Sea Duck Joint Venture
Final Project Report

Project Title: Sample size considerations for satellite telemetry studies using hypothetical and empirical data for sea ducks

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Project Description: The fifteen species of North American sea ducks (Tribe *Mergini*) winter mainly in coastal areas and the Great Lakes, and many breed in remote areas in the Arctic and boreal forests where it is difficult and costly to conduct field studies. As a result, life histories of sea ducks are not well understood, especially compared to other species of North American waterfowl. Satellite telemetry offers an opportunity to monitor long distance, seasonal movements and observe movements in remote areas such as the Arctic and offshore areas. Satellite telemetry has been promoted and used extensively by the Sea Duck Joint Venture (SDJV) as a way to gain insight into sea duck ecology. The most basic spatial questions managers and researchers are asking about sea ducks include: what is the overall breeding and wintering range?; what are the connections between breeding and wintering areas?; and, are there distinct breeding and wintering populations or sub-groups that monitoring efforts and management decisions (e.g. harvest) need to consider?

Satellite telemetry is expensive (e.g., the current total cost for equipment, field effort, and data access runs to approximately US $4000/bird, T. Bowman, pers. communication), and capturing individuals to outfit with transmitters can be difficult. The investment in, and risks of, a telemetry study are worthwhile when it is possible to collect sufficient high quality data to resolve the questions that are motivating the study. However, there is little guidance on the sample sizes necessary to address specific research questions using telemetry data. Lindberg and Walker (2007) explored sample size requirements as a function of the number of population “states,” either geographic or temporal, when membership in a state is unambiguous. They asked what sample sizes are necessary to ensure a high probability of sampling an individual belonging to each state and estimating the proportion of the population in each known state. They suggest that a minimum sample size of 25 is needed if there are two population states, and 75 for three states (see Appendix 1 for an extension of the analysis of Lindberg and Walker [2007]). Their analysis, however, did not address what sample sizes are needed to identify population states when they are unknown, as is the case for many questions about sea duck distribution and population structure.

Determining the appropriate number of sea ducks to capture and outfit with satellite transmitters in order to understand sea duck distributions and identify distinct sea duck breeding areas requires researchers to define the specific analysis they plan to perform: sample size determination is directly tied to calculations of power or precision (the probability of detecting a true effect, distinguishing true differences, or having some degree of certainty about the size of an effect). Another consideration in evaluating necessary sample size is the difference between the total number of birds tagged with transmitters and the smaller “effective sample size” available to address a specific question. Transmitters can fail during the period of interest, some birds die prematurely, and only data from a specific age or sex cohort may be relevant, so that the resulting sample relevant for inference is less than the number of birds originally outfitted with satellite transmitters (effective *n* < tagged *n*).

We explored the effective sample sizes needed to accurately characterize the breeding distributions of sea ducks and considered the particular problem of identifying spatially distinct breeding areas. Unlike Lindberg and Walker (2007) who examined sample sizes needed to describe the distribution of birds among different states, we were interested in determining the number and boundaries of those states. Our analysis used existing telemetry data from three species of sea ducks to create biologically realistic breeding distributions, from which we sampled to explore the relationship between sample size and the strength of the resulting inference about the characteristics of the breeding distribution.

Objectives: Our objective was to determine a range of sample sizes needed to most closely mimic a realistic distribution of breeding birds, considering both the entire breeding range and core breeding areas.

Methods: We used satellite telemetry data from three species of sea ducks: Barrow's goldeneye (*Bucephala islandica*) captured while wintering on the Pacific coast of the United States and Canada (S. Boyd unpublished data); black scoter (*Melanitta americana*) captured staging and wintering along the Atlantic coast of the U.S. and Canada (S. Gilliland unpublished data); and, surf scoter (*Melanitta perspicillata*) captured wintering along eastern (SDJV, BOEM, S. Gilliland, C. Lepage, BRI, unpublished data) and western North American coasts (S. Boyd, S. de la Cruz, J. Evenson, J. Takekawa, D. Ward, unpublished data). Data from these three species were originally collected for a variety of purposes, mostly related to annual movements.

These three species illustrate distinct cases for consideration in a sample size analysis, and data for each species represent a different capture strategy, spatial scale, and movement pattern. Barrow's goldeneye were captured while wintering on the Pacific coast of the United States and Canada (Figure 1) to investigate sex- and age-specific site fidelity of birds and to determine important breeding or wintering sites to direct habitat conservation efforts. Wintering areas represented in this data set do not encompass the entire wintering range of the species thus the observed breeding distribution may be biased towards birds from specific wintering areas rather than their entire Pacific Coast range. Compared to the other sea duck species in our analysis, these data best represent a small continental breeding range. Genetic mixing is seen in parts of their Pacific Coast range (Pearce et al. 2014) and Barrow's goldeneye appear to migrate west to east between coastal wintering areas and interior forest breeding areas (S. Boyd, unpublished data).

Black scoters were marked at a spring staging area in Bay de Chaleur, between New Brunswick and Quebec (Figure 1). Black scoters caught in Bay de Chaleur represent birds breeding in eastern North America and are treated as a separate management population than black scoters breeding in western North America. Eastern population of black scoters migrate from wintering areas along the Atlantic Coast of North America from Newfoundland to Florida to breeding areas in eastern Canada and the boreal forest.

Surf scoters were marked at wintering areas on both the Pacific and Atlantic coasts of North America (Figure 1). Captures on the Pacific Coast were conducted as far south as Baja Mexico (30^{o} N latitude) and as far north as Baynes Sound, British Columbia (50^{o} N latitude). Atlantic Coast captures were conducted from Pamlico Sound in North Carolina (35^{o} N latitude) to the Saint Lawrence Estuary (48^{o} N latitude). Surf scoters are currently thought of as separate east and west populations based on wintering areas on the Atlantic or Pacific coasts, but data indicate there may be substantial overlap in breeding distribution in central Canada (Sea Duck Joint Venture 2015). No surf scoters marked on breeding areas in Alaska were included in this analysis.

Since the determination of breeding ranges of the birds is likely conditional on capture locations and sample sizes of the original studies, we do not suggest that the distributions used in our analyses represent the actual breeding distributions of the three species; we use the locations to draw biologically realistic breeding distributions and inform sample size calculations. Raw telemetry data were obtained from the primary investigators of the original studies. For this analysis we considered only adult females. We removed males from the analysis because we cannot reliably distinguish breeding males from non-breeding males. We used only locations occurring during the nesting season after data were processed through the Douglas filter (Douglas et al. 2012) to remove low quality and questionable locations. The dates defining the nesting period varied by species and were drawn from expert opinion and previous analyses of telemetry data (Sea Duck Joint Venture 2015; Table 1). Locations during the nesting period were further subset to a group of chronologically sequential points after one large movement that indicated arrival to breeding grounds and before a second large movement that indicated departure due to completed or failed breeding. The distance that defined a large movement varied by species (Table 1). We then calculated the centroid of these apparent breeding locations for each individual each year. To avoid pseudoreplication, when we had breeding data for an individual in more than one year, we used only the final year in the subsequent analysis, which also reduced potential capture effects as data were further removed from the capture date. Choosing only the final year with sufficient breeding data may introduce bias compared with selecting a random year, as it selects for older females, but for the purpose of developing a realistic breeding distribution we prioritized the reduction of possible impacts of capture and transmitter application.

