

**A multi-faceted evaluation of a reintroduced waterfowl  
species:**

**Migration ecology, ecotoxicology, and population genetics of trumpeter swans in the  
Midwest**

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## **Dedication**

To John Iverson, who opened my eyes to wildlife ecology and inspired countless students to follow their passions. To my parents, Linda and Marty Wolfson, for their unwavering support. And to Sylvie and Leland.

## Abstract

Trumpeter swans (*Cygnus buccinator*), the largest waterfowl species in North America, were widespread throughout much of the continent prior to European colonization. Due to unregulated market hunting, trumpeter swans were nearly extirpated, and reached an estimated low of ~70 individuals in the lower 48 U.S. states during the 1930s. The creation of new protected areas coupled with management efforts allowed trumpeter swan abundance to recover, and they were successfully reintroduced to the western Great Lakes region in the late 20th century to re-establish the Interior Population (IP). However, a general lack of information about IP trumpeter swan ecology has hindered conservation decision-making. I partnered with agency biologists from seven U.S. states and one Canadian province to gather information on IP trumpeter swan annual movements and migration patterns, the prevalence of lead (Pb) in free roaming swans, and the genetic makeup of trumpeter swan populations in North America.

Before addressing the previously mentioned aspects of trumpeter swan ecology, in Chapter 1, I provide a review of piecewise regression, a flexible type of breakpoint analysis. I provide an overview of piecewise regression and then describe six case-studies, using piecewise regression on a variety of datasets that include a range of species, data types, ecological responses, statistical signals, and timeframes.

In Chapter 2, I use the methodology described in Chapter 1 (i.e., piecewise regression) to quantify annual movements and migration patterns in IP trumpeter swans. We (multiple state agency biologists and other collaborators) deployed 133 GPS-GSM transmitters on trumpeter swans across the current IP breeding range (i.e., the greater Midwest) during 2019–2022. Individual tracking data revealed that IP trumpeter swans are partial migrants, with a continuum of strategies each year, from local movements to long-distance migration. Much of the variability in movement patterns was related to factors tied to natural history demands (e.g., breeding status) and response to environmental conditions (e.g., through associations with breeding latitude).

In Chapter 3, I present a baseline assessment of the prevalence of lead in all trumpeter swans associated with the dissertation and an additional flock in Nebraska. I

estimated blood lead concentration for 119 IP trumpeter swans and detected lead in all individuals. However, 91% of swans had blood lead levels in the ‘background’ range (not considered to produce negative physiological effects), 7.5% of swans had blood lead levels in the ‘sub-clinical’ range, and only 1.5% of swans had blood lead levels in the ‘clinical’ or ‘severe’ range of lead toxicity.

Finally, in Chapter 4 I present a comparative assessment of the genetics of trumpeter swans in North America. I collected 150 genetic samples from IP trumpeter swans captured during 2019–2022 and also obtained 79 reference samples from the other two North American trumpeter swan populations. These samples provide evidence that all three populations are genetically distinct and that the High Plains flock of the IP has lower genetic diversity compared to the other groups, likely a result of smaller population size, relative geographic isolation, and potential founder effects.

## Table of Contents

Acknowledgments.....	i
Dedication .....	ii
Abstract.....	iii
List of Tables .....	vii
List of Figures .....	ix
Chapter 1: Using piecewise regression to identify biological phenomena in biotelemetry datasets .....	1
Synopsis .....	1
Introduction .....	2
Overview of piecewise regression and applications with the <i>mcp</i> package .....	3
Example applications .....	6
Identification of altered behavior post-capture .....	7
Pre-parturition movement.....	9
Physiological response to drone fly-over.....	10
Mortality signal from acceleration and temperature data.....	12
Migration phenology including stopovers .....	15
Segmentation of nesting stages.....	16
Discussion .....	17
Acknowledgments.....	19
References .....	32
Chapter 2: High variability of migration strategies in a re-established trumpeter swan population .....	49
Synopsis .....	49
Introduction .....	50
Methods.....	54
Study Area.....	54
Capture and Handling .....	54
Migration Phenology Classification.....	55
Migration Extent .....	58
Migration Phenology.....	59
Results.....	59

Migration Extent .....	60
Migration Phenology.....	60
Discussion .....	62
Conclusions .....	64
Acknowledgments.....	65
References .....	66
Chapter 3: The prevalence of lead (Pb) among Interior Population trumpeter swans .....	81
Synopsis .....	81
Introduction .....	81
Methods.....	84
Study site and sample collection .....	84
Laboratory analysis .....	84
Results.....	85
Discussion .....	85
Acknowledgments.....	88
References .....	89
Chapter 4: Comparative population genomics of trumpeter swans in North America.....	99
Introduction .....	99
Methods.....	103
Sample collection, DNA extraction, and sequencing.....	103
Bioinformatics .....	104
Genetic Analyses .....	105
Results.....	107
Discussion .....	109
Acknowledgments.....	111
References .....	112
Appendices.....	129
Chapter 2.....	129
Appendix A.....	129
Chapter 4.....	140
Appendix A .....	140



## List of Tables

Table 1.1 Leave-one-out cross validation used to compute the estimated log predictive density (ELPD) of two different models fit to movement data from a trumpeter swan ( <i>Cygnus buccinator</i> ); one fit with a single intercept and the other with two intercepts separated by a change point. The higher ELPD for the model with two intercepts indicates that it has a higher predictive accuracy.....	20
Table 1.2. A summary of output from the model looking at the black bear ( <i>Ursus americanus</i> ) heart rate response to a drone flyover.....	21
Table 1.3. Leave-one-out cross validation was used to compute the estimated log predictive density (ELPD) for seven models of migration phenology of a sandhill crane ( <i>Antigone canadensis</i> ), each with an increasing number of intercepts. The highest ELPD for the model with six intercepts indicates that it has the highest predictive accuracy. ...	22
Table 1.4. Leave-one-out cross validation used the compute the estimated log predictive density (ELPD) of three different models of trumpeter swan ( <i>Cygnus buccinator</i> ) activity; one fit with a single intercept, one with two intercepts, and one with three intercepts. The higher ELPD for the model with two intercepts indicates that it has a higher predictive accuracy.....	23
Table 3.1 Blood lead concentrations for 119 trumpeter swans ( <i>Cygnus buccinator</i> ) from July 2019 to August 2022. The number of swans and average lead concentrations are grouped by categories of lead toxicity.....	97
Table 4.1 Genetic samples, grouped by state or province. ‘Wild’ samples are from trumpeter swans ( <i>Cygnus buccinator</i> ) captured in the field; ‘archived’ samples were obtained from previous field studies by USGS collaborators or from the Burke Museum of Natural History and Culture. Abbreviations are PCP=Pacific Coast Population, RMP=Rocky Mountain Population, HP=High Plains flock, IP=Interior Population. ....	120
Table 4.2 Genetic diversity metrics for 224 trumpeter swans ( <i>Cygnus buccinator</i> ) sampled across North America estimated from 82,234 single nucleotide polymorphisms	

(SNPs). Group abbreviations given in Table 1. Other abbreviations include:  $H_o$  = observed heterozygosity and  $H_s$  = mean gene diversity, a metric similar to expected heterozygosity ( $H_E$ ), except  $H_s$  is a measure of average genetic diversity across all loci within a population, while  $H_E$  measures diversity at a particular locus..... 121

## List of Figures

Figure 1-1 Hourly net-squared displacement measured from the point of release of a trumpeter swan ( <i>Cygnus buccinator</i> ) after collar deployment. Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines. The posterior distribution for the change point is shown in blue on the x-axis.....	24
Figure 1-2 Hourly net-squared displacement measured from the point of release of a trumpeter swan ( <i>Cygnus buccinator</i> ) after collar deployment. Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines. The posterior distribution for the change point is shown in blue on the x-axis.....	25
Figure 1-3. A kernel density posterior predictive check compares the distribution of observed outcomes [displacement in kilometers by a pregnant moose ( <i>Alces alces</i> )], shown in the black line, against 50 distributions of replicated datasets produced by the fitted model, each shown as a light blue line.....	26
Figure 1-4. Heart rate of a black bear ( <i>Ursus americanus</i> ) in relation to a drone flight. The green box indicates the duration of a drone flight. Grey lines represent 25 draws from the posterior distribution of the mean response. Red dotted lines depict the 95% credible intervals for the mean response. The posterior distribution for the change point is shown in blue on the x-axis.....	27
Figure 1-5. The x-axis is an index of time since the start of the time series for Overall Dynamic Body Acceleration (ODBA) on the top figure, and temperature on the bottom figure for a trumpeter swan ( <i>Cygnus buccinator</i> ). Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines. The posterior distribution for the change point is shown in blue on the x-axis.....	28
Figure 1-6. An annual migration cycle of a sandhill crane ( <i>Antigone canadensis</i> ). Average daily displacement from the breeding territory (in kilometers) on the y-axis, and an index of time since the start of the time series on the x-axis. Grey lines show 25 draws	

from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines and 80% prediction intervals in green dotted lines. The posterior distributions for the change points are shown in blue on the x-axis. .... 29

Figure 1-7. The top plot shows the accelerometer sensor dataset for a nesting trumpeter swan (*Cygnus buccinator*), with time on the x-axis expressed in hourly time intervals and averaged ODBA values on the y-axis. The bottom plot shows the 19 data points from the visual observation dataset with the estimated hatch date (the midpoint between the last incubation observation and the first cygnet observation; purple dotted line). .... 30

Figure 1-8. Date is on the x-axis and average hourly Overall Dynamic Body Acceleration (ODBA) for a trumpeter swan (*Cygnus buccinator*) is on the y-axis. The colored markers just above the x-axis correspond to visual observations of the swan’s nesting status. The dashed vertical line in purple is the observed transition from incubation to cygnets, and the solid brown line is the estimated change point from the piecewise regression model. Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red lines. .... 31

Figure 2-1 Capture locations of Interior Population trumpeter swans (*Cygnus buccinator*) collared with GPS-GSM transmitters from July 2019–January 2022. .... 75

Figure 2-2 An example of extracting migration phenology metrics from a fitted piecewise regression model of an annual time-series of displacement values for an individual trumpeter swan (*Cygnus buccinator*). .... 76

Figure 2-3 Migration extent versus breeding/capture latitude with color indicating the probability of assignment to one of two migration strategies within a 2-component mixture model describing the relationship between latitude and migration extent. Gray areas depict the 95% credible intervals for each strategy. .... 77

Figure 2-4 Estimates and 95% confidence intervals for model coefficients from linear mixed effects models fit to autumn departure, spring arrival, and duration of non-breeding period. The covariate for sex represents the difference between male and female (the reference category). The coefficients for non-breeder and paired contrast these categories with breeder (the reference category). Blue results represent statistically

significant coefficient estimates and red results represent coefficient estimates that were not statistically significant. .... 78

Figure 2-5 Marginal effect plots for the relationships between breeding/capture latitude and autumn departure (A), spring arrival (B), and duration of non-breeding period (C). Red lines and shaded areas depict the predicted values and 95% confidence intervals, respectively. Points shown are the observed migration phenology metrics. .... 79

Figure 2-6 Migration phenology dates by breeding status (breeder, paired, and non-breeder). Boxes bound the 25th and 75th percentiles, solid lines within the boxes indicate the median, lines extend to 1.5 times the interquartile range, and points correspond to individual swan-years. .... 80

Figure 3-1 Empirical cumulative distribution function for the blood lead concentrations for 119 trumpeter swans (*Cygnus buccinator*, 100 adults and 19 cygnets) from July 2019 to August 2022. The y-axis represents the proportion of the total sample for each group (e.g., adults and cygnets) below any given threshold of blood lead concentration in parts per billion (ppb). .... 98

Figure 4-1 Approximate breeding range of trumpeter swans (*Cygnus buccinator*), as reported by the North American Trumpeter Swan Survey (NATSS) cooperators (Groves 2017). Black lines show approximate boundaries of the breeding ranges of the three populations. .... 122

Figure 4-2 Locations of trumpeter swan (*Cygnus buccinator*) genetic samples used in this study. Relative size of points indicate if multiple samples were collected in an area. Black lines show approximate boundaries of the breeding ranges of the three populations in North America. High Plains swans are technically considered part of the Interior Population but are given a unique category in this map. .... 123

Figure 4-3 The upper figure shows a map of North America with a pie chart imposed over each sampling region. The relative proportion of the four colors in each pie chart correspond with the proportional assignment of individuals from each sampling region to the four genetic clusters identified by STRUCTURE. The lower figure shows the model-

based clustering output from STRUCTURE for each of the 224 trumpeter swan (*Cygnus buccinator*) individuals. .... 124

Figure 4-4 PCA of 66,847 SNPs with 95% ellipses surrounding points grouped by trumpeter swan (*Cygnus buccinator*) population (IP = Interior Population, PCP = Pacific Coast Population, RMP = Rocky Mountain Population), and the High Plains (HP) flock. .... 125

Figure 4-5 Heatmap of pairwise genetic distance between sampling locations of trumpeter swans (*Cygnus buccinator*; RMP = Rocky Mountain Population, PCP = Pacific Coast Population, HP = High Plains flock, and IP = Interior Population) using 82,234 SNPs. Pairwise  $F_{ST}$  values (Weir and Cockerham's  $\Theta$ , 1984) are shown below the diagonal. Associated p-values for each  $F_{ST}$  index estimated using 100 bootstrap replicates are shown above the diagonal..... 126

Figure 4-6 Neighbor-joining network for 224 trumpeter swans (*Cygnus buccinator*) with branch lengths corresponding to the evolutionary relatedness between individuals (based on Nei's D genetic distance). Branches in the tree are colored based on the group from which the sample was obtained..... 128

# Chapter 1: Using piecewise regression to identify biological phenomena in biotelemetry datasets

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Data and code associated with this work have been deposited in the Data Repository for University of Minnesota (DRUM): <https://doi.org/10.13020/qbha-bs48>.

## Synopsis

1. Technological advances in the field of animal tracking have greatly expanded the potential to remotely monitor animals, opening the door to exploring how animals shift their behavior over time or respond to external stimuli. A wide variety of animal-borne sensors can provide information on an animal's location, movement characteristics, external environmental conditions, and internal physiological status.
2. Here, we demonstrate how piecewise regression can be used to identify the presence and timing of potential shifts in a variety of biological responses using GPS telemetry and other biologging data streams. Different biological latent states can be inferred by partitioning a time-series into multiple segments based on changes in modeled responses (e.g., their mean, variance, trend, degree of autocorrelation) and specifying a unique model structure for each interval.
3. We provide five example applications highlighting a variety of taxonomic species, data streams, timescales, and biological phenomena. These examples include a short-term behavioral response (flee and return) by a trumpeter swan (*Cygnus buccinator*) immediately following a GPS collar deployment; remote identification of parturition based on movements by a pregnant moose (*Alces alces*); a physiological response (spike in heart-rate) in a black bear (*Ursus americanus*) to a stressful stimulus

- (presence of a drone); a mortality event of a trumpeter swan signaled by changes in collar temperature and Overall Dynamic Body Acceleration; and an unsupervised method for identifying the onset, return, duration, and staging use of sandhill crane (*Antigone canadensis*) migration.
4. We implement analyses using the *mcp* package in R, which provides functionality for specifying and fitting a wide variety of user-defined model structures in a Bayesian framework and methods for assessing and comparing models using information criterion and cross-validation measures. This approach uses simple modeling approaches that are accessible to a wide audience and is a straightforward means of assessing a variety of biologically relevant changes in animal behavior.

## **Introduction**

Recent technological advancements in the field of biotelemetry have greatly expanded the potential for remotely monitoring animals (Cagnacci et al., 2010; Hebblewhite & Haydon, 2010; Tomkiewicz et al., 2010). Historically, animals were tracked using very-high frequency (VHF) telemetry, which requires a receiver in close proximity to an animal to triangulate its location (Craighead, 1982). Global positioning system (GPS) transmitters, now the standard for most wildlife studies, are small enough to be placed on bats and songbirds weighing less than 20 grams (Cvikel et al., 2015). Current GPS technology provides copious fine-scale data over long study durations and spatial coverages. Other biologging sensors such as accelerometers, temperature-depth recorders, geolocators, and even heart-rate monitors, also produce valuable, high-frequency data on physiological and external conditions affecting animals (Cooke et al., 2004; Jonsen et al., 2007; Wilmers et al., 2015). Such data can elucidate behavioral patterns (foraging, migration, predator/prey dynamics) and provide knowledge about demographic parameters such as fecundity and survival; however, processing high-volume data can be complex and time-consuming (Brooks et al., 2019; Hamilton et al., 2017; Kramer et al., 2018). Simple and flexible tools are necessary to facilitate efficient



analysis of animal biotelemetry data to examine behavior over time and response to external stimuli.

When inferring behavior, raw measurements (e.g. location, acceleration, body temperature) can be used, or these data may be first converted into metrics that provide additional insights into biological events or phenomena (e.g. net-squared displacement for migration, overall dynamic body acceleration for energy expenditure: Wilson et al., 2006; Bunnefeld et al., 2011). Data collected at a high frequency provide autocorrelated time series, which can preclude some modeling approaches, such as resource selection functions, that require observations to be statistically independent. Past approaches for accounting for autocorrelation have focused on filtering data, which results in potential loss of information. More recent approaches now directly model the correlation structure to provide additional inference without loss of information (Fleming et al., 2015, Fleming et al, 2016).

Analyses of biotelemetry data collected at a high frequency often begin by partitioning datasets into homogeneous segments that correspond to different behavioral states (Edelhoff et al., 2016). Some common methods for partitioning datasets include clustering algorithms that minimize a cost function associated with statistical properties of a time series (e.g. the pruned exact linear time (PELT) algorithm or the penalized contrasts method; Lavielle 2005; Killick et al., 2012) or parametric state-space models such as hidden Markov models (Patterson et al., 2008). Often, these methods require restrictive assumptions, are computationally intensive, or are restricted to a single type of data stream (Morelle et al., 2017). Here, we demonstrate how piecewise regression can be used to analyze a wide range of biotelemetry data sets, offering a flexible and user-friendly approach with results that are easy to interpret.

### **Overview of piecewise regression and applications with the *mcp* package**

Piecewise regression is a common statistical method used to model ecological thresholds (Toms & Lesperance, 2003) and can be used to identify change points that signify potential shifts in the relationship between response and explanatory variables (Muggeo, 2003; Toms & Villard, 2015). Piecewise regression is an extremely flexible

modeling framework due to the ability to specify unique model structures for each segment between change points. Transitions between segments can be abrupt, if segments are disjunct, or smooth, if segments are joined.

Herein, we focus on a Bayesian formulation of piecewise regression following the formulation of Stephens (1994). Let  $\mathbf{y} = (y_1, \dots, y_n)$  be the realization of a sequence of random variables  $Y = (Y_1, \dots, Y_n)$  of length  $n$ , with a change point at  $r(1 \leq r \leq n)$ . We can write the distribution of  $Y_1, \dots, Y_n$  as:

$$Y_1, \dots, Y_r \sim [Y_i | \theta_1]_1$$

$$Y_{r+1}, \dots, Y_n \sim [Y_i | \theta_2]_2$$

where  $\theta_1$  and  $\theta_2$  are parameter vectors describing the data-generating process before and after the change point, respectively. Values of  $\theta$  can describe various characteristics of the response distributions, including their means, variances, or correlation structures. Inference is made via the posterior distribution of  $r$ ,  $\theta_1$ , and  $\theta_2$ , denoted by  $[r, \theta_1, \theta_2 | Y]$ . From Bayes's theorem,

$$[r, \theta_1, \theta_2 | Y] \propto [Y | r, \theta_1, \theta_2][r][\theta_1][\theta_2]$$

where  $[\theta_1]$ ,  $[\theta_2]$ , and  $[r]$  are prior distributions for the parameters describing the data-generating process. This approach easily extends to multiple change points,  $r_1, r_2, \dots, r_k$  with unique parameter vectors,  $\theta_1, \dots, \theta_k$ , describing the likelihood of  $Y$  within each segment:

Consider a simple example represented by separate intercepts before and after a single change point. Let  $r$  be the location of the change point along the x-axis, where the expected value of  $y_i$ ,  $\mu_i$ , changes from  $f_1(x, \beta_1)$ , to  $f_2(x, \beta_2)$ :

$$\mu_i = \begin{cases} f_1(x_i, \beta_1) & \text{if } x_i < r \\ f_2(x_i, \beta_2) & \text{if } x_i \geq r \end{cases}$$

$f_1(x, \beta_1)$  and  $f_2(x, \beta_2)$  can represent combinations of either additive or multiplicative effects of covariates on segments using the generalized format  $f_k(x_i, \beta_k) = \beta_{k,0} + x_i \cdot$

$\beta_{k,1} + \dots x_i \cdot \beta_{k,p}$  or  $f_k(x_i, \beta_k) = \beta_{k,0} \cdot x_i \cdot \beta_{k,1} \cdot \dots x_i \cdot \beta_{k,p}$  with parameters  $(\beta_{k,0}, \beta_{k,1}, \dots, \beta_{k,p})$  and  $k$  segments resulting from  $k - 1$  change points. For a more detailed description see Lindeløv (2020).

The *mcp* package uses indicator functions to map elements of a dataset (i.e., segments) to statistical models specific to each segment. For a dataset with  $y$  as the response variable and  $x$  as the predictor variable, the two-intercept case can be written as:

```
model=list(  
  y~1, # first intercept / plateau  
  ~1 # second intercept / plateau  
)
```

Specifying a model structure with a joined segment is also straightforward:

```
model=list(  
  y~1, # plateau  
  ~0 + x # joined slope  
)
```

The ‘~0’ term specifies that the second segment shares the same intercept, whereas the ‘+ x’ term assigns a slope term associated with the variable  $x$  such that the slope is 0 before the change point and  $\beta \cdot x$  afterwards.

The *mcp* package makes it easy to specify custom model structures for each segment and allows for a wide range of statistical distributions and link functions. Model parameters are assigned suitable vague prior distributions, though these can easily be altered (Lindeløv, 2020). Hierarchical models with random effects can be specified by allowing change points to covary within a group, thereby allowing the estimation of individual-specific and population-level change point parameters.

Another advantage of the *mcp* package is the combination of a user-friendly interface connected to a robust Bayesian modeling backend. The model structure for each segment is expressed using the familiar syntax of the *lme4* and *brms* packages, then is converted ‘on-the-fly’ to JAGS code (Bates et al., 2014; Bürkner, 2017). Support for fitting models using Stan is currently in development. The *mcp* package returns samples from the marginal posterior distributions for all change points and other model parameters, which can be used to visualize model output, for example with the *ggplot2*

and *bayesplot* packages and produce credible intervals (Gabry, 2017; Gabry et al., 2019; Wickham, 2011).

