Appendix F

Evans (2016) St. Louis River AOC Semi-aquatic Mammal Report

REPORT: STATUS OF SEMI-AQUATIC MAMMALS IN THE ST. LOUIS RIVER AREA OF CONCERN

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Beaver (*Castor canadensis*)

River otter (*Lutra canadensis*)

Mink (*Neovison vison*)

Muskrat (*Ondatra zibethicus*)

Abstract

This document summarizes the findings from research conducted to address Beneficial Use Impairments in the United State EPA designated St Louis River Area of Concern, specifically *BUI 2: Degraded Fish and Wildlife Populations* and *BUI 9: Loss of Wildlife Habitat* in regards to native mammals. Methods include aerial surveys for beaver, muskrat and otter sign, and trail camera surveys for beaver, muskrat, otter and mink.

The research was funded by a Great Lakes Restoration Initiative grant #GL-00E01312 Sub 3. It is a collaboration between the University of Wisconsin-Madison and the Wisconsin Department of Natural Resources, along with the Minnesota Pollution Control Agency, the Fond du Lac Band of Lake Superior Chippewa, and numerous local, state and federal agencies and staff. The data are also the focus of a master's thesis in the Department of Forest and Wildlife Ecology at UW Madison.

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1 Introduction

Expansion of human populations and industrial activity have impacted ecosystems and compromised their ability to support both natural and human wellbeing (Mills 2013). Functional riverine and wetland systems provide numerous ecosystem services, including water purification, flood control, wildlife habitat and aesthetic value, but since the turn of the twentieth century 50% of North American wetlands have undergone moderate to severe modification (Millennium Ecosystem Assessment 2005, Thorp et al. 2010). The St. Louis River, which flows into Lake Superior and forms the harbor between Duluth, Minnesota and Superior, Wisconsin, is significantly degraded as a result of human activities (MPCA and WDNR 1992*a*). Land use changes beginning in the 1860's included timber clearing and milling and installing railroad facilities (Kellner et al. 2000). The expansion of the transshipment industry created incentive for extensive modifications of the harbor and estuary, and as early as 1873 dredging for channels contributed to habitat degradation and sediment loading that are cause for concern to date (Kellner et al. 2000, MPCA 2015). Effects of industrialization in the area include extensive loss of wetlands, transformation of the benthic environment, and physical and chemical pollutants which damaged both the physical structure and ecological functioning of the river and estuary (MPCA and WDNR 1995, MPCA 2013).

In 1987, the St. Louis River was designated as an Area of Concern (AOC) in the Great Lakes Water Quality Agreement between the United States and Canada MPCA and WDNR 1992). Nine Beneficial Use Impairments (BUIs) were listed, and both rehabilitating human use and returning natural stability to the system were identified as management goals. Two BUIs pertain specifically to wildlife management: BUI 2 *Degradation of Fish and Wildlife Populations*, and BUI 9 *Loss of Fish and Wildlife Habitat* (MPCA 2013). There have been numerous collaborative remediation projects within the AOC, including work targeted to restore spawning habitat for Lake sturgeon and beach improvements for Piping plover (MPCA 2013), but to date there has been no direct assessment of the status of native mammals in the system.

The primary objective of this research was to determine if the St. Louis River AOC currently supports populations of native mammal species in similar abundances as areas with less extensive impairment. Complete population censuses are generally not feasible in wildlife research; but by performing surveys and estimating a probability of detection, relative occupancy estimates can be generated to answer similar ecologically relevant questions (MacKenzie et al. 2002, 2006). For this study, the state variables addressed by occupancy modeling (primarily the proportion of sampling units occupied by species of interest, MacKenzie et al. 2006) will be sufficient to meet the objectives set in place by the Remediation Action Plan (RAP, MPCA 2013). Regarding the Degradation of Fish and Wildlife Populations BUI the RAP states that “Removal of this BUI is not dependent on specific small aquatic mammal population numbers. However, to support development of concurrence among state resource management agencies, a small mammal survey will be conducted in the estuary to verify that populations are not limited by physical habitat, food sources, water quality, or contaminated sediments” (MPCA 2013).

To thoroughly assess the status of mammal populations in the area, four native species dependent on aquatic resources were selected to study: river otter (*Lontra canadensis*), mink (*Neovison vison*), beaver (*Castor canadensis*) and muskrat (*Ondatra zibethicus*). I collected data on occurrence of these species using both motion triggered camera surveys and aerial sign surveys, with the two goals of 1) estimating relative occupancies of all four species to provide adequate information for assessing the BUIs and 2) comparing between survey methods for relative costs, efficacy and potential biases.

2 Semi-aquatic Mammals

The four target species for this research span several trophic levels and have distinct roles within aquatic ecosystems, which presents both unique risks for species decline linked to industrialization as well benefits linked to species recovery. Both river otter and mink are carnivorous and therefore dependent upon access to reliable prey resources to occupy an area permanently (Buskirk and Zielinski 2003). They are two of the three mammals listed as representative species by the Great Lakes Water Quality Initiative wildlife criteria (United States Environmental Protection Agency 1995) because of their sensitivity to heavy metals and polychlorinated biphenyls (PCBs) in the environment. In an area with a history of chemical pollution such as the AOC, these compounds can bioaccumulate and lead to decreased survival and reproduction (Wren 1987, Poole et al. 1998, Mayack and Loukmas 2001). Buskirk and Zielinski (2003) compiled multiple lines of evidence (lesions, absence from areas with contaminants) showing that carnivores in aquatic systems are at potentially great risk from chronic exposure pesticides, heavy metals and other pollutants, and diminished populations have been demonstrated within other Areas of Concern (Letteros et al. 2008, Strom 2013). Otters are piscivores primarily, although supplementing with amphibians or small mammals, and incorporating crayfish in potentially large proportions seasonally (Roberts et al. 2008). The smaller mink eat a wider variety of prey including fish, crustaceans, and mammals, with muskrats being especially important during the winter (Kurta 1952, Melquist et al. 2003). Mink can also provide an indication of contamination in aquatic ecosystems, being high on the trophic chain, yet relatively short lived and occupying small home ranges increases their sensitivity (Larivière 2003, New York State 2010).

All of the four target species are regulated as furbearers, and experienced extensive reductions in population size following European settlement of the area (WDNR 2012). Increasing regulation of trapping began to emerge in the late 1800’s, and species have recovered in many areas and are routinely monitored using multiple methodologies (Kohn and Ashbrenner 1984, Rolley and MacFarland 2012, WDNR 2012).

Beavers were nearly extirpated from much of the great lakes region by 1900 due to unregulated trapping for the fur trade (WDNR 2012). Once trapping was regulated, populations throughout the area rebounded without human intervention; by 1990 beavers in northern Wisconsin were abundant enough that subsidies were offered to trappers to assist the Wisconsin DNR in reducing the population size (WDNR 2015). Beaver management zones were established to balance between the negative consequences of their habitat modifying behavior on trout populations and flooding of roadways, crops and private property with the benefits provided, particularly for waterfowl species (WDNR 1990). Beaver populations are monitored using specialized questionnaire replies from resident trappers to provide harvest information (Dhuey and Olson 2014a) and in the in the northern management zones, which have higher beaver densities, by using aerial surveys (Rolley et al. 2011). In recovering ecosystems beaver reintroductions have been used as a restoration tool to increase habitat complexity, connectivity and retention of water during drought conditions (Hood and Larson 2015).

Muskrats and mink are now also trapped extensively, with no bag limit lengthy trapping seasons in Wisconsin (late October/early November to early March, WDNR 2014). Populations within the state are tracked using questionnaires from registered trappers, which indicate that around half of surveyed trappers target muskrats, resulting in annual harvests around two to three hundred thousand, while fewer trappers target mink and harvest between ten and twenty thousand (Dhuey and Olson 2013, Dhuey and Olson 2014b). Muskrats are a major food source for mink and are prey for raccoons and terrestrial predators - as a prey species they reproduce more swiftly than the other species of interest to this research and are capable of bearing litters of young each year (Erb and Perry Jr. 2003).

3 Study Areas

The St. Louis River AOC in total encompasses the lower 63 kilometers (39 miles) of the St. Louis River, the associated watershed containing multiple tributaries and adjacent streams, and the Nemadji river watershed. The majority of remediation work has occurred on the stretch of the St. Louis River below the Fond du Lac dam, and in the Nemadji River watershed, where the Koppers Company lumber processing plant at Crawford Creek caused point source pollution from as early as 1928 to today, though since 1991 waste water has been transported offsite for treatment (MPCA 1992, 2013; MPCA and WDNR 1992). Therefore this research focused on those areas, specifically the St. Louis River from the Fond du Lac dam to the Bong Bridge, and the Nemadji River from six miles above Crawford Creek to its outlet in Allouez Bay (see Figure 1).

Because there is no information available on semi-aquatic mammal population status in the St. Louis River estuary prior to degradation, this study design uses reference areas in order to determine if the current population status for each target species meets the recovery requirements designated by the EPA. I and fellow researchers located two distinct reference sites to adequately reflect the diversity of habitats and flow regimes present in the St. Louis AOC: the Boulder Lake Reservoir in northeastern Minnesota, as an example of a relatively unimpaired lentic system, and the St. Croix River on the north central border between Wisconsin and Minnesota, to represent a relatively unimpaired lotic system. Both of these areas were deemed sound representations of the ecological potential of the AOC by meeting the criteria that they 1) possess similar habitat types to the St. Louis River estuary and are likely to support populations of the target species, based on expert opinion, 2) have minimal anthropogenic impacts including development and point sources of pollution along the shoreline, 3) are at least partially open to public trapping and are accessible by several means and 4) are geographically close to the AOC (<100 kilometers maximum linear distance) without being contained within it. Because the St. Louis AOC has an industrial component that will not be removed or restored, the reference areas populations will not be used as specific goals for AOC populations, but rather provide points for comparison and ultimately establish a basis for the consensus decision by resource managers about the status of semi-aquatic mammal populations in the AOC.

3.1 St. Louis River and Estuary

To ensure consistent effort and draw accurate inferences, I defined the boundaries for semi-aquatic mammal surveys within the AOC to extend from the Fond du Lac dam downstream to the Richard I. Bong Memorial Bridge. This area encompasses the diversity of flow regimes and habitat types in the AOC, and corresponds to several of the remediation projects (e.g sediment removal and wetland restoration at Mud Lake; shoreline restoration at Chamber's Grove. MPCA 2013). Immediately below the dam the St Louis river follows a narrow channel with relatively faster flow (channel width ranging 50-400m). The river then widens into a slower, shallower channel and forms Mud Lake and Spirit Lake (maximum width over 2,000m). On the Wisconsin (southeast) side several small tributaries form Pokegama Bay, an estuary system with meandering channels and dense vegetation. North of Pokegama Bay, the habitat transitions into moderate residential development and progressively becomes more industrialized as one approaches the Bong Bridge. Beyond this point the shoreline is intensively modified and provides very little natural habitat suitable for semi-aquatic mammals.

3.2 Nemadji Watershed and Allouez Bay

The Nemadji River water lies just southeast of the St. Louis River, and enters Lake Superior adjacent to Wisconsin Point and Allouez Bay. The river channel is relatively sinuous and narrow (typically 25-50m) and may provide habitat for wildlife that is distinct from the St Louis River. To investigate the potential impact of industrial point source contamination this study area extends from 10 kilometers (roughly 6 miles) upstream of the Crawford Creek confluence and downstream to the river's mouth, approximately 12 kilometers. Allouez Bay (including the interior, southern shoreline of Wisconsin Point) is also included in this portion of the study area. The bay is also vulnerable to industrial pollution and has been targeted for wildlife habitat restoration (MPCA 2013).

