; Bluett et al. 2005). Age data were from necropsies performed on 332 river otters (199 M, 133 F) collected mostly as road kills or incidental harvest in Illinois during 1996 - 2008. The sex of all specimens was determined and the uterine tract examined

in females to quantify fecundity rates based on placental scarring and presence of embryos. However, due to insufficient fecundity data due to poor resolution of placental scars, rates were based on those in previous models (Hamilton 1998; Woolf and Nielsen 2001; Bluett et al. 2005). The age of all specimens was determined through inspection of cementum annuli from lower canine teeth (Crowe 1972).

Model Structure

My global model consisted of two sub-models with the first sub-model consisting of a Bayesian uncertainty analysis (Moore and Read 2008), called as such given there are several different combinations of demographic parameters that may lead to the same overall growth rate (Moore and Read 2008). The uncertainty analysis was used to estimate survival rates and population growth. The second sub-model of the global model consisted of a state-space model (Kalman 1960). The state-space model was used to estimate current abundance and incorporate varying harvest strategies.

Modeling Current Population Abundance and Growth Rate

The first sub-model I developed was used to determine an overall population growth rate based on likely survival and fecundity rates for river otters. Fecundity rates were based on a joint estimate using rates provided by Hamilton (1998) and the more conservative Woolf and Nielsen (2001; Table 1). For survival rates, I used a competing risk model where mortality was age-based and dependent on three combined hazard equations, one each for juvenile, adult, and senescing animals, respectively (Siler 1979; Skalski et al. 2005; Moore and Read 2008). I used the age-at-death data from necropsies to determine these parameters for the hazard equations. Upon estimating survival parameters, I combined survival estimates with fecundity estimates within the Euler equation (Moore and Read 2008) to determine the population growth rate. I also included a conservative error term allowing my estimates to vary by 10%.

I began with the population estimate for river otters in 2001 (N=1,830; Woolf and Nielsen 2001) and then projected population growth forward to 2009 using a state-space model. The original model started only with the 346 river otters released in the Illinois, Kaskaskia, and Wabash river basins in Illinois during 1994-97 (Woolf and Nielsen 2001). The population growth rate was modeled in the state process using a Lefkovitch matrix model (Lefkovitch 1965, Caswell 2001), while stochasticity was modeled in the observation process. I modeled stochasticity in the observation process to allow for individual variation between years and for simplicity when harvest effects were incorporated thereafter.

Table 1. Breeding parameters used to determine fecundity in a model of river otter population growth in Illinois.

Parameter	-------- Breeding Probabilities --------		
	Hamilton (1998)	**Woolf and Nielsen (2001)**	**Average Probability**
Yearling	0.350	---	0.350
Adult	0.700	---	0.700
Female Young	1.750	0.875	1.313

Modeling Population Response to Harvest

My goal in modeling river otter response to harvest was to be conservative, as to not affect considerable declines in river otter numbers if my assumptions were inaccurate. I modeled harvest as an additive source of mortality (i.e., as a direct reduction in survival rates) and assumed harvest affected all stage-classes equally. I modeled annual harvest rates of 0% (i.e., assuming no harvest whatsoever and maintaining current growth levels), 5%, 10%, and 20%. I assumed that regulated harvest would replace incidental take (i.e., from beaver trappers who once accidentally killed river otters but would now be able to harvest them) in a compensatory manner (i.e., I did not include any additional parameters for accidental or illegal take). Population growth and response to harvest was projected five years into the future, until 2014, in one-year increments beginning with

the year 2009. I again used a state-space model similar to that used for estimating population abundance in 2009, this time including an equation in the observation process representing yearly additive harvest mortality.

Model Assumptions

My population model must be understood within the confines of a few important assumptions that I was unable to measure. The Lefkovitch model has the assumptions of a stable and stationary population with no measurement error in stage-classification (Skalski et al. 2005). The Lefkovitch model also assumes that all carcasses have the same probability of harvest and that the sample is representative of mortality within the species due to the use of pooled data (Skalski et al. 2005). The model further assumes that it is both appropriate to classify the population by stage-class and that there is no within-class variability (Caswell 2001).

Model Solutions

I used Bayesian analysis and MCMC to solve both subsections of these models (Moore and Read 2008, Yamamura et al. 2008). The MCMC analysis was carried out using the program WinBUGS (Lunn et al. 2000). Bayesian analysis requires the use of prior distributions for parameters (Tables 2 and 3). Each model was run using three separate Markov chains and convergence was determined using the Gelman-Rubin statistic (Gelman and Rubin 1996, Spiegelhalter et al. 1999). I also examined autocorrelation within my chains; convergence and autocorrelation analysis was conducted using the R package BOA (Smith 2007).

Results and Discussion

The Bayesian uncertainty analysis indicated a growing river otter population in the absence of harvest during 2000 - 2009 ($\lambda = 1.11 \pm 0.09$, SE throughout). Using the survival estimates provided by the uncertainty analysis and the joint fecundity estimate, the state-space model projected a river otter population abundance of 8,408 ± 1,934 in Fall 2009 (Figure 1). The 90% credible interval (similar to confidence interval) increased to roughly 21% of the mean value by 2009 (Figure 1) due to increased model uncertainty over time.

Table 2. Prior distributions for the Bayesian uncertainty analysis used to determine survival and population growth rate for river otters in Illinois.

Parameter	Distribution (Values)	Description
a_1	uniform (0, 3) [a]	juvenile mortality
a_2	uniform (0, 3) [a]	adult mortality
a_3	uniform (0, 3) [a]	senescent mortality
b_1	uniform (0, 5) [a]	decrease in juvenile mortality
b_3	uniform (0, 5) [a]	increase in adult mortality
f_1	beta (1, 0.27) x 0.88	fertility of yearling individuals
f_2	beta (1, 0.25) x 1.855	fertility of adult individuals
l_{nat}	uniform (0, 0.01)	survivorship to oldest age represented in the data

[a] Place-holder values for which data were not initially available due to modeling technique.

Table 3. Prior distributions for the state-space model used to estimate river otter abundance in Illinois.

Parameter	Distribution (Values)	Description
s_j	uniform (0.84, 0.91)	survival of juvenile animals
s_y	uniform (0.76, 0.83)	survival of yearling animals
s_a	uniform (0.73, 0.81)	survival of adult animals
f_j	uniform (0, 0)	fecundity of juvenile animals
f_y	uniform (0.4, 0.9)	fecundity of yearling animals
f_a	uniform (2.0, 2.6)	fecundity of adult animals

Harvest estimates were based on the mean population abundance value provided by the state-space model for the year 2009. The model continued to predict an increase in river otter abundance in the absence of harvest through 2014 to 23,599 animals (a 281% increase over 2009 levels). Harvest levels at 5% and 10% still resulted in population growth of river otters by 2014 (Figure 2). At the 5% and 10% harvest rates, river otter population abundance was 18,852 (a 224% increase over 2009) and 15,331 (a 182% increase over 2009), respectively. Population growth was nearly zero with the 20% harvest scenario, but not negative despite the 11% annual growth calculated during 2000-2009 (Figure 2). This is likely because of the slight J-shape in the population growth curve (an artifact of the particular modeling process used) exhibited during 2009-2014 (Figure 1). Annual harvest numbers ranged from 446-1,786 in 2010 to 884-2,614 in 2014 (Table 4).

The state-space model indicated that river otter populations will continue to grow in Illinois, and that they can certainly withstand modest harvest levels. I believe the current estimate of 8,408 river otters is likely conservative because I originally began modeling in 1996 with only reintroduced individuals (Woolf and Nielsen 2001). Immigrants have likely entered Illinois from nearby states (i.e., Missouri and Kentucky) that had growing river otter populations prior to Illinois otter reintroductions, and remnant otters likely were present in portions of Illinois before reintroductions in the mid-1990s.

Otter population growth was estimated to be 11% during 2000 - 2009, which is less than the 25% growth rate modeled during 1996 - 2001 (Woolf and Nielsen 2001). This disparity may be explained by slight density-dependent influences (which I could not truly assess due to relatively low annual sample sizes of necropsied animals) and/or considerable differences in modeling techniques. Woolf and Nielsen (2001) used simpler models that lacked the rigor of the state-space modeling framework (Moore and Read 2008, Yamamura et al. 2008), and growth rates of >25% were also noted in river otters in Missouri (Hamilton 1998) based on similar data sources and accounting-based models. Conversely, Iowa river otter populations were thought to be growing at a 7% annual rate, with a statewide population of 7,000 animals (Iowa DNR 2016), results that are more similar to those found in this study. Regardless, Illinois river otter populations are growing and could support a harvest season, which would provide recreational benefits to trappers while possibly reducing river otter damage to native wildlife populations. This is

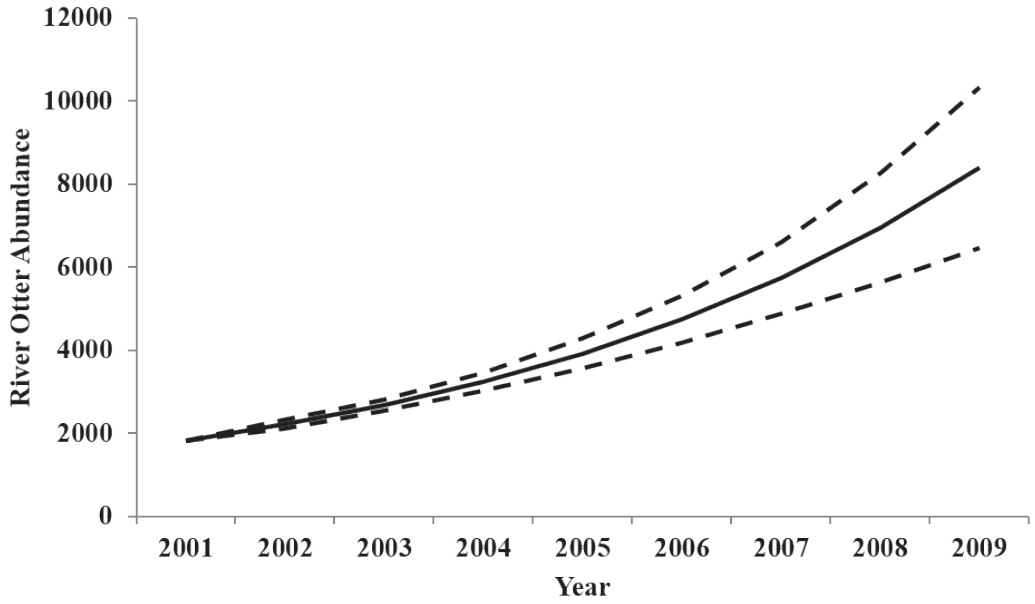


Figure 1. Projected population growth of river otters in Illinois during 2000 - 2009, including the 90% credible interval.

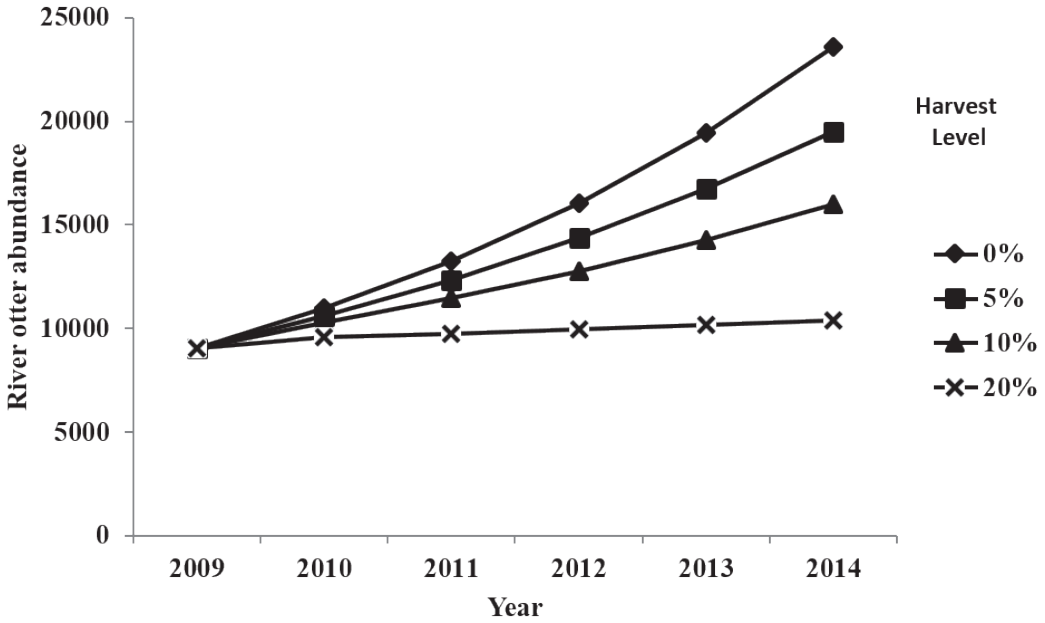


Figure 2. Simulated harvest levels based on population growth projections for river otters in Illinois during 2009 - 2014.