Lastly, the *mcp* package allows for a robust suite of methods for model assessment and comparison, hypothesis testing, and data simulation. Markov-chain Monte Carlo (MCMC) sampler performance can be checked via the Gelman-Rubin statistic, effective sample size, and visual assessment of MCMC chains. Posterior predictive checks ensure that the fitted model is consistent with the data-generating process (i.e., simulations from the fitted model resemble the original data; Gelman et al., 1996; Gelman & Shalizi, 2013). The influence of the priors can be evaluated by comparing prior-posterior overlap (Youngflesh, 2018). The predictive performance of multiple models can be compared with leave-one-out cross validation (LOO-CV), either using WAIC (Widely Applicable Information Criterion) or ELPD (Expected Log Predictive Density), which can be calculated with the *loo* package (Gelman et al., 2014; Vehtari et al., 2017). If the appropriate number of change points is not known *a priori*, LOO-CV allows the user to compare the predictive performance of multiple models to determine the optimal number, or to compare different model structures and prior distributions. Additionally, hypothesis testing can be performed using point Bayes factors (i.e., the prior to posterior ratios associated with specific parameter values; Verdinelli & Wasserman, 1995; Wagenmakers et al., 2010). Bayes factors can also be used to compare models that represent alternative hypotheses and for model averaging (Hooten & Hobbs, 2015; Kass & Raftery, 1995). Bayes factors  $> 10$  are commonly used to indicate strong evidence that the data support one model over another (De Santis, 2004; Schönbrodt & Wagenmakers, 2018).

## **Example applications**

We demonstrate the use of piecewise regression using the *mcp* package with five example applications highlighting a variety of taxonomic species, data streams, timescales, and biological phenomena. We have provided R code associated with each example at the publicly available Data Repository for University of Minnesota.

## **Identification of altered behavior post-capture**

Effects of capture, handling, and transmitter deployment are not well understood for many wildlife species, especially in the short-term period immediately post-capture. Efforts to quantify animal response have predominantly focused on the impacts of the transmitter itself (i.e., the weight and aerodynamics of the unit, usually in birds: Evans et al., 2020) or on the physiological effects from chemical immobilization (typically in large mammals: Barron et al., 2010; Brivio et al., 2015; Thompson et al., 2020). Most researchers have sought to quantify effects on vital rates, such as survival and fecundity (Casas et al., 2015; DelGiudice et al., 2005; Lameris & Kleyheeg, 2017), or short-term ethological responses such as changes in the time spent grooming (Kölzsch et al., 2016; Rachlow et al., 2014). It is generally accepted that events such as capture and handling may result in a short-term period of altered movement behavior. Thus, it is common to remove data from the first week or two post-capture, though often without biological or empirical justification for the threshold used to filter the data.

Arbitrarily filtering data without knowing the existence and duration of capture effects presents several issues for movement-related analyses. Removing data potentially discards useful information, which may already be scarce in studies using VHF telemetry or with small sample sizes (Girard et al., 2002). Alternatively, quantifying behavioral responses post-capture can provide species-specific information on altered behavior and inform future studies (Dechen Quinn et al., 2012). For example, Stabach et al. (2020) examined effects of GPS collar deployment on scimitar-horned oryx (*Oryx dammah*) to quantify the short-term responses in activity, behavior, and stress levels, and the length of time before these effects subsided. Visual observation showed headshaking significantly increased post-capture but returned to pre-capture levels within 3 days, and random forest classification of tri-axial accelerometer data indicated a 480% increase in headshaking compared to stable baseline levels that resumed after 24 hours. Using piecewise regression, Stabach et al. (2020) found that fecal glucocorticoid metabolite levels were elevated for five days following collar deployment, suggesting a stress response, before returning to baseline levels.

The effects of capture and handling on movement may also provide insights into how individuals respond to risky situations (e.g., capture, predation). For example, animals may exhibit a “flee and return” movement response, the strength of which may be indicative of their tolerance of risk as it relates to a fecundity-survival trade-off (Ghalambor & Martin, 2001; Montgomerie & Weatherhead, 1988). DelGiudice et al. (2015) documented that capture of moose (*Alces alces*) neonates caused some mothers to abandon their calves, and Obermoller et al. (2019) found that adult female moose frequently fled when their calves were predated and then returned after the risk had subsided. These responses suggest that many adult female moose favor individual survival over protection of their young.

Other studies have found similar “flee and return” movements in response to human activity such as hunting or helicopter-based capture (Jung et al., 2019; Sunde et al., 2009; Thurfjell et al., 2013). In an ongoing study of trumpeter swan (*Cygnus buccinator*) movement ecology, we found some swans exhibited a distinctive “flee and return” response immediately post-capture (Wolfson unpublished data). Figure 1 shows the results of a piecewise regression model fit to the relationship between Net-Squared Displacement (NSD) and time since capture:

```
model=list(  
  NSD~1, # distance of 'flee' response from capture site  
  ~1 # stable return distance from capture site  
)
```

The NSD value of the first intercept quantifies how far the swan fled and the change point reveals when a return to the capture site occurred. This model, which has two segments with different intercepts and a single change point, had a better fit using LOO-CV to estimate ELPD, a goodness-of-fit measure that estimates the predictive accuracy of a model while balancing the trade-offs of bias and variance, than an alternate model with a single intercept, therefore suggesting the presence of an altered state representing a flee response (Table 1).

## Pre-parturition movement

Accurate estimation of vital rates and their relative contributions to population dynamics is an essential tenet for population management (Coulson et al., 2005). Signals in movement data can reveal important biological events such as parturition in large ungulates, which relate to vital rates. Although fecundity is a key vital rate, detection of the occurrence and timing of parturition can be difficult, especially in ‘hider’ species that have low parental care of neonates (Lent, 1974; Ralls et al., 1986).

Pregnant ungulates typically make a long-distance movement immediately preceding parturition and then remain sedentary for some period (McGraw et al., 2014). Patterns in movement data thus offer a cost-effective means for identifying parturition in radio-collared ungulates (Asher et al., 2014; DeMars et al., 2013; Nicholson et al., 2019; Peterson et al., 2018; Severud et al., 2015). Although these methods can be effective at inferring parturition events, many are sensitive to parameter choices or involve visual observation of movement metrics, which can be time-intensive (Dettki & Ericsson, 2008).

To demonstrate remote identification of a confirmed parturition event, we provide an example of displacement of a pregnant moose (Figure 2). Severud et al. (2015) confirmed the parturition period by locating and collaring the twin calves shortly after they were born. In this case, the first segment is modeled with an intercept and slope of zero, representing typical moose movement, and the second segment is modeled with a change in the mean displacement (i.e., a different intercept) and also a change in variance (“sigma” in code below) that is representative of a shift to more sedentary movement at time of parturition.

```
model<-list(  
  NSD~1,      # baseline moose NSD  
  ~1+sigma(1) # movement spike and decreased variability  
)
```

LOO-CV of this model versus one without a change in variance reflected that this model is a better fit to the data, thus illustrating the flexibility in adapting model syntax

for each segment that corresponds to the biological situation. Prediction intervals illustrate that the addition of a change in variance for the second segment provides increased precision, therefore allowing the model syntax to closely mirror the biological situation of increased sedentary behavior (Figure 2).

Parturition events can be monitored in real time for studies involving the capture of neonates (Figure 2; Obermoller et al., 2019), or events can be identified post-hoc for retrospective analyses of ungulate breeding and fecundity (Bonar et al., 2018; Long et al., 2009). Previous studies have used piecewise regression to identify change points indicative of ungulate parturition based on movement, but implementation of the *mcp* package can provide additional analytical options (Berg et al., 2021).

Posterior predictive checks, a common method used to test goodness of fit, generate data from the fitted model by simulating from the posterior predictive distribution to visualize how well a model matches the observed values. Using the fitted model for moose movement, we demonstrate a graphical method to compare  $y$ , the observed response dataset, and  $y^{rep}$ , replicated datasets simulated from the fitted model. (Figure 3). The two peaks at 3 km and 12 km correspond to the two intercepts fit by the model and overall, the observed data conform well to the replicated datasets.

Prior field research can inform the ideal time window for change-point movement analysis and the scale of long-distance movement preceding parturition (allowing for a more informed prior).

### **Physiological response to drone fly-over**

Remotely piloted aircraft systems (hereafter drones) allow increased opportunity for remotely monitoring wildlife populations, and recent reductions in their cost have led to widespread adoption as an alternative to traditional aerial surveys using planes and helicopters (Watts et al., 2010). Drones instrumented with remote sensing technologies are now commonly used to estimate animal abundance in remote locations, collect fine-grain aerial imagery, and monitor poaching activities (Anderson & Gaston, 2013; Linchant et al., 2015; Mulero-Pázmány et al., 2017). The use of drones can decrease fieldwork costs and eliminate the need for hazardous fieldwork, and they can be coupled



with computer vision methods to increase the quality and precision of data collection (Chabot & Francis, 2016; Hodgson et al., 2018; Seymour et al., 2017).

Despite the advantages of using drones for field data collection, their presence can influence behavior or illicit a physiological response in the study species, especially when they are flown at low altitudes (McEvoy et al., 2016; Mulero-Pázmány et al., 2017). Response to anthropogenic stimuli such as noise has been shown to have detrimental effects on wildlife species at both immediate (e.g., increased vigilance and decreased foraging behavior) and long-term scales (e.g., decreased reproduction and population declines: Shannon et al., 2016; Blickley & Patricelli, 2010; Senzaki et al., 2020). The response of animals to drones has focused on external responses, such as altered movement and behavior. Most research on internal physiological responses have been limited to quantifying levels of glucocorticoids (e.g., cortisol and corticosterone: Baker et al., 2013; Bennitt et al., 2019; Millspaugh & Washburn, 2004; Vas et al., 2015; Wasser et al., 2000).

More recently, biotelemetry data have been paired with physiological data, allowing for new insights into the response of animals to anthropogenic stimuli. For example, Ditmer et al. (2015) measured changes in movement and heart rate levels of black bears (*Ursus americanus*) affixed with GPS collars and internally-implanted cardiac biologgers during drone flights. Although drones rarely elicited a behavioral response in black bears, heart rate levels were strongly correlated with proximity of drones overhead (Figure 4).

Figure 4 shows the relationship between drone presence and black bear heart rate in beats per minute (bpm) before and after controlled flights (Ditmer et al., 2015).

```
model<-list(  
  HeartRate~1, # intercept for stable background HR  
  ~1+time) # HR spike and slope of recovery rate
```

The initial segment prior to the change point includes an intercept capturing the baseline heart rate of the bear. The second segment contains an intercept and a disjointed slope term reflecting the heart rate gradually decreasing after the initial spike caused by the drone flight.

The flexibility of piecewise regression to accommodate different model structures for each segment allows for identification of the timing, magnitude, and acclimation rate of the stress response caused by the drone presence. For example, the change point, `cp_1`, is estimated to occur approximately 65 (95% CI=63-66) minutes into the observation period, the baseline heart rate, `int_1`, is estimated to increase by 116 bpm (`int_2-int_1`) once the drone appears, the heart rate then decreases by the slope of 0.78 bpm (`minutes_index_2`) and if the recovery rate is static, the black bear will return to baseline heart rate in approximately 2.5 hours ( $(int\_2-int\_1)/minutes\_index\_2=149$  minutes). Lastly, `sigma_1` captures the variability in heart rate about the overall trend.

```
## Family: gaussian(link = 'identity')
## Iterations: 9000 from 3 chains.
## Segments:
## 1: HeartRate ~ 1
## 2: HeartRate ~ 1 ~ 1 + minutes_index
##
## Population-level parameters:
##      name mean lower upper Rhat n.eff
##      cp_1 64.77 62.89 66.000 1 1357
##      int_1 43.83 39.86 47.792 1 4939
##      int_2 160.14 150.22 169.427 1 547
## minutes_index_2 -0.78 -1.08 -0.463 1 491
##      sigma_1 10.92 8.95 13.069 1 4222
```

### **Mortality signal from acceleration and temperature data**

Accurate estimates of vital rates such as survival and fecundity are necessary for estimating population growth rates. Survival rates can be estimated using mark-recapture and mark-resight methods. However, for wide-ranging migratory species, individuals in subsequent years may not be resighted due to the combination of three separate possibilities: mortality, emigration, or missed detection by observers (Anders & Marshall, 2005). Because it is difficult to separate these components, biologists are often forced to estimate ‘apparent’ survival, as opposed to true survival (Lebreton et al., 1992). Additionally, mark-recapture and mark-resight methods do not provide information on specific times or locations of mortalities.

Many studies have illustrated the importance of evaluating vital rates over the entire annual cycle (Rushing et al., 2017; Sillett & Holmes, 2002). The miniaturization of GPS attachment devices and increased use of radiotracking now allows for a more direct accounting of survival during the breeding, migration, and overwintering seasons (Kays et al., 2015). Increased precision in vital rates during different seasons (and of different age classes) can better inform ecological studies considering life-history tradeoffs between survival and fecundity, especially concerning survival during migration periods (Buechley et al., 2021; Cheng et al., 2019; Flack et al., 2016).

Despite the advantages that GPS telemetry can offer to survival rate estimation, mechanical failure of transmitters can obscure whether the true fate was mortality or equipment failure. Some degree of failure is an unfortunate reality of most GPS tracking studies (Klaassen et al., 2014). Recovery of a GPS transmitter after a mortality event is often logistically unfeasible for species that migrate long distances. Therefore, a variety of methods have been employed to infer mortality versus transmitter failure from remotely obtained transmitter signals (Buechley et al., 2021; Sergio et al., 2019). The most common method applied is a visual assessment of whether locations appear to be stationary. However, this approach may lead to different conclusions depending on who makes the visual assessment (Koczur et al., 2017; Nygård et al., 2016; Rotics et al., 2017).

Sensor data such as battery voltage, temperature, and accelerometry are increasingly being used as diagnostic criteria for determination of mortality events versus transmitter failure (Burnside et al., 2016; Ely & Meixell, 2016; Hewson et al., 2016). Typically, mortalities coincide with shifts in the trend or variability in these data. Piecewise regression is therefore a quick and easy method that can be used to identify breakpoints that may be indicative of mortality. This approach can also be extended to monitor nest success with temperature loggers (Hartman & Oring, 2006; Sutti & Strong, 2014; Zangmeister et al., 2009). As part of a study of trumpeter swan movement ecology, we evaluated whether we could remotely detect mortality using piecewise regression models

fit separately to Overall Dynamic Body Acceleration (ODBA), a proxy for energy expenditure, and temperature data from a confirmed mortality event (Figure 5).

The ODBA model includes a change point separating two segments with differing means and variances.

```
model<-list(  
  ODBA~1,      # baseline energy expenditure level  
  ~1+sigma(1)) # activity drop and decreased variability
```

The temperature model has a change point separating the first segment, which is modelled with an intercept and a first-order autoregressive residual term, and the second segment, which is modelled with an intercept and a third-order autoregressive residual term.

```
model<-list(  
  temp~1+ar(1), # collar temp tracks live swan temp  
  ~1+ar(3)) # collar temp tracks ambient air temp
```

A  $k$ th order autoregressive term models the correlation in residuals from  $k$  previous values in the time series and is ideal for data such as temperature that is highly temporally correlated. We allow for autocorrelation by assuming the residuals of the first segment area a function of residuals at time  $t-1$ , and the residuals of the second segment area a function of the residuals at time  $t-1$ ,  $t-2$ , and  $t-3$  (see the full annotated code and a short tutorial on choosing autoregressive terms in the open-access data repository; Wolfson et al. 2022).

Both models showed a clear distinction at the change point representing mortality of the collared swan (Figure 5). There are advantages to being able to identify true mortality using multiple auxiliary data sources. Many transmitters are not equipped with tri-axial accelerometers, either to save costs or because accelerometer data is not a focus of the study. Additionally, deriving ODBA estimates from raw sensor data adds an additional analytical step to the process of estimating mortality.

## **Migration phenology including stopovers**

Seasonal migration allows species to optimize energetic budgets and undergo reproductive cycles while avoiding harsh environmental conditions and low food availability (Newton, 2010). Knowledge of migration phenology throughout the annual cycle inform management activities such as timing of water drawdowns and annual surveys to occur during peak migration, advance understanding of disease dynamics based on timing and overlap with other species, and reveal a species' capacity to adapt their migratory timing in response to climate change to preserve optimal breeding conditions and peak food availability (Donnelly et al., 2019; Moller et al., 2008; Newman et al., 2009; O'Neal et al., 2012; Thurber et al., 2020).

Despite the importance of understanding migration phenology, consistent reproducible methods for determining departure and arrival dates has not yet emerged (Cerritelli et al., 2020; Soriano-Redondo et al., 2020). A simple repeatable approach is to segment phases of the migration cycle based on date ranges informed from prior studies (Takekawa et al., 2010; Wolfson et al., 2017a). Other approaches include using a spatial threshold, based on either the absolute distance from the capture origin, breeding territory, or last location; a spatio-temporal threshold, based on a certain distance moved within a period; or a crossing of a chosen latitude or landmark (Flack et al., 2016; Giunchi et al., 2019; Rotics et al., 2016). Although these approaches may yield useful estimates of migration phenology, often they rely on arbitrary criteria or choices that are either species-specific or highly subjective, and therefore hard to generalize to other study systems.

Model-based methods, including non-linear theoretical movement models fit to NSD, are also commonly used to estimate migration phenology (Börger & Fryxell, 2012; Bunnefeld et al., 2011; de Grissac et al., 2016; Spitz et al., 2017). Using piecewise regression to segment an annual cycle based on NSD allows for a model-based approach but is more flexible than typical NSD modelling *sensu* Bunnefeld et al. (2011) and can also provide additional information on the timing and duration of stop-overs that is not attainable using traditional NSD-based methods.

We demonstrate the utility of piecewise regression in assessing movements during the annual cycle of a migratory bird using a NSD time-series of sandhill crane (*Antigone canadensis*) locations in North America (Figure 6; Wolfson et al., 2017b; Wolfson 2018). Although a basic three-intercept model would sufficiently discriminate the summer and winter periods, adding additional intercepts reveals each major staging area that the crane used.

```
model<-list(  
  NSD~1, # summer breeding territory in Minnesota  
  ~1, # first fall staging area in Nebraska  
  ~1, # second fall staging area in Oklahoma  
  ~1, # over-wintering area along Texas coast  
  ~1, # spring staging area in Nebraska  
  ~1) # summer breeding territory in Minnesota
```

The ideal number of stopover sites can be visually examined or empirically derived using cross-validation to compare multiple models with differing number of change points. We demonstrate a data-driven model selection method by fitting seven separate models with increasing numbers of intercepts. Table 3 shows that the model with six intercepts (representing two spring staging areas and one autumn staging area) is the most well supported using ELPD as a model comparison metric and that adding additional intercepts does not increase the predictive accuracy.

### **Segmentation of nesting stages**

The timing of nest creation, incubation, and hatching events can inform our understanding of nest success, juvenile survival, and local recruitment. Climate change is advancing the onset of spring, especially in the Arctic, where earlier snowmelt allows some species to adapt their breeding cycles to match changing conditions (Lameris et al., 2018; Nolet et al., 2020). Although data on the timing of breeding are critical to understanding reproduction, locating and regularly monitoring active nests is labor-intensive and unfeasible in many remote locations (Schreven et al., 2021).

As part of an ongoing trumpeter swan study (D. W. Wolfson, unpublished data), we collected visual observations on a focal sample of collared swans several times a week during the breeding season to determine nesting status (building a nest, incubation,

eggs hatched and cygnets present). We also collected fine-scale accelerometry data from each observed swan's GPS-GSM collar to evaluate if we could infer nesting status from ODBA values, representing activity levels. We used the visual observation dataset to ground-truth model output from piecewise regression applied to a collared swan in the following case study.

Between 1 April and 7 July, 2021, we collected 19 observations of nesting status and 735,311 tri-axial accelerometer readings taken in 3-second bursts at 10Hz every 5 minutes that were converted to average hourly ODBA values (Fig. 7). We predicted that a shift to higher activity levels would occur once cygnets were present and expected that the change point for a model with two segments with varying intercepts would accurately predict the time of egg hatching.

```
model<-list(  
  ODBA~1, # intercept for activity levels during incubation  
  ~1) # intercept for increased activity levels post-incubation
```

Table 4 shows that the model with two intercepts was the best choice at fitting the data based on LOO-CV using ELPD. The piecewise regression model was able to estimate the transition based on a shift in mean activity levels using remotely sensed data (Fig. 8).

## Discussion

As we demonstrated with examples of birth, death, migration, and behavioral responses (both internal and external), piecewise regression is a flexible tool for identifying a wide variety of biological phenomena across different taxa and data types. When analyzing data, we recommend fitting a limited number of models that attempt to capture specific biological hypotheses in the style of Chamberlin's framework of multiple working hypotheses (Chamberlin, 1890; Elliott and Brook, 2007). For example, in the first case study, we highlight two model syntaxes that represent different biological responses (one intercept represents the absence of a flee response, two intercepts indicate a flee and return). ELPD or WAIC, calculated using cross validation can then be used to evaluate support for each hypothesis. The *mcp* package also provides functionality for

null-hypothesis testing and interval estimation for any model parameter within a fitted model object (Lindeløv, 2020).

Piecewise regression, and change point detection in general, is most appropriate when the objective is to partition a dataset into a limited number of heterogeneous segments that may correspond with different biological states. Alternative approaches, such as HMMs or Behavioral Change Point Analysis (BCPA), may be more appropriate when the goal is to identify recurring behavioral states (e.g., resting and foraging) using information derived from movement metrics (e.g., step lengths, turning angles). Although piecewise regression could be used in these situations, it will often be slower due to the computational challenges of estimating a large number of change points. Similar to BCPA, piecewise regression would also require a two-step approach in which segments are first identified and then later grouped into homogeneous categories. Unlike BCPA, however, the response data do not have to be Normally distributed when using *mcp*.

Many R packages can detect change points, though each has unique pros and cons. Several require a user-defined threshold to detect change points, such as *changepoint* (Killick and Eckley, 2014), *bcp* (Erdman and Emerson, 2007), and *ecp* (James and Matteson, 2014), but the selection of a threshold value may often be species-specific and hard to generalize. The *changepoint* package, which only identifies change points based on abrupt changes in the mean or variance, may be a good option for a simple segmentation analysis, but it does not estimate uncertainty or provide methods for model evaluation or comparison. Other packages provide more flexibility in specifying a regression model, such as *strucchange* (Zeileis et al., 2002) and *segmented* (Muggeo, 2008), but still offer limited options relative to *mcp*.

The *mcp* package offers rigorous statistical methodology, flexibility, ease of use and reproducibility. It allows the user to specify a unique model syntax for each segment between change points, detect changes in mean, variance, and autocorrelation, and it also allows for robust inference using full posterior distributions for all parameters and change points. Previous knowledge of the study system can be directly incorporated when



specifying parameter ranges and prior distributions. Although computational performance may slow with very large datasets, *mcp* includes parallel processing to increase computational efficiency. We provide a suite of worked examples and encourage others to consider using piecewise regression for identifying signals in biotelemetry data.

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Table 1.1 Leave-one-out cross validation used to compute the estimated log predictive density (ELPD) of two different models fit to movement data from a trumpeter swan (*Cygnus buccinator*); one fit with a single intercept and the other with two intercepts separated by a change point. The higher ELPD for the model with two intercepts indicates that it has a higher predictive accuracy.

<b>Model Syntax</b>	<b>ELPD Difference</b>	<b>SE of Difference</b>
Two Intercepts	0.0	0.0
One Intercept	-213.3	64.2

Table 1.2. A summary of output from the model looking at the black bear (*Ursus americanus*) heart rate response to a drone flyover.