We used method “optics” from the package dbscan (Hahsler 2016) in Program R (R Core Team 2016) to calculate the number of clusters to best represent the breeding centroids for each species. Optics is an algorithm that performs density-based spatial clustering of points with noise, thus it accounts for uneven distribution in space and is not constrained to assign every point to a cluster (Hahsler 2016, Ankers et al. 1999). The method identifies spatially distinct clusters of breeding centroids, with the possibility that some centroids are not members of any cluster.

We also used the breeding centroids to create a kernel density layer for each species breeding range using the R package adehabitatHR (Calenge 2006) with the least squares cross validated (lscv) smoothing parameter (h) constrained at the 99% isopleth. This kernel describes the spatial extent of, and population density within, the breeding area for each species and is considered the “original” layer in the remainder of the analysis. We estimated the core area of the breeding distribution for each species following Vander Wal and Rodgers (2012). Their method entails plotting the breeding area defined by successive kernel isopleths against the corresponding probability of a breeding centroid in the kernel within that isopleth. The core area is represented by the isopleth for which the slope of the curve is equal to one: at this point, the area begins to increase at a greater rate than the probability of an additional breeding location. Thus, the core represents the smallest area of the breeding range with the highest probability of a breeding bird being present.

To sample from the original breeding distribution for simulations described below, the kernel density layer was transformed into a breeding probability raster with each pixel’s value calculated as its proportional kernel density. We sampled points from the raster probability layer to generate simulated breeding locations. We used the optics method to calculate the number of clusters to best describe each of these sets of simulated points and estimate a new, simulated, kernel density on the same grid as the original breeding kernel density. We also calculated the 99% kernel and the core area for each simulated breeding distribution using the Vander Wal and Rodgers (2012) method.

To quantify the similarity of breeding distributions between the simulated kernels and the original kernel, we calculated the Pearson’s coefficient of correlation between each simulated kernel density and the original breeding kernel. We measured the spatial similarity of the 99% kernel and the core areas by calculating the percent that each simulated area overlapped the original, with 1 being a perfect match. We repeated the simulation 1000 times for sample sizes from 20 to 200, in increments of 10.

For each sample size, we calculated the mean correlation, mean 99% kernel area overlap, and mean core area overlap for the 1000 simulations along with the percent change in each from the previous sample size to the current sample size. We considered that the relationship between the simulated and observed results had approximated an asymptote when the mean correlation or area of overlap value changed by less than 1%; we also report for mean change < 5%. This quantifies the point at which there is diminishing information gained by increasing sample size. We also report the mean number of clusters identified as a function of sample size, which illustrates how identification of the number of discrete breeding areas changes with increased sampling.

Results: After applying our rules for estimating breeding centroids, we calculated a kernel density raster for Barrow’s goldeneye breeding locations using an effective sample size of 70 birds from an original data set of 79 winter-captured adult female Barrow’s goldeneye fitted with satellite transmitters (Figure 1; Table 2). Our cluster analysis determined these points were comprised of two clusters (Figure 2). Mean correlation between the original raster and 1000 simulation rasters calculated at each sample size reached an asymptote (i.e., <1% change) at a sample size of 130 (sample size of 90 for < 5% change; Table 3). Overlap of estimated kernel area from the samples over the original kernel area reached an asymptote (<1% change) at a sample size of 120 (60 for <5% change). The core area of the original kernel was 80%. The asymptote of the core area comparison occurred at a sample size of 150 (80 for <5% change; Figure 4).

The black scoter telemetry data set included 54 adult females captured and fitted with satellite transmitters at spring staging areas in Bay de Chaleur; our rules allowed for calculation of kernel density estimates of breeding areas for 36 birds. The subsequent density and cluster analysis determined two breeding clusters (Figure 1). There are two areas of high breeding density visible in the black scoter kernel (Figure 3a). The average number of clusters from simulated points suggest two clusters with sample sizes of 20-70, but with larger sample sizes the breeding area resolves into a single cluster. This is due to larger sample sizes including an increased number of points in between the two primary breeding areas. The average correlation between the original and simulation rasters reaches an asymptote (<1% change) at a sample size of 80 (40 for <5%; Figure 5). Overlap of estimated kernel area from the samples over the original kernel area had an asymptote of <1% at a sample size of 90 (70 for <5%; Table 3). Mean overlap of the original core area (77%) and the 1000 samples at each sample size reached <1% change at sample sizes of 100 (60 for <5% change; Figure 5).

We compiled surf scoter telemetry data sets for a total of 202 adult females; 119 of those birds met our criteria and were used in our analysis. The cluster and kernel density analyses found two clusters (Figure 1) indicating two areas of high use for breeding (Figure 3b). Over the range of sample sizes considered, the average number of clusters had a mean near two with a decrease in accuracy with increasing sample size (Figure 6), a result not seen in the other two species. Mean correlation between the original kernel and the simulated kernels changed <1% at a sample size of 130 (60 for <5% change; Figure 6). The overlap of the entire kernel with simulations had an <1% asymptote at a sample size of 70 (60 for <5%; Table 3). Core area of the surf scoter breeding centroids was represented by the 75% kernel. The average overlap of simulated core areas and the original core area reached <1% change at a sample size of 150 (60 for <5% change).

Summary and Discussion: Our simulation results suggest that the minimum necessary sample size to characterize the breeding range of Barrow’s goldeneye and continental surf scoter is 130 females, while the minimum sample size for the same calculations for eastern black scoter is 80. Based on our calculated reduction from raw data to effective sample size in these studies, the capture and outfitting of close to 150 Barrow’s goldeneye, 220 surf scoter, and 120 black scoter wintering females would be required to obtain the minimum effective sample sizes (Table 2). Ensuring that the data provide sufficient information for core area delineation requires somewhat larger raw sample sizes: 170 for Barrow’s goldeneye, 250 for surf scoter, and 150 for black scoter. Sample sizes needed to provide sufficient information to identify the broad distribution (99% kernel) were generally smaller than the correlation or core area values. Sample size requirements were reduced by about one-quarter to one half when the asymptote of 5% was used, but these samples produced poorer matches to the original distributions (Figures 4-6).

The differences in sample size requirements between black scoters and the other two species may be due to the spatial scale being considered: for Barrow’s goldeneye, the analysis is characterizing the details of a reasonably compact regional distribution and for surf scoters the two clusters are very defined over a large geographic area. In contrast, the black scoter data regional distribution is both much larger and less complex than that of the Barrow’s goldeneye but clusters are less distinct than surf scoters. The apparent number of black scoter breeding clusters decreases from two to one and becomes more certain with increasing sample size, while in contrast the surf scoter cluster calculation does not trend to one.