3.3 Boulder Lake Reservoir

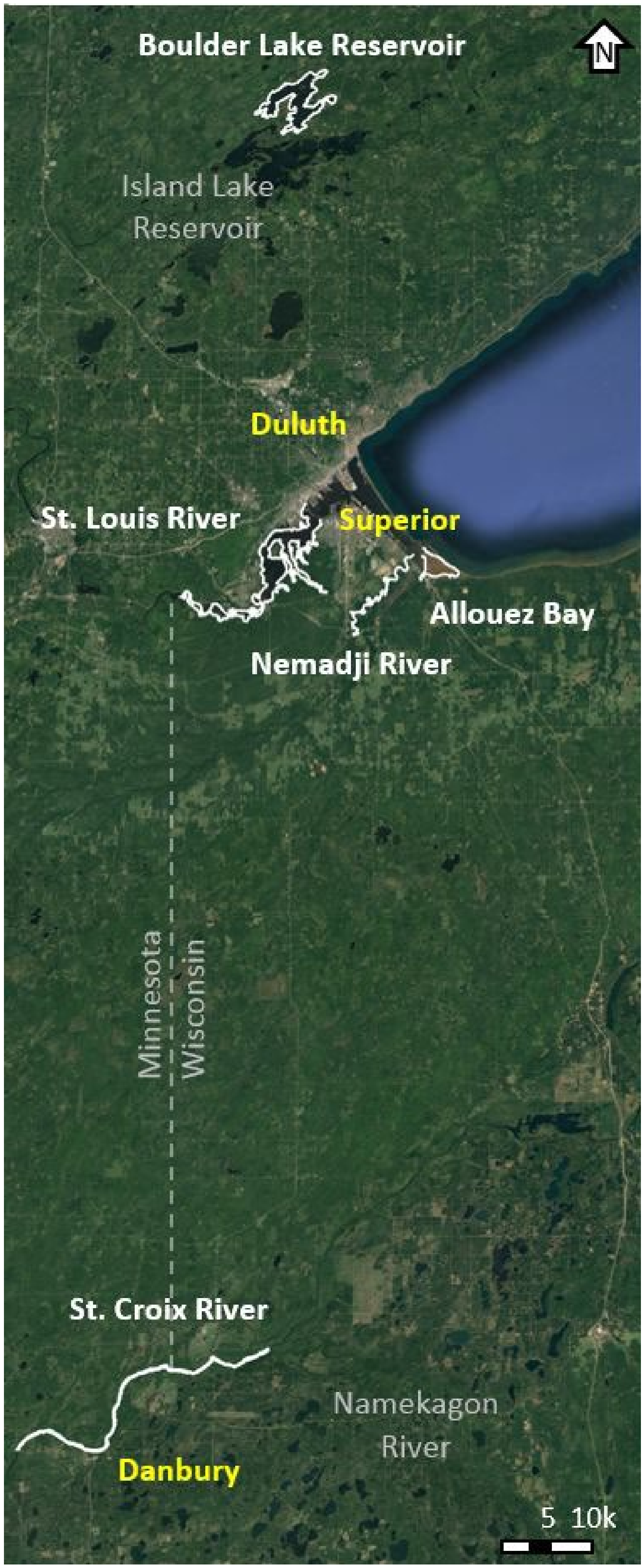
The Boulder Lake Reservoir, in northeastern Minnesota, is owned by Minnesota Power, ALLETE Inc and supports one small dam, but is otherwise minimally disturbed. The land is managed by the Boulder Lake Environmental Learning Center through the University of

Minnesota-Extension (www.boulderlake.org). Boulder Lake Reservoir was selected as a reference site out of several lakes in northeastern Minnesota because it has low levels of private development along the shoreline and can be assumed to represent minimally degraded habitat. Furthermore, Boulder Lake has open public access for boating, camping, fishing and hunting recreation which both mimics the St. Louis study area and facilitates access for this research. Specific access and logistic support for the project was granted through the Boulder Lake Environmental Learning Center by the program director.

3.4 St. Croix River

The St. Croix River runs from northwestern Wisconsin, and forms the border between Minnesota and Wisconsin until joining the Mississippi River below Minneapolis. It is designated as a National Scenic Riverway, and as such there is public access to a relatively undisturbed shoreline both by foot and by canoe. It is managed by the National Park Service (NPS), and while hunting is allowed trapping is prohibited on portions owned by the park (NPS 2006, WDNR 2014). However, trapping is allowed on other publically owned segments in both Minnesota and Wisconsin, and in Minnesota water sets between the mean high tide mark and the center of the channel(NPS 2006, MDNR 2016). Trapping is also allowed for privately held land with the owner's permission and for tribal trappers exercising treaty rights. Furthermore, the removal of nuisance beaver is conducted by USDA Animal and Plant Health Inspection Service for safety concerns along the waterway as well as to enhance trout habitat (NPS 2006, WDNR 2012). As such, the trapping regime should be similar to that of the St. Louis River estuary and does not impede comparison between the two sites. This reference area includes the stretch of river north of Danbury, Wisconsin from the confluence of the Namekagon River downstream approximately 26 kilometers to Thayer's Landing at the HWY 77 bridge / MN 173 junction. This portion of the river offers several points of access both on foot and by canoe, and is sufficient in size for comparable survey effort as the Boulder Lake reference area.

Figure 1. Map of AOC study areas (St. Louis River, Nemadji River, Allouez Bay) and reference sites (Boulder Lake Reservoir, St. Croix River)



4 Field Methods

Wildlife species are often cryptic and elusive, which make their populations difficult to quantify (O'Brien 2011). Complete counts of all individuals of a species are nearly impossible to obtain, and partial count data must be adjusted to account for imperfect detection (White 2005). For species that are harvested, some relevant data can be gathered through registration of carcasses or reports from hunters and trappers, but such data can be unreliable due to variation in harvest effort as recreational interest and market values change (Kohn and Ashbrenner 1984) and the often non-linear relationship between density and harvest (Van Deelen and Etter 2003). The natural resource management agencies of both Wisconsin (WDNR) and Minnesota (Minnesota Department of Natural Resources, MDNR) have used various techniques to monitor population trends for beaver, otter, mink and muskrat. Current monitoring techniques for these species typically combine harvest reports and related demographic data (Dhuey et al. 2015, Erb 2016) and winter sign surveys both on the ground and from aircraft for beaver and otter (Erb 2013, Rolley et al. 2011, Rolley et al. 2013). While historic and contemporary harvest information can be useful for tracking population trends around the AOC, more detailed and precise information was needed to meet the mandate of this research. Because aerial surveys are already used by management agencies for beaver and otter, aerial surveys in fall and winter were included in the study design to collect data on the status of these two species within the St. Louis AOC.

However, while muskrat sign is also visible from the air, there is no established protocol to collect information on muskrat or mink by aerial surveys. Furthermore, relying on a single methods for which estimates of precision and bias are not available could lead to flawed inference, therefore I investigated the use of multiple survey methods. Using more than one approach allows for simultaneous investigation of all four species, and offers quality control against potential flaws in a single approach, as well as providing information to management agencies on efficacy should future surveys be desired. Motion triggered cameras are increasingly popular in wildlife research, and rigorous methods for study design and analysis are widely accepted (O'Connell et al. 2011). Using trail cameras is effective for collecting data on multiple species simultaneously (Lesmeister et al. 2015), enables data collection beyond the seasonal restrictions for aerial surveys for beaver and otter, and can circumvent some potential biases present in other non-invasive methods. For example, other methods for surveying otters include scat surveys (Jeffress et al. 2011) which typically assess only one species at a time (but see Williamson and Clark 2011), can be impacted by seasonal differences in detection probability (Fusillo et al. 2007, Parry et al. 2013), and may have bias introduced by distance from anthropogenic structures or limited area coverage to account for false absences (Swimley et al. 1998, Crimmins et al. 2009). Cameras are also able to collect data at a fine spatial scale and can incorporate information on habitat features at microsites within the area of interest. Several dozen Reconyx brand trail cameras (Reconyx, Holman WI) were available for research use from the WDNR, and based on the above benefits I selected this approach for semi-aquatic mammal surveys.

Other non-invasive techniques considered were baited track plates (which have been used for many non-aquatic species, Zielinski et al. 1995) and floating rafts (often used for mink surveys in Europe, Schooley et al. 2012). After considering these techniques I determined that track plates would not encompass all the species of interest and could have complications in close proximity to water, and that although there is interest in floating rafts to assess recovery of mink in the Sheboygan Area of Concern (Natalie Miller, personal communication), that approach would be logistically infeasible to meet the objectives of this project.

4.1 Aerial survey methods

In order to efficiently track beaver populations on the landscape, many agencies in both North America and northern Europe have used aerial surveys (Payne 1981). Differences in detection success vary between fixed wing, helicopter and ground survey methods (e.g. Robel and Fox 1993). To conduct a thorough comparison of census-style flight surveys, Payne (1981) used both fixed wing (Super Cub) and helicopter surveys flown in a single day, and tested the results against “ground truthed” data from reliable trapper surveys. The results indicated that helicopters missed 19% of beaver lodges and fixed wings missed 39%. Aerial surveys within Wisconsin are included in the current ten-year beaver management plan, and include both helicopter surveys of selected quadrats and fixed wing flights over trout streams (WDNR 2015). However, no data exists on the survey efficacy for this approach in Wisconsin.

For this study, flight surveys used a Cessna fixed-wing four person aircraft provided by WDNR and flown by an agency pilot with experience conducting wildlife surveys. Although many wildlife surveys use fixed wing aircraft (Walsh et al. 2010, Jacques et al. 2014) they do have certain flight speed and height limitations that could theoretically impact detection rates for such surveys. To account for any resulting inadequacies with the data gathered from fixed wing surveys, one comparison survey was completed in a helicopter. Helicopters have increased maneuverability and the ability to fly slowly and hover, which allows for more precise surveys.

However, helicopters are significantly more expensive than fixed wing aircraft and as such specific insight into the feasibility of less expensive techniques is desirable.

Ideal timing for aerial surveys of beaver lodges is in the window between leaf-off and ice up, ranging in October and November, while otter sign surveys require ice cover and recent snow fall (WDNR 2015). The majority of our fixed wing flights occurred in synchrony with fall camera trapping sessions, which also corresponds to the protocol followed by Wisconsin DNR for beaver and river otter population estimates (Rolley et al 2011, Rolley et al 2013). An additional late flight in February 2016 (after closing camera stations) was conducted due to poor conditions for otter sign on earlier winter 2015/2016 flights.

Data collected on the flights included a GPS track logs of the flight path; starting and stopping locations and times for each segment of the surveys; and waypoints for any sign recorded. Observable sign for beaver consisted of lodges, food caches, and wood chips and downed trees as a result of chewing activity. Muskrat sign consisted of “push-ups” in the fall, which were generally not observable after snow had fallen. Otter sign consisted of tracks in snow, which were only observable during periods of ice cover and were discernable from other animal tracks by the distinctive sliding pattern in the snow.