<b>Model Parameter</b>	<b>Mean</b>	<b>Lower</b>	<b>Upper</b>	<b>R-hat</b>	<b>Effective Sample Size</b>
Change Point 1	64.77	62.89	66.00	1	1,374
Intercept 1	43.83	39.86	47.79	1	5,159
Intercept 2	160.14	150.22	169.43	1	537
Time (in minutes)	-0.78	-1.08	-0.46	1	505
Sigma	10.92	8.95	13.07	1	4,325

Table 1.3. Leave-one-out cross validation was used to compute the estimated log predictive density (ELPD) for seven models of migration phenology of a sandhill crane (*Antigone canadensis*), each with an increasing number of intercepts. The highest ELPD for the model with six intercepts indicates that it has the highest predictive accuracy.

<b>Model Syntax</b>	<b>ELPD Difference</b>	<b>SE of Difference</b>
Six Intercepts	0.00	0.00
Five Intercepts	-0.48	0.83
Seven Intercepts	-19.59	32.82
Four Intercepts	-24.73	9.52
Two Intercepts	-57.39	14.01
Three Intercepts	-324.69	16.93
One Intercept	-336.17	16.73

Table 1.4. Leave-one-out cross validation used the compute the estimated log predictive density (ELPD) of three different models of trumpeter swan (*Cygnus buccinator*) activity; one fit with a single intercept, one with two intercepts, and one with three intercepts. The higher ELPD for the model with two intercepts indicates that it has a higher predictive accuracy.

<b>Model Syntax</b>	<b>ELPD Difference</b>	<b>SE of Difference</b>
Two Intercepts	0.0	0.0
Three Intercepts	-9.9	7.3
One Intercept	-188.4	19.0

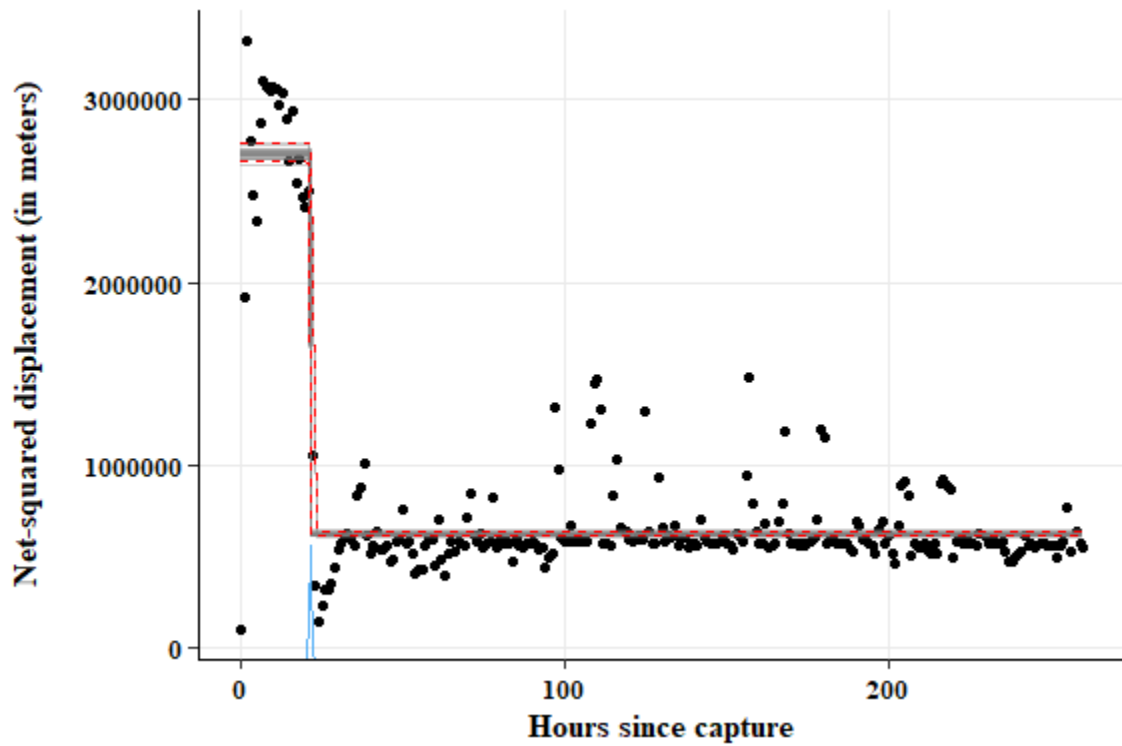


Figure 1-1 Hourly net-squared displacement measured from the point of release of a trumpeter swan (*Cygnus buccinator*) after collar deployment. Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines. The posterior distribution for the change point is shown in blue on the x-axis.

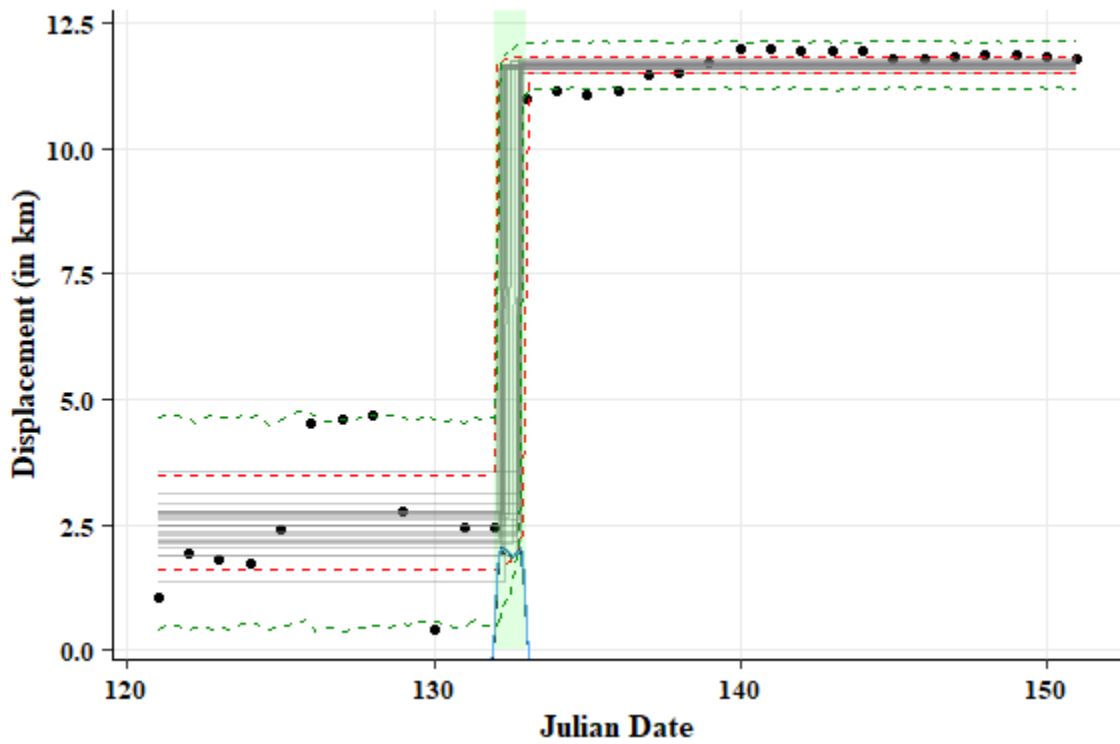


Figure 1-2 Hourly net-squared displacement measured from the point of release of a trumpeter swan (*Cygnus buccinator*) after collar deployment. Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines. The posterior distribution for the change point is shown in blue on the x-axis.

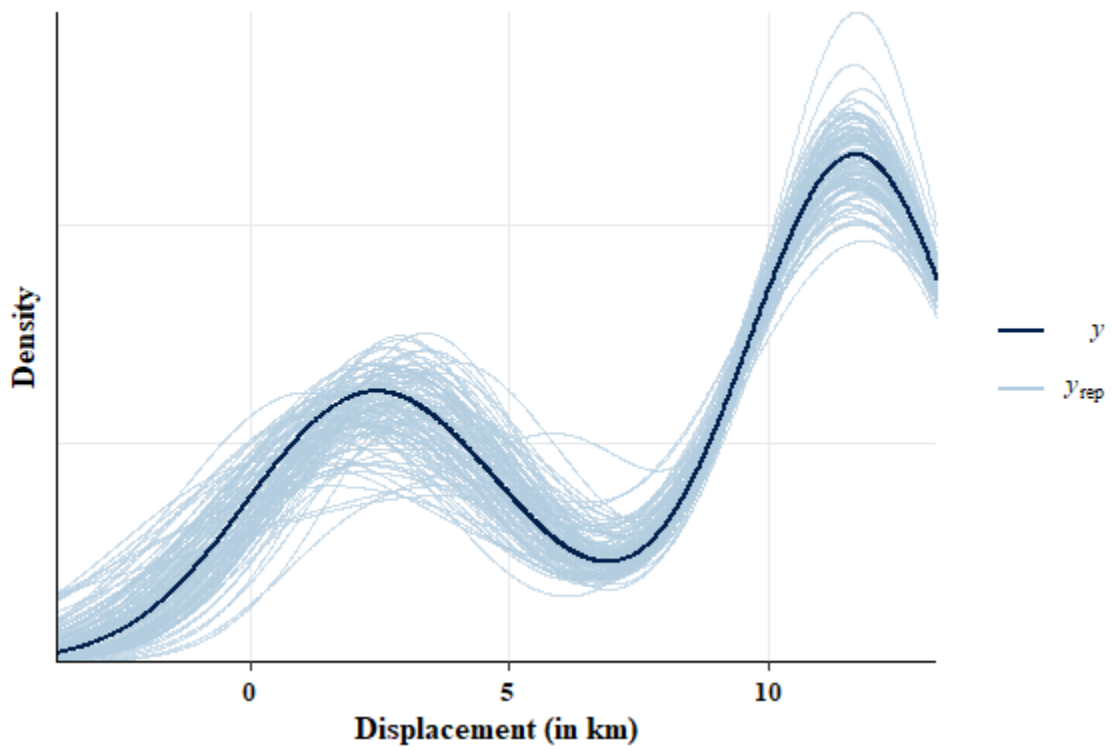


Figure 1-3. A kernel density posterior predictive check compares the distribution of observed outcomes [displacement in kilometers by a pregnant moose (*Alces alces*)], shown in the black line, against 50 distributions of replicated datasets produced by the fitted model, each shown as a light blue line.



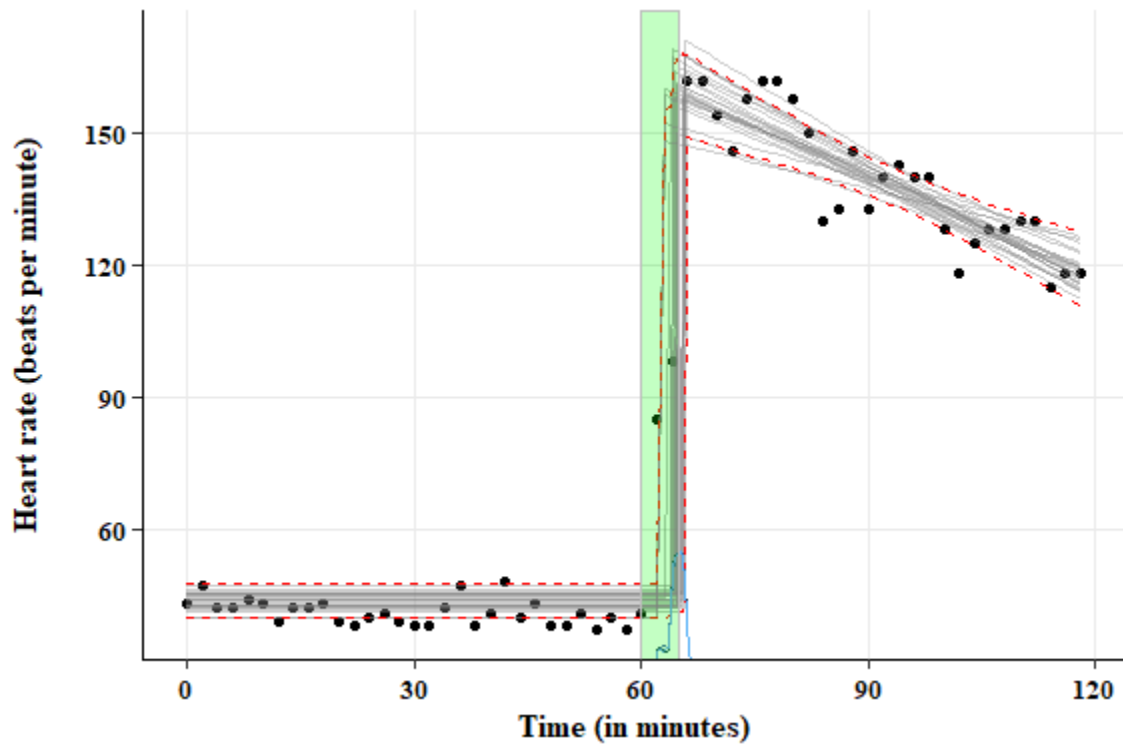


Figure 1-4. Heart rate of a black bear (*Ursus americanus*) in relation to a drone flight. The green box indicates the duration of a drone flight. Grey lines represent 25 draws from the posterior distribution of the mean response. Red dotted lines depict the 95% credible intervals for the mean response. The posterior distribution for the change point is shown in blue on the x-axis.

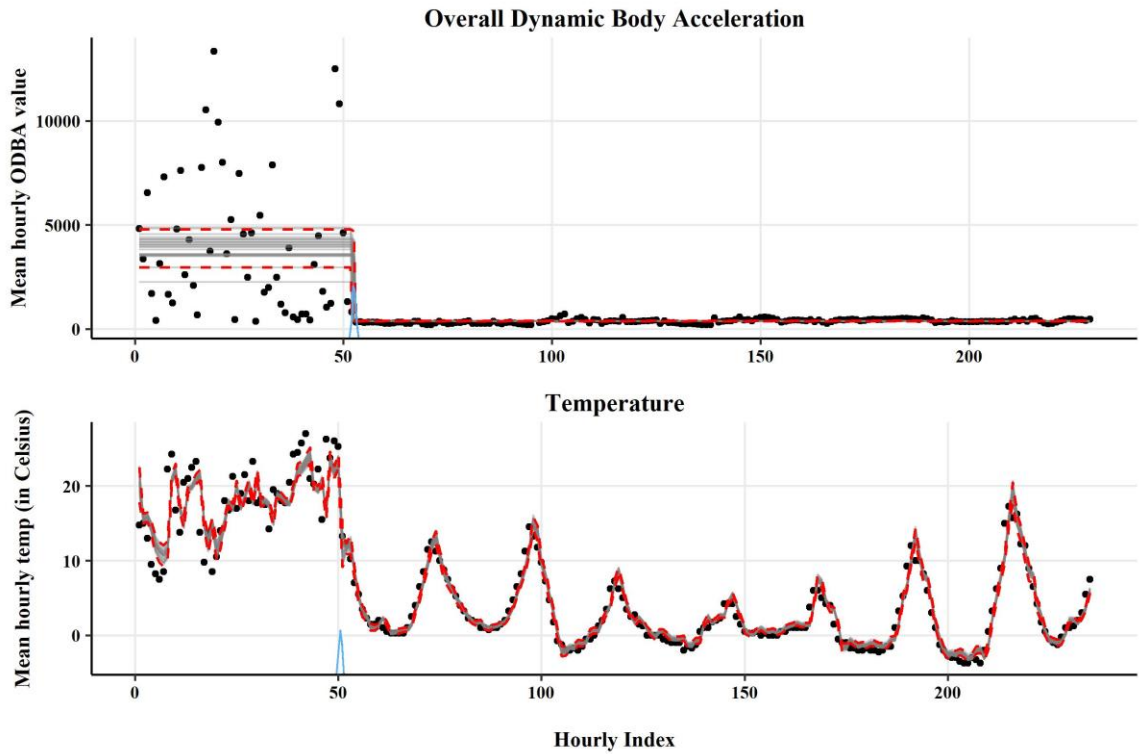


Figure 1-5. The x-axis is an index of time since the start of the time series for Overall Dynamic Body Acceleration (ODBA) on the top figure, and temperature on the bottom figure for a trumpeter swan (*Cygnus buccinator*). Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines. The posterior distribution for the change point is shown in blue on the x-axis.

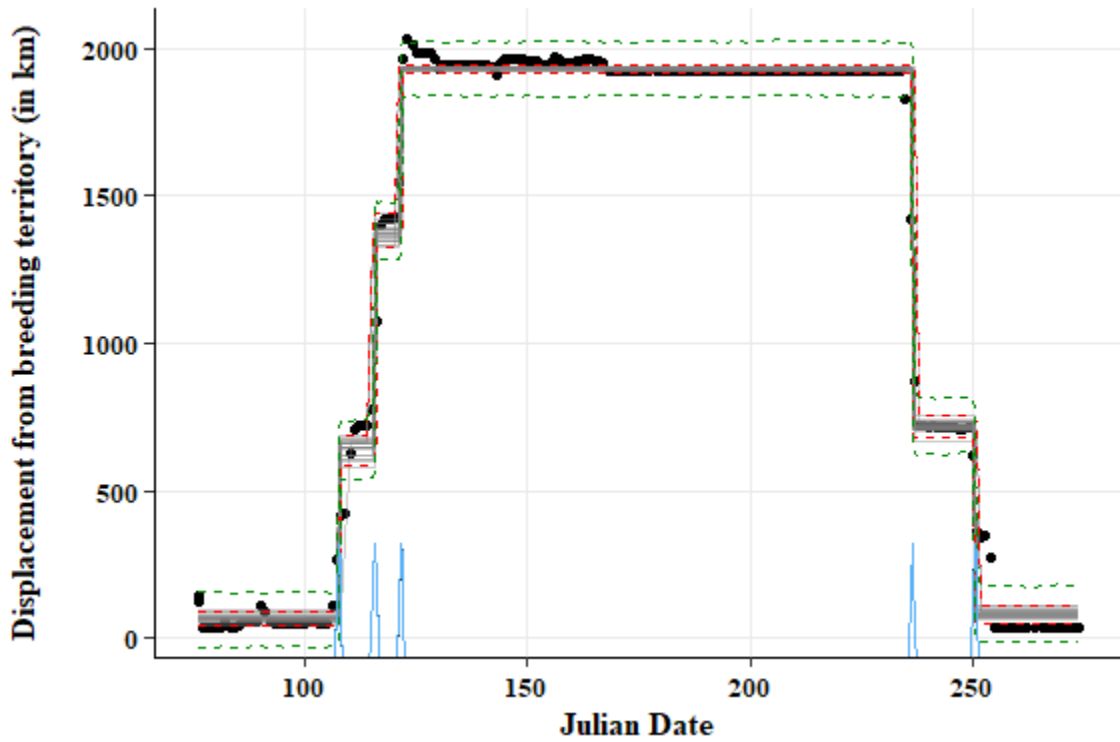


Figure 1-6. An annual migration cycle of a sandhill crane (*Antigone canadensis*). Average daily displacement from the breeding territory (in kilometers) on the y-axis, and an index of time since the start of the time series on the x-axis. Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red dotted lines and 80% prediction intervals in green dotted lines. The posterior distributions for the change points are shown in blue on the x-axis.

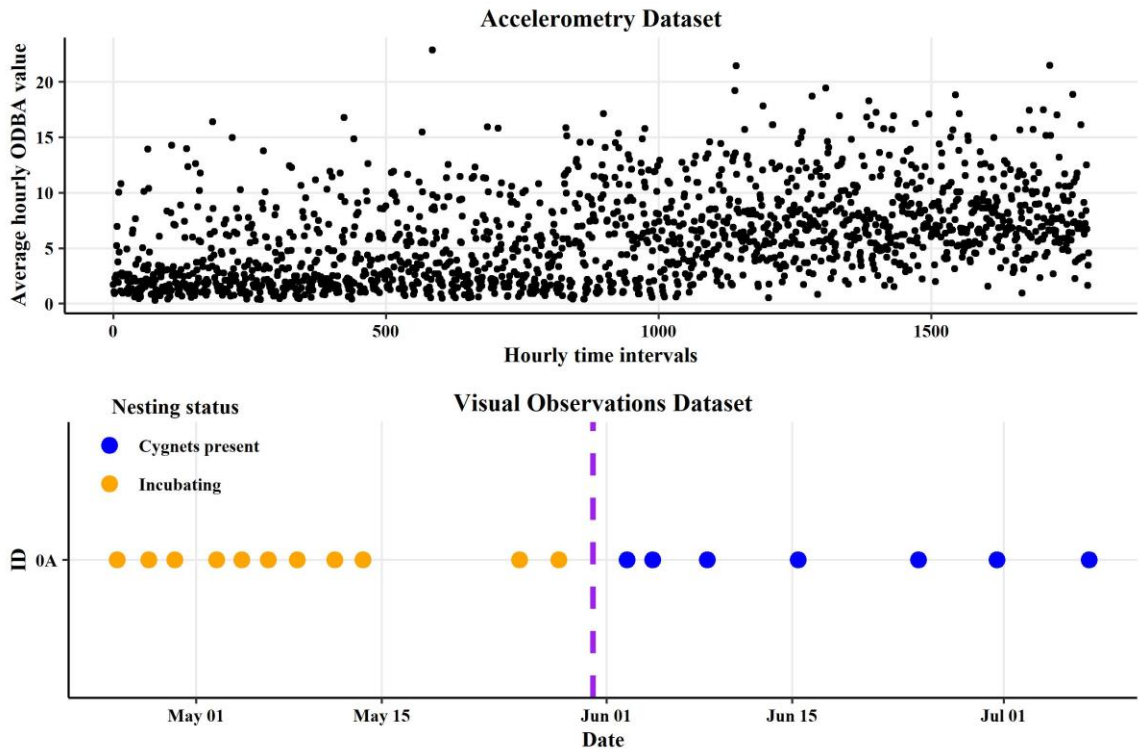


Figure 1-7. The top plot shows the accelerometer sensor dataset for a nesting trumpeter swan (*Cygnus buccinator*), with time on the x-axis expressed in hourly time intervals and averaged ODBA values on the y-axis. The bottom plot shows the 19 data points from the visual observation dataset with the estimated hatch date (the midpoint between the last incubation observation and the first cygnet observation; purple dotted line).