Our analysis suggests sample size recommendations from Lindberg and Walker (2007) do not adequately address population delineation questions for sea ducks. This reflects the differing assumptions of the two analyses: Lindberg and Walker (2007) assumed that states are identifiable as soon as a single individual occupies the state, while we do not make that assumption. As a result, information from more birds is needed to address our questions. Lindberg and Walker (2007) suggest that to detect all states or outcomes in a two-state system, a sample size of 25 is needed, and a sample size of 75 would be needed to detect all states in a three-state system. Sample sizes requirements when there are more than three states are difficult to meet with current methods. Powell et al. (2000) suggest a sample size of 25 is needed in each state to model movement among states (Powell et al. 2000), suggesting an overall sample size of 50 for movements between two states. In a more theoretical exercise, Thompson (1987) found a sample size of 177 is needed to estimate parameters of a model with four states with an alpha level of 0.5, much lower precision than is typically desired. Generally, identifying the number of distinct breeding or wintering areas is complex and requires a clear management definition and likely supplementary data on population connectivity such as banding data or genetics (Webster et al. 2002).

The SDJV has set target sample sizes of 40 adult females for population delineation in their recent study of sea ducks on the Atlantic Coast and Great Lakes. Based on our analysis, this sample size would give only an approximation of the structure of the distribution and the total range. When the costs of satellite telemetry remain high, it might be more cost effective to augment the information from satellite telemetry with other types of data such as surveys, banding data, stable isotopes, or genetics (Webster et al. 2002). For example, Pearce et al. (2014) used banding and genetics data to examine potential subpopulations of cavity nesting sea ducks.

Studies involving satellite telemetry have provided ground breaking information about species distribution and behavior (Weimerskirch et al. 1993, Hatch et al. 2000, Mosbech et al. 2006). The data from Atlantic black scoter used in this exercise (S. Gilliland) resulted in expanded range maps and the description of new breeding areas, information vital to designing population surveys and understanding habitat and harvest management. Our analysis illustrates, however, that as we move to addressing specific questions from telemetry data, we will require focused objectives and substantially larger sample sizes.

Multiple statistical assumptions underlie our analysis, and any study drawing inference about distributions based on telemetry data. A key assumption is that the sample of marked birds produces observed breeding distributions that are representative of the true distributions, which implies that the sample of birds captured and outfitted are representative. This requires careful consideration of capture locations and timing. Heterogeneous capture probabilities are common, resulting in birds of poorer condition, or otherwise unrepresentative of the population, being used to inform population level metrics. Sampled birds should be independent of one another and come from a variety of locations and from separate groups to best represent a random sample. Catching a single age/sex cohort of sea ducks is difficult and researchers might chose to fit satellite transmitters on all birds captured to avoid being left with unused transmitters, even though the resulting information may not be useful to the primary question of the study. It is also common to outfit multiple birds captured in the same time and place, violating the assumption that selected birds represent a random selection from the larger population. Other studies have demonstrated that sampling methods impact how representative individual birds are of a population (Raveling 1966, Weatherford and Greenwood 1981). Location accuracy is also an important consideration in satellite telemetry studies. There is some indication that smaller scale questions, such as how many states exist within a single wintering area, may not be suitable for satellite transmitter studies (Britten et al. 1999), though continuous advances in technology should be considered and tested. Finally, the transmitters should not impact the behavior and survival of individuals. This assumption is difficult to mitigate while it is possible implanted transmitters have effects on survival, migration, or other behaviors (Hupp et al. 2006, Murray and Fuller 2000).

While clearly articulating the goals and objectives of an assessment or monitoring program is a vital first step in developing a sampling scheme (Stem et al. 2005), there is often little guidance on how to determine an effective sample size. Simulation studies of the sort we describe here are a critical component in determining necessary effort prior to investing in monitoring efforts. Our calculations suggest that to properly characterize sea duck breeding distributions we require confidence in the breeding locations of approximately 80-130 females, with the consequent outfitting of about 120-220. At current costs ($4,000/bird), this translates to a possible US $480,000-880,000. This large investment requires careful consideration of the benefits of the resulting information, and careful pre-study planning to ensure success. To date, information from satellite telemetry has provided critical information on key sea duck habitats, migration timing and routes, and site fidelity from a relatively small numbers of birds (Petersen et al. 1995, Loring et al. 2014, Sea Duck Joint Venture 2015). Our analyses suggest, however, that characterizing habitat use and distribution at the population level using strictly satellite telemetry data will require substantial additional investment.

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Tables

Table 1.

Criteria used for calculating breeding centroids for three species of sea ducks to be used in further analysis of sample size consideration with a biologically realistic genesis. Points within a range of first and last day of breeding seasons for each species were further reduced based on additional criteria. We subset time periods within the breeding season to locations between long-distance movements for Barrow’s goldeneye and black scoter. For example, we calculated daily movement rate from 1 April to 1 July for each female Barrow’s goldeneye and took the average location between the first and second day that had a movement rate of 20 km/day. Definition of a long-distance movement varied by species due to differences in duty cycles and bird behavior, and was measured by the rate of movement between successive points.

	Date of breeding season		
	First	Last	Selection criteria
Barrow's goldeneye	1-Apr	1-Jul	More than 4 points, all points between the first and second long-distance movement of >20 km/day.
Black scoter	1-May	15-Jul	More than 4 points, all points between the first and second long-distance movement >100 km/day.
Surf scoter	30-May	24-Jun	More than 4 points, inclusive dates, points with a latitude >50.

Table 2. Sample sizes of adult females of three species of sea ducks marked during the non-breeding season and used to describe species breeding distributions. Breeding distributions were used in simulation analyses to determine sample sizes needed to recreate original distributions. Females that attempt to nest are needed to define breeding distributions so we eliminated from our analysis birds that did not make the migration to breeding sites (spatial data problems) and birds that were unable to be tracked into the breeding season due to death or transmitter failure (temporal data problems). Ratios of birds marked to birds used in analysis can inform capture effort of future studies.

	# Original	Temporal or Spatial problems	# Birds Used	% Used	
Barrow's goldeneye	79	9	70	89%	Both spatial and temporal data problems
Black scoter	54	18	36	67%	Most birds removed due to spatial data problems
Surf scoter	202	83	119	59%	Most birds removed due to temporal data problems

Table 3. Sample sizes needed to most closely approximate original kernel density layers based on data from three species of sea ducks. Approximations were based on change in additional information of <1% and <5% from one sample size to the next. Three measures were monitored: the mean correlation of cell values between the original and simulation, the mean overlap of the 99% kernel between the original and simulation, and the mean overlap between the original core area and the simulation. Values for the asymptote are displayed.