4.2 Camera survey methods

Reconyx brand HyperFire HC500, PC800 and PC900 cameras (Reconyx, Holman WI) were placed within the AOC and the two reference sites for two month-long pilot deployments in fall/winter 2014/2015 and one eight-month full deployment from summer 2015 to winter 2016. Sampling in multiple seasons should maximize detection probabilities of species more active on land prior to freeze (spring/summer 2015), enabling analysis of best management practices for using these techniques in the future, while also collecting data concurrent with the aerial surveys conducted by the Wisconsin Department of Natural Resources (fall/winter 2015).

The number of cameras allocated to each study area was proportional to the total miles of shoreline in each. Within the larger and more complex Area of Concern, distinct habitat types were stratified and effort was allocated accordingly to ensure complete, unbiased coverage (O’Connell et al. 2011). The habitat strata designated within the AOC were a) the upper river section, b) the central channel and Mud Lake, c) Pokegama bay, d) the suburban eastern shore, e) Allouez bay, and f) the Nemadji river (see appendix A.1 for a detailed map).The more homogenous reference areas were treated as a single habitat type.

To adequately space cameras for independent sampling and to avoid selection bias in the field, target locations were established before placing cameras in the field. First the shoreline of each study area was broken into 1 kilometer long segments, which were then randomly ranked. The first ranked segment was assigned a “target location” in the center of the segment. The process continued down the ranked segments, with either a point being assigned or censured such that no two adjacent segments were sampled simultaneously. During the first iteration for cameras deployed in November 2014, the minimum distance between two target locations was set at 1.5 kilometers. When randomizing for the second deployment of cameras in December 2014, target locations were at minimum 1.5 kilometers from another target site and 1 kilometer of any previously sampled camera site. For the third deployment in summer 2015, additional cameras were available such that I was not required to move them to ensure adequate coverage. I prioritized resampling prior locations, and followed the same process to select target locations for the remaining cameras in segments a minimum of 1 kilometer apart.

Cameras were deployed in the field by navigating to the target location as closely as possible, contingent on access and that the habitat provided at least some adequate structure for securing the camera. The area was then searched for sign of animal passage to the water and specific indications of use by target species, such as scat, tracks, lodges or feeding sign. Data collected at the selected site included GPS coordinates; terrestrial and emergent aquatic vegetation species and percent cover; diameter at breast height of the camera tree; bearing, angle and height above the ground for the camera; distance from the camera to the water; and camera make, model, and battery status. Cameras were revisited every 1-2 months to collect data cards, exchange batteries, and rectify any issues with the camera or site. In the event that site characteristics had changed substantially, either due to vegetation growth or rises in water level, cameras were re-angled or moved entirely (<30m) to compensate, and new covariate data were collected if applicable.

5 Statistical Methods

5.1 Aerial survey Kruskal Wallis tests

Analyses of aerial data were conducted by compiling all data points collected on each flight into species detections: beaver sign (lodges, food caches, and chewing sign), muskrat sign (push-ups), and otter tracks (tracks and slides in snow). A confidence score was assigned for potentially ambiguous points and two datasets were maintained of only *certain* data points or *combined* with lower confidence points. All counts were then divided by either the total kilometers of flight distance or the minutes of flight time for each study area completed in the survey to account for variable sampling effort. For estimated relative abundance, only a single observer present on all surveys was used for consistency. These data were then assessed on a single species basis with a Kruskall Wallis test against the null that median values would be equal between the AOC study areas and the reference sites. The non-parametric test does not require data to be normally distributed (Zar 2010), and although a Poisson distribution can be used for aerial data (Hodgson et al. 2016) the transformation was not universally helpful for these data.

5.2 Occupancy modeling framework

To accurately assess overall status across multiple camera stations, the pattern of detections (positive identified images) for each species can be modeled to inform both detection probabilities and occupancy estimates (MacKenzie et al. 2002, 2006, Royle and Nichols 2003). Each species of interest was recorded as either detected (1) or not detected (0) for every 24-hour period that a camera was functioning, to create a detection history. Even for sites that detected species, there were typically many days of non-detection, resulting in detection histories with many 0 events. To increase the modeling power I collapsed all daily data into one-week observation periods, such that a week in which a species was detected once or more is observed as 1, and never detected is observed as 0. These 0-1 observations result in 11 weekly detection history in each season, for each species and camera site.

Example: Mink detection history at camera site SLE3-1 in Season 1: h = 00001011110

Occupancy modeling leverages several ecological and mathematical features of the detection histories to obtain robust estimates of their underlying biological processes (MacKenzie et al. 2002, 2006, Royle and Nichols 2003). The first of these is that an observation period recorded as 1 must result from two conditions being met: the site is occupied by the species, and the species is detected successfully. The true occupancy state of a site, denoted z_i, is either 0 (unoccupied) or 1 (occupied). The probability of a site being occupied is then denoted as $\Psi = P(z_i = 1)$, and this is the value which can be modeled to infer species occupancy patterns. Because successful detection must also occur, the probability of any single observation *j* at site *i* recording a 1 is given as the probability of occupancy (Ψ) multiplied by the probability of detection (*p*):

$$P(obs_{ji} = 1) = \Psi p$$

The second characteristic is that a non-detection, or 0, observation can result from two different scenarios: either the site is truly unoccupied, or the site is occupied but the species was not successfully detected (false-absence). Because both these potential states are dichotomous, the probability of one event is simply one-minus the probability of the alternative (ie non-detection is one-minus-detection, or 1-*p*). Thus the probability of an observation being 0 can be modeled accounting for both scenarios:

$$P(obs_{ji} = 0) = (1-\Psi) + \Psi(1-p)$$

(Royle and Nichols 2003, Royle and Dorazio 2008)

The uncertainty in non-detections can be considered as a nuisance parameter and ignored, but this can result in severely biased conclusions (see White 2005). Instead, explicitly incorporating the underlying source of uncertainty in any wildlife research that depends on imperfect observations will improve the accuracy of the state variable of interest and strengthen inferences of the ecological processes involved (MacKenzie et al. 2002, 2006).An important assumption for these models is that the true occupancy state of a site, z_i, does not change over the monitoring period. I subdivided the full 2015/2016 season data into three 11-week seasons to ensure that this assumption was met. Models can be further strengthened by incorporating habitat and observations covariates that can influence both detection and occupancy probabilities. Because detection and occupancy state variables are binomial (one of two options), and informative parameters are typically continuous or categorical, they are linked with a logit transformation. This allows linear combinations of explanatory factors, but means resulting estimates must be back transformed if values in the original units are of interest. Modeling occupancy probably with covariates 1 to *x* gives

$$\text{logit}(\Psi) = \ln\left(\frac{\Psi}{1\text{-}\Psi}\right) = \beta_0 + \beta_1 + \dots \ \beta_x$$

(MacKenzie et al. 2006)

5.3 Detection probability modeling

I used a sequential modeling approach (Burnham and Anderson 2002, MacKenzie et al. 2012, Lesmeister et al. 2015) to first select only the parameters which were informative for the detection process. I speculated *a priori* that the following seven site-level factors might influence detection rates: study area in which the camera was located (AREA), distance from camera to water (DIST.WATER), percentage of canopy closure on land near the camera (TREE.COVER), percentage of emergent aquatic vegetation cover (MARSH.COVER), slope of the camera set (SLOPE), height of the camera from the ground (CAM.HEIGHT) and diameter at breast height of the focal tree (CAM.DBH). For modeling the effect of AREA, I differentiated between the St Louis river estuary (SLE) and the Nemadji river and Allouez bay (ALZNMJ), and between the two reference sites, Boulder Lake reservoir (BLR) and the St Croix river (SCR). The St Louis river estuary was modeled as the intercept (baseline for comparison), and the other three areas were incorporated as alternative states. This increases the number of variables (k) from only one (for the other site-level covariates) to three, which will play a role in the eventual AIC model ranking process.

In additional to site-level features, observation-level covariates could affect the probability of detecting species at cameras during each week-long period. These included temperature data which were collected twice each day at 2:00AM and 2:00PM when timelapse images were automatically recorded. Temperature measurements were then either averaged over each observation period (AM.Ave, PM.Ave), or sorted for weekly maximum and minimum temperatures (AM.Max, AM.Min, PM.Max and PM.Min). Lastly, the total number of days within the observation period for which each camera was operational was calculated (ACTIVE). Differences in the active period for cameras are the result of logistic realities deploying and checking scores of stations over three distinct areas, and of camera failures caused by filled SD cards, depleted batteries, water and ice cover, or other circumstances.

Table 1. Detection Model Suite

Site Covariates		Observation Covariates	
NULL	TREE.COVER	ACTIVE	AM.Min
AREA	MARSH.COVER	AM.Ave	PM.Min
DIST.WATER	SLOPE	PM.Ave	AM.Max
CAM.HEIGHT			PM.Max

These site-level and observation-level covariates were each run independently as the only explanatory factor for detection, with occupancy held null, in a suite of models for each species within each season. I used Program R (R Core Team 2014), the package unmarked (Fiske and Chandler 2011, Fiske et al 2016), and the function *occu* to fit the single season occupancy model described by MacKenzie et al. 2002. By using Aikaike's Information Criterion model ranking (Burnham and Anderson 2002) I determined which covariates may have meaningful influence on detection probabilities by sequentially including only the highest ranked models until I obtained a cumulative model weight ≥ 0.90. I noted the ΔAIC at each step, but did not follow a strict cut off at Δ = 2 (see Arnold 2010), because of both the non-nested nature of these models and the penalty assigned for additional parameters, which could put models including AREA at a disadvantage due to the dummy variables differentiating among study areas. This approach generated a set of detection covariates that were specifically relevant to each species and season, and only these were then included in the suite of models to investigate occupancy itself (MacKenzie et al. 2012).

5.4 Occupancy probability modeling and weighted estimates

To assess occupancy differences between camera sites and study areas, I examined the following variables in the second phase of sequential modeling to determine their influence on the presence or absence of a species: AREA, TREE.COVER, and MARSH.COVER. These variables were assembled into a full model suite (eight models), where each was present individually and in additive combination. I did not consider interactions due to the large number of competing models this approach would generate, and for each species and season the detection parameters indicated in the first phase were used.

Table 2. Occupancy Model Suite

Without AREA	With AREA
NULL	AREA
TREE.COVER	AREA + TREE.COVER
MARSH.COVER	AREA + MARSH.COVER
TREE.COVER + MARSH.COVER	AREA + TREE.COVER + MARSH.COVER

Top models were selected following similar rules for inclusion as for top detection models: keeping top ranked models up to a cumulative weight > 0.90, but not discarding suites with a highest model weight < .50. Those ranked as top models were then assessed with parametric bootstrapping to avoid incorrectly assuming that inclusion in top models indicated a good model (MacKenzie and Bailey 2004). I used *parboot* to refit 1000 simulated data sets back to the model and calculating the chi-square test statistic for goodness of fit and the $\hat{c}$ overdispersal factor (MacKenzie and Bailey 2004, Fiske et al. 2016).

Each of the occupancy models, by incorporating information from all of the included covariates, calculates a fitted occupancy estimate ($\hat{\Psi}$) for each observation period of each camera site. Using a model averaging approach (Burnham and Anderson 2002) I took the occupancy probability generated by each of the top models and averaged across observation periods within a season to obtain the predicted overall occupancy for each site for each model, $\hat{\Psi}_{MODEL}$. Finally, I multiplied that average for each site by the proportion of the cumulative AIC weight which that model accounted for, and then summed all top models together, giving

$$\hat{\Psi}_{WEIGHTED} = \text{Sum}\left(\frac{\hat{\Psi}_{MODEL} \times \text{ AIC weight}_{MODEL}}{\text{AIC weight}_{CUMULATIVE}}\right)$$

In this way the variation of the results from different covariates being present in different models is preserved but the final outcome is weighted towards those with the greatest explanatory power. This generated a data set of weighted occupancy estimates for each site, allowing comparison between the AOC and the reference areas (appendix).