Table 4. Forecasted mean river otter harvest levels in Illinois, 2010 - 2014.

Harvest Rate	---------- Number of River Otters Harvested / Year ----------				
	2010	**2011**	**2012**	**2013**	**2014**
5%	440	513	588	667	751
10%	881	999	1117	1236	1355
20%	1762	1903	2028	2132	2220

especially important given that river otters are top carnivores in aquatic ecosystems with relatively few natural predators in Illinois.

I modeled several harvest levels, assuming the goal of harvesting river otters to maintain zero percent or greater population growth. Harvest rates of 15 to 20% are common in river otter populations (Erb 2009), and my scenarios indicated that even a 20% harvest rate did not result in population declines. However, with slower population growth, Iowa allowed harvest rates of only 6% (Iowa DNR 2016).

Population models represent abstractions of reality, and although my models were based on relatively sound empirical data (with the exception of somewhat low annual sample sizes), these results must be interpreted with some caution. One of my assumptions was that density-independence was not acting on river otters, although slower growth during 2001 - 2009 relative to 1996 - 2001 (Woolf and Nielsen 2001) may indicate some density dependence. Although no empirical data were available to test the assumption of density-independence explicitly, I believe this analysis was appropriately conservative to ensure against overharvest, especially given that the starting estimate in 1996 was likely biased low. Thus, during the study period (i.e., 2000 - 2009) I believed it appropriate to model river population growth in the absence of density-dependence, and was not compelled to do so five years further into the future when incorporating harvest. This resulted in a 281% increase in river otter populations to 23,599 in the absence of harvest, which may be an overestimate of population growth potential, given that density-dependence may begin operating as populations grow further.

Management and Policy Implications

River otter populations continue to grow in Illinois, and as suggested previously (Woolf and Nielsen 2001), can sustain a regulated harvest. Harvest should occur close to the months of November-February (i.e., in conjunction with the beaver season when river otters are currently incidentally trapped) and employ bag and possession limits until the quota is filled, as in Iowa (Iowa DNR 2016) and Minnesota (Minnesota DNR 2009). These recommendations were ultimately employed by the IDNR, and as a result, a trapping season was enacted in Illinois in 2012 with a five otter/trapper limit. Data from harvested otters will be used to increase annual sample sizes, which will allow for the further testing and revision of the model presented in this study. Specifically, I will attempt to test for density dependence and differences in age- and sex-specific parameters used in the model.

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Wildlife Habitat Is Similar at Mined Versus Unmined Sites 30 Years Following Surface Mining for Coal in Southern Illinois

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Abstract: Riparian ecosystems provide important habitat for wildlife and serve as corridors for animal movements. Surface mining activities clearly impact wildlife habitat by removing vegetation needed by wildlife. However, few studies have assessed longer-term success of habitat restoration in riparian ecosystems following surface mining. During 2012-13, we evaluated microhabitat quality at three stream segments restored 30 years ago following surface mining for coal and one unmined stream segment in Perry County, Illinois. Microhabitat differences were very minor among restored and control stream sites. Of the 41 habitat variables measured, 14 differed among sites, four of which were uncorrelated: canopy cover, overstory hard mast, bare ground, and herbaceous ground cover; thus, restored riparian buffers were comparable to unmined sites, indicating that habitat restoration efforts were successful. Our analysis from data collected at Burning Star 4 mine in Perry County, Illinois, is of value to land managers for it shows how the reclamation process has successfully jump-started wildlife habitat in buffers around streams affected by mining.

Keywords: *habitat restoration, riparian, surface mining, wildlife habitat*

Large-scale surface mines in southern Illinois extracted coal from depths of 70 to 95 feet or more during the late 1970s and 1980s. The resulting effects were an altering of the landscape, reconstruction and/or redirection of the watershed, and in some instances, large bodies of water known as final-cut impoundments that were in some cases >1.25 miles in length (Nawrot et al. 2010). While active mining is being conducted, the entire upstream watershed is always diverted around the acreage of the active mining operation. As mining proceeds forward and reclamation progresses afterward, replacement and restoration of stream channels coincides with the processes of soil replacement, revegetation, and the establishment of farm fields, pastures, and upland wildlife habitats. Lands to be affected by mining are bonded and then reclaimed following an approved reclamation plan that requires the restoration of pre-mining capabilities which may include restoration of stream channel systems and riparian zones, crop fields, pasture lands, impoundments, wetlands, forests, and wildlife habitat. Bonds are released when capability standards as specified in the approved plan and the reclamation law are achieved.

Post-disturbance monitoring of the physical, chemical, and biological processes of the stream community is required through the Federal Surface Mining Control and Reclamation Act of 1977 (PL 95-87) (Office of Surface Mining Reclamation and Enforcement 2016). When surface mining disrupts the riparian vegetation and wetland habitat, it must be enhanced, restored, or at least partially replaced to promote fish and wildlife habitat along the affected stream (§. 816.97) (Office of Surface Mining Reclamation and Enforcement 2016). Streams and riparian zones support important functions for wildlife, even if their forms have been altered from historic conditions (Nawrot et al. 2010; Walton 2012). Wildlife habitat is affected directly and indirectly by the ecological processes that take place above and beneath the ground. Healthy

stream corridors aid in physical (hydrological and geomorphological), chemical, and biological landscape-level processes such as transporting water from the watershed to the channel; traveling through the channel, floodplains, and sediment; and eventually delivering water to the local landscape (Fischenich 2006; Scott et al. 2009). Geomorphological processes slowly change and rearrange riparian landscapes by moving woody debris and sediment to create new land forms and provide structural habitat for wildlife (Church 2002; Brierley et al. 2006). Biological processes produce and sustain diverse habitats to support vigorous aquatic and riparian biotic communities, aiding in maintaining predator-prey relationships and genetic diversity, thereby helping to preserve healthy physiological conditions in riparian zones (Ehrenfeld 2000; Fischenich 2006).

Succession describes the natural process of how plant and animal communities change over time after a disturbance, and knowledge of successional changes to riparian zones following restoration is important. Changes in vegetation structure and age promote biodiversity and ecological vigor, which is important to the long-term adaptation of ecosystems. By planting native species important to wildlife in the surrounding area, restoration efforts often "jump-start" succession (Walton 2012). Wildlife will then colonize particular habitats once the vegetation within those areas meets their habitat requirements. Through establishment of native plant communities and construction of similar substrates, it is reasonable to expect that wildlife will utilize previously mined and restored habitats as they would similar habitats on undisturbed sites (Zipper et al. 2011; Walton 2012). One way of gauging whether reclamation was successful is to compare the vegetative communities of reclaimed land with those of nearby land that was not disturbed by mining.

We quantified habitat characteristics for wildlife in restored stream corridors using a focal group of southern Illinois wildlife species to indicate a set of commonly-measured habitat variables that are important to those species. Wildlife species and species-groups chosen include mammals, birds, reptiles, and amphibians. Birds, of course, are not restricted to any one habitat patch but move about freely. The other species groups include carnivores, herbivores, and omnivores; may be terrestrial, aquatic, or aerial; may use both in-stream habitats and wooded corridors; or may spend part of their lives in water and part on land. This set of species and variables includes wildlife that have large and small home ranges and different life history traits. Species included are white-tailed deer (*Odocoileus virginianus*), bobcat (*Lynx rufus*), river otter (*Lontra canadensis*), beaver (*Castor canadensis*), white-footed mouse (*Peromyscus leucopus*), deer mouse (*P. maniculatus*), water snakes (*Nerodia spp.)*, testudines (terrestrial and aquatic turtles), anurans (frogs and toads), migratory birds, ground-nesting birds, raptors, and bats.

Here, we review specific habitat needs for these focal species and species-groups; variables mentioned are those measured in this study. Some focal wildlife species are habitat generalists, while others have more specific habitat requirements. Canopy cover, for instance, is important for a wide array of different species and has been measured for habitat studies of bobcats (Kolowski and Woolf 2002), beaver (Cox and Nelson 2009), white-footed mice (Nupp and Swihart 2001), *Nerodia* species (Cross and Peterson 2001; Pattishall and Cundall 2009), salamander species (Faccio 2003), birds (Saab 1999; Batten and Lawler 2006), and bats (Watrous et al. 2006). Canopy cover has been shown to be an ecological indicator and is useful for distinguishing different plant and animal habitat, and assessing forest floor microclimate and light conditions (Jennings et al. 1999; Lowman and Rinker 2004).

Ground cover such as grasses, herbaceous plants, leaf litter, woody debris, rocks, and bare ground provide substrate, foraging areas, and low cover for many smaller wildlife species such as the white-footed mouse (Adler and Wilson 1987), salamanders (Faccio 2003), *Nerodia* species (Cross and Peterson 2001; Pattishall and Cundall 2009), anurans (Anderson et al. 1999), and both aquatic and terrestrial turtles (Fuselier and Edds 1994; Converse and Savidge 2003; Rizkalla and Swihart 2006). As the summer days get longer and hotter, terrestrial turtles have an increased need for thermoregulatory sites; herbaceous ground cover and shrub structures can help provide shade (Converse and Savidge 2003). Ground cover also

has been measured for bobcats (Kolowski and Woolf 2002), river otters (Bowyer et al. 1995), and migratory birds (Saab 1999).

Variation in vertical foliage structure is a measurement of the density of vegetative cover for habitat suitability for wildlife and can be used for assessing habitat quality for deer (Griffith and Youtie 1988), white-footed mice (Nupp and Swihart 2001), and bobcats (Kolowski and Woolf 2002). Dense foliage under the forested canopy makes flight less navigable, so tall vegetation may exclude some avian species, such as larger raptors, from areas with dense understory.

Understory and overstory stem densities are important habitat characteristics selected by river otters and beaver (Bowyer et al. 1995; Cox and Nelson 2009). Reforested areas on restored streams provide higher-quality habitat than areas with agricultural and grasslands for the river otter. Jeffress et al. (2011) found that river otter occupancy increased with a higher proportion of increasing forested areas present along riparian corridors. Otter presence may be positively correlated with the presence of fish, their main prey, because fish abundance often increases in areas of woody debris (Angermeier and Karr 1984), which is linked to the amount of forested cover along banks and within riparian areas. Trees and the hard or soft mast they produce provide sustenance for ground-nesting birds such as wild turkey (*Meleagris gallpavo*), small mammals like the white-footed mouse, and larger herbivores such as white-tailed deer. Peak acorn production and densities of white-footed mice were highly correlated indicating the importance of mast to small mammals (Ostfeld et al. 1996).

Ectothermic species such as aquatic turtles, water snakes, salamanders, toads, frogs, and other herpetofauna have smaller home ranges, limited dispersal capabilities, and require heterogeneity within their habitat so that they may move from areas of higher or lower temperatures for thermoregulation (Walton 2012). Complexity of stream bank substrates have been measured for water snakes (Cross and Peterson 2001; Pattishall and Cundall 2009) and aquatic turtles (Fuselier and Edds 1994), as these provide cover and basking areas. Submerged aquatic vegetation at the edge of banks also provides cover for aquatic turtles, along with seasonal breeding habitat for toads, frogs, and salamanders. A variety of habitat features such as herbaceous ground cover, leaf litter, and decaying logs provide salamanders with the necessary microclimate conditions on the forest floor (Maser et al. 1979; Faccio 2003).