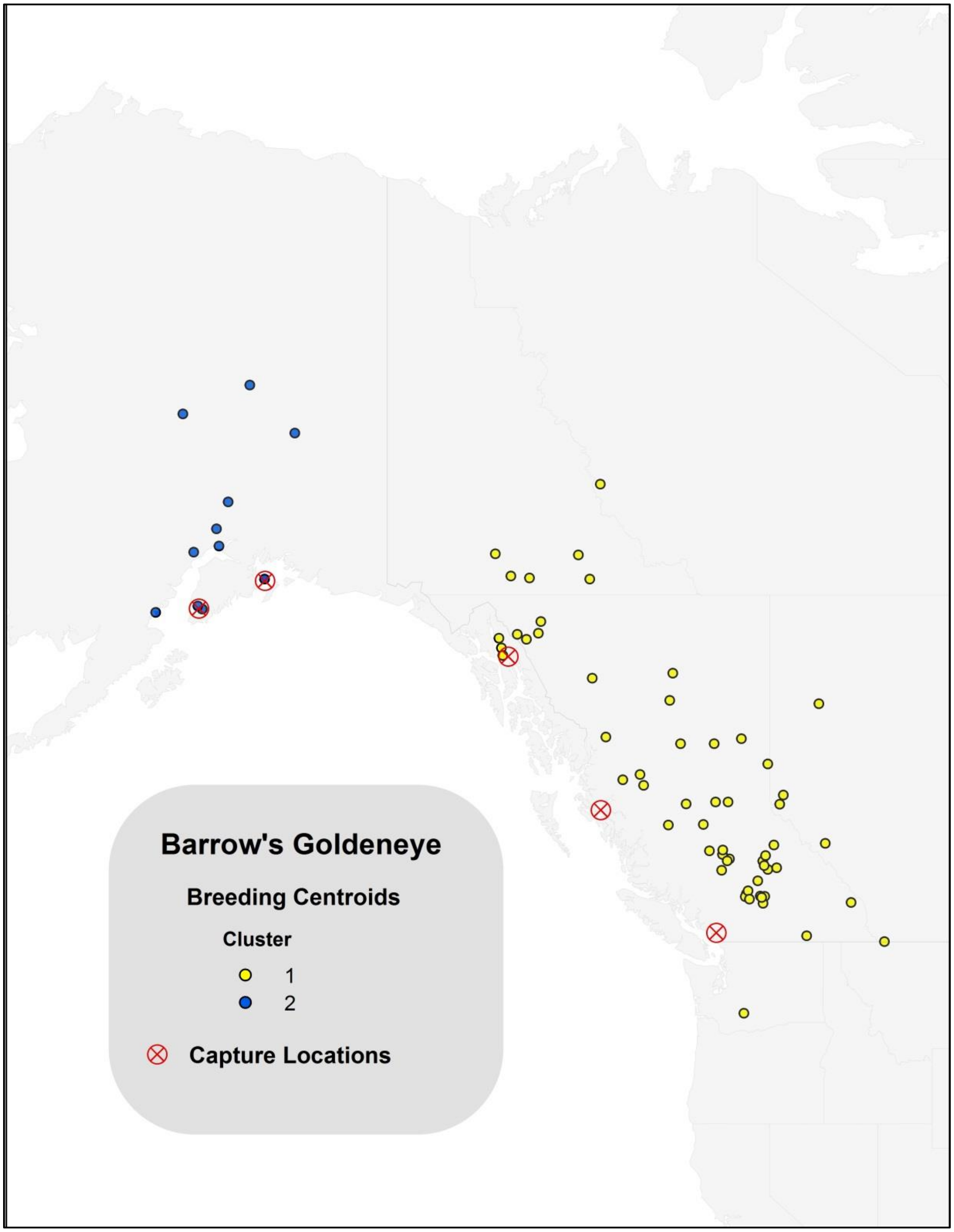
	Barrow's goldeneye			Black scoter			Surf scoter		
Asymptote	Correlation	99% KD	Core (80% KD)	Correlation	99% KD	Core (77% KD)	Correlation	99% KD	Core (75% KD)
1%	130	120	150	80	90	100	130	70	150
5%	90	60	80	40	70	60	60	60	60
	Value	Overlap	Overlap	Value	Overlap	Overlap	Value	Overlap	Overlap
1%	70%	66%	45%	82%	98%	66%	84%	93%	62%
5%	65%	75%	40%	78%	96%	61%	74%	90%	51%

Figures

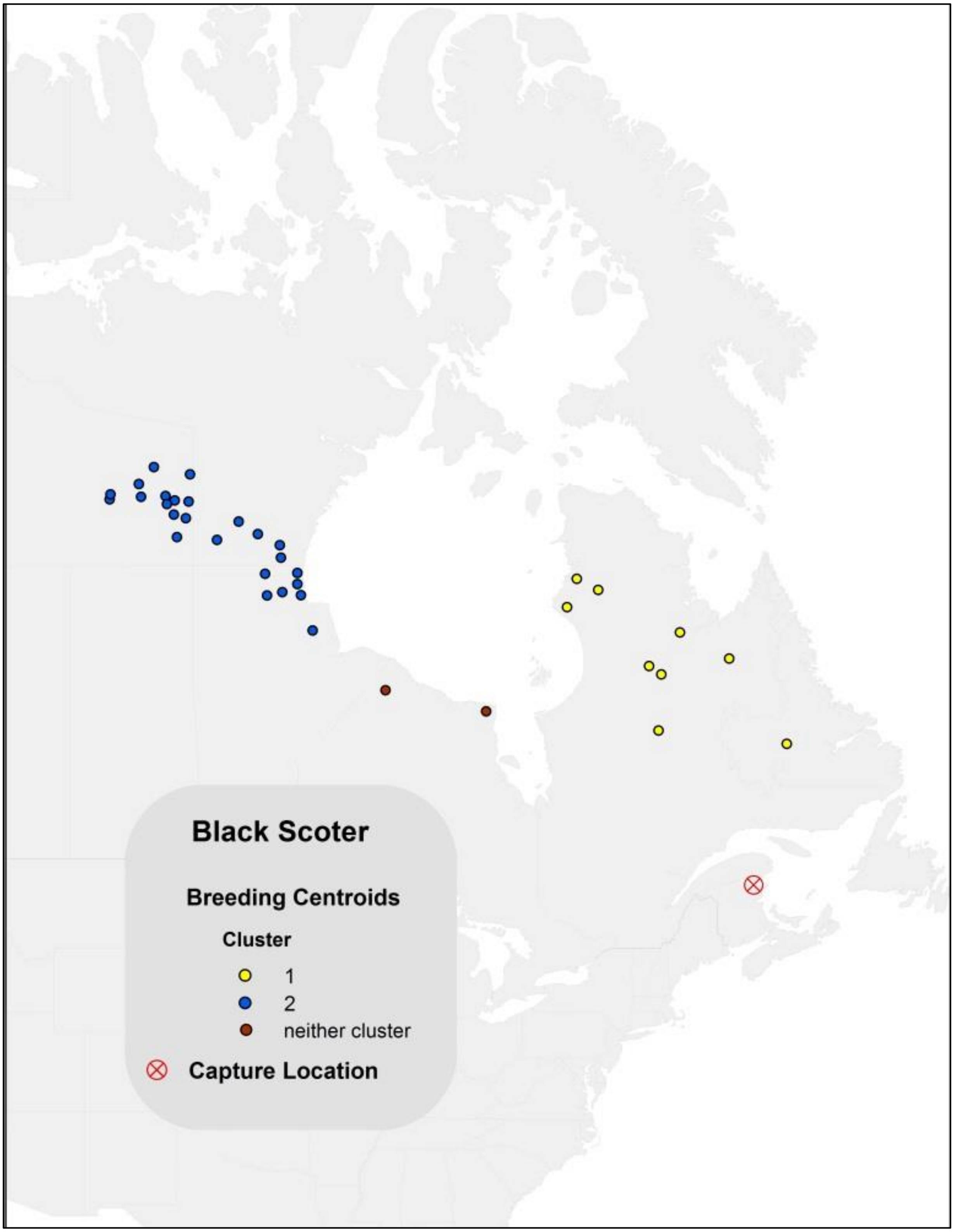
Figure 1.