5.5 Equivalency tests

Equivalency tests operate under the null assumption that two populations being compared will have different distributions or mean values (Wellek 2010). This is a departure from traditional hypothesis testing in which the null assumption is that there is no difference, and this emphasis makes it a potentially preferable method for assessing situations such as environmental remediation (Manly 2001). Placing the burden of proof on demonstrating the equality of a degraded system and a control system, rather than failing to find evidence against equality, is recommended by the US Environmental Protection Agency (EPA 1994) and there are examples of statistical exploration in the natural sciences (Robinson and Froese 2004, Robinson et al. 2005).

I used the R package *equivalence* version 0.7.2 (Robinson 2016) to test for differences in weighted occupancy estimates for each species and season between AOC and reference areas. The test *Rtost* is a robust two one-sided test that is appropriate even if assumptions of normally distributed data cannot be met.

6 Field Results

6.1 Aerial survey results

A total of six fixed wing flights and one helicopter flight were successfully conducted within the St Louis AOC and reference sites (table 3). Of the six fixed wing flights one was a training flight, and another was forced to terminate before completion due to mechanical problems with the aircraft. Numerous additional flights were attempted but either canceled or terminated prior to completion due to inclement weather or scheduling conflicts. Of the four complete fixed wing surveys three were conducted with the pilot and a single observer (BE, similar protocol to Johnston and Windels 2015). One fixed wing and the helicopter survey were conducted with multiple observers to help assess detection rates (Walsh et al. 2010).

One test flight was conducted on September 25 2014 to assess visibility of sign at typical fixed wing speed and height, and flight protocol was finalized based on those observations. In the 2014 season, one aerial survey was completed on November 20, and a second survey was partially performed on December 18. However, the second survey was terminated prior to completion due to safety concerns over a malfunction of aircraft altimeter equipment, and additional attempts were canceled due to weather. During the 2015 season similar conflicts with weather and prior commitments for flight time restricted the number of flights performed, with one conducted on November 13 and another on December 4. No additional flights in December were possible despite several attempts, and due to the early timing the snow cover was not consistent between all three study areas. To obtain more complete coverage of the study areas a later winter flight was completed on February 17 2016.

The timeline for contracting with a new helicopter service provider delayed the comparison helicopter survey until April, 2016. Once a contract was in place with Brainerd Helicopters Services Inc, a complete survey was conducted with three observers in a Bell 206B3 Jet Ranger helicopter. Total flight time was six hours and survey conditions were excellent for detecting beaver sign, however this survey occurred outside of the typical sampling season.

Table 3: Aerial surveys completed from fall 2014 to spring 2016 recorded the greatest number of observations occurred in early winter, with a thin layer snow on the ice to facilitate identification of otter tracks without obscuring beaver lodges. Fall fixed wing flights and the spring helicopter survey conditions also allowed for recording of beaver food caching and chewing activity.

Date Observers	Flight time (h:mm)	Distance (km)	Data Points	Conditions
09/24/2014 BE, NR	N/A	N/A	N/A	Beaver sign was abundant, but no snow for otter tracks. BE trained with NR. Data not included into analyses.
11/20/2014 BE	2:03	341	174	Light snow present for otter sign, larger beaver sign detectable but decreased visibility for muskrat sign.
12/18/2014 BE	(1:44)	(202)	(88)	PARTIAL FLIGHT: Allouez Bay, Nemadji River and St. Louis study areas flown, then error with the flight altimeter required landing early.
11/13/2015 BE	2:18	340	254	Excellent conditions for beaver and muskrat sign.
12/4/2015 BE	2:33	393	323	Partial snow conditions allowed for otter sign detection at Boulder Lake Reservoir consistently, in some portions of the St. Louis study area, and only in patches along the St. Croix River.
02/17/2016 BE, NF	2:40	365	198	Consistent snow cover for otter sign in all study areas. NF experienced observer.
		Helicopter Survey		
04/26/2016 BE, NF, MW	2:55	254	174	All ice cover melted, signs remaining of winter beaver food caches and fresh spring activity detectable as well as muskrat sign. MW trained.

6.2 Camera survey results

Motion triggered trail camera surveys were conducted from fall 2014 to early winter 2016 in three distinct deployments. Deployments 1 and 2 each spanned approximately one month from November to December 2014, and December 2014 to January 2015, and consisted of 28 and 29 cameras. In deployments 1 and 2 a total of 8,244 and 27,594 images were recorded, of which 369 and 9,706 were classified as false triggers, leaving 7,875 and 17,888 images triggered by people or animals. These deployments together are considered the *pilot season*, and although detections of target species were sparse they were valuable to assess proof of concept and establish successful research protocol. While all of the four target species were detected, only otter were detected at enough sites for preliminary analyses, and due to the short time span these data are not considered in further detail for the purposes of this report.

Deployment 3 spanned a total of eight months from June 2015 to February 2016, with up to 65 cameras, and these cameras were able to remain active at a site continuously unless changes in habitat characteristics (especially fluctuations in water level) resulted in the camera being moved to a new “site” within 30m of the original location. Some sites were pulled early if access would be unsafe following ice formation, but the majority remained deployed and usable data were collected through January 2016 (the cameras left in the field later into 2016 were not representative of all study areas and although some target species were detected, data analysis only include timeframes for which all areas can be compared). 585,106 total images were recorded, of which 388,164 were false triggers, leaving 196,942 images triggered by people or animals. These data comprise the *full season* of camera research, and to enable analyses over the extended time period deployment 3 was further divided into three roughly equal 11-week long ‘seasons’ (table 4).

During the pilot season, cold weather and precipitation caused some cameras to fail in the field, although the majority remained active and unobstructed. Over deployment 3, cameras were checked on a monthly basis whenever possible to reduce data loses from camera failures. Despite frequent revisits, SD cards were in some cases filled by false triggers (either moving vegetation or waves) which created gaps in the opportunities for a camera to detect target species. This potential difference in trap nights of effort was addressed in the occupancy modeling step. Despite camera failures, numerous species were detected throughout the three deployments with a high diversity of mammalian species (see appendix A.5).

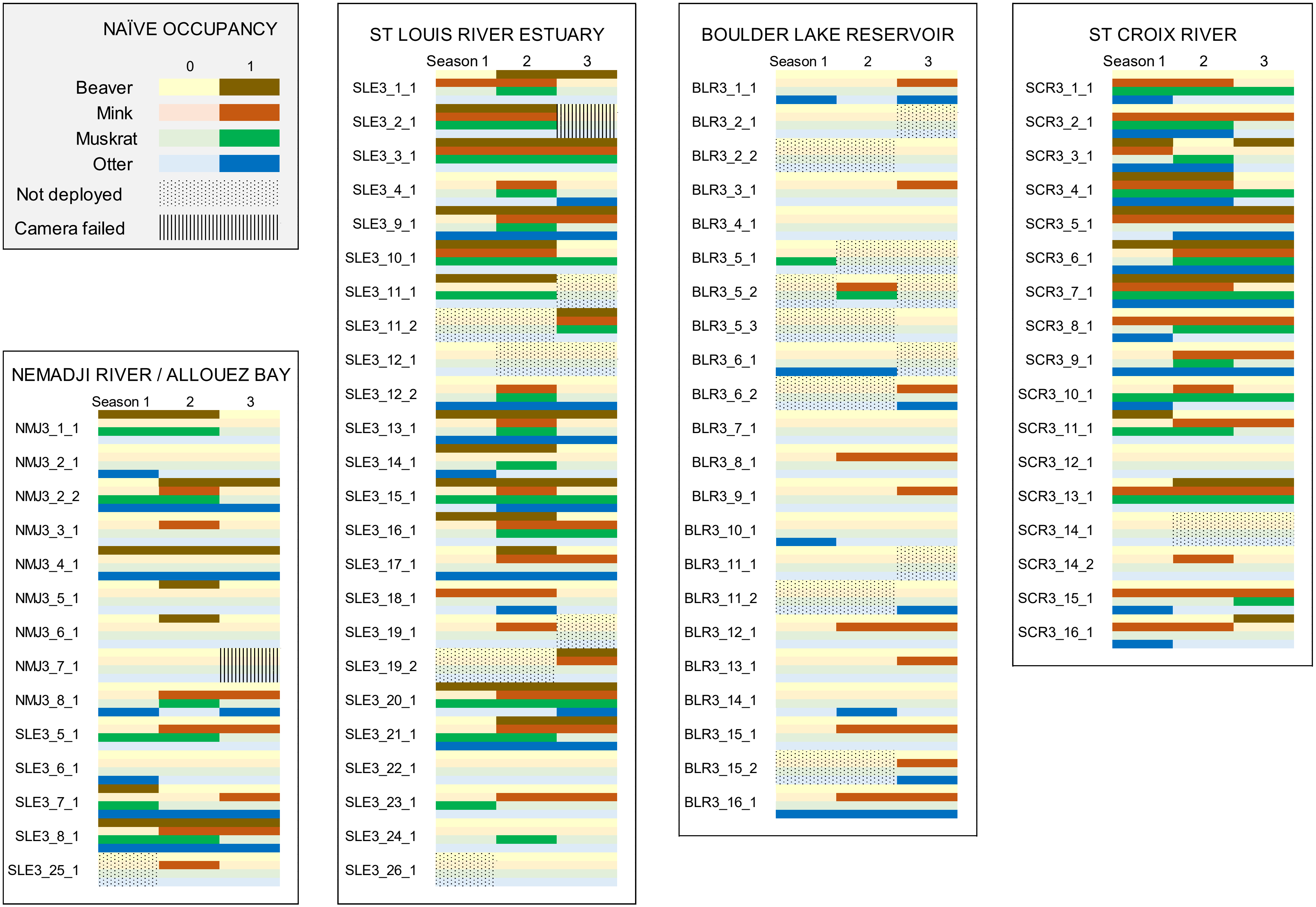
Table 4: Camera deployments from fall 2014 to winter 2016

Deployment	Start	End	Cameras
1	11/4-14/2014	12/2-12/8/2014	28: two malfunctioned; one did not record images.
2	12/2-7/2014	1/10-1/13/2015	29: two failed due to battery/ weather problems; two did not record images.
3.1	5/29/2015	8/14/2015	66: 4 moved to accommodate spring vegetation growth.
3.2	8/15/2015	10/30/2015	65: one was stolen by a beaver and no data was retrievable.
3.3	10/31/2015	1/15/2016**	57 total due to winter access concerns.

* *Setting, checking and adjusting cameras as-needed basis*
**Numerous cameras were not retrieved until early February 2016, and one camera remained deployed until 4/27/2016 due to unsafe ice conditions. Although these time frames cannot be included in analyses without other cameras to reference against (not all study areas are represented), target species were detected during the mid-winter and even early spring months, and the data are maintained for future reference.

Camera sites varied in the detection of target species between seasons, and the variation in weekly detections is further modeled in the occupancy analyses. Figure 2 shows naïve occupancy, simply the successful detection of each species at a camera site, over the course of the study.

Figure 2. Detections / non-detection history (naïve occupancy) of each target species at each camera site over all three seasons.