Aerial species such as birds and bats are not necessarily dependent on any specific habitat patch given their ability to fly, but still have specific habitat needs. Trees provide bats with roosting sites in the form of natural and excavated cavities, exfoliating bark, top-outs, splits, and fissures. Live tree species supporting higher number of bat roosts include shagbark hickory, black locust, and sugar maple, while American elm and oak trees were selected for when dead (Waltrous et al. 2006). Migratory birds and raptors also use snags for perching and roosting. Other significant habitat features for Neotropical migrants include the amount of canopy cover, understory and overstory stem densities, and the presence of cavities (Saab 1999; Batten and Lawler 2006).

Research on stream restoration has been ongoing by the Cooperative Wildlife Research Laboratory at Southern Illinois University since the early 1980s (Nawrot et al. 2009), but our knowledge is yet incomplete about how wildlife respond to reclamation in riparian areas. Scientists and land managers must understand the roles that habitat quality and landscape connectivity play in the movement of wildlife over the greater landscape as a naturally-functioning riparian ecosystem provides important habitat and serves as movement corridors (Boutin et al. 2003; Lees and Peres 2008). As part of a larger project assessing stream restoration efforts (Williard et al. 2013), our objective was to compare habitat characteristics of mined vs. unmined stream corridors in Perry County, Illinois, 30 years after the initiation of habitat restoration activities. We wished to assess the success of habitat restoration while providing information to land managers in which to guide future stream restorations and riparian management projects.

Landscapes that have been subject to surface mining and reclamation where the vegetation has been stripped away and later replaced may have been altered by past land use and be of a different quality for wildlife habitat than that of unmined

riparian buffers. We predicted that mined areas would be comprised of more grassland, have smaller trees, and lack the leaf litter and log-wood ground cover compared to unmined riparian buffers. Lack of mature forests also presents issues of hard-mast availability, which species like deer, turkey, and mice rely on for sustenance. Many wildlife species select for stream corridors that are comprised of more forested habitat, therefore restored riparian buffers may not adequately support all of the species of wildlife that would inhabit, disperse, or migrate through the area.

Study Area

Wildlife habitat was assessed at Burning Star 4 (BW4) mine (Figure 1; BS4 consists of Galum Creek and Bonnie Creek); Pipestone Creek; and Little Galum Creek, an unmined reference site in Pyramid State Park; in Perry County, Illinois. On the western portion of BS4, the 4.3-mile Galum Creek restoration was completed in 1980, and on the eastern side, the 3.7-mile Bonnie Creek restoration was completed in 1997. In 2001, Galum Creek and Bonnie Creek were reconnected to their local watershed with the streams meeting at a confluence at the southern end of the property. The restoration of 4.6-mile Pipestone Creek was completed in 1991 and is now managed, along with Little Galum Creek, as part of Pyramid State Park.

Potential wildlife habitat on restored streams consists of agricultural lands, reforested riparian buffers, and numerous wetlands. During the restoration process, riparian buffers were planted with trees and grasses. Dominant tree species within the riparian buffers included, but are not limited to: oak (*Quercus spp.*), hickory (*Carya spp*.), silver maple (*Acer saccharinum*) and boxelder (*Acer negundo*). Dominant herbaceous plants and grasses observed were goldenrod (*Solidago spp*.), sunflower (*Helianthus spp*.), bush clover (*Lespedeza spp*.), broomsedge (*Andropogon virginicus*), and Virginia wild rye (*Elymus virginica)*.

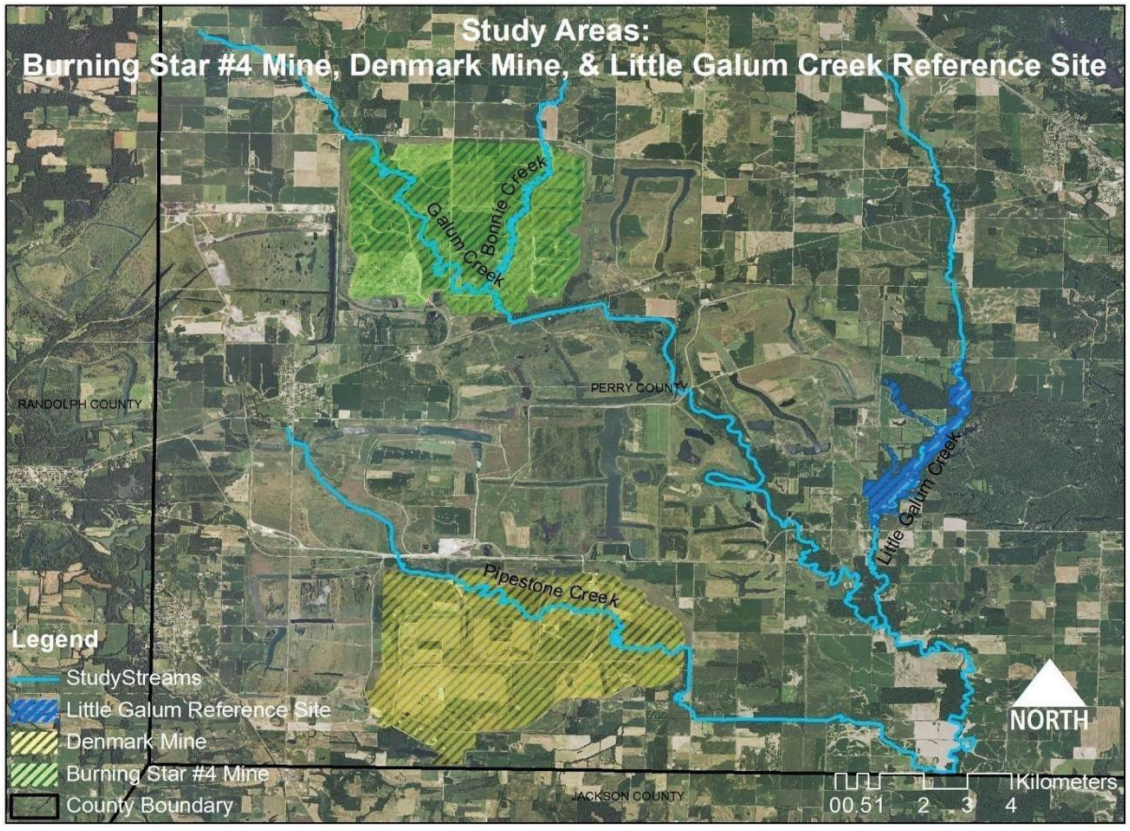


Figure 1. Study sites for wildlife habitat measurements at restored and unmined stream sites in Perry County, Illinois, 2012-13.

Methods

A list of focal wildlife species known to inhabit the study area was used to determine a set of 41 habitat variables to measure (Table 1). Habitat measurements were taken during July-September 2012 and 2013 at 200 plots at Galum Creek, Bonnie Creek, Pipestone Creek, and Little Galum Creek sites. Ground cover was measured by a modified version of the step-point method (Evans and Love 1957); steps were walked on three parallel 20-m transects spaced 10-m apart. Each step was classified to one of the eight following cover types: bare ground, herbaceous (i.e., forb and grass), rock, woody debris, grass, shrub (stems < 1.5 m in height), ground litter, or phragmites. Percent canopy cover was measured with a densiometer at the center of plots, held at elbow height, and facing each cardinal direction. Visual obstruction was measured by the average percent of a 1.5-m cover pole obscured from 10 m away in each cardinal direction (Griffith and Youtie 1988). The cover pole was divided into three sections: low (< 0.5 m), mid (0.5 - 1.0 m), and high (1.0 - 1.5 m) with each section consisting of 10 5-cm sections. Understory and overstory stem densities were measured by the number of woody stems > 1.5 m in height and > 7.5 cm dbh for overstory trees or < 7.5 cm dbh for understory trees. Trees counted were within arm's length (82 cm) of the same three transects as walked for ground cover. While categorizing overstory and understory stem densities, we also categorized whether the tree produced hard mast or soft mast. Hard-mast species were oak, hickory and American beech (*Fagus grandifolia*); other masting trees fell into the soft mast category. Downed logs within an arm's length along each transect were classified as either > 7.5 cm or < 7.5 cm and given a decay class ranking from 1 - 5 (adaptation from Maser et al. 1979). Class 1 logs had intact bark with twigs present and a portion of the tree was still elevated on support points above the ground. Class 2 logs had intact bark, twigs were absent and the texture of the tree was slightly softened; the tree was still elevated but sagging slightly. Class 3 logs had traces of bark, but no twigs, with the tree resting on the ground. Class 4 logs had no bark or twigs with the entire tree on the ground. Class 5 logs were oval-shaped, soft and powdery, and red-brown to dark-brown in color with the entire tree on the ground. Tree species with exfoliating bark, cavities, fissures, snags, and top-outs also were noted because they provide habitat for birds, bats, and other wildlife. Bank vegetation was categorized as the cover type that was the most prominent of the eight cover types used as categories for ground cover.

Differences in variable values among the four study sites were first tested for using Kruskal-Wallis ANOVAs and the Wilcoxon Two-Sample Test for pairwise comparisons when overall ANOVAs were significant ($\alpha = 0.05$ throughout). Correlations were then assessed among significant habitat variables using Spearman rank correlations, to further consider only uncorrelated variables. All statistical analyses were performed using SAS 9.3.

Results

Overall, microhabitat differences were very minor among sites. Of the 41 microhabitat variables measured, 27 did not differ among sites ($0.0593 \leq P \leq 0.8825$). Fourteen variables differed among sites ($0.0001 \leq P \leq 0.0140$): canopy cover, low vertical vegetative cover, mid-height vertical vegetative cover, overall vegetative cover, understory and overstory stem densities, understory soft mast, overstory soft mast, overstory hard mast, bare ground, herbaceous ground cover, log-woody debris ground cover, grass cover, and leaf litter cover. Four of those variables were uncorrelated (Table 2): canopy cover, overstory hard mast, bare ground, and herbaceous ground cover. Canopy cover was lowest at Bonnie Creek, moderate and similar at Galum Creek and Pipestone Creek, and highest at Little Galum Creek. Herbaceous ground cover did not differ among Bonnie Creek, Pipestone Creek, and Little Galum Creek, and was significantly lower at Galum Creek. Bare ground cover was similar at Galum Creek, Little Galum Creek, and Bonnie Creek, with Bonnie Creek and Pipestone Creek values overlapping. Overstory hard mast was similar between Little Galum Creek and Galum Creek, but Galum Creek overstory hard mast overlapped with Bonnie Creek and Pipestone Creek.

Table 1. Habitat variables and description of sampling methods conducted at restored and unmined stream sites in Perry County, Illinois, July–September 2012-13.