Breeding centroids and approximate winter capture locations of the three populations of sea ducks used in simulation analysis: a) Barrow's goldeneye b) black scoter c) surf scoter. Colors of breeding centroids (points) represent the cluster they belong to as determined by cluster analysis.

a)



b)


Black Scoter
Breeding Centroids
Cluster
1
2
neither cluster
Capture Location

c)

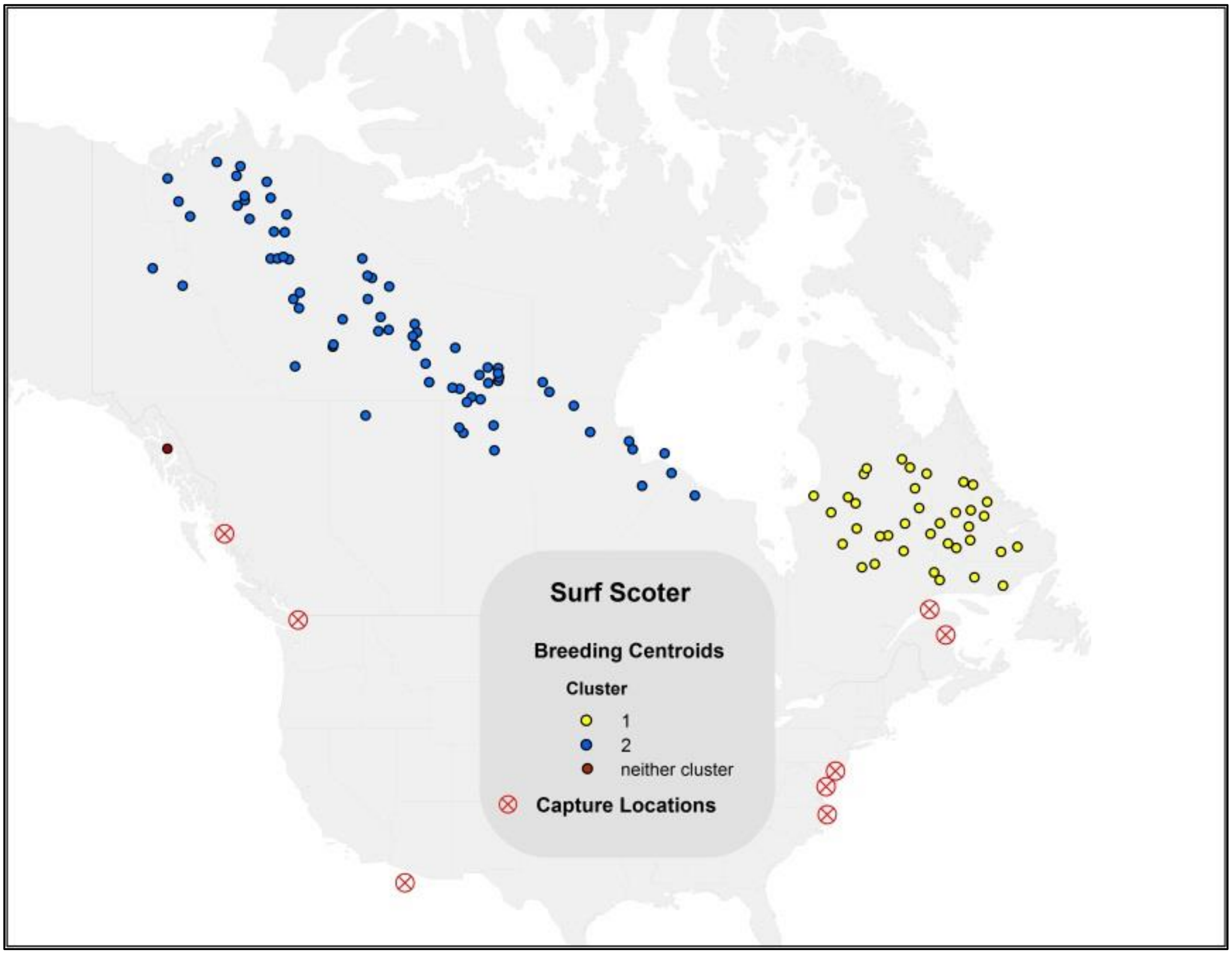

Surf Scoter
Breeding Centroids
Cluster
1
2
neither cluster
Capture Locations

Figure 2.

Barrow's goldeneye data (n = 70) and two examples of simulation results (for n=20 and n=80). The top left panel illustrates the original estimated breeding centroids with clusters identified by the optics method represented by color (red vs. black) and the top right panel displays the kernel density associated with these original breeding centroids: this kernel density was used to generate locations for the simulations. The second row represents the centroids, clusters, and resulting kernel density estimate for a single n = 20 simulation; the third row represents the same for an n = 80 simulation. The resulting correlations to the original kernel density are included on the figure. Sample sizes 20-200 in increments of 10 were each simulated 1000 times.