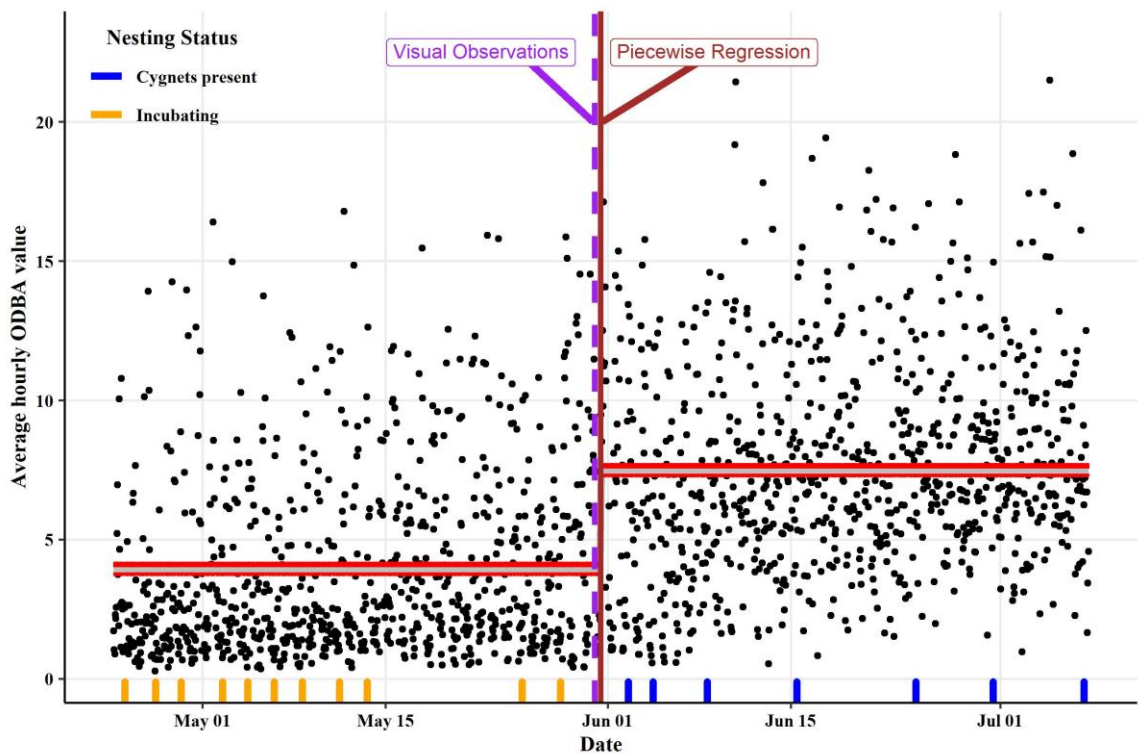


Figure 1-8. Date is on the x-axis and average hourly Overall Dynamic Body Acceleration (ODBA) for a trumpeter swan (*Cygnus buccinator*) is on the y-axis. The colored markers just above the x-axis correspond to visual observations of the swan’s nesting status. The dashed vertical line in purple is the observed transition from incubation to cygnets, and the solid brown line is the estimated change point from the piecewise regression model. Grey lines show 25 draws from the posterior distribution, with 95% credible intervals for the mean response shown as red lines.

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## Chapter 2: High variability of migration strategies in a re-established trumpeter swan population

### Synopsis

1. The Interior Population (IP) of trumpeter swans (*Cygnus buccinator*), formerly extirpated by market hunting, was re-established in eastern North America by releasing individuals from both migratory and non-migratory populations; their current annual movement patterns are largely unknown. Our goal was to describe their seasonal movements and quantify the proportion of the IP that is migratory, the extent and phenology (e.g., departure and arrival dates) of seasonal movements, and associations between movement patterns and breeding status and breeding location.
2. We deployed 113 GPS-GSM transmitters on IP trumpeter swans in six U.S. states and one Canadian province across the current IP breeding range. Using data from 252 'swan-years', we estimated dates of migration events by segmenting the annual cycle using piecewise regression models fit to each yearly time-series of displacement from the breeding site. We fit a latent state model to characterize population-level associations between breeding latitude and maximum extent of migration, and linear mixed models to quantify associations between individual characteristics (e.g., breeding status, sex) and migration phenology.
3. At the individual level, 59% of swans moved to distant non-breeding period areas (long-distance migration defined as moving >100 km from the breeding site); 16% exhibited regional migration (>25 km and <100 km from breeding site); 19% exhibited non-migratory, local movements (<25 km from breeding site); and 6% exhibited multiple migration strategies across years. Migration extent was best described using a 2-component mixture model in which migration extent was

linearly associated with breeding latitude for some individuals and was much lower and exhibited a non-linear association with breeding latitude for others. Swans breeding at more northern latitudes departed their territories earlier in autumn and returned later in the spring than those breeding at more southern latitudes. Breeding swans departed later in the autumn than non-breeders, but breeding status did not have a strong association with arrival in the spring.

4. IP trumpeter swans are partial migrants, with a continuum of strategies each year, from local movements to long-distance migration. Much of the variability in movement patterns was related to factors tied to natural history demands (e.g., breeding status) and response to environmental conditions (e.g., through associations with breeding latitude).

## **Introduction**

Migration is a behavioral mechanism widely used by all major vertebrate groups (e.g., birds, mammals, fishes, reptiles, amphibians) that allows individuals to track seasonal availability of resources to increase short-term survival, and by optimizing migration phenology, maximize long-term fitness (Barker et al., 2022; Fryxell et al., 1988; Milner-Gulland et al., 2011; Winger et al., 2019). Despite its prevalence as an ecological process and a large body of research involving migration, how population-level migratory traditions are established is not well understood (Abrahms et al. 2021). In part, that lack of understanding is hindered by challenges associated with making population-level inference from observations of individuals, quantifying migratory movements along a continuum of variability, and the relative scarcity of successful reintroductions of formerly rare migratory species (Mueller et al., 2011; Senner et al., 2020).

Similar to other large, long-lived avian species such as geese, cranes, and storks, adult swans provide care for their young during the first year of life, providing access to food and protection, and guiding them on their first migration cycle (Chernetsov et al., 2004; Mueller et al., 2013). As a consequence, cultural transmission during the first year



is thought to be the primary mechanism that dictates the learned migratory strategy used in subsequent years (Palacín et al., 2011; Sutherland, 1998). Although this transfer of information is an effective mechanism for preserving migratory patterns through generations, how these trends become established after a population is reintroduced on a landscape from which it had previously been extirpated is unclear. Jesmer et al. (2018) found that newly translocated ungulate populations initially lost their migratory tendencies, and it took many generations to re-establish such patterns but that migratory trends eventually stabilized.

Trumpeter swans (*Cygnus buccinator*), the largest waterfowl species in North America, were widespread throughout much of the continent prior to European colonization (Alison, 1975). Due to widespread hunting for meat, skins for powder puffs, and feather quills for writing instruments, trumpeter swans were nearly extirpated in the lower 48 U.S. states and southern Canada, and reached an estimated low of 70 individuals in the 1930s (Banko, 1960). In 1935, low numbers of trumpeter swans led to the establishment of Red Rock Lakes Migratory Waterfowl Refuge (renamed Red Rock Lakes National Wildlife Refuge [RRLNWR] in 1961) in the convergence of Montana, Wyoming, and Idaho, which was the last vestige of a breeding swan population in the U.S. (outside of Alaska; Shea et al., 2002). As trumpeter swan numbers at RRLNWR started to rise, this population became a source for reintroduction efforts in other parts of their historical breeding range. Trumpeter swans from RRLNWR were translocated to several U.S. states to augment and increase the abundance and distribution of the diminished Rocky Mountain Population (RMP) in the inter-Mountain West and to restore the Interior Population (IP), which had been extirpated in eastern North America (Shea et al., 2002). In the 1950s, aerial surveys in central Alaska revealed another population of trumpeter swans (the Pacific Coast Population [PCP]), and this group subsequently provided the majority of swans used for reintroductions within the IP (Hansen et al., 1971; Matteson et al., 1988). An important distinction between these source populations is that PCP swans breeding in Alaska migrate to British Columbia and the northwestern

United States each winter, whereas RMP swans from RRLNWR are non-migratory (Baskin, 1993; Oyler-McCance et al., 2007).

Estimates of IP abundance have increased dramatically since reintroductions began in the 1960s, and both population size and distribution have expanded significantly (Groves, 2017). Trumpeter swans currently breed throughout most of the western Great Lakes region, including in Minnesota, Wisconsin, Michigan, Iowa, Manitoba, Ontario, and Ohio. Beyond estimates of population size and trends, however, there is relatively little recent information about their ecology, including seasonal movements and migration patterns. For example, it is not known what proportion of the IP remains resident on their breeding range during the non-breeding period, the extent (i.e., distance) of movement for those swans that do leave their breeding territories, the timing of migratory periods (e.g., autumn departure, spring arrival), and the magnitude of intra- and inter-individual variability in migration behavior. A more comprehensive understanding of when and where IP swans move throughout the annual cycle, including any differences related to breeding status or latitude will better elucidate habitat requirements and inform optimal study design for surveys to index abundance.

Many factors likely influence an individual swan's decision to leave its breeding territory during the non-breeding period, the distance migrating individuals travel, and the degree of among-individual variability in movements for swans breeding at locations in close proximity to one another; these factors include life history requirements, knowledge transfer of migratory traditions, and inherent genetic control of migration (Alerstam et al., 2003; Chapman et al., 2011; Dingle & Drake, 2007). Arriving early in the spring allows individuals to re-establish and defend a breeding territory and lay and incubate eggs early during the breeding period when nest success and survival of offspring is generally highest (Anderson et al., 2001; Blums et al., 2002; Dawson & Clark, 2000). Similarly, there are advantages to staying on breeding territories longer in the autumn to allow cygnets time to learn to fly and develop sufficient fat reserves to migrate to areas used during the non-breeding period (Lok et al., 2017; Winger & Pegan, 2021). Survival rates of migrant birds are typically lowest during the non-breeding period

of the annual cycle due to challenges associated with navigating relatively unfamiliar landscapes and the high energetic demand of migration (Rushing et al., 2017; Sillett & Holmes, 2002). Yet, by migrating to a more temperate area during the non-breeding period, swans can increase their access to food and other resources that allow them to avoid harsh winter conditions in their breeding territories, thereby balancing potential costs of migration with the benefits of increased resource availability (Alerstam et al., 2003; Somveille et al., 2015).

It is likely that the drivers of migration vary within the IP based on the location of a swan's breeding territory. Cues for migration can include declines in food availability, which may also be affected by density-dependent intra-specific competition (Rappole, 2013). Swans that spend the summer at different latitudes experience varying environmental conditions that may influence arrival in spring, such as the timing of ice melt and vegetative greenness (La Sorte et al., 2014; La Sorte & Graham, 2021), and departure in the autumn, such as ice formation on shallow wetlands and plant senescence (Schummer et al., 2010; Xu & Si, 2019). Swans breeding farther from the equator contend with shorter growing seasons and greater pressures for offspring to sufficiently develop flight before environmental conditions dictate migrating to non-breeding areas (Stafford et al., 2014). The 'push' to avoid harsh winter conditions varies substantially throughout the IP breeding range, with concurrent implications for decisions related to migration propensity and timing. Many populations include some individuals that migrate each year whereas others are residents (i.e., partial migration), which can result in higher overall fitness (Chapman et al., 2011; Lundberg, 1988, 2013). We expected the propensity for trumpeter swans to migrate to be higher in more northern latitudes.

To better understand current movement ecology of the re-established IP trumpeter swans, we marked a sample of swans with GPS-GSM transmitters and monitored their movements over multiple annual cycles. Our primary goal was to better understand how a re-established population of trumpeter swans derived from different source populations uses a novel landscape. Our specific objectives were to quantify 1) the proportion of the IP that is migratory and the extent of migratory movements, 2) migration phenology (e.g.,

timing of autumn departure and spring arrival), and 3) associations between annual movement patterns and breeding status and breeding location.

## **Methods**

### **Study Area**

We captured IP swans from 2019–2022 throughout their current breeding distribution (Groves, 2017) and during the breeding period, except for 4 swans captured during the non-breeding period in Arkansas (Fig. 1, Fig. S1). We deployed transmitters on IP trumpeter swans as far north and west as southern Manitoba (51.1° N, 99.7° W), as far south as Arkansas (35.5° N, 91.9° W), and as far east as Ohio (41.4° N, 80.7° W). Capture locations occurred in a mix of Laurentian Mixed Forest, Prairie Parkland, Eastern Broadleaf Forest, and Aspen Parklands (Cleland et al., 1997).

### **Capture and Handling**

We captured all swans during the definitive prebasic molt period (except for 4 captured in Arkansas using snares during the non-breeding period) when adult swans replace their remiges, and are therefore flightless, using a combination of jon boats, airboats, step deck transom boats, and square-stern canoes. We primarily used long-tail mud motors (Powell Performance Fab, Hutchinson, Minnesota, USA) to navigate shallow wetlands where swans were located, though some swans were captured using boats with surface-drive motors (Gator-Tail, Loreauville, Louisiana, USA) or non-motorized canoes and kayaks. We hand-captured most swans using shepherd's crook poles or large landing nets (Eltringham, 1978; Hindman et al., 2016) and captured swans in Michigan using a shoulder-fired net gun. We predominantly targeted adult swans, which have higher survival rates than juveniles, to maximize the longevity of telemetry data collection (Lockman, 1990). Protocols for capturing and marking trumpeter swans in the United States were approved by the University of Minnesota Animal Care and Use Committee (protocol no. 1905-37072A), the U.S. Fish and Wildlife Service (Research & Monitoring Special Use Permits no. K-10-001, M-20-002, and M-21-014), the U.S. Geological

Survey Bird Banding Laboratory (Federal Bird Banding Permit no. 21631) and state-specific permits approved by each state wildlife agency involved.

We marked swans with two types of collars; 55-g neck collars with GPS-GSM transmitters incorporated into the collar housing (Model OrniTrack-N62 3G, Ornitela, Vilnius, Lithuania) and 140-g GPS transmitters (Model CTT-ES400, Cellular Tracking Technologies, [CTT], Rio Grande, New Jersey, USA) that were adhered to 64-mm neck collars (Haggie Engraving, Crumpton, Maryland, USA). All collar and transmitter types weighed <3% of the marked individual's body weight, as per animal care requirements. Both types of neck collars contained a unique alpha-numeric code for visual identification. Swans captured in Michigan were fit with CTT collars and all other swans in the study were fit with Ornitela collars. All transmitters were programmed to collect GPS locations at 15-min intervals throughout the 24-hr daily period. In the U.S., we leg-banded each swan with a U.S. Geological Survey butt-end aluminum band and in Canada we banded each swan with a stainless steel locking-tab leg band. We determined sex via cloacal examination and assigned a breeding status to each swan at the time of capture depending on if a mate and cygnet were present ('breeder' status), if a mate was present but no cygnets were observed ('paired' status), or if neither a mate nor cygnets were present ('non-breeder' status). Due to logistical constraints, we were unable to continuously monitor the breeding status of swans in subsequent years, and we retained the initial breeding status designation for all analyses. We are not aware of published information on the probability of trumpeter swans nesting in subsequent years. Previous research on mute swans (*Cygnus olor*) documented the probability of nesting in subsequent years at >90% (Birkhead & Perrins, 1985; Ellis & Elphick, 2007). We use the term breeding (e.g., breeding latitude, breeding territory) to indicate either breeding territory for successful breeders, or capture location for non-breeding swans.

### **Migration Phenology Classification**

One of our key objectives was to accurately quantify IP trumpeter swan migration phenology. Given the size of the dataset (~6M locations over 252 swan-years), we sought an efficient workflow to segment location data into periods of the annual cycle and

estimate migration metrics. Many migration segmentation approaches are based on spatiotemporal criteria that require subjective species-specific decisions, reducing reproducibility and limiting the potential to generalize to other studies, especially given the complexity of migratory behaviors many species exhibit (Soriano-Redondo et al., 2020; van de Kerk et al., 2021).

To more objectively quantify migration phenology, we used a model-driven approach with displacement from the breeding site used to segment the annual cycle into stationary periods that correspond with breeding, stopover, and non-breeding areas. We first calculated annual time-series of Net-Squared Displacement (NSD) values for each swan (referred to as a ‘swan-year’ dataset), using 1 July (a time of year that swans are relatively stationary) as a cutoff date between years for individuals with multiple years of GPS data and an origin point for NSD values, and then thinned datasets to contain a single averaged displacement value per day (Hansen et al., 1971; Wolfson et al., 2022). After excluding 11 swan-year datasets with <30 days of data, we iteratively fit a series of 7 intercept-only piecewise regression models to each time-series (Lindeløv, 2020; Wolfson et al., 2022). The syntax of each model corresponded to an increasing number of intercepts included (from 1–7) for average displacement values throughout the time series separated by breakpoints in time where the intercept values transitioned; therefore, intercepts represent stationary segments in time corresponding to periods of the annual cycle, and breakpoints are the transitions between these segments that provide information on the timing of migration events such as autumn departure and spring arrival (Fig. 2).

We fit all piecewise regression models in JAGS (Plummer, 2003) using the *mcp* package (Lindeløv, 2020) in Program R version 4.0.2 (R Core Team, 2022), using its default priors and 15,000 iterations and a burn-in period of 10,000. We used the *future* R package to run all scripts in parallel via the Minnesota Supercomputing Institute using a partition with 24 cores and 50GB RAM per core (Bengtsson, 2021). We evaluated Markov-chain Monte Carlo (MCMC) convergence via the Gelman-Rubin statistic ( $\hat{R}$ ) and excluded models containing any parameters with a value of  $\hat{R} > 1.1$  from further

consideration (Brooks & Gelman, 1998). Through extensive testing, we found that models with MCMC samples that failed to converge in distribution after 15,000 samples typically provided a poor fit to the data (e.g., single-intercept models despite multiple migration periods or models with multiple breakpoints during a stationary period). If all parameters in a model passed the  $\hat{R}$  threshold, we evaluated model fit and predictive performance using leave-one-out cross validation (LOO-CV) with Pareto smoothed importance sampling to estimate the Expected Log Predictive Density (ELPD), using the *loo* package (Gelman et al., 2014; Vehtari et al., 2017). The ELPD values reflect the ability of the model to predict the posterior density of withheld data. We used LOO-CV to choose the ideal number of breakpoints (and thereby segments that correspond to migratory periods) for each swan-year dataset.

We qualitatively inspected the visual fit of each best-supported model (based on ELPD) for each swan-year dataset and removed 11 (out of 241 total datasets) that were obvious poor fits such that information from the breakpoints and intercepts would not adequately describe annual migration phenology. To avoid confusing short-distance and temporary relocation, we also excluded all segments <2 km from the previous segment and all changepoints <2 days from the previous changepoint. We then extracted parameter values to represent the movement metrics of interest (Fig. 2). To focus on swans that made obvious movement away from breeding areas, we extracted autumn departure dates for individuals that moved >100 km from breeding locations by 31 December (Fig. S2). We extracted spring arrival dates for individuals that moved >100 km from breeding locations during the non-breeding period and that returned within 30 km of their previous breeding territory (Fig. S2). We designated movement categories based on each swans' annual migration extent (farthest distance moved from breeding/capture territory during the non-breeding period), and assigned categories of local movement (<25 km), regional migration (25–100 km), and long-distance migration (>100 km) for each swan-year dataset (Fig. S3).

## Migration Extent

An exploratory analysis of annual movement data suggested a strong linear association between breeding latitude and migration extent for many individuals, whereas others moved a much lesser extent, especially at lower latitudes. It is possible that these patterns reflect two different migratory strategies with one segment of the population consistently migrating to lower latitudes and a second segment of the population using areas with open water closer to the location of their breeding site during the non-breeding period.

To describe these potential relationships between breeding latitude and migration extent, we used a 2-component mixture model (i.e., a model with two different groups of individuals, each following a different response pattern). Let  $Y_{ij}$  and  $x_{ij}$  represent the migration extent and breeding latitude, respectively, for individual  $i$  in year  $j$ , and let  $z_i$  be a latent variable representing the group membership of each individual. We assumed migration extent was linearly related to breeding latitude for individuals with  $z_i = 1$  and was much lower and non-linearly related to breeding latitude for individuals with  $z_i = 0$ :

$$Y_{ij}|z_i \sim \mathcal{N}(\mu_{ij}, \sigma_i^2)$$
$$\mu_{ij} = \begin{cases} \alpha + \beta \cdot x_{ij} & \text{if } z_i = 1 \\ c + \gamma \cdot x_{ij}^\delta & \text{if } z_i = 0 \end{cases}$$

In addition, we assumed the variance of  $Y_{ij}$  depended on the latent state,  $z_i$ .

$$\sigma_i^2 = \begin{cases} \sigma_1 & \text{if } z_i = 1 \\ \sigma_2 & \text{if } z_i = 0 \end{cases}$$

The latent state  $z_i$  (0 or 1) for assignment to a group (i.e., migration strategy) was modeled as:

$$z_i \sim \text{Bernoulli}(\pi_i)$$

We used normal priors for  $\alpha \sim \mathcal{N}(400, 0.001)$ ,  $\beta \sim \mathcal{N}(150, 0.001)$ , and  $\gamma \sim \mathcal{N}(30, 0.001)$ , parameterized in terms of their mean and precision ( $1/\text{variance}$ ). We used uniform priors for  $c \sim \text{Uniform}(0, 100)$ ,  $\delta \sim \text{Uniform}(0, 30)$ ,  $\pi \sim \text{Uniform}(0, 1)$ ,  $\sigma_1 \sim \text{Uniform}(1, 200)$ , and  $\sigma_2 \sim \text{Uniform}(1, 200)$ . We used priors



partially informed by the data for the intercepts of each group,  $\alpha$  and  $c$ , based on the assumption that group 1 migrates a greater extent than group 2.

We implemented the model in JAGS via the *R2jags* package using 3 MCMC chains with 30,000 iterations, a burn-in of 10,000, and a thinning rate of 10 (Plummer, 2003). We determined convergence of chains by assessing trace plots and using the Gelman-Rubin statistic with  $\hat{R} < 1.1$  (Brooks & Gelman, 1998).

### **Migration Phenology**

We visualized observations of migration phenology grouped by breeding status with boxplots showing the medians, inter-quartile ranges, and outliers. To test associations between predictor variables and migration phenology, we fit separate linear mixed models (LMM) using the *lme4* package to each response (autumn departure date, spring arrival date, and duration of non-breeding period), with a random intercept for each individual to account for repeated measures (i.e., multiple migrations by the same individual), and fixed effects for sex, breeding status, and breeding latitude (Bates et al., 2014). We assessed model assumptions using the *performance* R package and plotted estimates of coefficients and associated 95% confidence intervals using the *sjPlot* R package (Lüdtke, 2018a; Lüdtke et al. 2021). To visualize the relationship between breeding latitude and each migration metric, we plotted estimated marginal effects from the linear mixed models using the *ggeffects* package (Lüdtke, 2018b). We used the *emmeans* package to conduct post-hoc pairwise comparisons involving the categorical levels of breeding status (i.e., breeder, paired, non-breeder), with Tukey's Honest Significant Difference (HSD) to adjust  $p$ -values and confidence intervals for each comparison (Lenth et al., 2023). We approximated the degrees of freedom for the HSD test of the spring arrival LMM using the Kenward–Roger method (Kenward & Roger, 1997).

### **Results**

We deployed 113 collars with GPS-GSM transmitters on 126 trumpeter swans (including 13 redeployments using collars recovered from mortalities) from July 2019–

August 2022, resulting in 252 unique ‘swan-year’ telemetry datasets (Fig. S1). Of the 126 swans, 78 were female and 48 were male; 73 were breeding adults (cygnets present), 22 were adults with mates present but no cygnets observed at time of capture, 24 were non-breeding adults captured while in large groups, and six were cygnets at the time of capture (however, data from cygnets were not used in analyses).

### **Migration Extent**

Annual movements of IP trumpeter swans were highly variable. After filtering out 31 swan-year datasets from 27 individuals with incomplete coverage to estimate annual trends, 59% ( $n=68$ ) of remaining IP swans ( $n=116$  individuals) made seasonal movements to distant non-breeding areas (long-distance migration defined as moving >100 km from breeding site during the non-breeding period), 16% ( $n=19$ ) underwent regional migration (>25 km and <100 km), 19% ( $n=22$ ) exhibited local movements (<25 km), and 6% ( $n=7$ ) exhibited multiple migration strategies across different years: regional and long-distance ( $n=3$ ), and local and regional ( $n=4$ ). The mixture model applied to migration extent classified all individuals to a group with high probability, so it was unnecessary to set a user-defined threshold to determine group assignment. There was a strong association between breeding latitude (mostly between 43 and 53 degrees North latitude) and the extent of migration (Fig. 3). Yet, many swans with breeding sites between 40 and 45 degrees North latitude exhibited minimal movement during the non-breeding period. We considered these individuals to exhibit local seasonal movements, with most of these swans leaving their breeding territory or increasing their overall space use during the non-breeding period, likely to increase access to ice-free water or access to food.