7 Statistical Results

7.1 Aerial survey results

Kruskal Wallis non-parametric tests found that there was no combination of species, area (reference or AOC), and method (detections per kilometer or per minute) which provides evidence against the null that aerial survey results are equal between the AOC study areas and the reference sites. Both beaver sign and muskrat sign were recorded more frequently in the AOC versus reference sites (359 versus 142 and 311 versus 108 detections) while otter tracks were less frequently recorded in the AOC (69 versus 134), likely a result of excellent visibility and large areas of open ice on Boulder Lake Reservoir.

Due to the somewhat sparse data, tests were pooled across the two AOC areas and the two reference areas. I found no evidence of significant ($p < 0.05$) differences in the relative abundance of beaver or otter sign between the AOC and the reference sites. Flight sample sizes may be insufficient to further model any heterogeneity in detection linked to season, weather or observers, but the data available do not indicate any reduced abundance in the Area of Concern.

Table 5: Results of Kruskal Wallis non-parametric tests for aerial survey data.

	Detections per kilometer			Detections per minute		
	AOC Median	Reference Median	Kruskall Wallis p-value	AOC Median	Reference Median	Kruskall Wallis p-value
Beaver sign	0.563	0.404	0.1984	0.463	0.232	0.4097
Muskrat sign	0.387	0.121	0.3342	0.425	0.126	0.4665
Otter tracks	0.281	0.631	0.2996	0.357	0.407	0.3640

7.2 Detection probability modeling results

The AIC top model ranking results for testing all parameters which could impact detection by each species and season are shown in the appendix (A.6). With the exception of otter in season 2, the AIC ranking process selected one or more top models, and the variables present in them were concluded to be informative parameters for the underlying biological process of species detection (table 6). The sign of the β slope value for temperature was typically positive, indicating that warmer measurements increased detection probability, while distance to water was negative, suggesting that for species which included that parameter cameras closer to water would have greater detection success. The slope for the camera height variable was overall negative, which may reflect the quality of camera sites in which a lower attachment location is possible. However, speculation on detection processes is not the focus of this research. Inclusion of only relevant parameters strengthens the models used to assess occupancy, but are not otherwise examined further.

Table 6. Selected detection parameters by species and season

	Beaver		Muskrat
1	AMave + PMmin	1	DIST.WATER
2	ALZNMJ + BLR + SCR	2	DIST.WATER
3	AMave + AMmin	3	AMmax + PMave + AMave
	Mink		**Otter**
1	ALZNMJ + BLR + SCR + DIST.WATER	1	CAM.HEIGHT
2	DIST.WATER + AMmin	2	*Null*
3	AMmax + AMave	3	PMmax + PMave

7.3 Occupancy probability modeling results and weighted averages

Results of AIC model ranking for assessing occupancy processes are included in the appendix (A.7). Top model weights were more evenly distributed than for detection model ranking, so more models were included to obtain the cumulative weight cut off of 0.9. I conducted parametric bootstrapping for all top models to assess model fit, because ranking among top models cannot be assumed to mean that good models are present (MacKenzie and Bailey 2004). Overall I found excellent fit in terms of chi-square tests, with the mean probability of $p(\chi^2) = 0.635 \pm 0.272$, which does not raise concerns that observed values are abnormally far from expected values. The overall overdispersion factors $\widehat{C} = 1.101 \pm 0.163$ are also very close to 1, indicating the variance within the data are neither much higher nor lower than expected. Only the top two models for otter in season 2 showed an indication of fitting poorly, the chi-square tests bordered on an extreme value at the alpha = 0.05 level (highest model $p(\chi^2)$ = 0.065 and second highest model $p(\chi^2) = 0.045$), and there was some evidence that the observed variation was lower than that expected by the model ($\widehat{C} = 0.877$ and $\widehat{C} = 0.834$) although these are not extreme departures from 1 and clear cutoffs are not defined (Zar 2010). The next three models included in the suite demonstrated slightly better fit, and by using a weighted average approach conclusions should be reasonably good. The data for otters in season 2 were the only case were the detection model ranking rules I followed produced a null result, suggesting unexplained variation in the detection process may contribute to the poorer fitting occupancy models.

In regards to the ranking of the different study area parameters among top models for occupancy, the trend is that the AREA variables are rarely included if they are already present in the detection formula. Looking at the structure of the models when the study area is explicitly modeled for occupancy, in beaver season 2 and mink season 1, the β coefficients are slightly negative for the Allouez bay/Nemadji river factor, very negative for Boulder Lake, and positive for St Croix River. This indicates that the occupancy values St Louis estuary, modeled as the intercept, fall between either extreme. For more rigorous assessment of the differences between study areas, I used the weighted site occupancy values to statistically assess the equivalency between AOC and reference areas for all species and seasons.

While the full weighted occupancy output is extensive, an average value across all top models is presented below (table 7) and indicates the overall high occupancy probabilities within the AOC. The range shown is the minimum to maximum for all models and all sites with a study area, regardless of AIC weighting.

Table 7. The average, weighted occupancy value for each species at all sites in a study area (by AIC model weight), and the range of values at sites prior to averaging (all models).

BEAVER

	Season 1		Season 2		Season 3	
	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range
BLR	1.38×10^{05}	(9.65×10^{7}, .147)	6.97×10^{6}	(5.03×10^{7},1.98×10^{5})	9.16×10^{6}	(1.21×10^{6}, .217)
SCR	0.103	(.019, .160)	0.126	(.035, .175)	0.142	(.047, .258)
SLE	0.159	(.019, .252)	0.219	(.094, .364)	0.124	(.041, .227)
ALZNMJ	0.098	(.042, .164)	0.156	(.073, .218)	0.105	(.055, .227)

MINK

	Season 1		Season 2		Season 3	
	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range
BLR	3.67×10^{6}	(4.59×10^{12}, 2.27×10^{4})	0.072	(.002, .283)	0.107	(.057, .302)
SCR	0.140	(.008, .293)	0.278	(.103, .416)	0.138	(.088, .276)
SLE	0.056	(.004, .190)	0.216	(.099, .306)	0.108	(.043, .199)
ALZNMJ	2.10×10^{6}	(1.10×10^{11}, 1.31×10^{5})	0.170	(.058, .322)	0.135	(.056, .265)

MUSKRAT

	Season 1		Season 2		Season 3	
	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range
BLR	0.026	(8.77×10^{8}, .252)	0.034	(9.46×10^{7}, .458)	2.16×10^{06}	(1.33×10^{8}, .189)
SCR	0.117	(.007, .280)	0.160	(.023, .480)	0.164	(.031, .345)
SLE	0.086	(.007, .256)	0.209	(.023, .469)	0.065	(.033, .201)
ALZNMJ	0.095	(.003, .230)	0.191	(.007, .411)	1.22×10^{05}	(4.45×10^{7}, .178)

OTTER

	Season 1		Season 2		Season 3	
	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range	$\bar{\Psi}$	Range
BLR	0.058	(.009, .183)	0.076	(.013, .135)	0.099	(.040, .220)
SCR	0.182	(.037, .315)	0.115	(.032, .166)	0.150	(.035, .323)
SLE	0.077	(.030, .269)	0.098	(.031, .147)	0.123	(.044, .222)
ALZNMJ	0.169	(.003, .327)	0.096	(0.0468, .135)	0.158	(.072, .321)

7.4 Equivalency tests

Weighted estimates for the occupancy probability at each camera site ranged widely from <0.001 for some estimates of beaver at Boulder Lake to 0.416 for muskrat at several sites along the St Croix river. All site estimates for each species and season were grouped by either REF if in a reference area or AOC if in the area of concern, and treated as the sample of populations of interest for assessing any significant difference. The equivalency test calculates the mean difference $\bar{d}$ and then uses the standard error of the reference site to determine the region of equivalence. If the sample distributions fall within that region, than the null assumption that they are different can be rejected (table 8).

Table 8: Results of robust TOST (two one-sided tests) against the null that the means of weighted occupancy at AOC sites and at reference sites are different ($\bar{d} \neq 0$). Alpha = 0.05, region of equivalence = 20% SE of reference data. All tests reject the null.

	$\bar{d}$	SE	90% CI	df	P-value
Beaver 01	-0.086	0.016954	(-0.11495, -0.05779)	38.3	$4.10\text{x}10^{-16}$
Beaver 02	-0.136	0.019661	(-0.16921, -0.10224)	27.0	$8.86\text{x}10^{-10}$
Beaver 03	-0.065	0.017592	(-0.09501, -0.03538)	31.5	$2.71\text{x}10^{-15}$
Mink 01	0.019	0.018976	(-0.01370, 0.051049)	25.8	$8.65\text{x}10^{-15}$
Mink 02	-0.030	0.033653	(-0.08794, 0.027254)	23.8	$8.50\text{x}10^{-09}$
Mink 03	0.005	0.010463	(-0.01344, 0.022374)	23.8	$1.92\text{x}10^{-20}$
Muskrat 01	-0.027	0.019957	(-0.06115, 0.006271)	35.3	$2.00\text{x}10^{-16}$
Muskrat 02	-0.091	0.041396	(-0.16090, -0.02018)	28.7	$5.65\text{x}10^{-06}$
Muskrat 03	0.011	0.017577	(-0.01857, 0.041031)	31.1	$1.39\text{x}10^{-17}$
Otter 01	0.010	0.021818	(-0.02665, 0.047184)	33.1	$1.55\text{x}10^{-15}$
Otter 02	0.002	0.008095	(-0.01191, 0.015366)	38.9	$1.02\text{x}10^{-32}$
Otter 03	-0.016	0.007827	(-0.02908, -0.00271)	39.3	$1.04\text{x}10^{-32}$

The successful rejection of the null hypothesis for all species and season tests indicate that no statistically significant differences are detectable between the AOC and the reference sites. Visual representation via boxplots show that although there are outliers the majority of site estimates do overlap (see appendix A.8).

8 Discussion

8.1 Methods to compare between Area of Concern and reference study areas

The use of two distinct survey methods enabled comparison between the study areas despite challenges presented by the diversity of habitat types, logistic constraints and year to year variation experienced. This is illustrated especially by the low occupancy estimates from camera surveys for Boulder Lake reservoir for the third deployment, which I speculate were ultimately caused by unusually low water levels altering space use by target species. While cameras obtained fewer detections overall, and none of beaver, the aerial surveys were less susceptible to this short-term fluctuation. Because trail camera success is closely tied to the quality of the site selected, during periods of time when animal movement patterns may have shifted (e.g. due to weather anomalies or disturbance) the boarder spatial scale provided by aerial surveys could prevent complete failure to detect animal sign.

However, the effort and cost required to conduct thorough fixed wing or helicopter surveys is extensive, and with the limited number of flights I was able to conduct there are limitations on the rigor of conclusions that can be drawn. Even with dedicated staff to ensure that every good weather day could be taken advantage of, it is still only possible to collect information on beaver, otter and muskrat, and quality observations are restricted to only a specific season each year. Cameras have the advantage for monitoring a broad suite of species, and with the caveat that multiple sites must be maintained and monitored to account for heterogeneous detection, they were able to collect sufficient data for more rigorous analyses.

8.2 Toxicant analyses

Legacy and ongoing contaminants can be a cause for concern throughout aquatic ecosystems, and therefore the scope of this project potentially included investigating levels from tissue samples within the AOC and the reference areas should evidence of suppressed populations arise. None of the data available from these surveys indicates that otter, mink, muskrat or beaver are abnormally restricted within the AOC, thus toxicant analyses are not considered to be a high priority at this time.