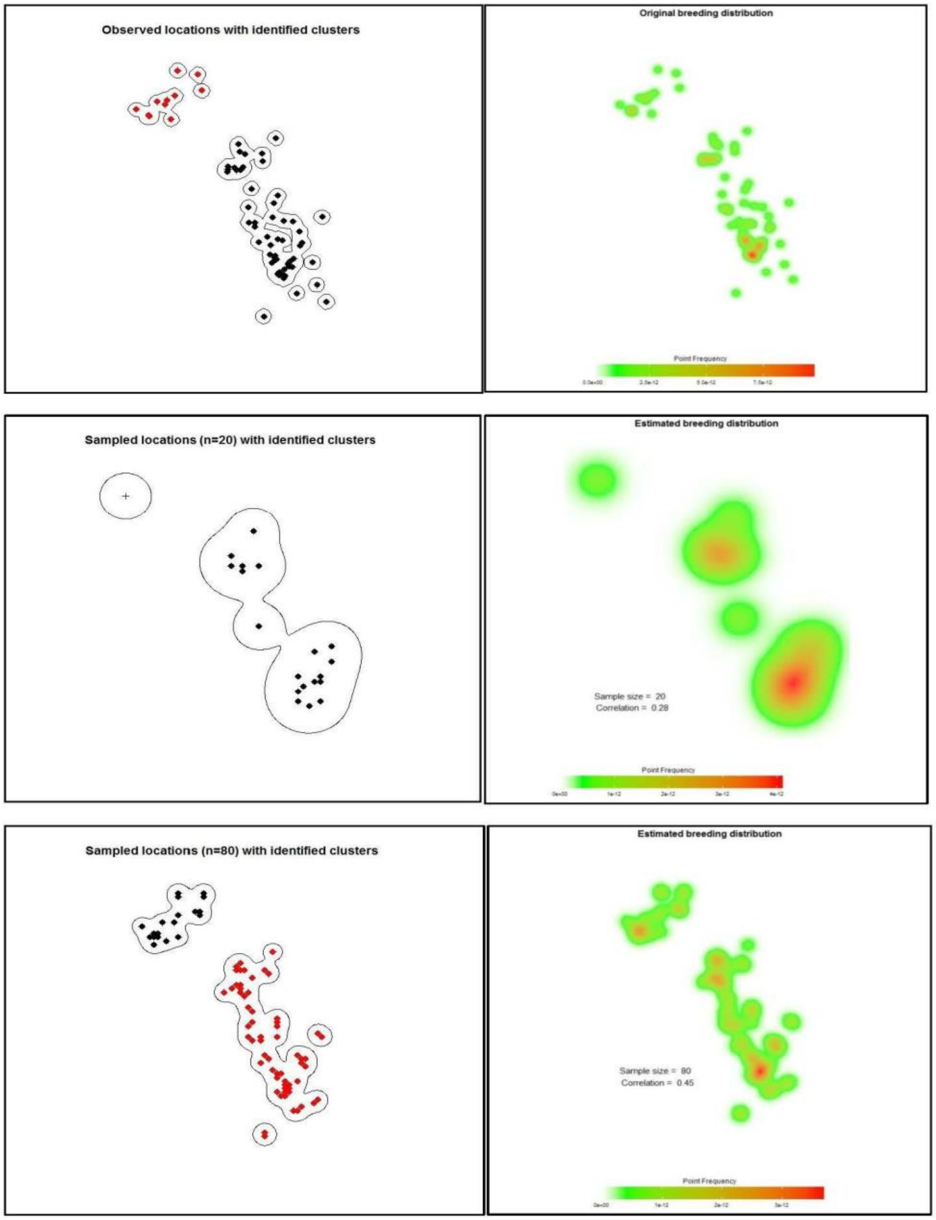
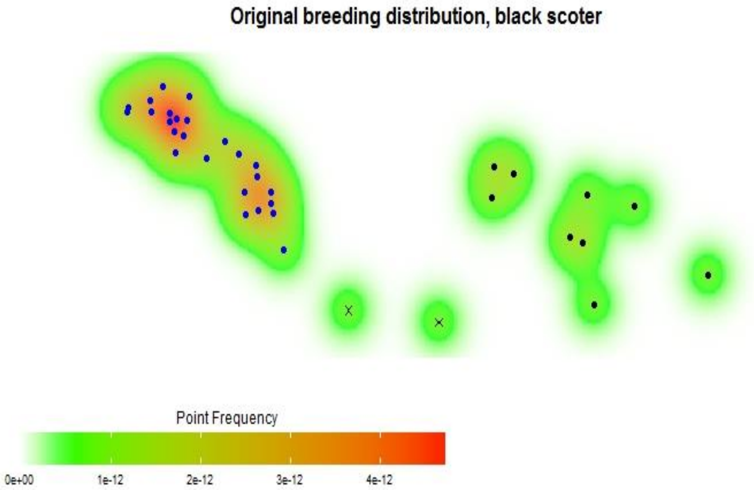


Figure 3.
Estimated breeding centroids with clusters represented by color (black vs. blue circles, black x indicates not assigned to a cluster) and original kernel densities used in simulations. Breeding centroids and kernel densities are derived from two species of sea duck, a) black scoter (n = 36) and b) surf scoter (n = 119)

a)



b)

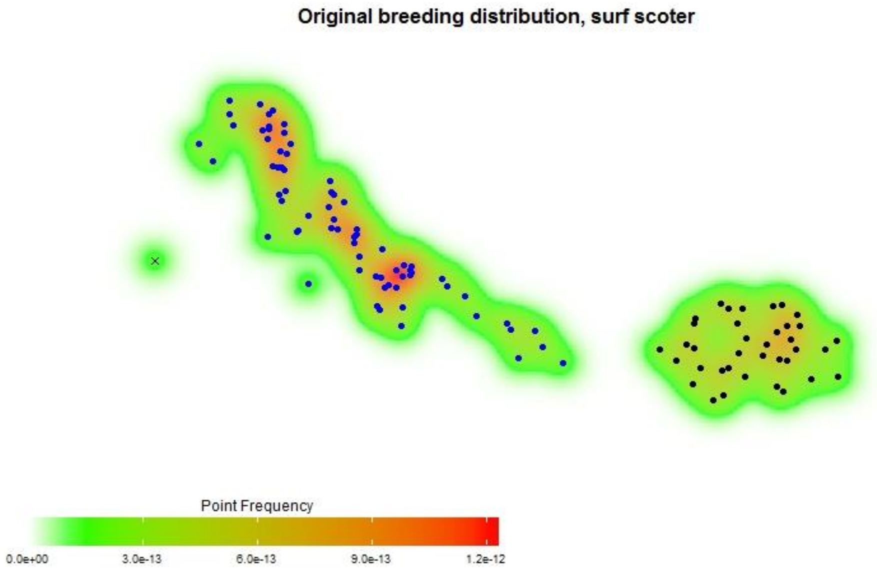

Original breeding distribution, surf scoter
Point Frequency
0.0e+00
3.0e-13
6.0e-13
9.0e-13
1.2e-12

Figure 4. Boxplots of the correlation between simulated rasters and the original raster, overlap of simulated total over original total area, and overlap of simulated core over the original core area as a function of sample size for Barrow's goldeneye. Also shown is mean number of clusters over the 1000 simulations at each sample size. The red lines on boxplots mark the asymptote as measured by <5% change in value and the blue lines mark the asymptote at <1% change in value.