### **Migration Phenology**

#### **Autumn Departure**

The average autumn departure date from breeding territories across years for all long-distance (>100 km) migrants was 7 November with a standard deviation of 25 days, and yearly averages ranged from 31 October to 17 November (Table S1, Table S2).

Long-distance migrants at higher latitudes left territories earlier in autumn than swans at lower latitudes (Fig. 5). On average, swans left 4 days earlier (95% CI = -6.2–1.9) for every increase of 1 degree of breeding latitude (corresponding to approximately 111 km; Fig. 4, Table S10). Autumn departure dates differed by breeding status (Fig. 6), and estimated autumn departure dates of breeding swans were, on average, 18 days (95% CI = 2.9–33.9) later than non-breeders, and 6 days (95% CI = -8.7–20.9) later than paired swans (Fig. 4, Table S11).

### **Spring Arrival**

The average spring arrival date across years for all long-distance (>100 km) migrants was 4 March with a standard deviation of 15 days, and yearly averages ranged from 2 March to 6 March (Table S4, Table S5). Swans that returned to within 30 km of their previous territory arrived earliest at southern latitudes (Fig. 5). On average, swans arrived 5 days (95% CI = 3.9–6.4) later for every increase of 1 degree of breeding latitude (Fig. 4, Table S10). Differences in spring arrival between breeding categories were minimal (Fig. 6), and confidence intervals spanned 0 for all two-way comparisons (breeding–non-breeder,  $\beta = -1.2$ , 95% CI = -12.9–10.5; breeder–paired,  $\beta = -1.1$ , 95% CI = -11.2–9.1; non-breeder–paired,  $\beta = -0.1$ , 95% CI = -12.9–13.2; Fig. 4, Table S12).

### **Duration of Non-Breeding Period**

The average duration of the non-breeding period for swans that had estimated autumn departure dates and also spring arrival dates the following year was 115 days with a standard deviation of 29 days, and yearly average ranged from 99 days to 119 days (Table S7, Table S8). On average, long-distance migrants at higher latitudes had higher durations of the non-breeding period, with every increase of 1 degree of breeding latitude equating to 6 additional days away from the breeding territory (95% CI = 2.6–8.9; Fig. 4, Fig. 5, Table S10). Long-distance migrants classified as breeders tended to have shorter durations of the non-breeding period (Fig. 6), but confidence intervals spanned 0 for all two-way comparisons (breeding–non-breeder,  $\beta = -14.5$ , 95% CI = -40.0–11.1; breeder–

paired,  $\beta = -13.3$ , 95% CI = -35.3–8.7; non-breeder–paired,  $\beta = -1.1$ , 95% CI = -27.3–29.6; Fig. 4, Table S13)

## **Discussion**

Annual movements of IP swans were highly variable in their extent and timing, with a continuum of movements exhibited each year. Much of this variability was related to factors tied to natural history demands (e.g., breeding status) and response to environmental conditions (e.g., through associations with breeding latitude). It is not immediately clear why IP swans seemed to exhibit multiple migration strategies even at similar latitudes where the effects of environmental conditions (e.g., temperature, precipitation) should also be similar, but other factors such as social dynamics, genetic lineage, and site-specific differences in the availability of open water and food likely play an important role. Fine-scale spatial variation, especially related to open water, may have a large influence on determining which swans undergo long-distance migration and which can spend the non-breeding period at higher latitudes.

The migration phenology of IP trumpeter swans appears similar to that of other long-lived avian migrants, with breeders leaving later in the autumn than non-breeders and spending less time away from the breeding territory during the non-breeding period (Conklin et al., 2010; Nilsson et al., 2013; van Wijk et al., 2012). Autumn departure, spring arrival, and duration of non-breeding period were also highly correlated with breeding latitude (Fig. 4), likely driven by variable access to open water. Breeding latitude was also associated with migration extent during the non-breeding period (Fig. 3). Individuals that breed in the northern part of the IP range all made relatively long-distance autumn migrations. Given the severity of winter conditions at higher latitudes, options for accessing open water created by currents on rivers or anthropogenic influences (e.g., below dams, on lakes with aerators) were likely not close to breeding locations. Autumn migration distances of swans breeding at mid-latitudes were variable, with some swans moving relatively long distances, whereas others only exhibited local or regional movements. These latter swans likely remained near their breeding locations during the non-breeding period if they were able to find open water and access to food.

Swans breeding at lower latitudes all remained close to their breeding locations year-round, as the local environmental conditions likely continued to provide open water and access to food.

Several other factors may influence movements and migratory patterns, including transmission of migratory information between generations genetically and through social learning (Åkesson & Helm, 2020). For some species with short lifespans (e.g., songbirds), migration is considered innate and primarily genetically controlled based on observations of individuals that complete their first migrations independently without parents or other conspecifics to guide them (Berthold, 1991). However, for species with long generation times and extended periods of parental care, such as trumpeter swans, social learning typically also plays an important role in forming migration strategies (Mueller et al., 2013). Collective knowledge has been shown to accumulate over generations to drive migration patterns and improve efficiency in flocking species with socially learned migration behaviors. In reintroduced populations of whooping cranes (*Grus americana*) that were initially trained (i.e., learned) to migrate by following aircraft, migratory efficiency of flocks rapidly increased when older individuals were present (Mueller et al., 2013; Sasaki & Biro, 2017). Thus, experience and familiarity with the landscape also likely play important roles in determining migratory movements. For swans, in particular, knowledge of sites with access to open water and food may be important for allowing swans to ‘escape’ migration in more northern latitudes, and this knowledge may be passed down through generations.

IP swans are unique because their reintroduction to central North America occurred relatively recently (<50 years) from a mix of source populations with varying migratory strategies (Ransler et al., 2011). Thus, there may exist heterogeneity within the population in terms of genetic makeup that influence swan movements. Although previous studies have considered genetic differences of IP source populations using microsatellites, contemporary research methods (i.e., population genomics) are needed to better evaluate the genetic structure of the IP as it relates to other trumpeter swan populations (Oyler-McCance et al., 2007; Ransler et al., 2011). Continued monitoring is

also necessary to determine whether population-level migration characteristics (e.g., annual proportion of IP swans that migrate) are still in flux or if conditions have stabilized akin to the environmental threshold model, in which certain parts of the population are obligate residents or migrants and annual environmental conditions determine the migration threshold that dictates the outcome of facultative migrants, a population-level paradigm thought to be maintained predominantly through genetic variation (Pulido, 2011). Ultimately, our study provides a snapshot in time of current IP trumpeter swan migration patterns ~50 years following initial reintroduction efforts, and population-level trends may still be stabilizing and exhibiting variation based on annual environmental conditions.

In addition, the history of IP reintroduction includes many years of anthropogenic influences, at the level of both wildlife managers and private citizens, that have likely influenced migration patterns, and it is not well understood to what extent these influences may be passed down through future generations (Shea et al., 2002). Some of these influences include intentional feeding during the non-breeding period at sites of high IP swan density and lake aerators that keep lakes ice-free throughout the winter to prevent winter kill of fish (Ballard et al., 2021; Ellis & Stefan, 1989; Slater, 2006). Although supplemental feeding has been discontinued by state and provincial management agencies throughout the IP distribution, the practice continues with some private citizens, and potential effects include not only curtailment of migration but also increased risk of pathogen transmission (Satterfield et al., 2018). There is also increasing evidence that many local groups of swans have discovered field feeding as an additional strategy to acquire resources, and this knowledge likely affects migratory behavior, though additional research is needed to quantify the spatial extent, intensity, and timing of field-feeding in trumpeter swans. (Baldassarre, 2014; Varner, 2008).

## **Conclusions**

Knowledge of current IP swan migration patterns can help inform IP swan conservation by providing current information on the limits of the non-breeding period

range and quantifying variability in migration strategies. Under current conditions, non-breeding-period habitat for swans occurs in all but the most northerly portions of the IP breeding distribution. Managers may need to conserve habitat during the non-breeding period for both long-distance migrants and residents that remain on their breeding territories year-round while also anticipating the effects of future climate scenarios (Reese & Skagen, 2017). It is not clear how changing climate conditions will influence future IP swan migration strategies, but currently, multiple migration strategies exist within the IP, providing a range of behavior as the basis for adaptation to changing conditions that may help offset potential negative effects of asynchrony between migration phenology and environmental conditions (Nussey et al., 2005). The variability present in IP annual movements may position IP swans to quickly adapt to conditions resulting from changing climate.

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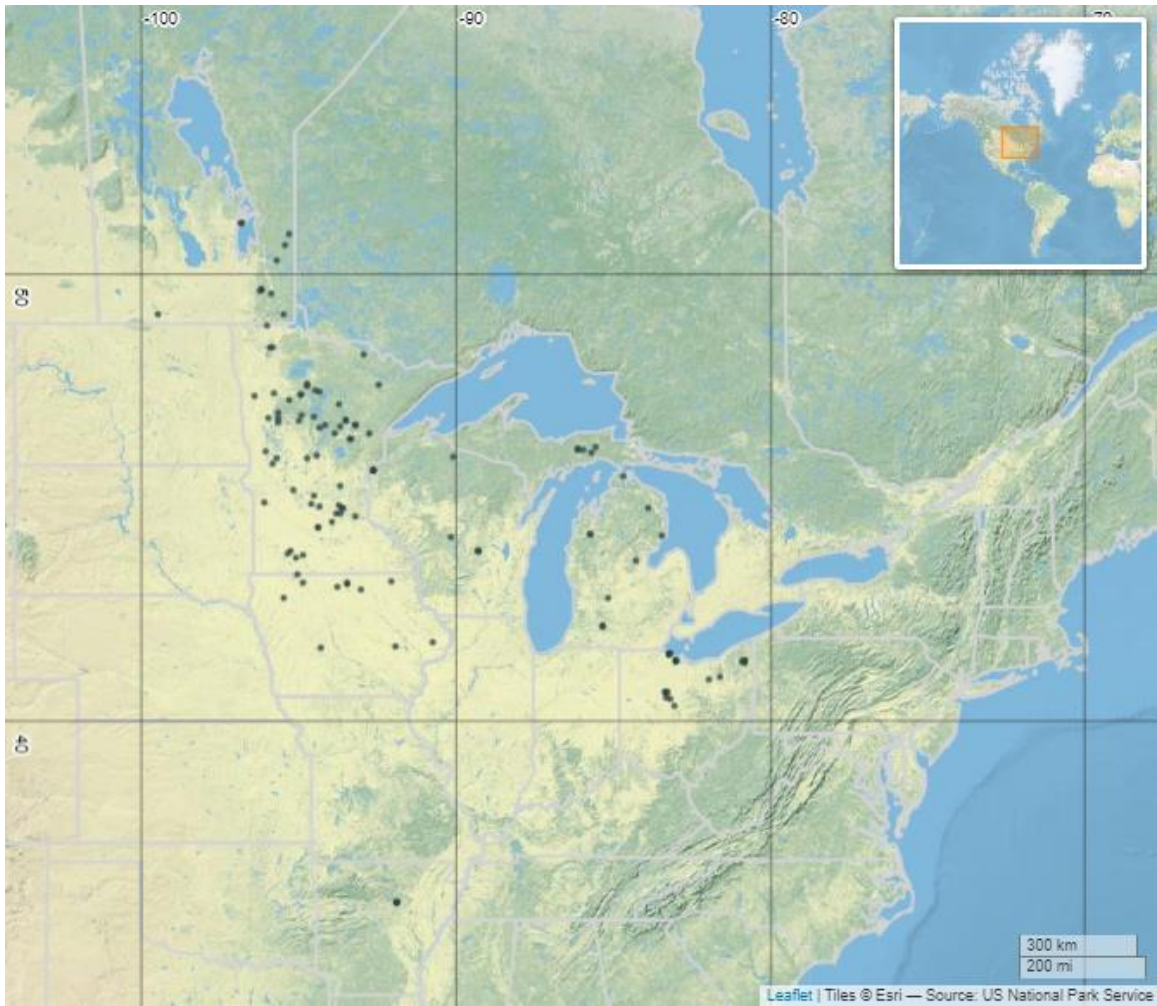


Figure 2-1 Capture locations of Interior Population trumpeter swans (*Cygnus buccinator*) collared with GPS-GSM transmitters from July 2019–January 2022.

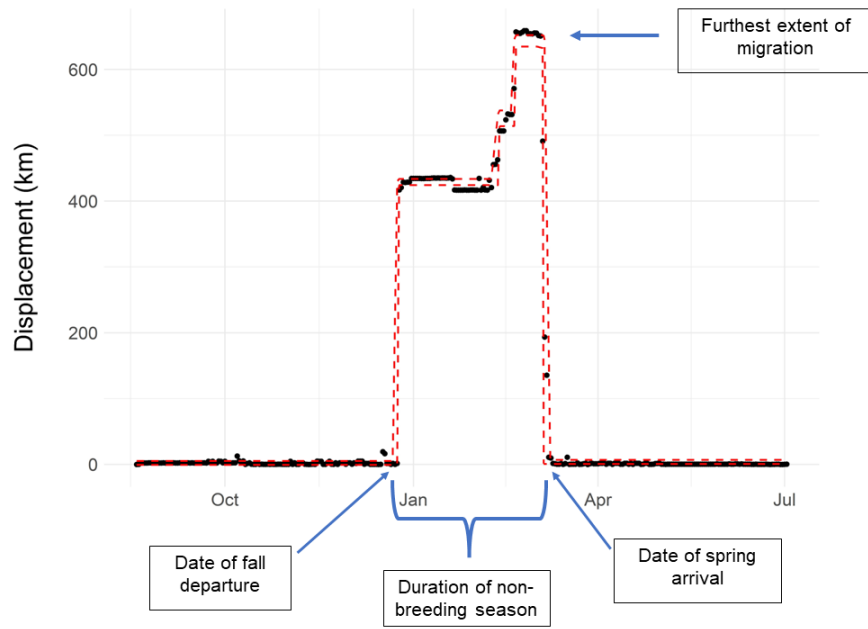


Figure 2-2 An example of extracting migration phenology metrics from a fitted piecewise regression model of an annual time-series of displacement values for an individual trumpeter swan (*Cygnus buccinator*).

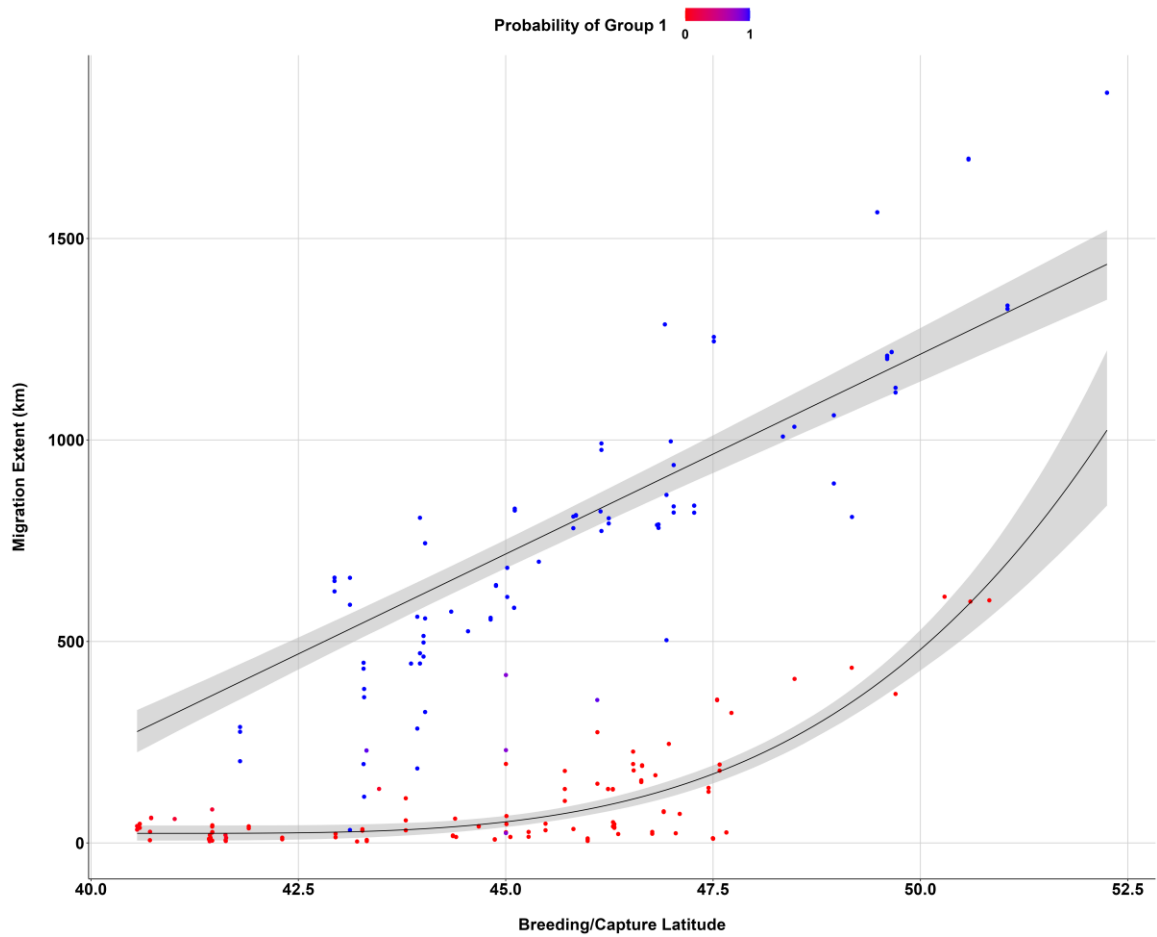


Figure 2-3 Migration extent versus breeding/capture latitude with color indicating the probability of assignment to one of two migration strategies within a 2-component mixture model describing the relationship between latitude and migration extent. Gray areas depict the 95% credible intervals for each strategy.

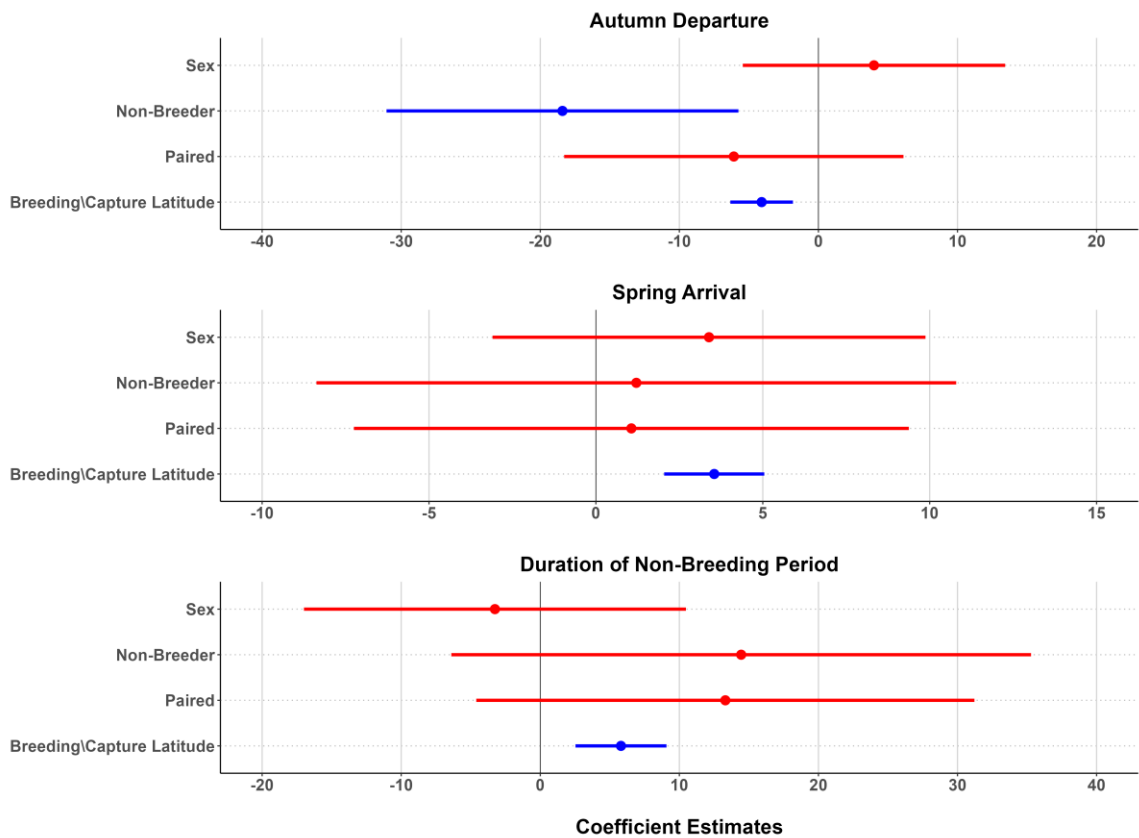


Figure 2-4 Estimates and 95% confidence intervals for model coefficients from linear mixed effects models fit to autumn departure, spring arrival, and duration of non-breeding period. The covariate for sex represents the difference between male and female (the reference category). The coefficients for non-breeder and paired contrast these categories with breeder (the reference category). Blue results represent statistically significant coefficient estimates and red results represent coefficient estimates that were not statistically significant.

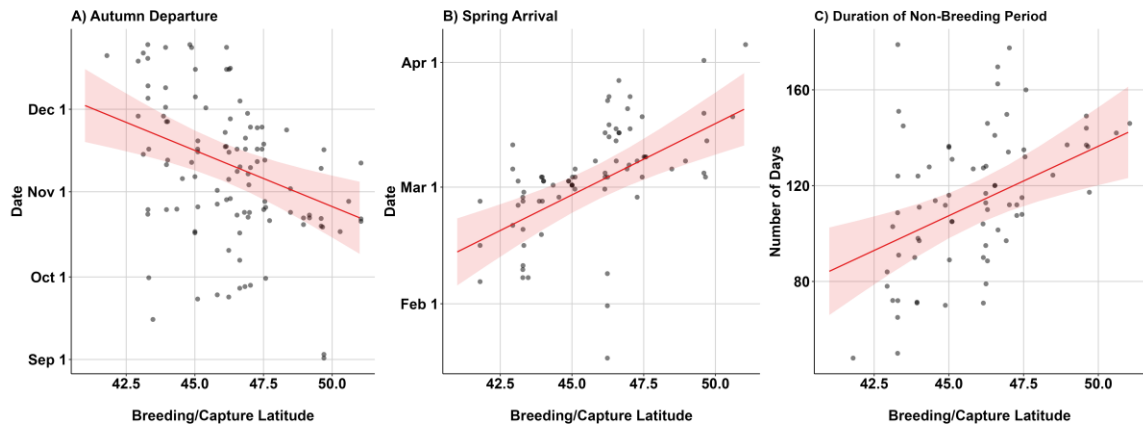


Figure 2-5 Marginal effect plots for the relationships between breeding/capture latitude and autumn departure (A), spring arrival (B), and duration of non-breeding period (C). Red lines and shaded areas depict the predicted values and 95% confidence intervals, respectively. Points shown are the observed migration phenology metrics.