Variable	Description and Method	Citation
Overall Vertical Vegetation	Avg. percent of a 1.5-m high cover pole obscured from 10 m away in each cardinal direction. Cover pole divided by 30 5-cm sections. Data recorded as percentage of 5-cm sections obscured (>50%) by vegetative or structural cover.	Griffith and Youtie 1988
Low Vegetative Cover	Avg. percent of low portion (<0.5 m) of cover pole obscured by vegetative or structural cover.	Griffith and Youtie 1988
Mid-Height Vegetative Cover	Avg. percent of middle portion (0.5-1.0 m) of cover pole obscured by vegetative or structural cover.	Griffith and Youtie 1988
High Vegetative Cover	Avg. percent of high portion (1.1-1.5 m) of cover pole obscured by vegetative or structural cover.	Griffith and Youtie 1988
Bare Ground Cover	Same as herbaceous cover except for bare ground.	Evans and Love 1957
Herbaceous Ground Cover	Hits/steps x 100 using step-point method, walked on three parallel, 20-m transects. Hits = steps along transect resulting in tip of shoe covering herbaceous ground cover.	Evans and Love 1957
Grass Cover	Same as herbaceous cover except for grass cover.	Evans and Love 1957
Rock Cover	Same as herbaceous cover except for rock cover.	Evans and Love 1957
Log/Woody Debris	Same as herbaceous cover except for log/woody debris.	Evans and Love 1957
Litter Cover	Same as herbaceous cover except for areas covered by leaf litter.	Evans and Love 1957
Shrub Cover	Percent cover of shrub stems measured on three 20-m transects. Sampled height range was 1.5 m.	Evans and Love 1957
Canopy Cover	Measured with densiometer at plot center; held 30-45 cm from body at elbow height, facing each cardinal direction.	Kolowski and Woolf 2002, Pattishall and Cundall 2009
Understory Stem Density	Number of woody stems >1.5 m in height and <7.5 cm dbh counted within same three arm's-length transects used for ground cover.	Kolowski and Woolf 2002
Overstory Stem Density	Number of woody stems >7.5 cm dbh counted within three arm's-length transects. Transects same as for understory stem density.	Kolowski and Woolf 2002
Downed Logs >7.5cm	Visual count, then assign decay class.	Saab 1999, Faccio 2003
Downed Logs <7.5cm	Visual count, then assign decay class.	Saab 1999, Faccio 2003
Log Decay Class	1 = freshly fallen, supported above soil by branch 2 = structurally sound, bark covered 3 = intact but rot and bark loss beginning, resting on soil 4 = soft with little bark remaining 5 = almost completely incorporated into soil	Maser et al. 1979
Hard/Soft Mast	Visual account of species. 0 = under mast producing age, 1 = soft mast, 2 = hard mast	Nupp and Swihart 2006
Roost Trees	Visual account of number of standing dead snags.	Watrous et al. 2006
Cavities/Bark/Split	Y/N; Presence of trees with loose bark, cavities, splits.	Watrous et al. 2006
Submerged Vegetation	Visual presence.	Fuseliar and Edds 1994
Bank Cover	Percentage; categories same used for ground cover.	Pattishall and Cundall 2009, Lesmeister and Nielsen 2011

Table 2. Differences in microhabitat variables among restored and unmined stream sites in Perry County, Illinois, July-September 2012-13. Little Galum Creek was the unmined control site. Different letters following mean ± SD values indicate pairwise differences among sites.

Variable	Galum Creek n = 45 plots	Bonnie Creek n = 35 plots	Pipestone Creek n = 47 plot	Little Galum Creek n = 73 plots
Canopy Cover	0.37 ± 0.34 B	0.22 ± 0.35 C	0.37 ± 0.40 B	0.85 ± .09 A
Herbaceous Ground Cover	0.09 ± 0.10 A	0.31 ± 0.24 B	0.21 ± 0.16 B	0.19 ± 0.10 B
Bare Ground Cover	0.07 ± 0.08 A	0.04 ± 0.04 A	0.01 ± 0.03 B	0.06 ± 0.10 A
Overstory Hard Mast	0.01 ± 0.01 A	0.01 ± 0.04 A,B	0.01 ± 0.02 B	0.01 ± 0.01 A

Discussion

This 30-year-post-mining assessment of wildlife habitat within the restored riparian buffers of Galum, Bonnie, and Pipestone Creeks in southern Illinois demonstrates that habitat conditions over that time period are suitable and highly desirable for many of the focal groups of southern Illinois wildlife that were used for the basis of the assessment. Previous research has shown that reclaimed mine lands can provide ample and suitable habitat for the many species of wildlife in the Midwest, including common, threatened, and endangered species (Lannoo et al. 2009; Zipper et al. 2011). Our results indicate that these restored riparian buffers are comparable to that which might be found on lands that have not previously been exposed to surface mining. These successful restoration processes, such as reforestation of the riparian buffers, combined with planting native grasses, shrubs, and herbaceous plants, aid in sustaining the wide diversity of wildlife species that disperse throughout, reside within, or migrate through southern Illinois. We recognize that choice of the control (i.e., not previously mined) stream segment (in our case, Little Galum Creek) was very important and that just because a stream segment is on unmined ground does not imply that it was unaffected by human activity. Certainly some habitat modifications, such as earlier tree cutting, prior channelization, and adjacent farming activities, made our unmined site less than pristine wildlife habitat, as such occasions are rare on the Illinois landscape. However, we believe our control site was certainly adequate for the comparisons presented herein.

Site-level microhabitat variables measured for this study were chosen for the general wildlife species and groups of species known to inhabit southern Illinois. Approximately 80-88% of the ground cover and forest understory at all four creeks was composed primarily of grasses, herbaceous plants, and litter cover which indicates they all provide important ground cover for small mammals, reptiles and amphibians (Adler and Wilson 1987; Cross and Peterson 2001; Nupp and Swihart 2001; Pattishall and Cundall 2009). Mammal runs were present at the northern end of Bonnie Creek, even in the mowed area, and predated turtle shells were also found within that area, providing evidence that those and other wildlife use this predominately grassy corridor. Overstory stem density is an important habitat characteristic for large mammals, small mammals, aquatic mammals, reptiles, amphibians, and birds (Anderson et al. 1991; Bowyer et al. 1995; Saab et al. 1999; Nupp and Swihart 2001; Kolowski and Woolf 2002; Pattishall and Cundall 2009), and did not differ between restored and unmined streams. We similarly found no differences among streams

for vertical vegetative cover, which is important for large, small, and aquatic mammals (Bowyer et al. 1995; Nupp and Swihart 2001; Kolowski and Woolf 2002).

The minor differences in habitat characteristics we observed among sites were likely due to differences in forest age among sites, such that the forest at the unmined Little Galum Creek site was considerably older than the restored stream sites. Little Galum Creek had the highest percentage canopy cover at 85%, more than double that of the restored streams, and there was a much greater likelihood of encountering snags, cavities, and mature trees with fissures and loose bark such as shagbark hickory (*C. ovata*), black locust (*Robinia pseudoacacia*), sugar maple (*A. saccharum*), and sycamore (*Platanus occidentalis*).

While taking field measurements around these restored wetland areas, we observed great egrets (*Ardea alba*) perched in mature baldcypress trees (*Taxodium distichum*), double-crested cormorants (*Phalacrocorax auritus*) drying their wings on snag structure that had been placed in the water, deer browsing on new growth, and birds perched on trees that will eventually become a closed-canopy forest. These restored riparian buffers contained many young oak trees that with time will mature into healthy bottomland hardwood forest similar to that observed at the reference site. Wildlife species will benefit at different times from early-successional habitat in restored stream corridors (Walton 2012). During succession, in general, grasslands change to mixed understory, and mixed understory transitions to mature closed canopy forest where soft mast recedes and hard mast tree species begin to shade out the ground vegetation. Some wildlife species will benefit from the change, move about, and begin to colonize new areas, and other species will leave and find early-successional habitat that will suit their needs over time (DeGraaf and Yamasaki 2003; Walton 2012). Had these areas been planted entirely to row-crop agriculture, wildlife habitat value would be considerably limited.

Management and Policy Implications

Our analysis from data collected at Burning Star 4 mine in Perry County, Illinois, is of value to land managers for it shows how the reclamation process has successfully jump-started wildlife habitat in buffers around streams affected by mining. Corridors at Bonnie Creek could be improved upon through additional reforestation, as they had the least canopy cover and the most herbaceous plants and bare ground cover. Corridors at Bonnie Creek also were the narrowest, so our recommendation for future restoration projects with wildlife habitat as a strong interest is to implement wider corridors since they provide more surface area for wildlife (Johnson and Buffler 2008). While canopy cover was much higher at the unmined control site, all streams had similar levels of hard mast trees within the overstory; as these forests grow, the restored streams will increase in the amount of wildlife habitat to further resemble a forest that has never been mined. For wildlife to persist in an area, all elements of habitat must be present for their specific and unique utilizations; rotting logs, an assortment of hard and soft mast trees of multiple ages and species, leaf litter, and herbaceous plants are all necessary to supply the local wildlife with habitat (Maser et al. 1979; Converse and Savidge 2003; Faccio 2003; Nupp and Swihart 2001). Over time and with proper land management, including proper plantings during reclamation and post-reclamation management, it is reasonable to believe that these restored forests will grow to have those similar characteristics of the unmined forests.

Lastly, 95% of land is privately owned in the state of Illinois and more private landowners should take advantage of incentives offered from Illinois Department of Natural Resources and the U. S. Department of Agriculture. These agencies provide opportunities for landowners in Illinois to enhance the wildlife habitat on their own private properties via grants, free trees, and/or yearly rental payments for restoring forests, grasslands, and wetland habitats on private property.

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Impacts of Pollutants on Beavers and Otters with Implications for Ecosystem Ramifications

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Abstract: Anthropogenic pollution has impacted ecosystems and organisms globally. Aquatic freshwater systems are of particular concern because of their importance to human health and livelihoods. Sentinel species can serve as indicators for both individual and population-level health risks to both wildlife and humans, and therefore facilitate the mitigation and prevention of such contamination. When such species are also keystone species and/or ecological engineers in aquatic ecosystems, understanding the influence of pollutants on their physiology and behavior acquires added importance. Given that river otters (*Lontra canadensis* and *Lutra lutra*) and beavers (*Castor canadensis* and *C. fiber*) serve such roles and are susceptible to a wide-range of anthropogenic pollution, this makes them prime candidates as indicators or sentinels of aquatic ecosystem health. We review published evidence on the toxicological impacts of pollutants in beavers and river otters and discuss the implications of exposure to their behavioral systems, conservation, and surrounding ecosystem.

Keywords: *bioindicator, keystone species, ecosystem engineers, sentinel species, toxicity*

Ecotoxicologists take an integrative approach to understanding the effects of contaminants in the biosphere at the individual, population, species, and community levels in both terrestrial and aquatic ecosystems (Rattner 2009; Relyea and Hoverman 2006). A particularly strong body of research exists that examines the vulnerability of aquatic ecosystems to pollutants ranging from trace to high concentrations (Carpenter et al. 2011; Douglas et al. 2012; Houde et al. 2011; Rosi-Marshall and Royer 2012; Woodward et al. 2012). Direct toxic exposure in an aquatic ecosystem can result in lethal or sublethal health effects on aquatic wildlife (Fleeger et al. 2003). Even natural chemical signals, such as kairomones, can have cascading ecological effects (Marino et al. 2015), revealing the sensitivity of these systems to chemical inputs. The presence of water-borne pollutants is particularly likely to initiate a trophic cascade if pollutants exert either lethal or sublethal effects on foundation species (e.g., primary producers or species that affect ecosystem structure, such as hard corals [see Angelini et al. 2012]), ecosystem engineers (e.g., in some cases herbivores that modify habitat like beavers or elephants [Jones et al. 1994]), or keystone species (such as terminal or near terminal trophic predators [Fleeger et al. 2003]). For this paper, we focus on the sublethal effects of water-borne pollutants and the roles of an ecosystem engineer, the beaver, and a keystone species, the otter, in freshwater habitats. Our tenet is that the interactions between two such ecosystem forces can potentially have profound impacts on the ecosystem that might not be predicted by considering only one.

Ecosystem engineers are those species that "directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials" (Jones et al. 1994, 373). Eurasian and North American beavers (*Castor fiber* and *C. canadensis*, respectively) are notable ecosystem engineers because they create wetlands, affecting sediments, organic matter, nutrient cycling, decomposition, water flow and temperature,

and plant and animal communities (Naiman and Melillo 1984; Naiman et al. 1986; Naiman et al. 1988; Naiman et al. 1994; Wright et al. 2002; Rosell et al. 2005). Similar to keystone species, ecological engineers can vary in their extent of impact based on location and habitat (Rosell et al. 2005; Moore 2006; Brzyski and Schulte 2009), requiring an understanding of abiotic, biotic, and anthropogenic inputs that affect ecosystem engineering. Beavers make their most profound impact on water flow and aquatic patch creation in their first two decades following colonization (Johnston and Naiman 1990). Thus, the flow of nutrients and pollutants within and through the system is dependent in part on the duration and extent of beaver-created wetlands and whether such systems go through their natural process of succession (Correll et al. 2000). While beavers themselves may be affected by pollutants, the ramifications of toxins on altering beaver activity as ecosystem engineers has the potential for much more profound ecological impacts.