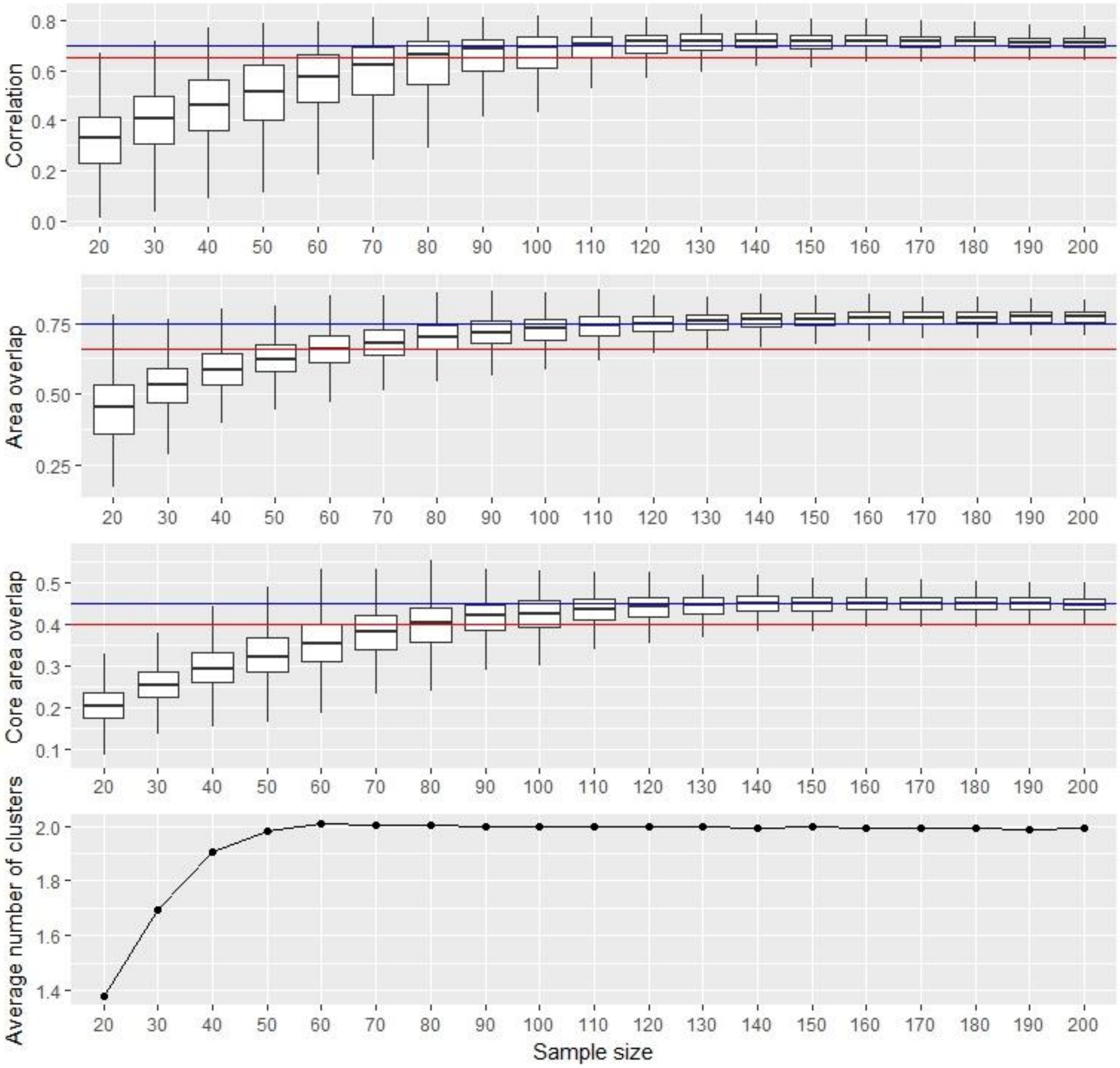


Figure 5. Boxplots of the correlation between simulated rasters and the original raster, overlap of simulated total over original total area, and overlap of simulated core over the original core area as a function of sample size for black scoter. Also shown is mean number of clusters over the 1000 simulations at each sample size. The red lines on boxplots mark the asymptote as measured by <5% change in value and the blue lines mark the asymptote at <1% change in value.