Over the course of this research myself and collaborators made efforts to collect carcasses of otters and mink from within the study areas, in the event toxicant analyses were deemed valuable and funds were available. While agency staff in both Minnesota and Wisconsin, as well as members of the trapping communities, were obliging, the sample sizes of animals that can be confirmed as harvested within the study areas are very small. Only two otters known to come out of the AOC were successfully collected by WDNR staff, and of those only one liver is available for analyses. Three otters were taken by a tribal trapper near the St Croix reference area, though the exact location is unknown. Additional livers were extracted from otters with the harvest county listed as either Burnett, Douglas or Washburn, but precise locations are unknown. No samples were available from the AOC and reference sites on the Minnesota side, but a trapper volunteered four otter and eight mink livers from Brimson MN. All samples that could be of potential value are currently frozen and stored at UW Madison.

8.4 Degraded Fish and Wildlife Populations BUI Status and Recommendations

From the analyses of aerial and camera surveys conducted for this report, I have not found evidence that beaver, mink, muskrat or otter detections within the St Louis River Area of Concern are significantly lower than those in reference sites. It is important to note that wildlife surveys are not the same as a population census, so while these data cannot provide an estimate of the number of individuals with the AOC, the surveys were designed to assess any differences in relative abundances of species. By assessing at the scale of detections per unit of survey effort in the flight data, and by modeling camera site data in an occupancy framework, results illustrate the presence of species and use of habitat resources throughout the areas of interest. A key assumption is that reference sites share many of the ecological features of the AOC, with only the history of degradation and remediation substantially differentiating them. From careful selection of reference sites, qualitative observations, and the quantitative results of the surveys, this appears to be a valid assumption despite weather anomalies.

The removal target for BUI 2 states *In consultation with their federal, tribal, local, and nonprofit partners, state resource management agencies concur that diverse native fish and wildlife populations are not limited by physical habitat, food sources, water quality, or contaminated sediments*. (MPCA 2013). This research assessed the status of one target group under the BUI; four native mammal species that are assumed to have been rare or extirpated during the most extreme degradation of the St Louis River ecosystem. This study has found that the removal objective for small semi-aquatic mammals is being met as no evidence that a current lack of suitable habitat, resources or pollution was impeding their ability to naturally repopulate the area. The data cannot ascertain if aspects of habitat, food availability, or water quality are sub-optimal, but there is support that the ecosystem is healthy to the degree required for these species to meet their life requirements at levels similar to areas without the same history of degradation.

Similarly, BUI 9 addresses the loss of habitat features required for wildlife to exist within the AOC, and while this research was not a direct assessment of habitat quality, the four target species span several trophic levels and have diverse habitat needs. The detection of beaver, muskrat, mink and otter in the St. Louis AOC indicate that sufficient resources are available to them. Although this research is not an exhaustive assessment of all wildlife species that should be present within the fully restored estuary, there is no indication that specific features require additional remediation attention before recovery can continue.

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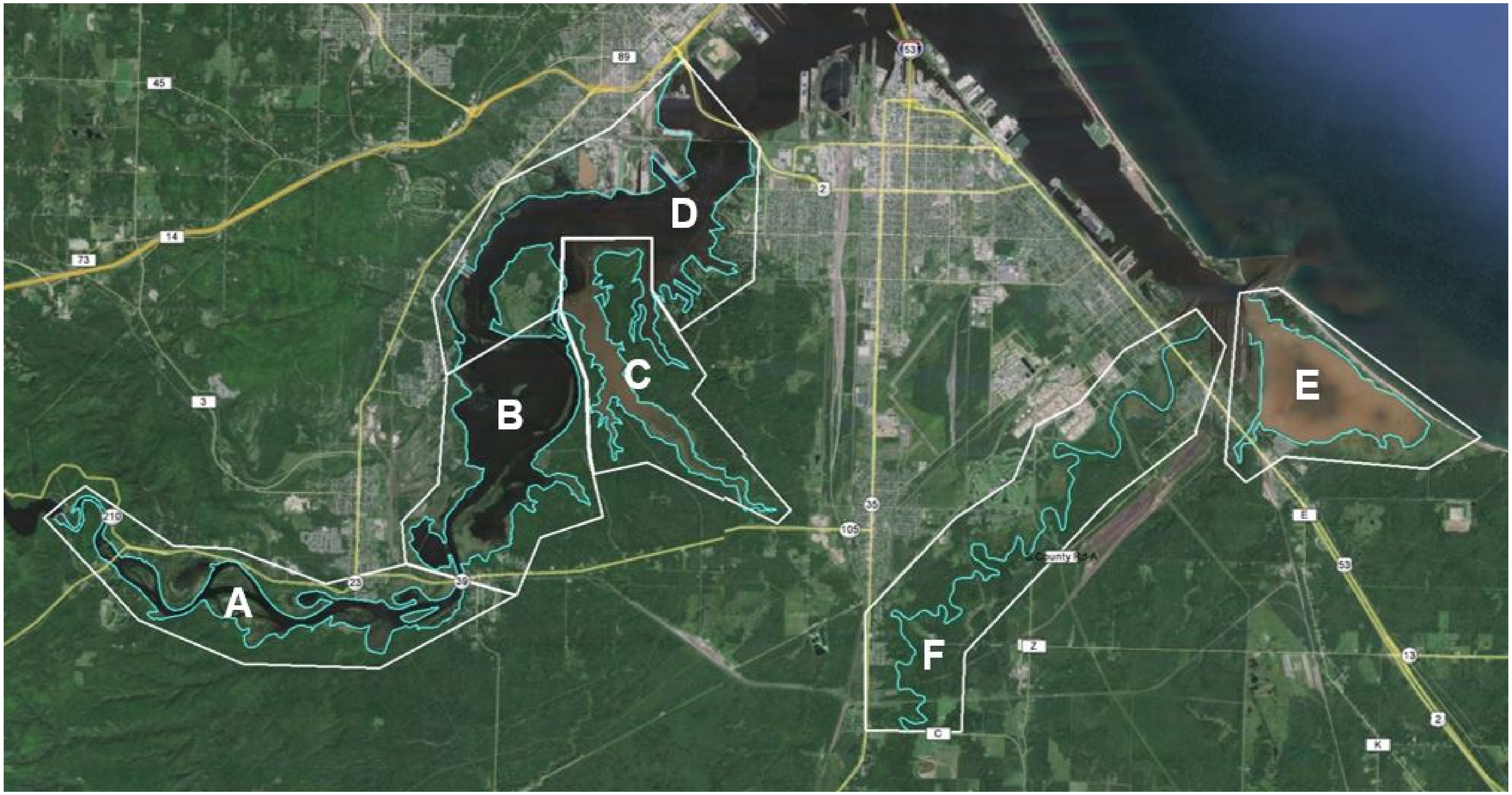
A Appendix

A.1 Map of zone differentiation for stratification by habitat type in the St Louis AOC and Nemadji / Allouez Bay study areas



Zone A: Riverine. Faster flowing narrowing channel from Fond du Lac Dam to Oliver Bridge near Mud Lake

Zone B: Undeveloped estuary. From Oliver Bridge to Spirit Lake, area includes remediation sites

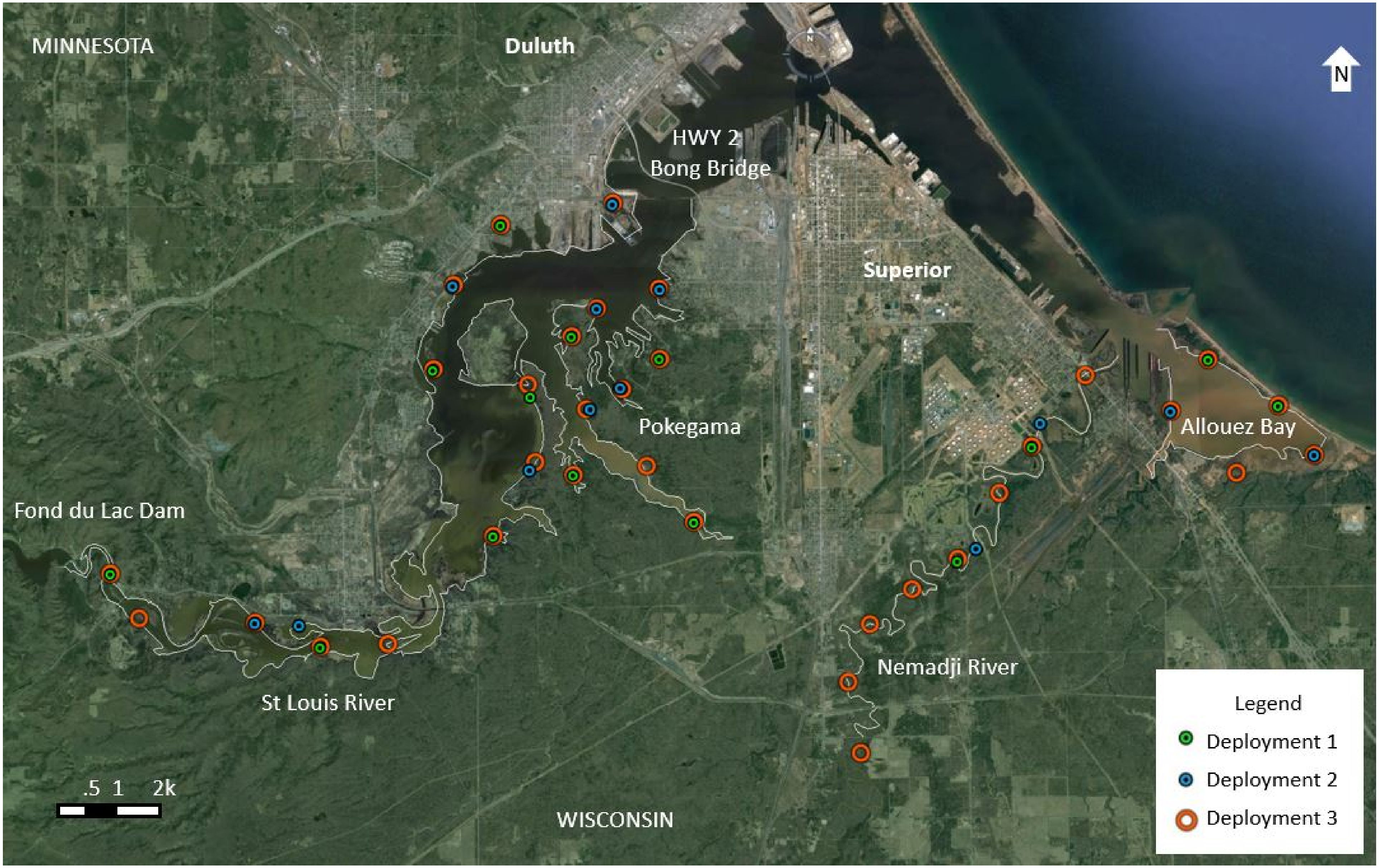
Zone C: Developed estuary. Morgan Park to Bong Bridge (west) and Pokegama estuary (east). Moderate to extensive industrialization