As a top predator in this system, and one that interacts with beavers in various ways, river otters (*Lutra lutra* and *Lontra canadesis*) serve as a keystone species (Estes and Duggins 1995; Roemer et al. 2009) and a sentinel species for freshwater health. The keystone species concept was introduced by Paine (1969) to describe the critical importance of a top predator (a starfish) in an intertidal habitat. The term lost some value because it became increasingly used for a variety of other species and ecosystem effects. Davic (2003) attempted to rectify the issue by maintaining the top-down ecological role of keystone species and adding a delimiter that the effects of a keystone species are large relative to its occurrence within its functional group (e.g., suite of prey species). The scale of impact necessary to be labeled keystone has not been firmly established, but modeling trophic webs using a mixed trophic matrix can help to identify the level of 'keystoneness' for a species or group of species (Libralato et al. 2005). A particular species may not always be keystone, but the species plus the particular ecosystem interactions determine the extent to which a particular species plays a keystone role (Libralato et al. 2005; Menge et al. 1994). The use of keystone has even been extended further to natural, defensive compounds, such as guanidine alkaloid neurotoxins, that affect community structure (Zimmer and Ferrer 2007). Thus, species and chemicals may exert a stronger effect on community structure, typically through trophic dynamics, than would be expected by their abundance, indicating a keystone role in the ecosystem.

Humans have long used other species as indicators of potential toxin threats to humans; these species are termed sentinel species (van der Schalie et al. 1999). Sentinel species may react to these toxins before major environmental problems ensue (Pritchard et al. 1997; Milne and Guillette 2008). In addition, they may be more sensitive than humans to exposure and/or respond in a similar fashion as humans (Pritchard et al. 1997; Milnes and Guillette 2008). In aquatic environments, widespread apex predators, such as bald eagles (*Haliaeetus leucocephalus*) and osprey (*Pandion haliaetus*) (Grove et al. 2009), marine mammals (Bossart 2011), and mustelids (Basu and Head 2008) serve well as sentinel species. A number of studies have examined the exposure of mustelids to a variety of contaminants including pesticides, heavy metals, cesium 137, polychlorinated biphenyls (PCBs), and crude oil (see Table 1 in Bowyer et al. 2003), providing a strong foundation for examining environmental impacts.

Species may be considered keystone, ecosystem engineers, sentinels or some combination of these three classifications. The commonality among species in these classifications is that such species exhibit strong interactions with the biotic and often abiotic components of their habitat (Soulé et al. 2005). The benefit of interactivity as a concept is akin to that of keystoneness (see above) – it suggests a continuum and not a duality in relative importance of species to ecosystem function. Clearly, all species have the potential to be detrimentally affected by contaminants, yet not all species can be effectively examined. Thus, those species with greater connectedness and impact on ecosystem operation provide a focus. When these species also are appealing to humans and parallel aspects of human physiology and behavior, they can be especially operative bellwethers of impending ecological damage.

In this paper, we examine beavers as ecosystem engineers and river otters as keystone species. We suggest that they might serve together as a sentinel species unit in light of aquatic contamination. Considering the two species is interesting because of their shared habitat yet different trophic levels. River otters are common in and around beaver ponds (LeBlanc et al. 2007) and have been considered competitors with beavers and sometimes predators on young beavers (Saunders 1988; Reid et al. 1994, 1998). In northern winters, otters will travel from one beaver pond to another, creating holes in dams for passage. This lowers the water level in a pond until the beavers can repair the dam. This periodic change in water flow even during winter could affect the movement of contaminants. In addition, the differences in trophic level (herbivorous beavers and carnivorous otters) and mobility (high site fidelity by beavers compared to otters) can influence the degree of biomagnification (Kidd et al. 2012) that may occur in these species and the environment. Together, these factors warrant considering the two species as a meaningful bioindicator unit and thus motivate their combined conservation (Kushlan 1993). We discuss the behavioral and toxicological impacts of anthropogenic pollutants on the conservation of beavers and river otters. To accomplish this, we first present the natural history of these species as a conservation concern. Second, we review the published evidence on the toxicological impacts of anthropogenic pollutants on these species and suggest potential behavioral abnormalities that may result as a consequence of exposure to anthropogenic pollution. Finally, we highlight the potential ecological implications of such exposure and behavioral abnormalities.

Natural History and Historical Conservation of Beavers and River Otters

The two extant beaver species are semi-aquatic rodents that belong to the order Rodentia and family Castoridae (Müller-Schwarze 2011). Prior to the nineteenth century, both species resided in a wide range of ecoregions, ranging from subtropical to subarctic throughout the globe, including the American, European, and Asian continents (Rosell et al. 2005). Due to overhunting for castoreum and fur, as well as agricultural expansion, beavers on a large portion of the North American continent were nearly extirpated by the end of the 20th century (Macdonald et al. 1995; Schulte and Müller-Schwarze 1999). Similarly, Eurasian beavers were over-hunted until there were approximately 1200 left in only a few isolated regions (Nolet and Rosell 1998). Since then, government-mandated artificial and natural recolonization of beavers have aided in the re-expansion of beavers on both continents. Beavers have been used to restore wetlands, improve stream dynamics, and reduce flooding (Müller-Schwarze 2011; Gibson and Olden 2014). The most recent estimate on the population size of the North American beaver is 6-12 million (Naiman et al. 1988) and the Eurasian beaver is estimated at 639,000 (Halley and Rosell 2003).

Similar to the beaver, river otters were once abundant throughout the waterways of Canada, the United States (Lariviere and Walton 1998), and Europe (MacDonald and Mason 1994). Drastic population declines of river otters (*Lontra canadensis*) in North America have been attributed to hunting for pelts, urbanization, changes in the availability of food sources, and pollution (Latch et al. 2008; Serfass et al. 1993). In the United Kingdom, reductions in the Eurasian otter (*Lutra lutra*) populations occurred starting in the 18th century, initially because of perceived competition with humans for fish and for sport, and later from pollution, persecution, habitat loss, and prey reduction (Jeffries 1989; Kruuk and Conroy 1991; Kruuk 1995). A similar pattern occurred for populations in mainland Europe (MacDonald and Mason 1994). Protection and recovery came slowly as by the early 1980s river otters were still rare in many locales west of the Mississippi (Jenkins 1983), while populations in Europe remained sparse or absent in many historic locations (MacDonald and Mason 1994). Over the next twenty years, reintroductions and improved habitats led to an expansion of river otters to much of their former range in North America (Raesly 2001), and in some places in Europe (Robataille and Laurence 2002). At least in coastal areas, natural range expansion can be relatively slow such that translocations may still be important for restoring populations (Blundell et al. 2002b).

Exposure to Anthropogenic Pollutants

Anthropogenic contamination has been considered to be one of the factors responsible for decreasing population sizes in otters in Europe (Chanin and Jefferies 1978; Gutleb 2000; Mason et al. 1986) and North America (Wren 1991), but the role of pollution in declining beaver populations on both continents is unknown. Exposure to PCBs and other organochlorides may be responsible for later twentieth century population declines in both American (Wren 1991) and Eurasian otters (Mason et al. 1986). River otters have been of particular interest to wildlife toxicologists due to their potential as biomonitors of environmental contaminants for humans, water quality, and riparian habitat conservation (Anderson-Bledsoe and Scanlon 1983; Basu et al. 2005a; Basu et al. 2005b; Kannan et al. 2002). On the other hand, there is little primary literature showing the effects of environmental contaminants in beavers for either *C. canadensis* or *C. fiber* (Fimreite et al. 2001; Giżejewska et al. 2014; Giżejewska et al. 2015; Hillis and Parker 1993; Nolet et al. 1994; Wren 1984; Zalewski et al. 2012).

Deaths resulting from direct poisoning are rare, potentially due to sampling limitations in the field. However, sublethal effects (low-level to high-level not fatal exposure) could cause organ failure and disease, as well as impact life history and behavioral systems responsible for successful reproduction and population dynamics. Furthermore, the widespread distribution of multiple pollutants at once increases the likelihood of synergistic effects. Together, these factors can result in population declines and prevent impacted populations from rebounding.

Both species accumulate various metals and organochlorine chemicals. In both otters and beavers, the focus of research has been on tissue and body burdens as a result of mercury exposure in river otters and cadmium exposure in beavers due to their food preferences. Accumulation of metals (arsenic, cadmium, lead, mercury, selenium, copper, manganese, and zinc) and PCBs in both species can be age-specific (Fimreite et al. 2001; Giżejewska et al. 2015; Hillis and Parker 1993), sex-specific (Walker et al. 2011), and tissue-dependent (Chadwick 2007; Kang et al. 2015; Wren 1991; Zalewski et al. 2012).

Beavers

For beavers, the focus has been on the accumulation of cadmium in tissues due to their herbivorous nature and consumption of plant material exposed to hazardous concentrations of this and other metals (Nolet et al. 1994). For example, mercury accumulates in both beavers (Giżejewska et al. 2014; Sheffy and Amant 1982; Wren 1984) and beaver wetlands (Driscoll et al. 1998; Painter et al. 2015). Beavers prefer an herbaceous and woody diet for both consumption and engineering of dams and dens (Bryzski and Schulte 2009), leaving them susceptible to pollution in the aquatic ecosystem and plant material.

Elevated levels of cadmium in tissues have been reported in multiple studies in both polluted areas and areas considered "ecologically clean" (Fimreite et al. 2001; Giżejewska et al. 2014; Giżejewska et al. 2015; Nolet et al. 1994; Zalewski et al. 2012). Fimreite et al. (2001) found that beavers' preferred plant species (aspen [*Populus* spp.], birch [*Betula* spp.], and rowan [*Sorbus* spp.]) were the highest accumulators of cadmium and that this likely induced the high cadmium concentrations found in beaver tissues in their study. Levels of cadmium in both the kidney and hair are correlated with concentrations of cadmium in both the bark and leaves of willow (*Salix* spp.) and poplar (*Populus* spp.) trees (Nolet et al. 1994). In the only known study of relocation of beavers into a contaminated area (Nolet et al. 1994), accumulation of cadmium in the kidney significantly increased in relation to the residential time, i.e. the length of presence in the contaminated ecosystem. In less than six months, there was a threefold increase in cadmium concentration in the hair (Nolet et al. 1994).

In addition, there is a relationship between fat content (an indicator of condition) and cadmium concentration in the kidneys; higher than normal cadmium concentrations may therefore reduce fat content in beavers (Fimreite et al. 2001). They store fat in their tails in an effort to decrease potential starvation during harsh winter conditions (Aleksiuk 1970). Therefore, cadmium exposure may increase their susceptibility to not only disease but also over-wintering starvation.

Otters

While otters consume a large number of species, they feed mostly on fish and shellfish (90% of their diet), and less so on other invertebrates, amphibians, birds, and mammals (Anderson-Bledsoe and Scanlon 1983; Carss 1995; Cote et al. 2008; Evans et al. 2000; Fretueg et al. 2015; Kean et al. 2013; Mason et al. 1986; Reid et al. 1994). This is of prominent conservation concern because the position of river otters at the top of the food chain increases their susceptibility to bioaccumulation of a wide-range of contaminants (Anderson-Bledsoe and Scanlon 1983; Evans et al. 2000; Kean et al. 2013; Mason et al. 1986).

Accumulation of mercury is well documented in mustelid tissues, particularly otters. Mercury poisoning is considered a risk factor for river otter survival because it is a ubiquitous neurotoxin that readily bioaccumulates in the food chain (Basu et al. 2005a; Basu et al. 2005b; Dornbos et al. 2013). Concentrations of mercury have been observed in various tissues in otters (Evans et al. 2000; Dornbos et al. 2013; Sleeman et al. 2010; Wren 1984; Wren et al. 1986; Giżejewska et al. 2014). Several authors have found quantifiable mercury levels in the brain (Dornbos et al. 2013: Evans et al. 2000), with significant mercury-associated neurochemical changes (Basu et al. 2005a; Basu et al. 2005b; Basu et al. 2007a; Basu et al. 2007b; see Basu and Head 2008 for a complete review; Dorbos et al. 2013). Thus, mercury exposure can have severe effects on the nervous system of river otters.