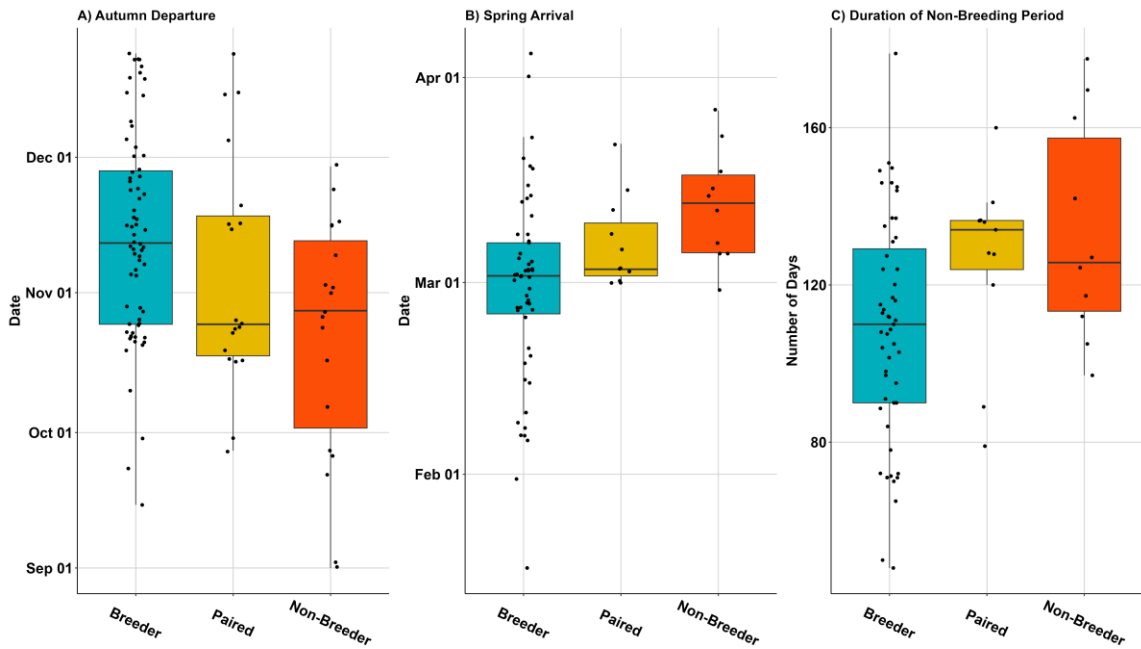


Figure 2-6 Migration phenology dates by breeding status (breeder, paired, and non-breeder). Boxes bound the 25th and 75th percentiles, solid lines within the boxes indicate the median, lines extend to 1.5 times the interquartile range, and points correspond to individual swan-years.

## **Chapter 3: The prevalence of lead (Pb) among Interior Population trumpeter swans**

### **Synopsis**

Lead (Pb) is a toxic heavy metal that has detrimental effects on wildlife populations, especially for species that use a muscular gizzard to digest food and those that forage in accumulated sediment at the bottom of lakes and streams. Lead poisoning from ingested lead shot or fishing sinkers has been a major source of mortality in trumpeter swans (*Cygnus buccinator*) across North America. We estimated blood lead concentrations for 119 trumpeter swans throughout the breeding range of the Interior Population. We detected lead in all samples but 91% of swans had blood lead levels in the ‘background’ range (<200 ppb) that are not considered to produce negative physiological effects, 8% of swans had blood lead levels in the ‘sub-clinical’ range (200–500 ppb), and 2% of swans had blood lead levels in the ‘clinical’ (500–1,000 ppb) or ‘severe’ (>1,000 ppb) range of lead toxicity. The swan with the highest blood lead level (1,076 ppb) was located 3 km from a major coal power plant, but we were unable to directly determine the source of lead in that individual. Our study provides useful baseline information on the prevalence and magnitude of blood lead levels among Interior Population trumpeter swans.

### **Introduction**

Lead, a toxic heavy metal with detrimental effects on wildlife, is pervasive throughout the environment due to anthropogenic activity (Pain 1996, Saaristo et al. 2018, Pain et al. 2019). Acting as a non-target toxicant, absorption of even trace amounts of lead can result in a variety of sublethal effects to health, reproduction, and behavior, and higher quantities can directly lead to mortality (Fisher et al. 2006, Monclús et al. 2020). Exposure to lead has been linked to reductions in long-term population growth, though the impacts of lead differ among demographic categories (e.g., age, sex, breeding status; Hanley et al. 2022, 2023).

Lead poisoning is a particularly harmful threat for waterfowl species that consume grit to facilitate breaking down food during digestion (Sanderson and Bellrose 1986, Pain et al. 2019). Ingested lead, consumed while foraging, is retained in the gizzard until it is broken down by the muscular grinding action and the acidic conditions (pH 2.5) in the stomach, forming soluble lead salts that are absorbed into the bloodstream and distributed throughout tissues in the body, primarily the liver, kidney, and bone (Bellrose 1961, Clemens et al. 1975, De Francisco et al. 2003).

There are two primary sources of lead exposure in waterfowl: spent ammunition and fishing tackle (Rattner et al. 2008). Lead exposure from ammunition used in waterfowl hunting has markedly declined since the ban of lead ammunition in aquatic habitats in the United States in 1991, but lead from historical use of toxic ammunition continues to be a serious threat to waterfowl populations because it degrades slowly and may become periodically available depending upon environmental conditions (Samuel and Bowers 2000, Flint and Schamber 2010, Check and Marteel-Parrish 2013). Despite demonstrable evidence of reduced waterfowl mortalities following lead fishing tackle bans in Europe, lead fishing tackle has only been banned outright in the U.S. in California, and just six other states have partial bans or other restrictions (Haig et al. 2014, Wood et al. 2019, Ellis and Miller 2023).

Most of our understanding of lead levels in wildlife comes from animals with lead poisoning that are brought into wildlife rehab facilities; much less is known about the levels of lead present in wild-roaming animals, the overall prevalence of bio-available lead on the landscape, and the degree to which avian populations can tolerate lead exposure without major deleterious effects (Monclús et al. 2020, Cox 2021). Critical knowledge gaps remain in understanding how subclinical (not producing apparent symptoms) and sublethal concentrations of lead exposure affect the behavior and physiology of large waterfowl (Haig et al. 2014, Kearns et al. 2019). Subclinical levels of lead exposure do not cause outwardly conspicuous effects, but can produce a range of negative physiological symptoms such as the inhibition of  $\delta$ -aminolevulinic acid dehydratase (ALAD), an essential enzyme in the heme synthesis cascade needed for red



blood cell production (Hoffman et al. 1981, Pain 1989). Clinical levels cause more noticeable signs, such as weakness, weight loss, regurgitation, loss of muscular coordination, change in vocalization, and abnormal feces (Routh and Sanderson 2009, Franson and Pain 2011).

Trumpeter swans (*Cygnus buccinator*) are especially susceptible to lead ingestion because their long necks allow them to forage for sub-emergent vegetation and grit at the bottom of shallow wetlands, thereby potentially ingesting lead shot and sinkers that accumulate in the sediment layer (Banko 1960, Blus et al. 1989, Degernes et al. 2002). The longevity of lead in wetland systems means that even historical accumulation of lead still poses a threat to swans, and lead poisoning is a major source of mortality, especially when swans concentrate in higher densities during the non-breeding period (Blus 1994, Lagerquist et al. 1994, Wilson et al. 1998, Degernes et al. 2006). Wilson (2009) found that lead shot was the cause of >80% of 2,577 swan mortalities in northwestern Washington and southwestern British Columbia during the winters of 1999–2008. Die-offs are particularly exacerbated during periods of drought, when low water levels allow waterfowl to reach lake sediment that is otherwise inaccessible (Degernes et al. 2006).

The Interior Population (IP) of trumpeter swans was extirpated from their former range in eastern North America due to unregulated market hunting (Banko 1960). Reintroductions to historical IP breeding range from other extant trumpeter swan populations began in the 1960s, and IP swans currently breed throughout the western Great Lakes region of the U.S. and Canada (Groves 2017). Although multiple studies have quantified lead levels for IP trumpeter swans with lead poisoning that were brought into rehabilitation clinics, no data are available on free-roaming individuals across the landscape (Strom et al. 2009, Degernes and Frank 2013, Cox 2021). Therefore, our main objective was to quantify concentrations of lead in trumpeter swans across the IP breeding range to provide a contemporary summary of the overall prevalence and magnitude of blood lead levels among wild IP trumpeter swans.

## **Methods**

### **Study site and sample collection**

We captured trumpeter swans across the breeding range of the IP, including the main contiguous Great Lakes areas and the ‘High Plains’ flock in western Nebraska from July 2019 through August 2022 (Groves 2017). We used a variety of methods to capture swans, including jon boats with long-tail mud motors, airboats, and step deck transom boats with surface-drive motors. We hand-captured swans using a shepherd’s crook pole (Eltringham 1978, Hindman et al. 2016). We collected whole blood samples from 119 swans (100 adults and 19 first-year ‘cygnets’) during the prebasic summer molt when adult swans are flightless and cygnets have not fledged, except for 4 swans captured during the winter with snares. We assigned age as either adult or cygnet. We collected 2 mL of whole blood from each captured swan via medial metatarsal venipuncture using a 23-gauge, 1-inch needle with a 3-mL syringe, stored samples in ethylenediaminetetraacetic acid (EDTA) blood collection tubes to prevent coagulation, and refrigerated tubes until they were received by the veterinary diagnostics lab. Protocols for capturing trumpeter swans in the United States were approved by the University of Minnesota Animal Care and Use Committee (protocol no. 1905-37072A), the U.S. Fish and Wildlife Service (Research & Monitoring Special Use Permits no. K-10-001, M-20-002, and M-21-014), the U.S. Geological Survey Bird Banding Laboratory (Federal Bird Banding Permit no. 21631) and state-specific permits approved by each state wildlife agency involved.

### **Laboratory analysis**

Lab technicians at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) used Inductively Coupled Plasma Mass Spectrometry (ICP-MS) according to internal standard operating procedures to quantify blood lead concentrations in parts per billion (ppb). Analysis was performed with a Plasma Quant Elite ICP-MS (Analytik Jena Inc. Woburn, MA, USA) in CRI mode with hydrogen as the skimmer gas and bismuth as the internal standard. Standards for elemental analyses were obtained from Inorganic

Ventures (Christiansburg, VA), and 15 mL centrifuge tubes and trace mineral grade nitric acid were obtained from Fisher Scientific (Pittsburgh, PA). Samples were digested in concentrated nitric acid, by adding 0.25 mL of acid to 0.25 mL of blood at 70°C for one hour in a 15 mL centrifuge tube. After digestion, the digest was brought to 5 mL with 18 MΩ deionized water, mixed and centrifuged. Sample digests were filtered through a 0.45 μm syringe filter. All samples were rigorously mixed, then analyzed by ICP-MS. For quality control, a control blood sample was analyzed with each analytical run and determined to be within 2 standard deviations of the expected concentration. The limit of detection (i.e., the lowest possible concentration at which the method can detect lead; LOD) using the equipment at ISU-VDL is 0.054 ppb, and the limit of quantification (i.e., the lowest possible concentration of lead that can be reliably detected and quantified; LOQ) is 2.0 ppb (Armbruster and Pry 2008). To compare results with other studies of blood lead concentrations reporting in μg/mL, 1000 ppb is equivalent to 1 μg/mL.

## **Results**

All 119 swans tested positive (i.e., above the LOD) for lead, and all concentration values were above the reported LOQ. Blood lead concentrations for Anseriformes are typically interpreted using the following categories: <200 ppb is considered background level (also referred to as ‘normal environmental exposure’), 200-500 ppb is considered subclinical lead poisoning, 500-1000 ppb is considered clinical lead poisoning, and >1000 ppb is considered severe clinical lead poisoning (Pain 1996, Fransom and Pain 2011). The majority of swans (100% of cygnets, 89% of adults) had blood lead levels within the background category (Table 1, Fig. 1).

## **Discussion**

All of our sampled swans had been exposed to lead, but the majority of swans had lead concentrations that were within the background exposure range. These findings are consistent with Ely and Fransom (2014), who sampled 653 flightless tundra swans (*Cygnus columbianus*) on their breeding range in northern Alaska from 2007–2008. Although lead concentrations within this range are considered below the threshold for

physiological effects, such as the inhibition of ALAD, chronic exposure to low levels of lead may cause subclinical effects, such as changes in behavior, physical growth, and brain development (Hoffman et al. 2000, Buekers et al. 2009, van den Heever et al. 2024). Additionally, Pain (1996) suggested that any concentration of lead will cause negative health impacts, and activities of certain enzymes are inhibited at blood lead levels as low as 50 ppb.

It can be challenging to link blood lead levels to the environments in which exposure took place. Although often retained in the gizzard for up to 6 weeks, lead shot can also be evacuated soon after ingestion, which may lead to minimal effects on blood lead levels (Pain 1996). Insufficient research exists to accurately estimate how long lead persists in the bloodstream of wild birds, further complicating potential inference (Williams et al. 2017). Anders et al. (1982) calculated that the half-life of blood lead is 13 days in pigeons (*Columba livia*), and Fry and Maurer (2003) also estimated a half-life of 13 days for blood lead in California condors (*Gymnogyps californianus*), but there have not been any quantitative estimates for waterfowl species, and Pain et al. (2019) merely state that blood lead remains elevated for ‘weeks or months after exposure’. Lead concentrations in this study are almost all from flightless swans (either adults during molt or pre-fledged cygnets), and likely reflect lead that swans ingested at or near the capture area; however, we can’t rule out the possibility that swans ingested lead prior to arriving at the molt site.

There are no other studies estimating blood lead concentrations for wild trumpeter swans to compare our results to. Instead, our measures of lead can serve as a baseline for trumpeter swans in the IP. Although other studies have quantified blood lead concentrations for trumpeter swans, their samples consist of either necropsies conducted on dead birds, or swans brought into wildlife rehabilitation facilities already exhibiting signs of lead poisoning, and therefore are not necessarily indicative of free-roaming wild populations (Blus et al. 1989, Katavolos et al. 2007, Strom et al. 2009, Degernes and Frank 2013). Our findings were consistent with Ely and Franson (2014), the only other study reporting the lead concentrations of a wild swan species in North America, who

sampled 653 flightless tundra swans on their breeding range in northern Alaska from 2007–2008.

Our results also highlight the potential for wildlife to serve as bioindicators across the landscape (Burger 2006a). For example, 4T, the swan with the highest lead level in this study (1,076 ppb), was captured ~3.5 km west of a large coal-fired power plant in Cohasset, MN, USA that produces >885 MW of electricity. Although the lead that 4T ingested was not necessarily a byproduct of the power plant, after completing molt of its primaries, 4T repeatedly made trips from its breeding territory to open waste ponds at the power plant. The breeding site of 4T, Little White Oak Lake, has a long history of fishing and waterfowl hunting, so lead is likely present in the accumulated lake sediment. Despite the relatively high blood lead levels at the time of capture, 4T did not display visible signs of lead poisoning at the time of capture, was observed with cygnets each subsequent year of the study (2020–2024), and migrated >800 km to southwestern Michigan each winter.

If tracing the source point of lead is a research priority, stable isotope analysis allows researchers to differentiate types of lead based on their unique isotope ratios (Rabinowitz and Wetherill 1972). Analysis of stable lead isotopes in wild birds has typically been used to contrast lead from ammunition as opposed to fishing tackle, but several studies have also compared isotopic signatures from leaded gasoline and toxic waste from mining operations (Scheuhammer and Templeton 1998, Meharg et al. 2002, Scheuhammer et al. 2003). Recent work from Wang et al. (2019) demonstrated that lead isotopes in coal fly ash can be differentiated from other major anthropogenic lead sources in the U.S.

The lack of representative samples for wild trumpeter swan blood lead levels makes it difficult to compare our results with other past studies, and we see these results as a first baseline measure for wild trumpeter swans in North America, especially for trumpeter swans in the IP. Although other studies have quantified blood lead concentrations for trumpeter swans, their samples largely come from necropsies conducted on dead birds, or swans brought into wildlife rehabilitation facilities already

exhibiting signs of lead poisoning (Blus et al. 1989, Katavolos et al. 2007, Strom et al. 2009, Degernes and Frank 2013). Research on whooper swans (*Cygnus cygnus*) in Europe found that 42% of 260 whooper swans captured in northwest England from 2010–2014 had blood lead levels above 200 ppb (O’Halloran and Myers 1988, Newth et al. 2016).

Long-term monitoring of biological populations can provide the opportunity to quantify trends over time in the prevalence and bio-accumulation of heavy metals and other harmful toxins (Burger 2006*b*). Researchers have used avian populations across a number of different ecosystems (e.g., urban systems, wetlands, marine systems) as heavy metal bioindicators (Burger and Gochfeld 2004, Zhang and Ma 2011, Cai and Calisi 2016). In addition to bone and tissues (e.g., blood, liver, kidney), heavy metal concentrations can be detected from feathers, eggs, and excrement of birds, allowing non-invasive sampling options that don’t require capture of live birds, and may be more logistically feasible (Denneman and Douben 1993, Berglund et al. 2011, Rave et al. 2014, Hashmi et al. 2015). Monzalvo-Santos et al. (2016) found that feathers from 3 passerine species had higher lead and arsenic levels at sites exposed to mining activities. Khwankitrittikul et al. (2024) found that heavy metal concentrations from wild bird liver and kidney samples showed interspecies differences but concentrations in feather samples did not. Collection of feathers during the prebasic molt period may provide the cheapest and least invasive sampling option, linking swans to the heavy metals present at the collection site.

Trumpeter swans in the IP are rapidly expanding in both abundance and distribution (Groves 2017). Baseline monitoring data on the overall prevalence and magnitude of lead toxicity provides useful information on the relative threat that lead poses to this population and provides an opportunity to track trends in blood lead levels over time.

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Table 3.1 Blood lead concentrations for 119 trumpeter swans (*Cygnus buccinator*) from July 2019 to August 2022. The number of swans and average lead concentrations are grouped by categories of lead toxicity.

<b>Lead category</b>	<b>Range (in ppb)</b>	<b>Number of swans</b>	<b>Average concentration (ppb)</b>
Background	<200	108	58
Sub-Clinical	200-500	9	262
Clinical	500-1000	1	542
Severe	>1,000	1	1,076

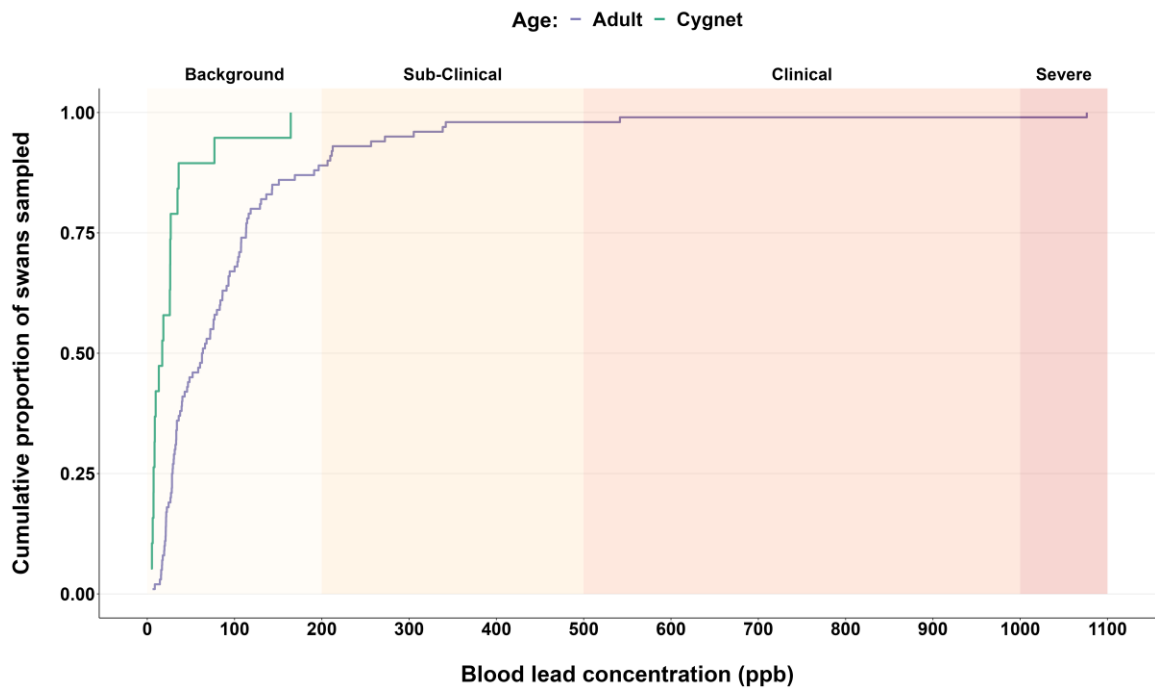


Figure 3-1 Empirical cumulative distribution function for the blood lead concentrations for 119 trumpeter swans (*Cygnus buccinator*, 100 adults and 19 cygnets) from July 2019 to August 2022. The y-axis represents the proportion of the total sample for each group (e.g., adults and cygnets) below any given threshold of blood lead concentration in parts per billion (ppb).



## Chapter 4: Comparative population genomics of trumpeter swans in North America

### Introduction

Wildlife managers possess a diverse array of tools to combat species loss and improve the functional connectivity of populations (Hoffmann et al. 2010; Luther et al. 2016). An often used management technique is human-assisted movement of animals into new areas, either translocations to supplement at-risk populations, or reintroductions to restore formerly extirpated populations (Griffith et al. 1989; Ewen et al. 2012). Reintroductions have helped rescue multiple wildlife species in North America from the brink of extinction, including California condors (*Gymnogyps californianus*), American bison (*Bison bison*), and black-footed ferrets (*Mustela nigripes*) (Russell et al. 1994; Ralls and Ballou 2004; Roe 2019).

When reintroducing wildlife to re-establish formerly extirpated populations, it's important to consider a range of factors affecting long-term conservation while managing budget and logistical constraints (Seaborn et al. 2021). Reintroduction plans often focus on meeting minimum population sizes or range distributions that will allow a population to attain long-term persistence (Ewen et al. 2014). Although there are many metrics to evaluate the status of a reintroduced population, including its abundance, survival rates, and fecundity rates; the role of genetic variability in both the source and destination populations has historically been given insufficient attention in initial wildlife reintroductions; and also as part of long-term monitoring following such events (De Barba et al. 2010; Weeks et al. 2011). Populations that increase in abundance and meet population goals can still experience genetic effects from reintroductions resulting from the generally small number of individuals in the founder population, including inbreeding and genetic drift, which can lead to an overall loss of genetic diversity and the fixation of deleterious alleles; ultimately decreasing fitness and the capacity to adapt to environmental change (Jamieson 2011).