Zone D: Pokegama estuary. Shallow water, dense vegetation

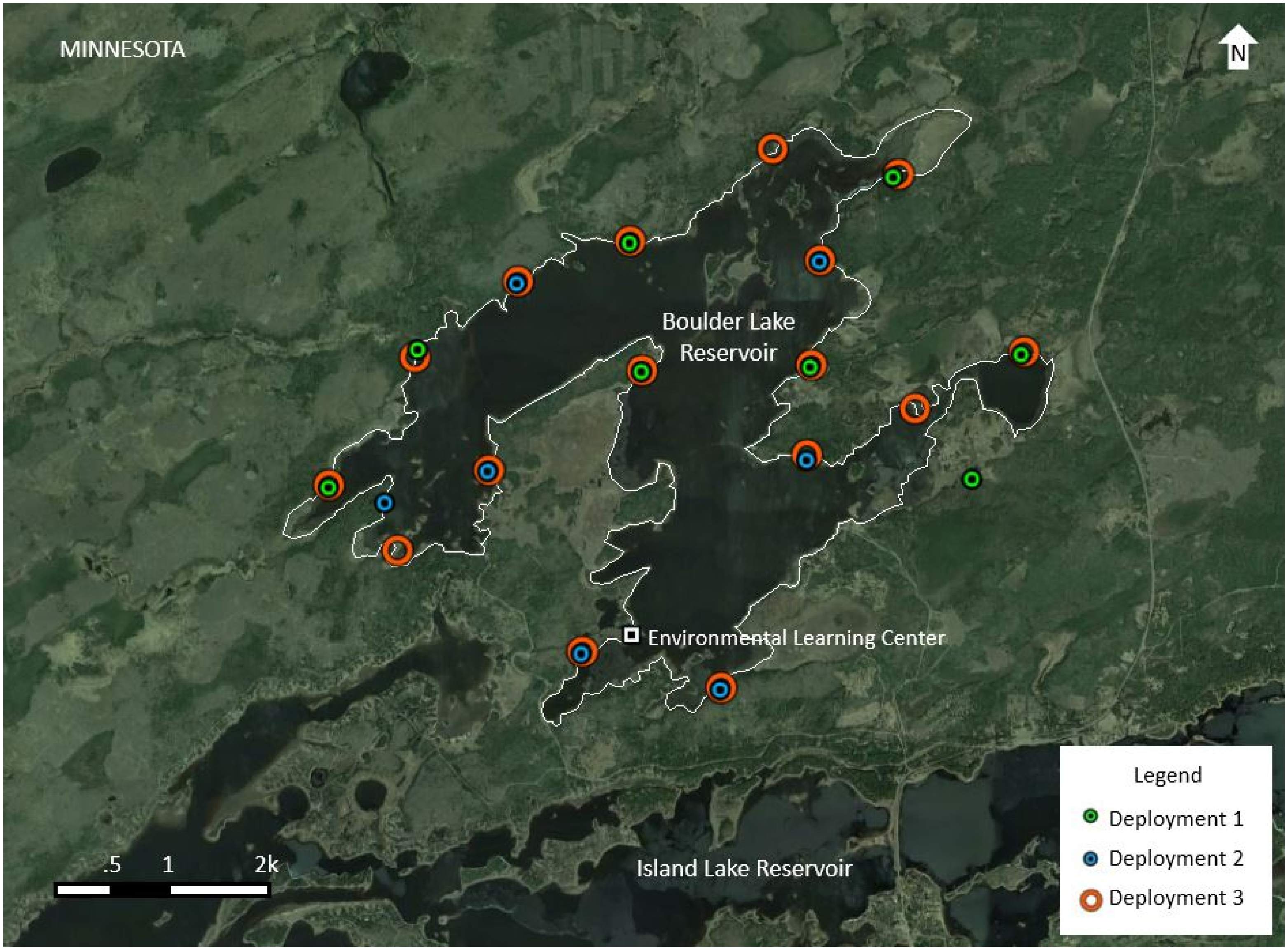
Zone E: Allouez Bay. Inside perimeter of Wisconsin Point, eastern habitat including extensive marsh

Zone F: Nemadji River. From mouth up 12 miles to bridge at E County Road CS

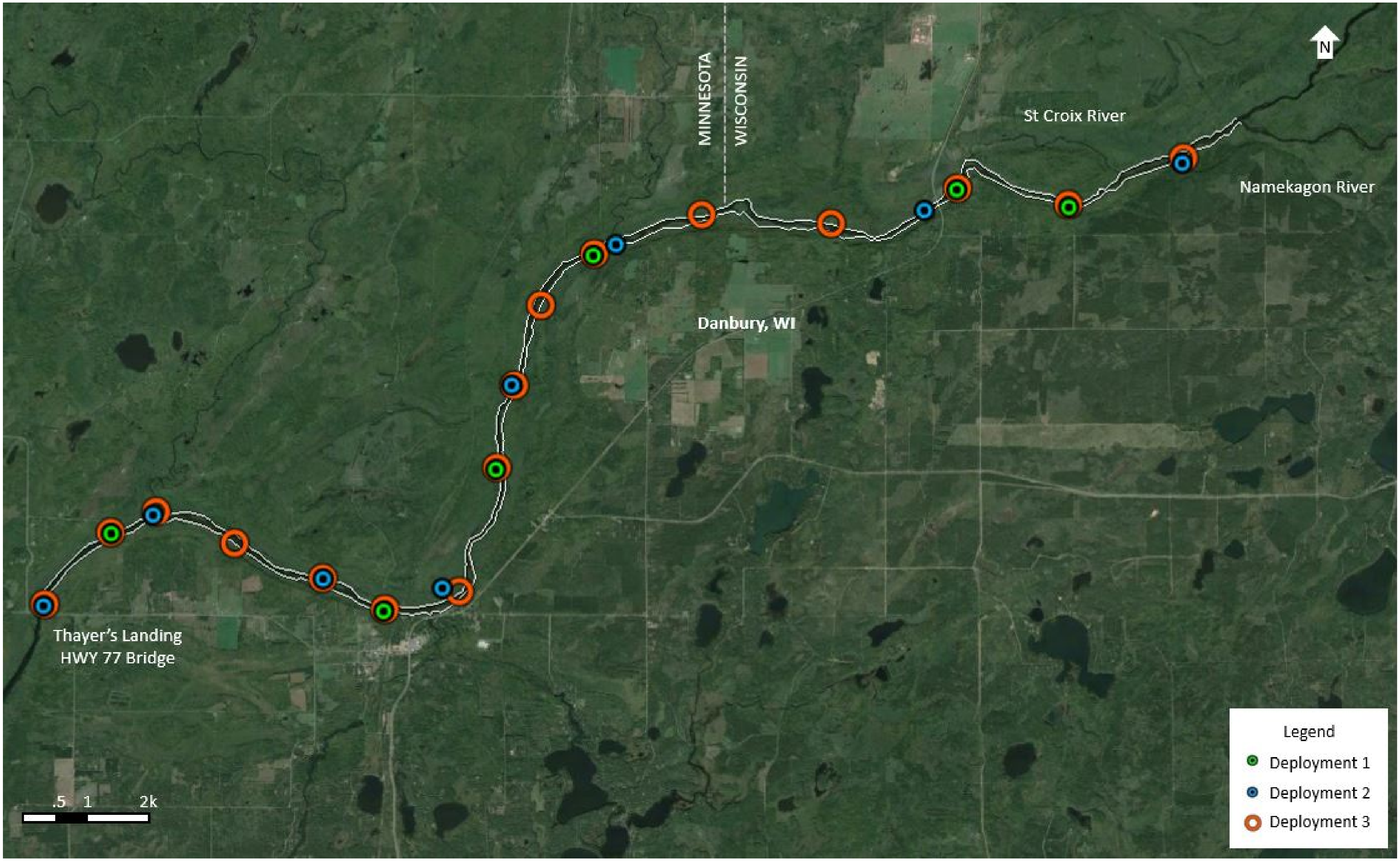
A.2 Camera locations for all deployments in the St Louis River AOC and Nemadji River / Allouez Bay study areas



A.3 Camera locations for all deployments in the Boulder Lake reference area



A.4 Camera locations for all deployments in the St. Croix River reference area



A.5 Pilot Season Species Detections: Early results indicated that all four target species could be detected with trail cameras, as well as a diversity of other species. There was a drop in sites with confirmed detections of target species in the second deployment.

Deployment one: 28 Cameras

Species	# Sites	Species	# Sites
Bird *all species*	11	People	3
Otter	**11**	**Beaver**	**3**
Squirrel	10	Black bear	2
Mouse	9	**Mink**	**3**
Coyote	8	Weasel	2
Deer	8	Domestic cat	1
Fox	8	Domestic dog	1
Rabbit	7	Fisher	1
Raccoon	6	Marten	1
Bobcat	4	**Muskrat**	**1**

Deployment two: 29 Cameras

Species	# Sites	Species	# Sites
Bird *all species*	6	People	7
Otter	**1**	**Beaver**	**0**
Squirrel	10	Black bear	0
Mouse	10	**Mink**	**1**
Coyote	9	Weasel	1
Deer	2	Domestic cat	0
Fox	10	Domestic dog	7
Rabbit	5	Fisher	0
Raccoon	15	Marten	0
Bobcat	8	**Muskrat**	**0**

Deployment 3 Detections: More cameras were deployed for an extended period of time, allowing sites to be modified as needed and providing more detections of target species.

Species	Season 1 *70 sites*	Season 2 *66 sites*	Season 3 *66 sites*
Beaver	20	24	18
Mink	15	41	31
Muskrat	21	32	14
Otter	28	21	22

Deployment 3 non-target species: Those in bold were not detected in the pilot year

Species detected at one or more camera sites across all three seasons						
Badger	Coyote	Duck / Geese	Groundhog	Mouse	Rabbit	Striped Skunk
Bald Eagle	Crane / Heron	Fisher	Grouse	Owl / Raptors	Raccoon	Turkey
Birds	Deer	Fox - Gray	Gull / Pelican	People	**Reptile**	Weasel
Black Bear	Dom. Dog / Cat	Fox - Red	Raven /Jay	Porcupine	Squirrel	**Wolf**

A.6 Ranking top detection models with site- and observation-level covariates: Of the seven site-level variables tested, four (TREE.COVER, MARSH.COVER, SLOPE, and TREE.DBH) were never present in a top model. Of the observation-level variables, ACTIVE was never present in a top model.

BEAVER

Season	Model Equation	AIC	Δ AIC	AIC Weight	Cumulative Weight
1	Ψ(.), p(AM.Ave)	313.2184	0	0.577113	0.577
	Ψ(.), p(PM.Min)	314.5877	1.369323	0.291015	0.868
2	Ψ(.), p(AREA)	405.57	0	0.988655	0.989
3	Ψ(.), p(AM.Ave)	234.0961	0	0.751067	0.751
	Ψ(.), p(AM.Min)	237.0452	2.94904	0.171911	0.923

MINK

Season	Model Equation	AIC	Δ AIC	AIC Weight	Cumulative Weight
1	Ψ(.), p(AREA)	225.5965	0	0.819405	0.819
	Ψ(.), p(DIST.WATER)	229.0399	3.443387	0.146479	0.966
2	Ψ(.), p(DIST.WATER)	604.6594	0	0.711929	0.712
	Ψ(.), p(AM.Min)	606.8938	2.234401	0.232939	0.945
3	Ψ(.), p(AM.Max)	336.52	0	0.578023	0.578
	Ψ(.), p(AM.Ave)	337.3379	0.817878	0.384012	0.962

MUSKRAT

Season	Model Equation	AIC	Δ AIC	AIC Weight	Cumulative Weight
1	Ψ(.), p(DIST.WATER)	280.9117	0	0.999895	0.9999
2	Ψ(.), p(DIST.WATER)	481.4915	0	1	1
3	Ψ(.), p(AM.Max)	141.7617	0	0.5223	0.522
	Ψ(.), p(PM.Ave)	143.0719	1.310182	0.27128	0.794
	Ψ(.), p(AM.Ave)	144.0001	2.238425	0.17055	0.964

OTTER

Season	Model Equation	AIC	Δ AIC	AIC Weight	Cumulative Weight
1	Ψ(.), p(CAM.HEIGHT)	373.5132	0	0.958728	0.959
2*	*Ψ(.), p(CAM.HEIGHT) – NULL*		*0*	*0.324404*	*0.324*
3	Ψ(.), p(PM.Min)	316.7077	0	0.658745	0.659
	Ψ(.), p(PM.Ave)	318.4363	1.728654	0.277553	0.936

* The top model for otter season 2 detection falls below the 0.5 AIC Weight cut off for consideration as a top model. The null detection model is therefore selected.