During and post oil spills, both river and sea otters are at risk of sublethal exposure to pollutant petroleum products in coastal marine regions via consumption of prey and during grooming. Run-off from engine oil discharges contributes to widespread contamination of hydrocarbons in freshwater ecosystems; this leads to chronic exposure to a host of chemical compounds that comprise gasoline (Mason 1989). Sublethal exposure induces many resultant health deficits in river otters, including increased haptoglobin (an indicator of tissue damage), decreased body mass, chronic inflammation, liver injury (Duffy et al. 1993; Kimber and Kollias 2000), and malnutrition (Kimber and Kollias 2000). Acute exposure reduces waterproofing of their fur, resulting in heat loss and hypothermia (Mason 1989; Stoskopf et al. 1997).

Oil spills in coastal regions can lead to changes in the diet of river otters (Bowyer et al. 1994) by reducing the variety of prey (Kimber and Kollias 2000). In a study by Ben-David et al. (2000), river otters experimentally exposed to oil from an oil spill were anemic relative to control otters; the oiled otters had higher oxygen consumption when foraging and dove for food less. In addition, exposure to petroleum products reduces buoyancy (Mason 1989) and increases the number of abandoned latrine sites (Kimber and Kollias 2000), which are important in social recognition and spacing (Oldham and Black 2009).

Potential Behavioral and Ecological Ramifications

Individual-level effects on behavior (such as movement and spatial use, foraging, social organization, and reproductive behavior) can impact biodiversity (Berger-Tal et al. 2011), particularly in this system due to the status of both the otter and beaver individually, and synergistically. Therefore, considering behavioral ramifications will be important in promoting conservation efforts for both species and the aquatic ecosystem at large.

Behavioral Ramifications

Toxicants can have a variety of physiological effects that can alter or eliminate behaviors important for survival and reproduction in the exposed generation (Clotfelter et al. 2004; Scott and Sloman 2004; Weis et al. 2001). As a result of low-level exposure to endocrine-disrupting chemicals (EDCs), a range of behaviors important for fitness and effective social organization of populations can be altered (including but not limited to: mating, aggression, territoriality, parental care, coordination and balance, memory, chemosensory communication, and motivation and activity; see Zala and Penn 2004 for a complete review). EDCs that adversely affect predatory behavior, parental care, and sociality, in particular, could potentially alter species interaction with the ecosystem and change the ecosystem dynamics via predator-prey interactions and use of latrine sites. In addition,

remediation of EDCs from the environment does not eliminate resulting behavioral modifications, as shown in studies testing transgenerational exposure (meaning that the parental generation is exposed and three subsequent generations are unexposed, see Anway and Skinner 2006 for a complete review; Crews et al. 2007). This means that even low-level exposure to EDCs can have long-lasting effects on conservation efforts, well past environmental remediation.

In otters, decreased appetite, decreased pup survival, and reproductive failure are a common response to a host of pollutants (Kean et al. 2013; Wren 1991 for a complete review). Kean et al. (2013) found a reduction in baculum weight and increases in reproductive abnormalities (such as cryptorchidism and cysts on the vas deferens), attributed to high levels of persistent organic pollutants. Reductions in male reproductive organs can potentially feminize males, inducing changes in cognitive processing, stress responses, sexual behaviors, and territorial behaviors, as shown in research in mammalian model systems (von Saal et al. 1995; Zuloaga et al. 2008). In response to cadmium toxicity, beavers exhibited a similar response in reproduction: decrease in reproductive fertility via abnormal sperm counts (Zalewski et al. 2012). Changes in reproductive viability are likely to have a cascading effect on continued conservation of these species and maintenance of their status as sentinels, keystone, and ecosystem engineers.

Ecological Ramifications and Synergistic Effects

The presence of otters often correlates with that of beavers (DePue and Ben-David 2010; Dubuc et al. 1990; Gallant et al. 2009; Newman and Griffin 1994; Swimley et al. 1999). Thus, the protection and reintroduction of beavers can be a positive contributing factor to the recovery and maintenance of river otter populations, at least in North America (Collen and Gibson 2001; LeBlanc et al. 2007). However, this also indicates that each species could not only be exposed to similar contaminants, but their individual and interactive action could affect the storage or flow of said contaminants.

The predatory behavior and the creation of latrines by river otters influence the dynamics of both their aquatic and terrestrial habitats. Piscivory by river otters facilitates the movement of nutrients, notably nitrogen and phosphorus between sea or freshwater systems and land, enhancing productivity of terrestrial vegetation (Ben-David et al. 1998; Lilleskov et al. 2001; Roe et al. 2010). The social nature of river otters also relates to the species of fishes caught, and the availability of particular fish species may affect the distribution of social otters. Male river otters are found in social groups more often than females who care for the young (Blundell et al. 2002a).

In coastal Alaskan waters, social otters cooperatively hunt on fast swimming pelagic fish, but sociality is not kin based (Blundell et al. 2004). Although typically having a diverse diet, river otters may be more restricted in some systems, such as their dependency on cutthroat trout in Yellowstone Lake (Crait and Ben-David 2006). In such situations, factors, including toxins, which adversely affect one or a few prey species, could have more immediate and dire consequences than for otters with a more diverse diet.

Latrine sites are areas that are scent-marked with feces, urine or anal gland secretions for either intra-group communication (marked more heavily in social groups) or mutual avoidance in nonsocial groups (Ben-David et al. 2005). Although otter diet improves the movement of nutrients and subsequently the productivity of vegetation (Roe et al. 2010) through increases in fish density at latrine sites (Ben-David et al. 2005), the physical activity of creating latrines can damage smaller plants (Roe et al. 2010). The influx of nutrients released at river otter latrine sites varies with respect to the social nature of the otters (Ben-David et al. 2005); less social otters use a relatively large number of different latrine sites with infrequent revisits, whereas more social otters visit fewer latrines frequently. Kimber and Kollias (2000) found increased abandonment of latrine sites post-petroleum exposure; higher levels of abandonment of latrines may indicate changes in sociality in otters but would subsequently decrease damage to smaller plants. However, abandonment would also result in decreased piscivory at latrine sites, causing a decrease in the productivity of the local terrestrial vegetation.

Contaminants can be moved in a similar fashion as nutrients. While we could find no work directly on the potential nutrient flow related to scent mound construction by beavers, the physical modification of habitat exacted by beavers could clearly influence the location of pollutants in the water and sediment. Destruction of dams by the U.S. Department of Agriculture Forest Service in Lake Tahoe occurs annually in the early fall in an effort to provide kokanee salmon (*Onchorynchus nerka*) spawning grounds (Muskopf 2007). The creation of beaver ponds reduced the total phosphorus concentration in the environment, potentially improving the water quality via nutrient retention. Roy et al. (2009) found that downstream aquatic communities with beaver ponds less than ten years old contained the greatest methylation efficiencies and methyl mercury concentrations, incurring high heterotrophic activity as a result. Painter et al. (2015) also found increased levels of methylmercury, periphyton, and invertebrates in the water downstream of beaver ponds. The conflicting nature of the phosphorous and mercury results indicates the potential impact of beaver on either the remediation or worsening of pollution. Because beaver ponds serve as habitat for salmonid fishes (Colleen and Gibson 2001), toxins may pass from these fish to higher predators such as otters or humans. Thus, further research is needed to determine the influence of beaver activity on environmental contaminants.

The environmental context can influence the ecological impact of a toxicant (Liess and Beketov 2008). For example, beavers and otters may show few effects under good conditions, but during times of stress (e.g., extended severe weather), contaminants could have a more pronounced impact. Thus, direct effects on a species can be modulated by conditions, but there also can be important indirect effects (see review by Fleeger et al. 2003). In some cases, the effects on a predator and a prey may offset, such that no net impact can be attributed to the toxicant (Bridges 1999). Yet, when the health and behavior of keystone species or ecological engineers are altered by contaminants, the trophic cascades and competitive releases have the potential to be dramatic, potentially reducing the quality of ecosystem functions and services (Cairns and Niederlehner 1994; Peters et al. 2013) that such species provide.

Conclusion

Given the importance of both beavers and river otters in the productivity and health of the freshwater aquatic ecosystems, it is important to understand the implications of the vast number of potential contaminants that pose both sublethal and lethal risks. Understanding resulting behavioral modifications due to exposure to sublethal levels still needs to be explored in more detail. Further wildlife studies are needed, particularly with a focus on behavior conservation to determine behavioral indicators and species management (see Berger-Tal et al. 2011 for the behavioral conservation framework). In particular, potential research avenues could include studies that focus on: 1) behaviors that relate to fitness and reproductive success, 2) impact of behavioral changes on ecosystem biodiversity and function, and 3) the interaction of otters and beavers as a unit to determine their impact as a synergistic unit on ecosystem toxicity or remediation. This will lend insight into how changes in the behavior of these keystone and engineer species will in turn modify the individual, population, and community-level responses.

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New Evidence of the Semi-Aquatic Nimba Otter Shrew (*Micropotamogale lamottei*) at Mount Nimba and in the Putu Range of Liberia – Uncertain Future for an Evolutionary Distinct and Globally Endangered (EDGE) Species in the Face of Recent Industrial Developments

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Abstract: New records of the endemic semi-aquatic Nimba Otter Shrew *Micropotamogale lamottei* (Tenrecidae, Potamogalinae) were obtained during recent Environmental Impact Assessments (EIAs) in Liberia. We discuss the future of this Evolutionary Distinct and Globally Endangered (EDGE) species in the face of large-scale landscape transformations. In an attempt to assess genetic diversity and genetic differentiation of *M. lamottei* throughout its distributional range, we examined two mitochondrial loci (cytochrome *b* and 12S rRNA) from five samples of Nimba Otter Shrews at three localities in Liberia and compared the 12S sequences with a GenBank sequence from Côte d'Ivoire. While all six individuals examined had unique mitochondrial haplotypes, the sequence divergence among these haplotypes was less than 1 %. However, the F_{ST} values indicated substantial differences between East Nimba and the other two populations. Recognizing that the genetic results are based on very small samples, the data suggest that the three populations experienced sufficient gene flow in the past, preventing sequence divergence, but currently there is substantially reduced gene flow with the East Nimba population. More targeted surveys in the Upper Guinea Region are recommended for this elusive species using aquatic traps in combination with an environmental DNA (eDNA) approach.

Keywords: *mammals, Afrotheria, Tenrecidae, Upper Guinea, West Africa, conservation*

Otter shrews (subfamily Potamogalinae) are unrelated to true shrews (family Soricidae, order Soricomopha), but belong to the African family Tenrecidae. They are part of a superordinal clade of anatomically and biologically quite dissimilar mammals of African origin, which, based on strong molecular evidence, are now united in the Supercohort Afrotheria (Stanhope et al. 1998b; Madsen et al. 2001; Kingdon et al. 2013). Within the Afrotheria, the Tenrecidae are combined with the golden moles (Chrysochloridae) in the African, or Gondwanan order called Afrosoricida (Stanhope et al. 1998a) or Tenrecoidea (Malia et al. 2002; Poux et al. 2008; Asher and Helgen 2010). Because of this independent African origin, golden moles present a convergent adaptive radiation to true moles (Talpidae) that are classified within the northern

Supercohort Laurasiatheria, whereas otter shrews have a vague resemblance to the laurasiatherian otters (Carnivora) and many of their semi-aquatic adaptations (Madsen et al. 2001).