Prior to European settlement, trumpeter swans (*Cygnus buccinator*) were thought to be widely distributed across much of North America (Alison 1975; Barrett and Vyse 1982). Overharvest due to unregulated market hunting caused the near extinction of trumpeter swans due to the high demands for swan feathers to make hats and writing quills, and skins to make powder puffs (Banko 1960). In 1935, low numbers of trumpeter swans led to the establishment of what became Red Rock Lakes National Wildlife Refuge (RRLNWR) at the intersection of Montana, Wyoming, and Idaho (Tri-State Area), which contained the last known flock of trumpeter swans in the United States (outside of Alaska; Shea et al. 2002). As trumpeter swan numbers at RRLNWR increased, this population became a source of individuals used to augment the diminished Rocky Mountain Population (RMP) in the inter-Mountain West and to restore the Interior Population (IP), which had been extirpated in eastern North America (Shea et al. 2002). In the 1950s, aerial surveys in central Alaska revealed another population of trumpeter swans (the Pacific Coast Population [PCP]), and this group provided another source of swans used for reintroductions within the IP (Hansen et al. 1971; Matteson et al. 1988). An important distinction between these source populations is that PCP swans breeding in Alaska migrate to British Columbia and the northwestern United States each winter, whereas RMP swans from RRLNWR were historically non-migratory (Baskin 1993; Oyler-McCance et al. 2007).

Trumpeter swan reintroductions to the IP started in the 1960s, but most individuals were released from 1980–2000 by state and provincial wildlife agencies in the greater Midwest (Shea et al. 2002). The High Plains (HP) flock in Nebraska was the first IP reintroduction, and unlike the rest of the IP, only received swans from a single source (RRLNWR) during the early 1960s (Johnson 1999). Trumpeter swans in the remainder of the IP were re-established via three types of translocations: 1) direct transfers of adults and cygnets from the Tri-State Area (RMP), 2) eggs taken from nests in Alaska (Pacific Coast Population [PCP]), and 3) captive-bred swans transferred from zoos and private breeders—a mix of both RMP and PCP origins (Johnson 1999; Shea et al. 2002). The demographic history of swans reintroduced from zoos and other breeders is unclear, as

information on the ancestral lineage and pedigrees of swans while in captivity weren't made publicly available (C. Henderson, pers. comm.). The cumulative numbers of each type of translocations are not available, but records indicate Alaskan eggs likely represented 36% of IP reintroductions (Johnson 1999). RMP swans (especially from the Tri-State Area) have remained at low abundance with little population growth for the last 50 years, whereas the PCP has been increasing during this period at an annual rate of ~5% (Moser 2006; Groves 2012).

The IP reintroduction program is widely considered to be a success, as population abundance is rising and breeding range is expanding each year (Shea et al. 2002; Groves 2017). The North American Trumpeter Swan Survey (NATSS), a partnership of state and federal agencies and other organizations, is the primary mechanism for monitoring trumpeter swan abundance, and has been conducted every 5 years from 1975–2015 across breeding trumpeter swan habitat (Fig. 1; Groves 2017). The latest NATSS in 2015 estimated that the IP is rapidly increasing, with an average annual growth rate of 14.4% from 1968–2015, although other groups, such as swans in the Tri-State Area (a subset of the RMP) are stationary, with an average growth rate of -0.1% over the same period. The NATSS was not conducted in 2020 due to the pandemic, and is expected to be discontinued due to logistical costs; therefore, it is necessary to use other approaches (e.g., genetics) to evaluate trumpeter swan population ecology in North America (Vrtiska and Anderson 2022).

Translocated populations typically have reduced genetic diversity compared to source populations, and this phenomenon may be exacerbated for trumpeter swans, which have lower species-wide genetic diversity compared to other swan and waterfowl species, likely due to a past species-level bottleneck (Mock et al. 2004; Oyler-McCance et al. 2007). Many of the swans used for reintroduction came from captivity (e.g., zoos and private holdings), and these individuals are expected to have even lower levels of genetic diversity due to inbreeding (Laikre and Ryman 1991; Sigg 2006). It is unknown whether and how much genetic structure is present within the reintroduced IP, to what extent each source population has contributed to the current gene pool of the IP, and how much gene

flow is present between different regions in the IP (including the geographically distinct High Plains flock in Nebraska). Characterizing the genetic structure of trumpeter swan populations in North America would allow managers to evaluate the functional connectivity among and within populations and their genetic potential for adaptation in the context of changing environmental conditions.

A better understanding of trumpeter swan genetics has been recognized as a research priority for >30 years (Mitchell 1993), but prior genetic analyses have been limited by small sample sizes and used lab methods (e.g., DNA fingerprinting, isozymes, microsatellites) that have now been superseded by improved technologies (Barrett and Vyse 1982; Meng et al. 1990; Pelizza and Britten 2002; Oyler-McCance et al. 2007; Ransler et al. 2011). Over the last 15 years, cost reductions in high-throughput sequencing technology and the widespread adoption of reduced-representation sequencing methods has revolutionized the field of genetic sequencing, allowing modern studies to produce orders of magnitude more sequencing reads than older methods such as those based on microsatellites (Baird et al. 2008). This proliferation of cost-effective sequencing methods that can be used on non-model species has greatly increased the precision and scope of genetic analyses that use ‘next-gen’ techniques such as restriction-site associated DNA (RAD) tags and allowed widespread progression to genome-scale analyses (Miller et al. 2007; Davey and Blaxter 2010; Peterson et al. 2012).

The objectives of this study were to quantify the population structure and genetic diversity and the degree of genetic differentiation among trumpeter swan populations in North America. Given the relatively recent reintroduction of the IP, we expected that IP swan genetics would reflect a mixture of the genetics of source populations. We predicted that the HP, due to its geographic isolation and a lack of evidence of long-distance dispersal, would be genetically distinct from the rest of the IP and have lower genetic diversity than all other groups of trumpeter swans because their reintroduction was accomplished using a single source population.

## Methods

### Sample collection, DNA extraction, and sequencing

We collected genetic samples from live IP trumpeter swans during the definitive prebasic molt period (except for three samples from swans captured in Arkansas using snares during the nonbreeding period), when adult swans replace their remiges, and are therefore flightless. Protocols for capturing and marking trumpeter swans in the United States were approved by the University of Minnesota Animal Care and Use Committee (protocol no. 1905-37072A), the U.S. Fish and Wildlife Service (Research & Monitoring Special Use Permits no. K-10-001, M-20-002, and M-21-014), the U.S. Geological Survey Bird Banding Laboratory (Federal Bird Banding Permit no. 21631) and state-specific permits approved by each state wildlife agency involved.

We collected 147 genetic samples from IP trumpeter swans captured during 2019–2022 throughout their current breeding range in Minnesota, Wisconsin, Michigan, Iowa, Ohio, and Nebraska and three during the nonbreeding period in Arkansas ( $n=150$ ). Although the HP flock in Nebraska is technically considered part of the IP, we consider the HP a separate population in subsequent analyses based on their re-establishment history and apparent lack of contact with the rest of the IP. We also obtained 53 archived genetic samples taken from PCP and RMP trumpeter swans during 2003–2013 in Alaska, Alberta, Idaho, and Wyoming via acquisitions from the USGS Fort Collins Science Center and 26 archived genetic samples taken from PCP trumpeter swans during 1997–2017 in Washington via acquisitions from the University of Washington Burke Museum of Natural History and Culture.

We extracted DNA from 229 feather, whole blood, and tissue samples using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) and quantified extractions using a Qubit Flex Fluoremeter (Invitrogen, Waltham, MA, USA). The University of Minnesota Genomics Center (UMGC, Minneapolis, MN, USA) prepared reduced representation DNA libraries for Illumina sequencing following a protocol suitable for double-digest restriction associated DNA (ddRAD) sequencing (Peterson et al. 2012). The UMGc digested genomic DNA with two restriction endonucleases (*MspI*

and *PstI*), ligated barcoded adapters, and then sequenced single-end (100-bp) reads using a P2 flow cell on an Illumina NextSeq 2000.

## **Bioinformatics**

After demultiplexing samples, we trimmed adaptor sequences using Cutadapt version 1.18 via a custom Perl script, retaining reads longer than 50 basepairs (Martin 2011). We used the STACKS v2.61 `process_radtags` function with the `-c` and `-q` flags to remove low quality reads and reads with uncalled bases (Rochette et al. 2019). We created an index of the *Cygnus buccinator* reference genome (NCBI identifier: GCA\_019232035.1) using program *bwa* v0.7.17 and then aligned all reads to the reference genome using the BWA-MEM algorithm (Li and Durbin 2009). We used *samtools* v1.16.1 to convert the `.sam` output files from *bwa* into `.bam` file format (Li and Durbin 2009). We used the *ref\_map.pl* program from STACKS v2.61 to run the *gstacks* and *populations* modules. First, *gstacks* reads in the `.bam` files and uses a sliding window algorithm (a de bruijn graph assembly) to create RAD loci (up to 1 kilobase in length) by aligning reads to the reference genome, using a minimum PHRED-scaled mapping quality of 10 to consider a read, a soft-clipping max level of 0.2, and a max allowed sequencing insert length of 1,000 base pairs (Rochette et al. 2019; Rivera-Colón and Catchen 2022). Then the marukilow Bayesian model, which uses the presence of a SNP at a particular loci in the entire metapopulation as a prior for genotyping each sample individually at that locus, is used to first identify SNPs and then call genotypes for each individual at each identified SNP using an alpha threshold of 0.05 for each of these two steps. The *gstacks* module then assembles SNPs into phased haplotypes using a read-based phasing method that uses co-occurrence information of alleles at multiple nearby SNPs to determine haplotype assignment (Rochette et al. 2019). We then filtered SNPs using the *populations* module by selecting a single SNP per RAD tag (to minimize issues of linkage) and excluding loci that were not genotyped in >75% of the individuals per population (e.g., IP, HP, RMP, PCP). We refer to this dataset as the ‘filtered SNP dataset’.

We also prepared a second version of the SNP dataset with additional filtering steps for analyses with stricter assumptions. We reran the *populations* module in STACKS and implemented a minimum allele frequency filter of 0.05, and then further thinned the SNP dataset using the *vcfR* and *SNPfiltR* R packages (Knaus and Grünwald 2017; DeRaad 2022). We used the *SNPfiltR::hard\_filter* function with a minimum depth of 3 for all genotype calls and a minimum genotype quality score of 30, and then pruned for linkage with the *SNPfiltR::distance\_thin* function by excluding additional SNPs on each scaffold unless they were >500 base-pairs away from the first SNP. We refer to this dataset as the ‘filtered unlinked SNP dataset’.

### **Genetic Analyses**

To quantify the genetic structure between trumpeter swan populations we used program Structure v.2.3.4, which selects distinct genetic clusters based on variation in allele frequencies (Pritchard et al. 2000). Structure clusters individuals into groups based on allele frequencies by minimizing the Hardy-Weinberg disequilibrium (HWD) within groups while maximizing it between groups (Pearman et al. 2022). To meet the model assumptions of Structure (e.g., Hardy-Weinberg equilibrium, linkage equilibrium), we used a subset of 1,000 randomly selected SNPs from the filtered SNP dataset. For all model runs, we used the admixture model with correlated allele frequencies among populations due to shared ancestry. We performed runs with 10 iterations per value of K, defined as the number of distinct clusters, for K=1–9, each with a burn-in period of 10,000 replications, followed by 10,000 MCMC replications. We uploaded Q-matrices (i.e., membership coefficients for all individuals to each of the K clusters) from all Structure runs to the CLUMPAK web server main pipeline (Kopelman et al. 2015). CLUMPAK uses program CLUMPP to align results from multiple STRUCTURE runs, check for multimodality, assign averaged Q values for each cluster, and generate consensus plots of the major modes of clustering for each value of K using the DISTRUCT program (Rosenberg 2004; Jakobsson and Rosenberg 2007; Kopelman et al. 2015). We used Structure Harvester within CLUMPAK to assess the optimal number of clusters (K) using two methods: the  $\Delta K$  method of Evanno (Evanno et al. 2005), which

looks to maximize the rate of change of log probability of the data given a value of  $K$ , and the  $\ln(\Pr(X|K))$  method, known as the ‘likelihood method’ of Pritchard et al. (2000), which selects for the asymptote of the relationship between mean log probability and increasing  $K$  (Earl and vonHoldt 2012). We used the averaged estimated group memberships from the best-supported  $K$ -value model runs to map admixture proportions onto a map of sampling locations using the *mapmixture* v1.1.0 R package (Jenkins 2024).

We used PCA, a nonparametric multivariate clustering method, to group individuals based on the overall variation in the SNPs without imposing prior population genetic assumptions. Because PCA assumes independence among samples, we used the filtered unlinked SNP dataset for this analysis. To prepare necessary inputs for PCA, we created a covariance matrix of allele frequencies from the filtered and thinned SNP dataset, and imputed missing values with the average allele frequency by population using the *adegenet* R package (Jombart 2008). We then estimated the eigenvalues of the covariance matrix, centering but not scaling values, using the *ade4* R package (Dray and Dufour 2007).

We estimated genetic diversity for the previously clustered groups from the Structure analyses (e.g., PCP, RMP, HP, IP) to quantify gene flow and characterize the genetic health of groups (i.e., risk of genetic drift due to isolation). We quantified the level of pairwise genetic differentiation among the groups by calculating the unbiased  $F_{ST}$  fixation index (Weir and Cockerham 1984) using the *StAMPP* R package with 100 bootstrap replicates used to estimate 95% confidence intervals (Pembleton et al. 2013).

We estimated observed heterozygosity ( $H_o$ ), mean gene diversity ( $H_s$ ), and rarefied allelic richness (i.e., number of alleles per locus corrected by the smallest sample size) at each locus, and also across all loci, using the *hierfstat* R package (Goudet 2005). We created an unrooted phylogenetic network using the filtered SNP dataset to visualize the evolutionary relationships between different groups of Trumpeter Swans using genetic distance to infer relatedness. We used the *StAMPP* package to create a pairwise distance matrix using Nei’s  $D$  (Nei 1972), and then estimated a neighbor-joining tree and



plotted the tree as a neighbor-net with each tip corresponding to an individual using the *ape* v5.7-1 R package (Saitou and Nei 1987; Bryant and Moulton 2004).

## Results

We sequenced 229 samples (150 collected from the field, 79 from museum archives) using ddRAD protocols. After reviewing quality control of raw sequencing data with FASTQC, we dropped five samples due to low quality reads. STACKS retained 86.2% of primary alignments with the reference genome and skipped 5.1% of alignments with insufficient mapping qualities, 1.3% of alignments that were excessively soft-clipped, and excluded 7.4% of reads because they were unmapped. Our initial bioinformatics pipeline, filtering only on excluding low quality reads (i.e., phred scores) and uncalled bases, produced 429,115 loci from 224 individuals, with an effective per-sample coverage of 10.8x (std. dev=4.6x, min=2.1x, max=28.8x), and an average of 90.5 sites per locus. After the second filtering stage in STACKS (i.e., single SNP per RAD-tag, retaining loci in >75% of the individuals per population, filtering for HWE), we retained 82,234 SNPs (referred to as ‘filtered SNP dataset’), and after the final filtering stage in SNPfiltR (depth of genotype coverage, minimum genotype quality, and physical proximity along the scaffold) we retained 66,847 SNPs (referred to as ‘filtered unlinked SNP dataset’).

The two methods we used to evaluate the optimal number of genetic clusters in STRUCTURE produced very different results. The  $\Delta K$  method selected 2 as the optimal number of clusters, but the  $\Delta K$  method has a tendency to overestimate  $K=2$  as the optimal  $K$  value, and is not necessarily a valid approach (Janes et al. 2017; Cullingham et al. 2020). Following the advice of Pritchard et al. (2009), who suggested to pick the optimal  $K$  as the smallest value before the  $\ln(\Pr(X|K))$  likelihood ‘plateaus’, we estimated the optimal value of  $K$  to be approximately 4, which better matched both our prior expectations based on trumpeter swan reintroduction history and also the visual representation of clustering among the different  $K$  model runs (see supplemental fig of structure output with  $K=2-5$ ). Using a  $K$ -value of 4, we found that swans genetically

clustered into a grouping that matched the three main populations with the High Plains flock representing a distinct fourth group (Fig. 3).

In the PCA using the filtered unlinked SNP dataset of 66,847 SNPs, the first two principal components (PCs) explained 4.1% and 1.8%, respectively, of the total genetic variance (Fig. 4). Clusters from the first two PCs confirmed results derived using STRUCTURE, with each of the populations forming distinct clusters. The IP cluster overlapped with its source populations (RMP and PCP), reflecting the reintroduction history. The HP, in a cluster by itself, provides evidence that its geographic isolation (along with genetic drift due to small population size) led to genetic differentiation between the HP and other groups of trumpeter swans.

Pairwise  $F_{ST}$  values between populations (including HP as a separate group) ranged from 0.036 to 0.135, with lowest values for comparisons between the IP and its source populations (Fig. 5). Comparisons involving the High Plains flock were the highest ( $>0.1$ ), potentially reflecting the geographic isolation of this subset of the IP. Confidence intervals for all  $F_{ST}$  estimates excluded zero, providing evidence at the  $\alpha = 0.05$  level to reject the null hypothesis that there is no genetic differentiation between groups.

The rarefied allelic richness, a measure of genetic diversity and adaptive potential, was similar for the three main populations but substantially less for the HP flock. Similarly, measures of observed and expected heterozygosity were comparable for the IP, PCP, and RMP, but lower for the HP (Table 2).

The phylogenetic network using Nei's D for genetic distance provides evidence for how distinct genetic clusters are related to each other. The branches of the phylogeny being colored by the same assignment of genetic clusters as inferred by STRUCTURE and PCA showed that origins of the reintroduced populations are more complex to decipher (Fig. 6). The HP is recovered as a single cluster with a close relationship to the RMP, as would be predicted given that the HP was solely derived from the RRLNWR (RMP). Individuals from the IP were grouped in several clusters and had close

associations with RMP and PCP, but not HP, corroborating what is known of their origins from both these source populations.

## **Discussion**

Using an extensive dataset representing all trumpeter swan populations, we used ddRAD sequencing methods to characterize patterns of genetic diversity and population structure for this charismatic species that has been re-established in portions of its prior distribution. Our work builds upon previous genetic studies on trumpeter swans in North America, but our increased sample sizes and contemporary methodology (i.e., high-throughput sequencing as opposed to microsatellites) provide improved accuracy and precision in comparisons of genetic differentiation between populations and genetic diversity post-reintroduction. Our main findings include that (1) the three populations of trumpeter swans (e.g., IP, RMP, PCP) and the HP are genetically distinct groups; (2) genetic differentiation between the IP and its source populations (RMP and PCP) is low, likely due to a diverse mix of reintroduction sources and the relatively recent divergence between source and reintroduced populations; and (3) the HP has lower genetic diversity than the other populations, likely a result of smaller population size, less interbreeding with other groups of trumpeter swans due to relative geographic isolation, and potential founder effects due to a one-time reintroduction with relatively few individuals.

Although trumpeter swans were thought to be widespread across North America as recently as 250 years ago, our current results show clear genetic substructure across the continent. Existing historical records of trumpeter swan occurrence and abundance are of limited scope and quality; therefore, it is unknown to what extent interbreeding among populations of trumpeter swans promoted gene flow prior to overharvest and in the case of the IP, extirpation, and if it was at a sufficient level for trumpeter swans across North America to be panmictic (Monnie 1966; Denson 1970; Alison 1975). Because trumpeter swans inhabit shallow waters with emergent vegetation, the arid sections of western North America east of the Rocky Mountains and west of the Prairie Pothole Region may have been a historical natural barrier genetically separating trumpeter swans from the western and eastern portions of the continent (Banko 1960).

Given the mix of source types (e.g., wild versus captive), source locations (e.g., Alaska, RRLNWR, zoos/private), and the wide variety in reintroduction timing and locations across the Midwest (e.g., many separate state/provincial efforts over ~40 years), reintroduced IP swans have likely had a high incidence of interbreeding among genetically diverse individuals, which may have contributed to discrete genetic population structure among the IP, RMP, and PCP. However, trumpeter swans don't typically breed until 4-6 years, and so the number of generations since the IP reintroduction started is few, which may contribute to relatively low  $F_{ST}$  values and genetic differentiation among the IP, RMP, and PCP (Banko 1960; Gale et al. 1987).

Genetic results from this study provide information that complements that from current research efforts on the movements of trumpeter swans across North America. In the last 5-10 years, multiple state and inter-agency collaborations have used GPS-telemetry to individually track the annual movements of trumpeter swans in the Inter-Mountain West, Nebraska Sandhills, and the greater Midwest (Poessel et al. 2022; Wolfson et al. 2022; Vrtiska et al. 2024). Motivations behind these research efforts include quantifying the degree of mixing between different groups within a population (RMP), estimating winter movements and site fidelity (HP), and characterizing the prevalence and magnitude of migratory behavior in a recently re-introduced population (IP). Similar to other waterfowl species, trumpeter swans form initial pair bonds during the non-breeding season; therefore, gene flow is likely influenced by which conspecifics are in proximity during this period (Robertson and Cooke 1999; Johnsgard 2010). The RMP is mainly comprised of two flocks (one in the Tri-State area, the other in Alberta/Yukon/British Columbia) that are spatially disjunct during the breeding season, but during the non-breeding season Canadian swans migrate south to overwinter with other RMP swans in the Tri-State area (Shea et al. 2002). The degree to which swans from these two flocks form pair bonds and interbreed has not been well documented with tracking data; however, quantifying rates of gene flows between these groups would help answer this question.

Fruitful future research efforts might involve combining genetic sequencing and individual tracking datasets to further understand drivers of migration and the steps involved in adapting population-level patterns of movement (Delmore et al. 2020). Factors influencing migration include hereditary genetics, phenotypic plasticity, and the transfer of knowledge between individuals (e.g., social learning; Berthold et al. 1991; Berthold and Pulido 1994; Pulido 2007; Leidvogel et al. 2011). The re-establishment of the IP set the stage for a natural experiment to assess relearning a population-scale migratory tradition (Berthold et al. 1992). Selective sweep mapping (e.g., screening for outliers with runs of homozygosity) of whole-gene sequencing datasets, alongside a reference genome containing annotated chromosomes detailing gene functionality, can pair with GPS-tracking data to characterize the movements of individuals containing regions under selection and evaluate if particular gene outliers correspond to differences in migratory phenotypes (Delmore et al. 2015; Andrade et al. 2024). By better understanding the genetic components of population-level behaviors, it may also be possible to unravel the mechanisms required for populations to respond to climatic or other anthropogenic environmental changes.

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Table 4.1 Genetic samples, grouped by state or province. ‘Wild’ samples are from trumpeter swans (*Cygnus buccinator*) captured in the field; ‘archived’ samples were obtained from previous field studies by USGS collaborators or from the Burke Museum of Natural History and Culture. Abbreviations are PCP=Pacific Coast Population, RMP=Rocky Mountain Population, HP=High Plains flock, IP=Interior Population.

State/Province	Group	N	Sample Type	Date Range
Alaska	PCP	18	archived	2003-2004
Washington	PCP	26	archived	1997-2017
Alberta	RMP	13	archived	2004-2004
Idaho	RMP	3	archived	2003-2003
Wyoming	RMP	19	archived	2003-2013
Nebraska	HP	15	wild	2020-2020
Minnesota	IP	60	wild	2019-2021
Iowa	IP	31	wild	2020-2022
Wisconsin	IP	7	wild	2020-2021
Michigan	IP	14	wild	2019-2020
Ohio	IP	20	wild	2020-2021
Arkansas	IP	3	wild	2021-2021

Table 4.2 Genetic diversity metrics for 224 trumpeter swans (*Cygnus buccinator*) sampled across North America estimated from 82,234 single nucleotide polymorphisms (SNPs). Group abbreviations given in Table 1. Other abbreviations include:  $H_o$  = observed heterozygosity and  $H_s$  = mean gene diversity, a metric similar to expected heterozygosity ( $H_E$ ), except  $H_s$  is a measure of average genetic diversity across all loci within a population, while  $H_E$  measures diversity at a particular locus.