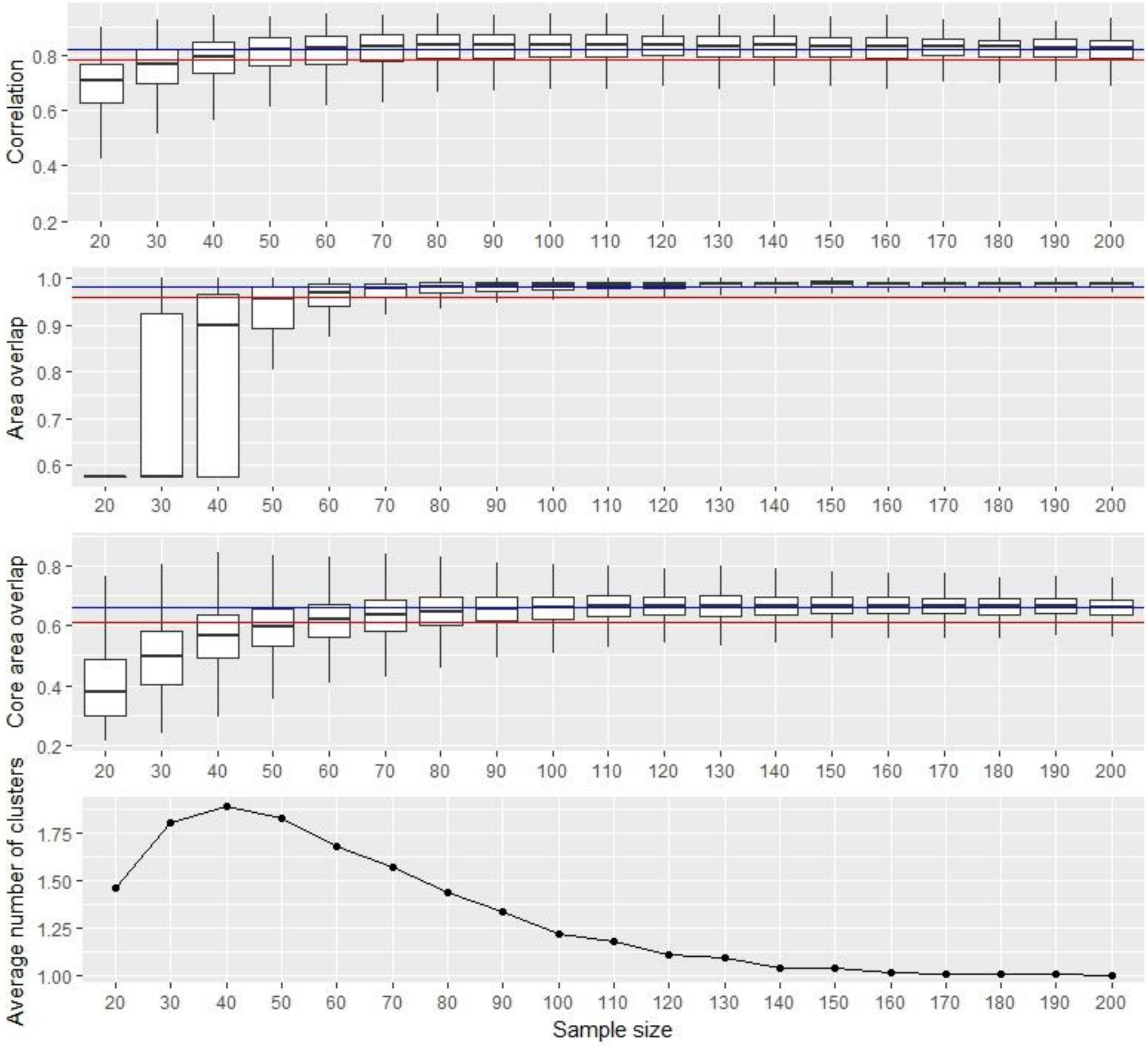
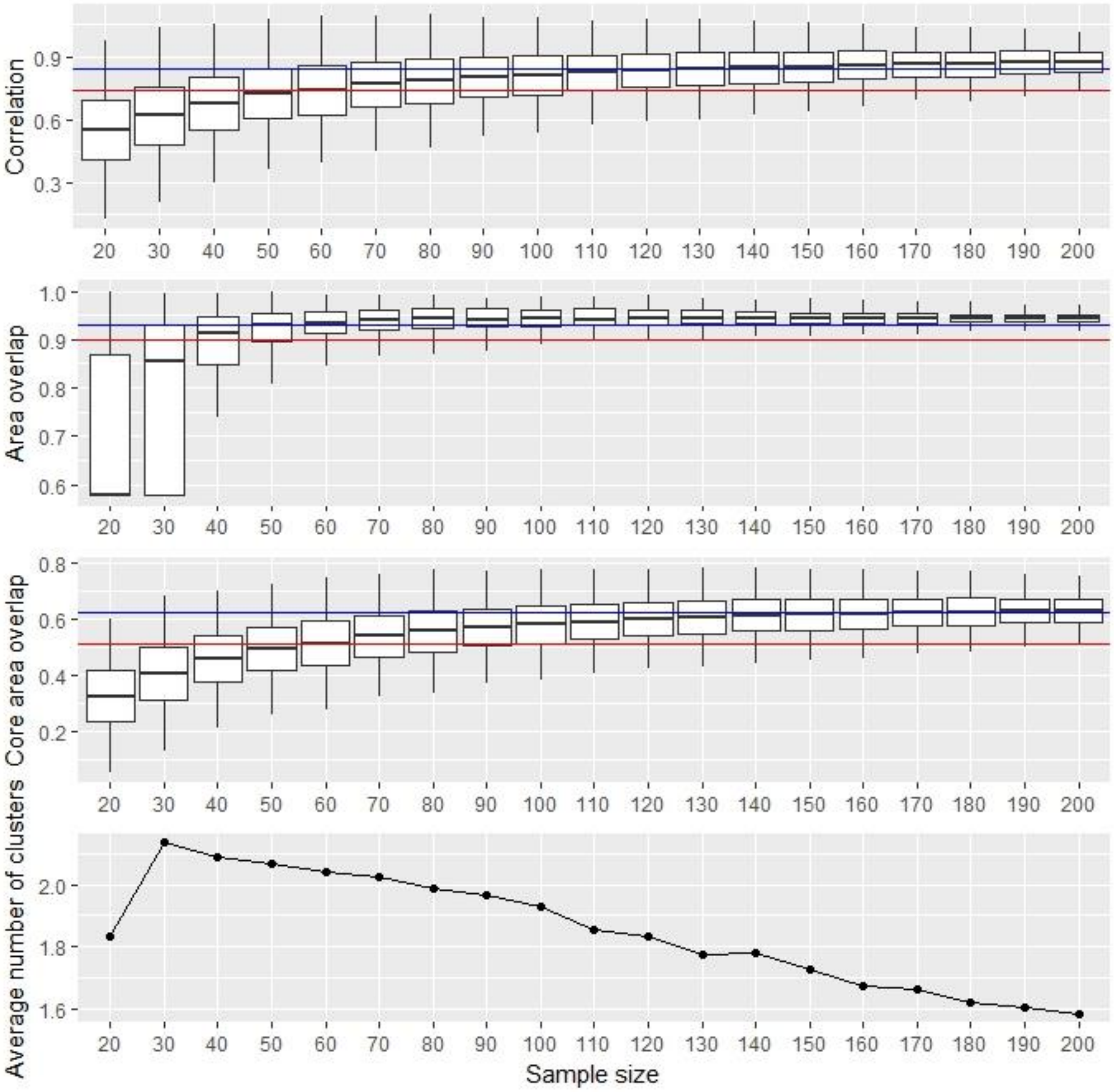


Figure 6. Boxplots of the correlation between simulated rasters and the original raster, overlap of simulated total over original total area, and overlap of simulated core over the original core area as a function of sample size for surf scoter. Also shown is mean number of clusters over the 1000 simulations at each sample size. The red lines on boxplots mark the asymptote as measured by <5% change in value and the blue lines mark the asymptote at <1% change in value.



Appendix 1.

We extended the analysis reported in Lindberg & Walker (2007), who calculated sample sizes needed to ensure that 95% confidence intervals estimating the proportion of the population in a given state (p = probability of affiliation) did not overlap with zero for three p values (0.05, 0.25, 0.5). These calculations can be recast to determine the sample size needed to ensure the confidence interval does not overlap zero as a function of p, for various confidence bounds and number of states using the following equation:

$n > q/p(CV)^2$

where CV is the critical value ($Z_{\alpha/2}$ for two states and $\square^{2}_{1,\alpha/k}$ for k>2 states). Note that Lindberg & Walker (2007) explored the sample size needed to detect a state under the assumption that the state was known. Our problem is different, as we are trying to identify the number of states (i.e., observing the location of the birds does not tell us what population they belong to until we delineate the populations).

According to our calculations based on Lindberg & Walker's (2007) approach, the sample size needed to ensure the confidence interval estimating the proportion of animals present in a known state does not overlap zero is 40 in the two-state case, as long as the proportion of population in each state is at least 10% (Fig. A1). If there are three or more states, a sample size of at least 185 is needed to ensure the confidence interval for a state with 10% of the population does not include zero.

Figure A1. Sample size needed to ensure the confidence interval estimating the proportion of animals present in a known state does not overlap zero for α = 0.5 (solid lines) and α = 0.10 (dashed lines) with number of states = 2 (black), 3 (blue), and 4 (red).