There are three species of semi-aquatic otter shrews, one in the genus *Potamogale* and two in the genus *Micropotamogale*. The larger *Potamogale* includes only one species, the Giant Otter Shrew *(Potamogale velox*), and occurs in Central Africa from Western Nigeria to northern Zambia. The smaller *Micropotamogale* includes two species, the Rwenzori Otter Shrew *(M. ruwenzorii*) limited to the Kivu and Ruwenzori regions of the Eastern DR Congo and western Uganda, and the Pygmy or Nimba Otter Shrew (*M. lamottei*) limited to a small area in the Upper Guinea region of West Africa straddling the borders of Liberia, Guinea, and Côte d'Ivoire (Vogel 2013). *Micropotamogale lamottei* is considered "less progressive" than the other two members of the Potamogalinae, based on its brain characteristics (Stephan and Kuhn 1982) and other morphological characters (Guth et al. 1959). The Nimba Otter Shrew was not discovered until 1951 at Ziéla, near Mount Nimba, Guinea (Heim de Balsac 1954). Most of the known records of *M. lamottei* cluster around Mount Nimba, with Sérédou in Guinea to the west and the Putu Mountains in east-central Liberia forming outliers to the main distribution (Figure 1). Kuhn (1964) first noted the presence of *M. lamottei* in the Saniquellie District (Deaple and Kahnple) on the Liberian side of Mount Nimba. Seven years later he reported an additional specimen from the Putu Mountains in east-central Liberia caught on 5 December 1970 in a crab-trap set in a mountain stream "near the camp of the Bong Mining Company near Peloken, Putu Range, Grand Gedeh County, Liberia" (Kuhn 1971, 477), which extended the then known distribution of the Nimba Otter Shrew by about 100 km to the southeast. It was not until almost 40 years later, on 10 November 2010, that three researchers (JD, CRG, JCG) caught another specimen in a minnow trap in a stream probably very close to the 1970 site, in the context of an environmental impact assessment (EIA) for a new iron ore mining project at Putu (Figure 2). The known point localities across the Nimba Otter Shrew distribution result in an extent of occurrence of 57,436 km^2 and an area of occupancy (based on actual habitat suitability) of only 10,851 km^2 (Boitani et al. 2008).

The core habitat of *M. lamottei* around Mount Nimba appears to be in hilly, forested country with many small creeks, swampy areas, and fallow rice fields, where it lives on fresh water crabs, primarily *Liberonautes* spp. (Potamonautidae), but also on ants (Formicidae) and fishes (Figure 3; Kuhn 1964; Vogel 1983). At Putu, the Nimba Otter Shrew was only found in small forest creeks. Ninety percent of all *M. lamottei* captures occurred in water, usually in some kind of fish capture device. Of these, 92 % of individuals were captured in rivers or creeks and only 8 % in swamps (Vogel 1983).

Due to its extremely limited distribution in the Upper Guinea Region of West Africa and various threats from habitat loss resulting from deforestation, farming, and natural resource extraction, the Nimba Otter Shrew has been listed as Endangered with a decreasing population trend on the IUCN Red List (Vogel 2008). It is also included in the list of Evolutionary Distinct and Globally Endangered (EDGE) species. EDGE species are scored according to the amount of unique evolutionary history they represent (Evolutionary Distinctiveness, or ED), and their conservation status (Global Endangerment, or GE; see Isaac et al. 2007 and http://www.edgeofexistence.org). It might be expected that the species also occurs between the main distribution at Mount Nimba and the two disjunct satellite areas of distribution at Sérédou (Ziama) in Guinea, and in the Putu Mountains of Liberia, but to date, evidence for this is lacking. Otter shrews belong to the roughly 70 out of 1,200 living genera of mammals worldwide that are freshwater-dependent, 18 genera (35 species) of which occur in Africa alone. The vast majority of these freshwater mammal species, including all three species of otter shrews, are in IUCN Red List categories higher than Least Concern (Veron et al. 2008), and thus deserving of particular conservation attention. In a phylogenetic and conservation priority analysis, *M. lamottei* ranked highest among Afrotheria for conservation priorities under all criteria used to transform IUCN categories to extinction risk (Kuntner et al. 2011).

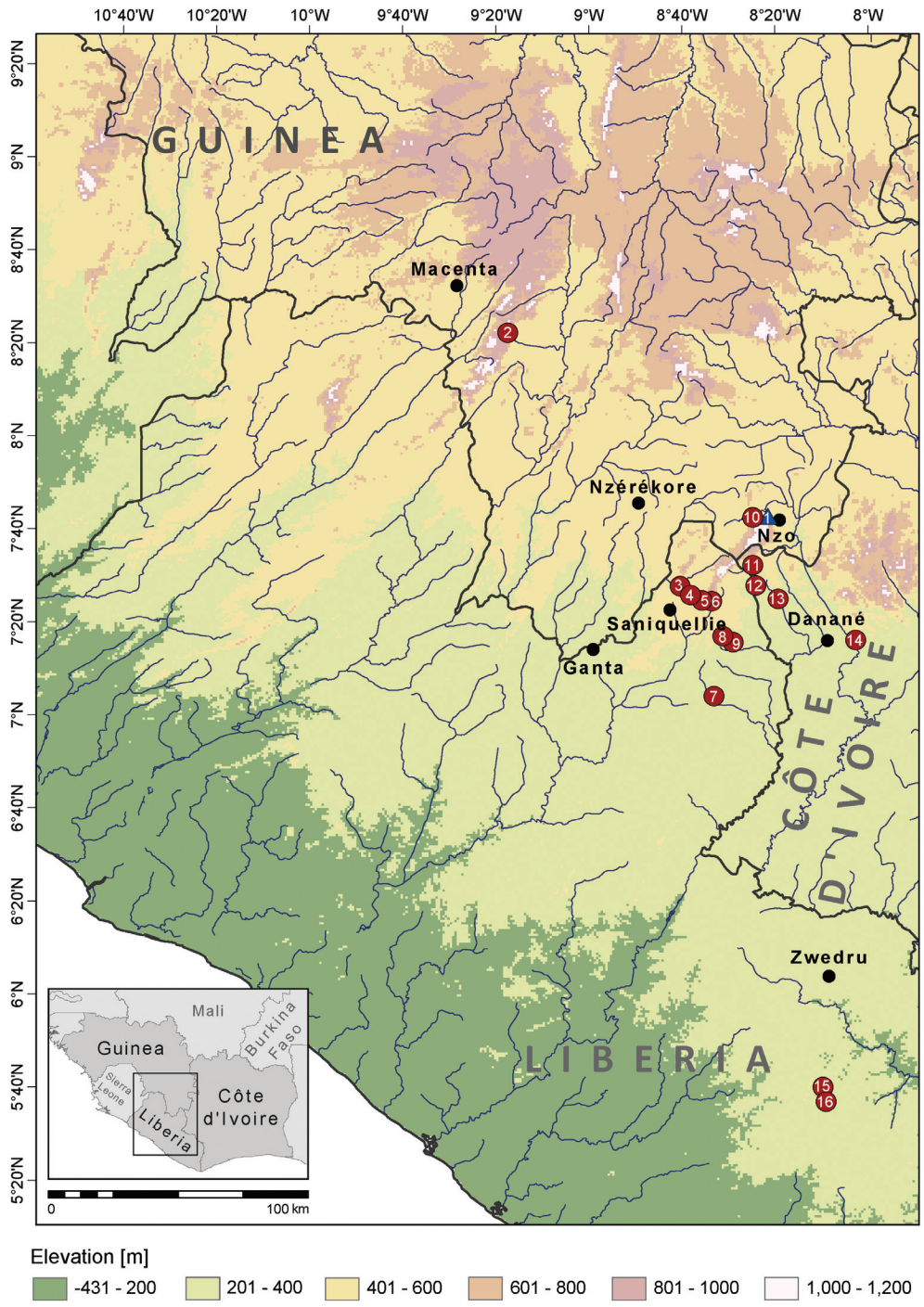


Figure 1. Map of *Micropotamogale lamottei* localities centered on Mount Nimba. Numbered Localities are: 1. Ziéla (= Type locality, alt. 550 m asl, 1951), 2. Sérédou, 3. Tokadeh, 4. Zolowee, 5. East Nimba Nature Reserve (ENNR), 6. Near East Nimba, 7. Deaple, 8. Kahnple, 9, Lapié, 10. Zouguépo, 11. Yéalé, 12. Gbantopleu, 13. Danyapleu, 14. Matonguiné, 15. Slabertsville (Putu), 16. Bong Mining Camp (Putu). Map drawn by Flora Ihlow, ZFMK.



Figure 2. J.C. Garteh holding a minnow trap with a freshly captured *Micropotamogale lamottei* at Putu. The palm nuts (*Elais guineensis*) used to pre-bait the traps to attract freshwater crabs or crayfish are visible within the trap (Photo: © J. Decher 2010).



Figure 3. Nimba Otter Shrew (*Micropotamogale lamottei*) captured at East Nimba and subsequently fitted with a radio-transmitter (Photo: © A. Monadjem).

Micropotamogale lamottei and Human Activities in Its Area of Occurrence - Do Our Assessment Data Matter?

The first detailed description of the Nimba Otter Shrew's natural history by Kuhn (1964) already pointed out that this species is affected by local mining. Kuhn (1964) reported that the Gio people had observed the animal falling into the holes of the diamond diggers (dug a few years earlier during the "diamond rush"). Our 2010 survey in the Putu Mountains re-confirmed the occurrence of this species next to a mining camp ("Slabertsville") on the East slope of the Putu Mountains. In fact, during a second survey in 2012, active bulldozing and clearing of forest was occurring adjacent to the stream habitat of *M. lamottei.* We were unsuccessful in verifying the species from elsewhere in or around the Putu Mountains including the northern edge of nearby Sapo National Park (J. Decher, unpublished data).

Unfortunately, relevant biological and ecological data necessary for policy decision-makers and specifically pertaining to the Nimba Otter Shrew are mostly lacking. For example, its total distribution is still incompletely known, probably due to the fact that it is so difficult to capture. Its population density and movement patterns are also poorly known. The population density was estimated as one otter shrew per 10 km^2 by Vogel (2013) based on drowned animals collected by fishermen in fish bownets. We suspect that the figure of one animal per 10 km^2 may have been a print error and that the intended figure may have been one animal per one km^2 , especially since Vogel (1983) tells us that between 1972 and 1976 villagers collected 84 specimens in fish traps just in the relatively small area in Côte d'Ivoire.

The impact of mining on the Nimba Otter Shrew has already been touched on above. The 1990 IUCN Action Plan (Nicoll et al. 1990) pointed to the devastating mining activities at Mount Nimba as a threat for *M. lamottei* and suggested further research and perhaps a captive breeding program based on the previous successes of keeping the species in captivity (Vogel 1983). Parallel surveys for the Nimba Western Range DSO Iron Ore Project in 2010 found specimens of *M. lamottei* at a proposed Tokadeh stockpile area, which overlaps with a swamp where *M. lamottei* and other globally threatened species occur, and in a small forest stream near Bassa Village less than two km from the East Nimba Nature Reserve (ENNR) (Arcelor Mittal & URS/ Scott Wilson 2010). The local Mah people refer to the otter shrew as "*beng-suno"* (in the Maawe language, *beng* = otter, while *suno* = shrew, so the local and English names have the same meaning; A. Manvell, personal communication). During a recent survey in 2008 of the Guinean side of Mount Nimba, no individuals were located in Ziéla. However, fishermen recognized this species as one that enters in their fish nets. Close to Saniquellie, villagers brought in a specimen and indicated it comes from "bas fond cultivé"(= cultivated depressions; C. Denys, unpublished). In his discussion of the species in the comprehensive *Mammals of Africa*, Vogel (2013) also pointed out that wetland rice agriculture introduced to the Nimba area in 1980 led to large-scale habitat destruction. Since then, more than a dozen individuals have been captured within a 20 km radius of Yekepa, on the Liberian side of Mount Nimba. To date, seven of these individuals have been fitted with radio-transmitters and tracked over several days (A. Monadjem, unpublished data).