A.7 Top model AIC ranking for occupancy estimation, using the detection parameters determined to be significant for each species and season. Parametric bootstrapping found excellent model fit with chi-square and overdispersal factors except the otter season 2 suite.

BEAVER

Season	Model #	Model Equation	k	n	AIC	Δ AIC	AIC Weight	R^2	Cumulative Weight	$p(\chi^2)$	$\hat{c}$
1	6	p(AMave+PMmin), Ψ(AREA+MARSH.COVER)	8	70	303.2	0	0.40	0.224	0.40	0.37	0.98
	2	p(AMave+PMmin), Ψ(AREA)	7	70	304.0	0.80	0.27	0.192	0.67	0.33	0.98
	8	p(AMave+PMmin), Ψ(AREA+TREE.COVER+MARSH.COVER)	9	70	304.7	1.45	0.20	0.230	0.87	0.28	0.96
	5	p(AMave+PMmin), Ψ(AREA+TREE.COVER)	8	70	305.7	2.50	0.12	0.196	0.98	0.25	0.96
2	1	p(AREA), Ψ(.)	5	66	405.6	0	0.29	0.000	0.29	0.65	1.02
	4	p(AREA), Ψ(MARSH.COVER)	6	66	406.4	0.84	0.19	0.018	0.48	0.89	1.97
	3	p(AREA), Ψ(TREE.COVER)	6	66	406.7	1.18	0.16	0.012	0.64	0.67	1.02
	7	p(AREA), Ψ(TREE.COVER+MARSH.COVER)	7	66	407.2	1.65	0.13	0.035	0.76	0.87	1.08
	2	p(AREA), Ψ(AREA)	8	66	408.2	2.68	0.08	0.049	0.84	0.44	0.99
	6	p(AREA), Ψ(AREA+MARSH.COVER)	9	66	408.3	2.71	0.07	0.077	0.91	0.61	1.03
	8	p(AREA), Ψ(AREA+TREE.COVER+MARSH.COVER)	10	66	409.1	3.56	0.05	0.093	0.96	0.55	1.03
3	2	p(AMave+AMmin), Ψ(AREA)	7	66	228.4	0	0.46	0.194	0.46	0.89	1.24
	6	p(AMave+AMmin), Ψ(AREA+MARSH.COVER)	8	66	229.5	1.13	0.26	0.205	0.71	0.89	1.26
	5	p(AMave+AMmin), Ψ(AREA+TREE.COVER)	8	66	230.3	1.98	0.17	0.194	0.88	0.87	1.26
	8	p(AMave+AMmin) ~ ALZNMJ + BLR + SCR + ZTREE.COVER + ZMARSH.COVER	9	66	231.5	3.13	0.10	0.205	0.98	0.89	1.30

MINK

Season	Model #	Model Equation	k	n	AIC	Δ AIC	AIC Weight	R^2	Cumulative Weight	$p(\chi^2)$	$\hat{c}$
1	4	p(AREA+DIST.WATER), Ψ(MARSH.COVER)	7	70	217.5	0	0.48	0.056	0.48	0.83	1.21
	1	p(AREA+DIST.WATER), Ψ(.)	6	70	219.3	1.81	0.19	0.000	0.67	0.96	1.23
	7	p(AREA+DIST.WATER), Ψ(TREE.COVER+MARSH.COVER)	8	70	219.4	1.97	0.18	0.056	0.85	0.75	1.15
	3	p(AREA+DIST.WATER), Ψ(TREE.COVER)	7	70	221.2	3.71	0.07	0.002	0.92	0.91	1.20
2	2	p(DIST.WATER+AMmin), Ψ(AREA)	7	66	591.4	0	0.25	0.087	0.25	0.91	1.10
	1	p(DIST.WATER+AMmin), Ψ(.)	4	66	591.5	0.03	0.25	0.000	0.50	0.91	1.09
	6	p(DIST.WATER+AMmin), Ψ(AREA+MARSH.COVER)	8	66	593.0	1.58	0.12	0.093	0.62	0.89	1.10
	4	p(DIST.WATER+AMmin), Ψ(MARSH.COVER)	5	66	593.2	1.72	0.11	0.005	0.73	0.91	1.10
	5	p(DIST.WATER+AMmin), Ψ(AREA+TREE.COVER)	8	66	593.4	1.97	0.09	0.088	0.82	0.89	1.09
	3	p(DIST.WATER+AMmin), Ψ(TREE.COVER)	5	66	593.5	2.03	0.09	0.000	0.91	0.92	1.09
	8	p(DIST.WATER+AMmin), Ψ(AREA+TREE.COVER+MARSH.COVER)	9	66	594.9	3.42	0.05	0.095	0.96	0.86	1.10
3	1	p(AMmax+AMave), Ψ(.)	4	66	338.1	0	0.38	0.000	0.38	0.96	1.12
	3	p(AMmax+AMave), Ψ(TREE.COVER)	5	66	339.1	1.02	0.23	0.015	0.61	0.92	1.11
	4	p(AMmax+AMave), Ψ(MARSH.COVER)	5	66	339.9	1.80	0.16	0.003	0.77	0.92	1.11
	7	p(AMmax+AMave), Ψ(TREE.COVER+MARSH.COVER)	6	66	340.5	2.39	0.12	0.024	0.88	0.82	1.08
	2	p(AMmax+AMave), Ψ(AREA)	7	66	342.5	4.33	0.04	0.025	0.93	0.91	1.12
	5	p(AMmax+AMave), Ψ(AREA+TREE.COVER)	8	66	342.8	4.66	0.04	0.050	0.96	0.77	1.09

MUSKRAT

Season	Model #	Model Equation	k	n	AIC	Δ AIC	AIC Weight	R^2	Cumulative Weight	$p(\chi^2)$	$\hat{c}$
1	1	p(DIST.WATER), Ψ(.)	3	70	280.9	0	0.41	0.000	0.41	0.63	1.15
	3	p(DIST.WATER), Ψ(TREE.COVER)	4	70	282.3	1.39	0.20	0.009	0.61	0.60	1.12
	4	p(DIST.WATER), Ψ(MARSH.COVER)	4	70	282.3	1.42	0.20	0.008	0.81	0.61	1.15
	7	p(DIST.WATER), Ψ(TREE.COVER+MARSH.COVER)	5	70	283.2	2.28	0.13	0.025	0.94	0.54	1.10
2	1	p(DIST.WATER), Ψ(.)	3	66	481.5	0	0.28	0.000	0.28	0.82	1.27
	3	p(DIST.WATER), Ψ(TREE.COVER)	4	66	482.6	1.11	0.16	0.013	0.44	0.81	1.25
	4	p(DIST.WATER), Ψ(MARSH.COVER)	4	66	482.6	1.12	0.16	0.013	0.60	0.90	1.27
	7	p(DIST.WATER), Ψ(TREE.COVER+MARSH.COVER)	5	66	483.2	1.72	0.12	0.034	0.71	0.81	1.25
	2	p(DIST.WATER), Ψ(AREA)	6	66	483.6	2.12	0.10	0.057	0.81	0.85	1.28
	5	p(DIST.WATER), Ψ(AREA+TREE.COVER)	7	66	484.1	2.56	0.08	0.079	0.89	0.85	1.29
	8	p(DIST.WATER), Ψ(AREA+TREE.COVER+MARSH.COVER)	8	66	484.6	3.10	0.06	0.099	0.95	0.84	1.33
3	2	p(AMmax+AMave+PMave), Ψ(AREA)	8	66	131.8	0	0.48	0.278	0.48	0.45	1.10
	5	p(AMmax+AMave+PMave), Ψ(AREA+TREE.COVER)	9	66	133.1	1.29	0.25	0.288	0.73	0.34	0.96
	6	p(AMmax+AMave+PMave), Ψ(AREA+MARSH.COVER)	9	66	133.8	2.00	0.18	0.278	0.91	0.45	1.05
	8	p(AMmax+AMave+PMave), Ψ(AREA+TREE.COVER+MARSH.COVER)	10	66	135.0	3.28	0.09	0.288	1.00	0.33	1.02

OTTER

Season	Model #	Model Equation	k	n	AIC	Δ AIC	AIC Weight	R^2	Cumulative Weight	$p(\chi^2)$	$\hat{c}$
1	2	p(CAM.HEIGHT), Ψ(AREA)	6	70	368.2	0	0.41	0.151	0.41	0.47	1.00
	6	p(CAM.HEIGHT), Ψ(AREA+MARSH.COVER)	7	70	369.4	1.27	0.22	0.159	0.63	0.31	0.98
	5	p(CAM.HEIGHT), Ψ(AREA+TREE.COVER)	7	70	369.7	1.57	0.19	0.156	0.82	0.44	1.01
	8	p(CAM.HEIGHT), Ψ(AREA+TREE.COVER+MARSH.COVER)	8	70	370.7	2.56	0.11	0.168	0.93	0.42	1.00
2	4	p(.), Ψ(MARSH.COVER)	3	66	355.9	0	0.43	0.059	0.43	0.07*	0.88
	7	p(.), Ψ(TREE.COVER+MARSH.COVER)	4	66	357.5	1.58	0.19	0.065	0.62	0.05**	0.83
	1	p(.), Ψ(.)	2	66	357.9	1.98	0.16	0.000	0.78	0.18	0.97
	3	p(.), Ψ(TREE.COVER)	3	66	358.7	2.83	0.10	0.017	0.89	0.20	0.97
	6	p(.), Ψ(AREA+MARSH.COVER)	6	66	360.2	4.33	0.05	0.082	0.93	0.22	0.93
3	4	p(PMmax+PMave), Ψ(MARSH.COVER)	5	66	318.2	0	0.36	0.038	0.36	0.62	0.88
	1	p(PMmax+PMave), Ψ(.)	4	66	318.7	0.54	0.27	0.000	0.63	0.18	0.97
	7	p(PMmax+PMave), Ψ(TREE.COVER+MARSH.COVER)	6	66	319.7	1.52	0.17	0.045	0.80	0.68	1.05
	3	p(PMmax+PMave), Ψ(TREE.COVER)	5	66	320.6	2.48	0.10	0.001	0.91	0.20	0.97
	6	p(PMmax+PMave), Ψ(AREA+MARSH.COVER)	8	66	322.7	4.56	0.04	0.059	0.94	0.38	0.98

* Significant at the alpha = 0.10 level
** Significant at the alpha = 0.05 level

A.8 Boxplots of mean value and outliers for weighted occupancy estimates by AOC and reference areas; species, and season.