Materials and Methods

Otter Shrew Capture Methods

Vogel (1983) succeeded in capturing otter shrews in Sherman traps in Côte d'Ivoire. At East Nimba Nature Reserve (ENNR), a researcher (BP) deployed three wire mesh funnel traps (= fish traps) and nine Sherman traps with live crab as bait, and captured one otter shrew in a fish trap at Tokadeh (Arcelor Mittal & URS/Scott Wilson 2010). At Putu, the first specimen from 1970 was captured accidentally "in a crab trap that was set in a mountain stream" (Kuhn 1971, 477). Based on this information, our second specimen was captured in 2010 in one of 12 commercially available wire-mesh "Gee Minnow" traps (http://www.memphisnet.net/) placed in the creek beds, usually in deep and quiet pools and under bank overhangs (Figure 4). Two researchers (CD & BK) obtained *M. lamottei* from traditional fishing baskets baited with living crabs quite close to



Figure 4. Small forest creek on the East slope of the Putu Mountains where *Micropotamogale lamottei* was caught (Photo: © J. Decher 2010).

villages and cultivated areas at Zolowee (LB4), Liberia (Figure 5). Likewise, at East Nimba one of us (AM) used similar locally made, funnel-shaped traps. These funnel-traps were placed in shallow streams, side by side from one bank to the next, effectively cutting off the path of an otter shrew traveling in the water.

Molecular Approaches to *Micropotamogale* Population Structure

Genomic DNA from five specimens of *Micropotamogale lamottei* (Table 1) was extracted from liver stored in 95 % ethanol using a modified Gentra Puregene (Qiagen, Valencia, CA) Mouse Tail extraction protocol or NucleoSpin 96 Tissue Kit (Macherey-Nagel, Bethlehem, PA) following the procedure of Vogelstein and Gillespie (1979). The quantity and quality of the extracted DNA was assessed using a NanoDrop 1000 spectrophotometer (NanoDrop, Bethesda, MD). The first 820 bp of the cytochrome *b* mitochondrial gene were amplified by PCR using the primers Cytb-A (L14115) and Cytb-F (H14949) described by Sullivan et al. (1997) and



Figure 5. Typical *Micropotamogale lamottei* habitat in cultivated areas at Zolowee (LB4), Liberia. (Photo: © C. Denys).

Table 1. Percentages of K2P genetic divergence between specimens of the Nimba Otter Shrew (*Micropotamogale lamottei*) from different regions of its area of occurrence in the Upper Guinea Region of West Africa, with the cytochrome *b* divergence in the white and the 12S rRNA divergence in the grey side of the diagonal.

Location			**Yeale**	**Putu**	**Zolowee Nimba**		**East Nimba**	
	Sample		IZEA 5038	ZFMK 2011.0044	LB4	LB149	Ara504	Ara535
		12S rRNA Accession ↓	**Cyt b Accession** →	KU697904	KU697905	KU697906	KU697907	KU697908
Yeale	IZEA 5038	AF390539	----	----	----	----	----	----
Putu	ZFMK 2011.0044	KU726977	0.110	----	0.864	0.616	0.490	0.616
Zolowee Nimba	LB4	KU726978	0.211	0.220	---	0.985	0.864	0.738
	LB149	KU726979	0.000	0.110	0.221	---	0.864	0.738
East Nimba	Ara504	KU726980	0.194	0.098	0.208	0.194	---	0.123
	Ara535	KU726981	0.316	0.331	0.420	0.316	0.000	----

the entire 12S rRNA (957 bp) was amplified with primers 1S (L39) and 3'GW (H1209) described by McNiff and Allard (1998). Double-stranded polymerase chain products were purified with an exonuclease and shrimp alkaline phosphatase digestion. Cycle sequencing was accomplished with dye terminators (ABI, PRISM ver. 3) and excess dye-labeled terminators were removed by Sephadex spin columns. Cycle sequencing products were fractionated and visualized on an Applied Biosystems 373 or 13730XL automated DNA sequencer (Applied Biosystems, ABI, Foster City, CA). Both 12S rRNA and cytochrome *b* sequences were aligned using Clustal X2 (Larkin et al. 2007) and the alignments were further examined by eye. The 12S rRNA sequences obtained were further compared to a sequence from specimen (IZEA 5038) from Yéalé, Cote d'Ivoire obtained from GenBank (Accession # AF390539). PAUP* (Swofford 2002) was used to determine the amount of sequence divergence (uncorrected p) and to calculate Kimura 2-parameter genetic distances (Kimura 1980) between pairwise comparisons of sequences. Arlequin (Excoffier et al. 2005) was used to calculate pairwise F_{ST} between populations.

Results

We captured a total of five (1-Putu; 2-Zolowee; 2-East Nimba) specimens with the various methods described above and then made a first attempt to assess the molecular geographic variation of *Micropotamogale lamottei* throughout its distributional range. Genetic diversity and genetic differentiation was examined from five samples of Nimba Otter Shrews from three localities in Liberia at two mitochondrial loci (cytochrome *b* and 12S rRNA). The 12S sequences were compared with a GenBank sequence (AF390539) from a specimen (IZEA 5038) collected by P. Vogel in Côte d'Ivoire. While all six individuals examined had unique mitochondrial haplotypes, the sequence divergence among these haplotypes was less than 1 %. The number of mutational steps (point mutations) between the composite haplotypes ranged from five to 12 (Figure 6). Estimates of pairwise F_{ST} ranged from 0.00 between Putu and Zolowee to 0.666 between Zolowee and East Nimba and 0.818 between Putu and East Nimba. None of the F_{ST} values were significantly different from zero presumably because of the small sample sizes. Indirect estimates of gene flow provided from these F_{ST} values suggest infinite gene flow between Putu and Zolowee, but only 0.11 and 0.25 migrants per generation between Putu and East Nimba and Zolowee and East Nimba, respectively. However, the unbiased estimates of F_{ST} result in values that are either equal to or less than the true value and thus may overestimate the amount of gene flow.

Although 12S rRNA sequences were incomplete for two of the specimens examined, Putu Specimen JD738 missing the first 50 bases and East Nimba specimen ARA 504 missing data between position 433 and 868, variation was observed at seven positions. Geographic variation and/or polymorphisms observed at these positions of the 957 bases of the 12S rRNA sequences included two insertion-deletions, one of a single base (position 857) and the other of a nine base segment, and five SNPs, four transitions and a transversion. The variations observed at 12 positions among the 820 bases of cytochrome *b* examined in five specimens of Nimba Otter Shrews were all transitions.

The level of genetic divergence (K2P distances) at the cytochrome *b* locus ranged from 0.123 % among specimens from East Nimba to 0.985 % between specimens from Zolowee Nimba (Table 1). The highest divergence was between the two specimens from Zolowee (Nimba), as well as between the one specimen from Zolowee and the specimen from Putu (Table 1). The lowest divergence was between two specimens caught in East Nimba. Genetic divergence was intermediate for other comparisons (Table 1). These cytochrome *b* K2P distances are typical of intraspecific comparisons reported for small mammals.

Less genetic divergence was observed among the 957 bases of the 12S rRNA gene, ranging from zero to 0.42 %, excluding the indels. The greatest genetic differentiation observed was between one of the sequences from Zolowee (LB4) and a sequence from East Nimba (ARA 535), which differed by 4 nucleotide substitutions and a 9 base insertion-deletion. The other sequence obtained from specimens from Zolowee (LB149) was

identical to the 12S rRNA sequence acquired from a specimen from Yéalé (from GenBank). Although one of the sequences procured from East Nimba was incomplete, no differentiation was detected from the two sequences obtained from this area. However, the two sequences from Zolowee differed by two point mutations and a one base insertion-deletion. The greatest genetic differentiation was observed among the specimens from East Nimba and the other three sites sampled with an average K2P genetic distance of about 0.25 % and a nine base insertion-deletion. Mean levels of differentiation were similar among the other three locations (Yeale, Putu, and Zolowee) ranging from a little over 0.1 % to 0.165 %.

Discussion

The difference between the low levels of sequence divergence (K2P) and the high F_{ST} values seen among sites suggests that these three populations were much more contiguous in the past but the East Nimba population is currently receiving relatively little, if any, gene exchange with either the Putu or the Zolowee populations. While the F_{ST} method clearly over-estimates the amount of gene flow, the impact of the small sample sizes on the F_{ST} values is unclear.

Future Direction of Nimba Otter Shrew Research and Conservation

We strongly urge that survey efforts for *M. lamottei* are continued in an increasing radius from Mount Nimba, as well as in the Putu Range and surrounding areas. At Putu, survey sites should include rice fields and low-lying marshy areas. Further sampling at Sapo National Park is necessary to determine the true extent of occurrence of the Nimba Otter Shrew, and to potentially locate additional outlying populations. This would be important for the establishment of offsets for areas that will most likely be lost to mining, while recognizing that offsets within existing protected areas may be insufficient to compensate for such losses (Maron et al. 2015). One researcher (AM) is involved in the implementation of the Nimba Otter Shrew Protection Plan to investigate its ecology, status, and distribution in Liberian Nimba. Methods included two types of locally made funnel traps and also camera traps. However, camera traps were not found to be an efficient means of verifying *M. lamottei* presence (Arcelor Mittal 2013). A three-pronged approach was implemented to gain more information about this elusive species: 1) More extensive habitat surveys were completed in both dry and wet season in all habitats within the

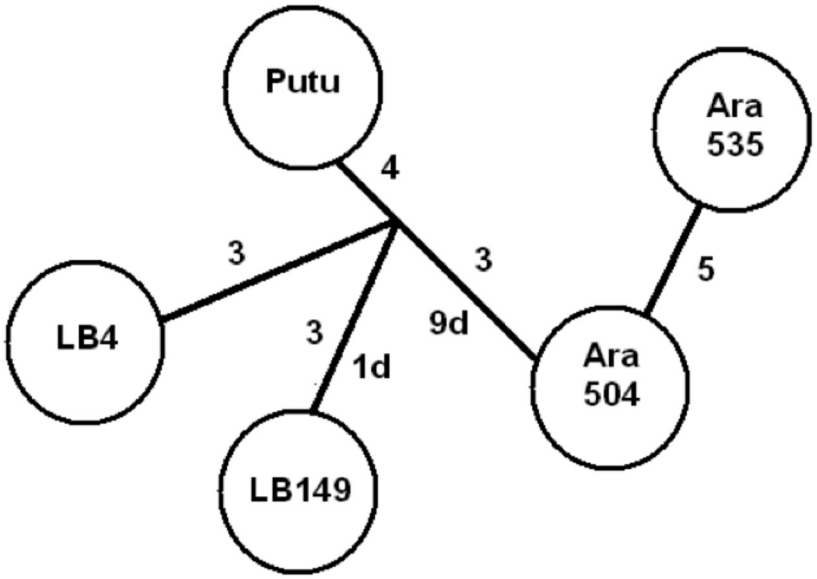


Figure 6. Minimal spanning network of concatenate haplotypes showing the number of point mutations and the one base (1d) and nine base (9d) insertion-deletion along each branch.

species distribution in the Arcelor Mittal mining concession; 2) A pioneering radio-telemetry study was applied that will reveal insights into the spatial ecology and habitat selection of *M. lamottei*; 3) A study was carried out on prey availability and the impact of mining operations on the prey of the Nimba Otter Shrew (mainly crayfish).

At Putu, more traditional trapping efforts using fish traps in the rivers, creeks, and swamps could be combined with an environmental DNA (eDNA) approach using filtered water samples potentially linked to next-generation sequencing (NGS) and eDNA metabarcoding (Goldberg et al. 2011; Taberlet et al. 2012) to assess the wider occurrence of this species in the watershed on the East slope of the Putu Mountains and beyond. A recent critique of the eDNA approach (Roussel et al. 2015) has reinforced the need to combine eDNA detection with other detection methods. By enacting public information campaigns and distributing educational materials to local schools, pride and local awareness of the presence and uniqueness of a globally endangered and extremely restricted species should be raised throughout all known and suspected sites of the distribution of the Nimba Otter Shrew.

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Ecology and Conservation of Semi-aquatic Mammals and their Habitats: Implications for Policy

Issue Editor
Clayton K. Nielsen

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