<b>Group</b>	<b>n</b>	<b>Allelic Richness</b>	<b><math>H_o</math></b>	<b><math>H_s</math></b>
HP	15	1.237	0.252	0.237
IP	134	1.302	0.302	0.302
PCP	44	1.292	0.293	0.292
RMP	31	1.304	0.311	0.278

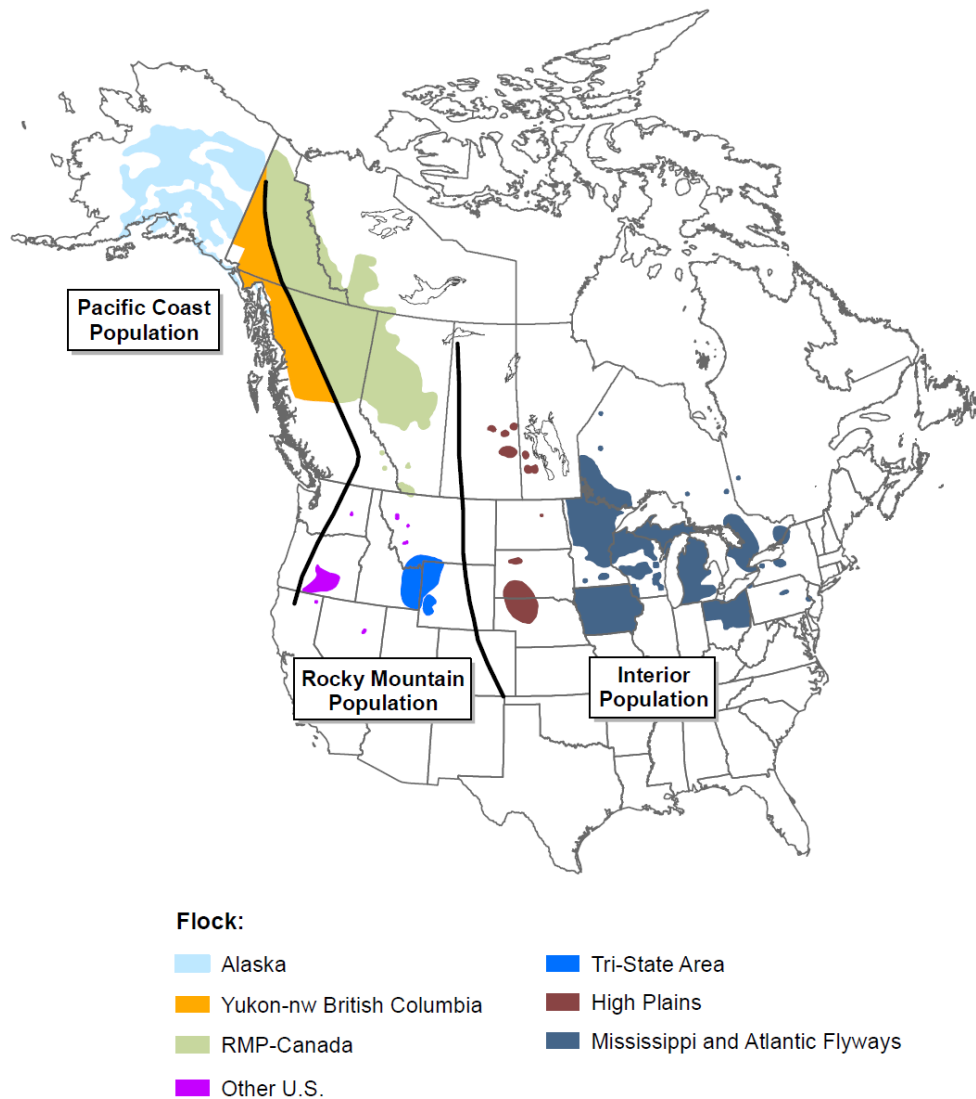


Figure 4-1 Approximate breeding range of trumpeter swans (*Cygnus buccinator*), as reported by the North American Trumpeter Swan Survey (NATSS) cooperators (Groves 2017). Black lines show approximate boundaries of the breeding ranges of the three populations.



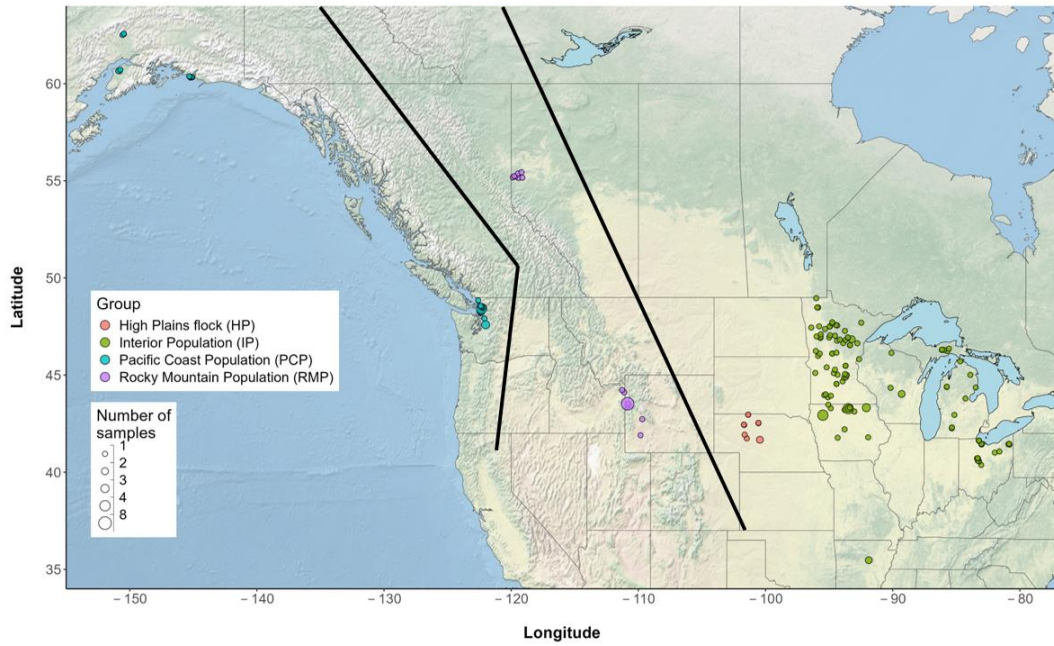


Figure 4-2 Locations of trumpeter swan (*Cygnus buccinator*) genetic samples used in this study. Relative size of points indicate if multiple samples were collected in an area. Black lines show approximate boundaries of the breeding ranges of the three populations in North America. High Plains swans are technically considered part of the Interior Population but are given a unique category in this map.

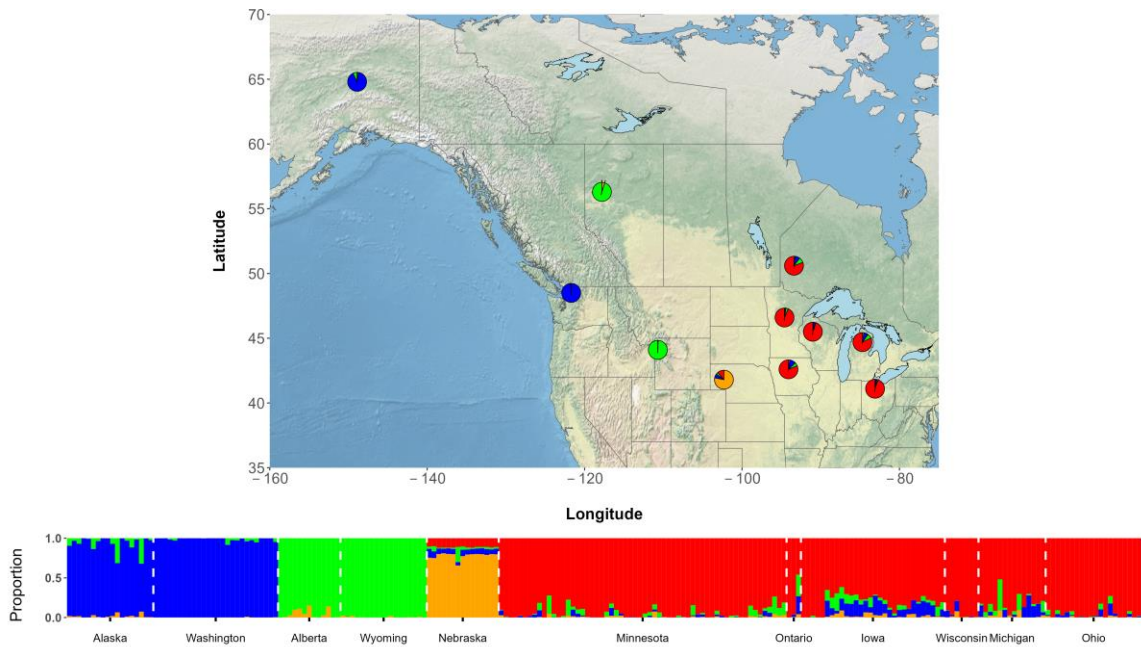


Figure 4-3 The upper figure shows a map of North America with a pie chart imposed over each sampling region. The relative proportion of the four colors in each pie chart correspond with the proportional assignment of individuals from each sampling region to the four genetic clusters identified by STRUCTURE. The lower figure shows the model-based clustering output from STRUCTURE for each of the 224 trumpeter swan (*Cygnus buccinator*) individuals.

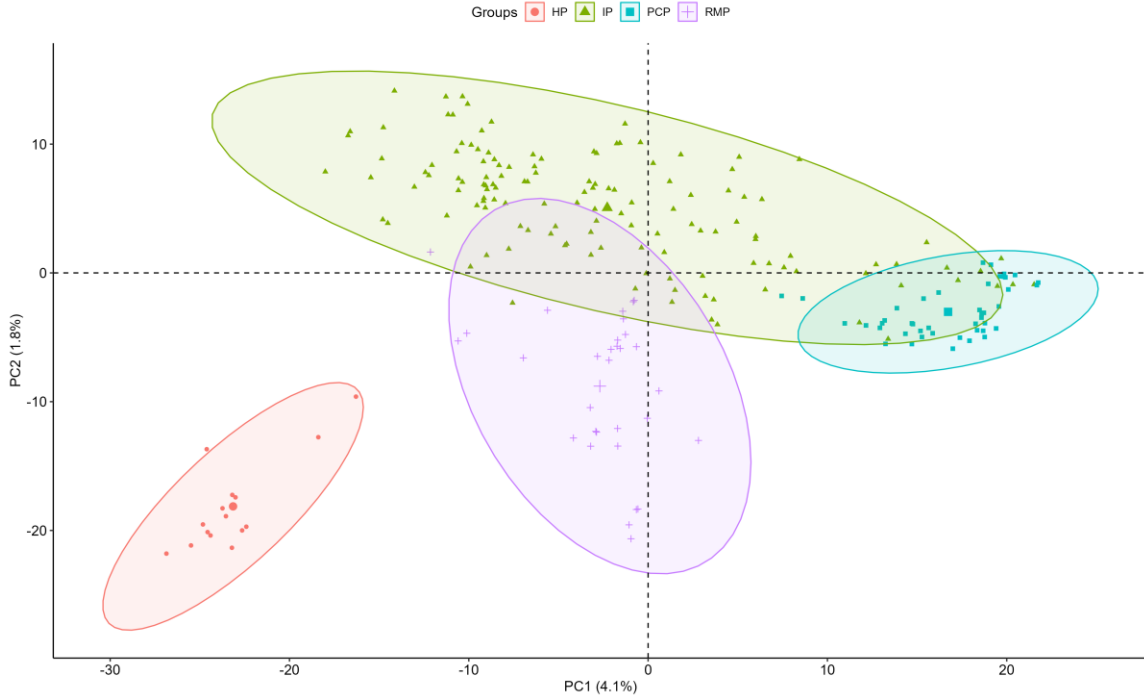


Figure 4-4 PCA of 66,847 SNPs with 95% ellipses surrounding points grouped by trumpeter swan (*Cygnus buccinator*) population (IP = Interior Population, PCP = Pacific Coast Population, RMP = Rocky Mountain Population), and the High Plains (HP) flock.

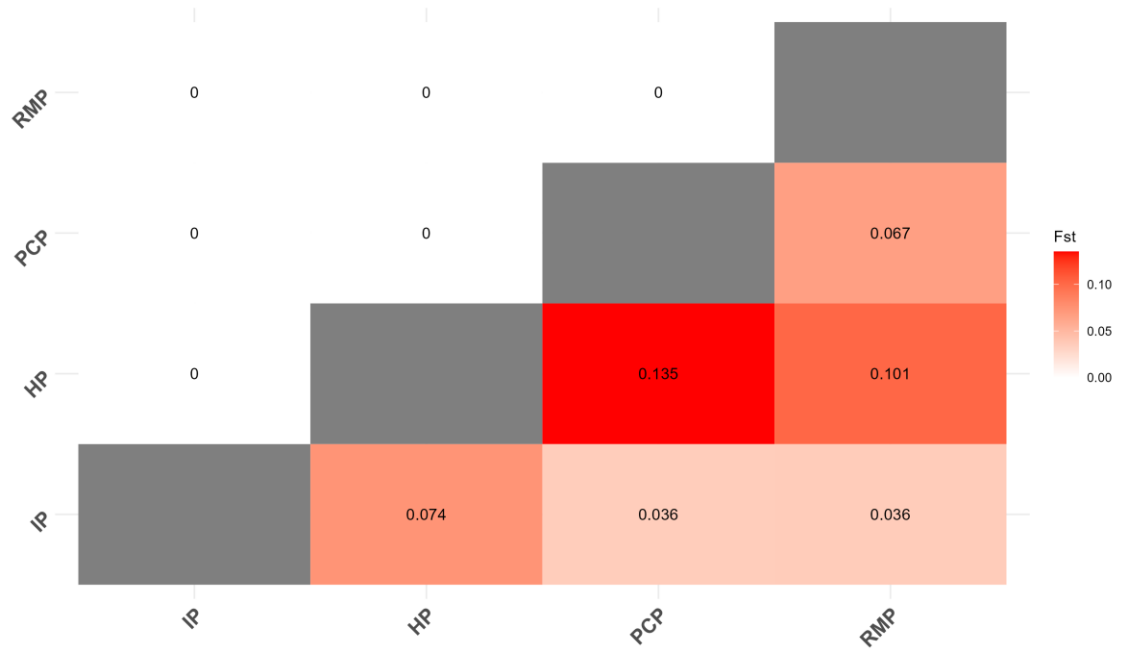


Figure 4-5 Heatmap of pairwise genetic distance between sampling locations of trumpeter swans (*Cygnus buccinator*; RMP = Rocky Mountain Population, PCP = Pacific Coast Population, HP = High Plains flock, and IP = Interior Population) using 82,234 SNPs. Pairwise  $F_{ST}$  values (Weir and Cockerham's  $\Theta$ , 1984) are shown below the diagonal. Associated p-values for each  $F_{ST}$  index estimated using 100 bootstrap replicates are shown above the diagonal.

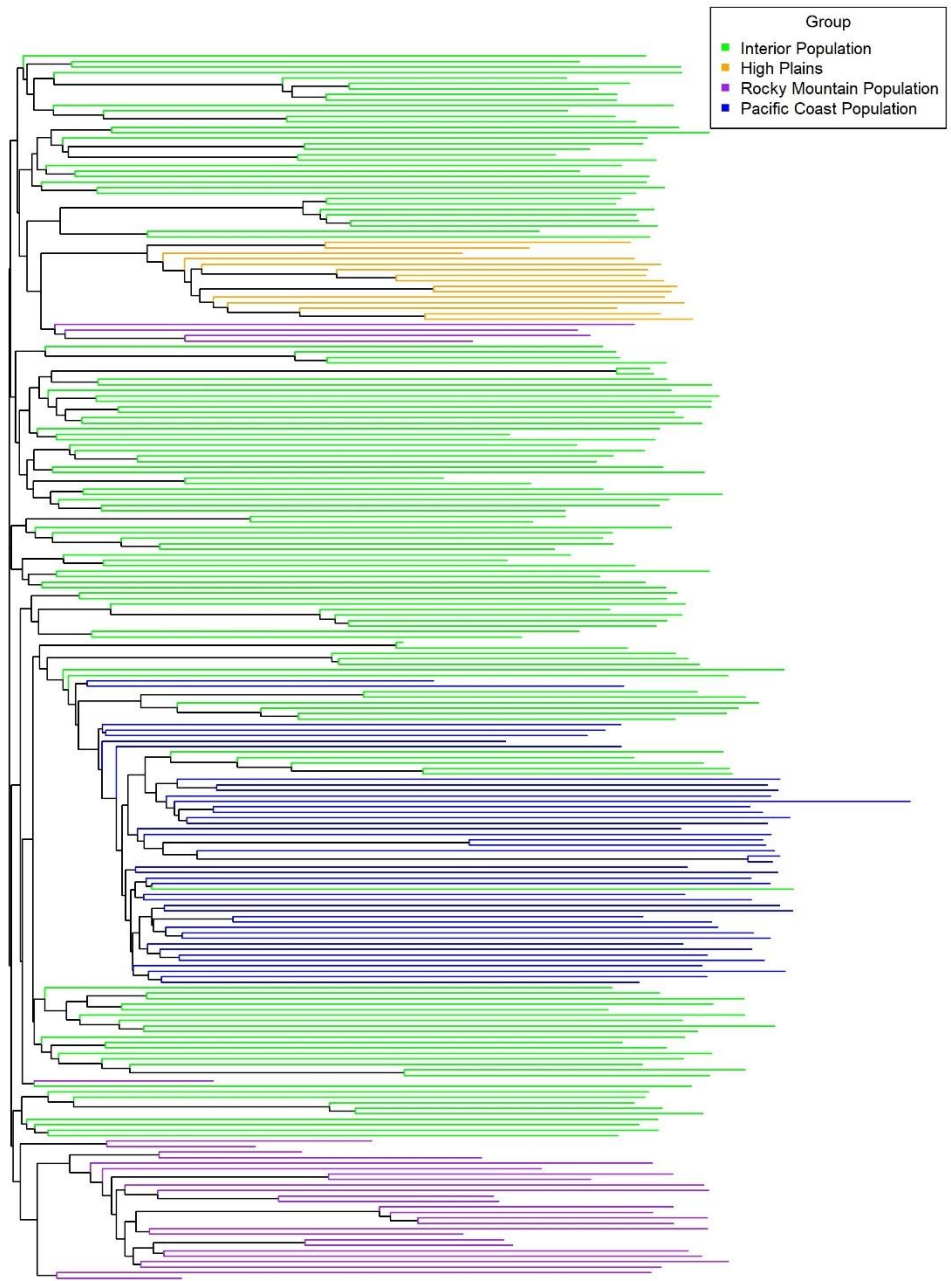


Figure 4-6 Neighbor-joining network for 224 trumpeter swans (*Cygnus buccinator*) with branch lengths corresponding to the evolutionary relatedness between individuals (based on Nei's D genetic distance). Branches in the tree are colored based on the group from which the sample was obtained.

## Appendices

### Chapter 2

#### Appendix A

### Migration Phenology Summary Statistics

#### Autumn departure

We estimated autumn departure dates for all swans that traveled >100 km from the breeding/capture territory by 31 December.

Table S1. Compiled migration phenology of all autumn departures from 2019–2022.

Total Swans Tracked	Number of Long-Distance Migrants	Number of Fall Departure Events	Average Autumn Departure	Standard Deviation (days)	Earliest Departure	Latest Departure
122	71	117	November 07	25	September 01	December 24

Table S2. Yearly summaries of migration phenology of autumn departures from 2019–2022.

Year	Total Swans Tracked	Number of Long-Distance Migrants	Average Autumn Departure	Standard Deviation (days)	Earliest Departure	Latest Departure
2019	17	7	October 31	7	October 21	November 08
2020	82	49	November 02	28	September 01	December 24
2021	86	38	November 09	24	September 15	December 23
2022	44	23	November 17	18	October 07	December 20

Table S3. Autumn departure dates of long-distance migrants by breeding status.

Breeding Status	Total Swans Tracked	Number of Long-Distance Migrants	Average Autumn Departure	Standard Deviation (days)	Earliest Departure	Latest Departure
Breeder	70	36	November 13	23	September 15	December 24
Non-Breeder	22	12	October 22	27	September 01	November 29
Paired	21	14	November 04	26	September 27	December 24



### Spring arrival

We estimated spring arrival for all swans that traveled >100 km from the breeding/capture territory during the non-breeding period, left their territory by 31 December, and returned to <30 km of their previous year's territory.

Table S4. Compiled migration phenology of all spring arrivals from 2020–2023.

Total Swans Tracked	Number of Long-Distance Migrants	Number of Spring Arrival Events	Average Spring Arrival	Standard Deviation (days)	Earliest Arrival	Latest Arrival
122	51	84	March 04	15	January 18	April 11

Table S5. Yearly summaries of migration phenology of spring arrivals from 2020–2023.

Year	Total Swans Tracked	Number of Long-Distance Migrants	Average Spring Arrival	Standard Deviation (days)	Earliest Arrival	Latest Arrival
2020	17	4	March 02	18	February 08	March 23
2021	82	35	March 03	8	January 31	March 23
2022	86	33	March 06	19	January 18	April 11
2023	44	12	March 02	20	February 06	April 08

Table S6. Spring arrivals of long-distance migrants by breeding status.

Breeding Status	Total Swans Tracked	Number of Long-Distance Migrants	Number of Spring Arrival Events	Average Spring Arrival	Standard Deviation (days)	Earliest Arrival	Latest Arrival
Breeder	70	29	54	February 29	15	January 18	April 05
Non-Breeder	22	8	10	March 12	9	February 29	March 27
Paired	21	8	11	March 06	7	March 01	March 22

### Duration of non-breeding period

We estimated duration of non-breeding period for all swans that had an autumn departure (i.e., traveled >100 km from territory) followed by a spring arrival the following year. This migration metric represents the span of time absent from the breeding/capture territory during the non-breeding period, and is calculated by the difference in days between spring arrival and the previous year's autumn departure.

Table S7. Compiled duration of non-breeding period for all swans from 2019–2023.

Total Swans Tracked	Number of Long-Distance Migrants	Number of Annual Cycles	Average Duration of Non-breeding Period (days)	Standard Deviation (days)
122	49	78	115	29

Table S8. Yearly summaries of duration of non-breeding period from 2019–2020 until 2022–2023.

Year	Total Swans Tracked	Number of Long-Distance Migrants	Average Duration of Non-breeding Period (days)	Standard Deviation (days)
2020	17	4	119	21
2021	82	34	118	29
2022	86	28	117	28
2023	44	12	99	33

Table S9. Summaries by breeding status of non-breeding period from 2019–2020 until 2022–2023.

Breeding Status	Total Swans Tracked	Number of Long-Distance Migrants	Average Duration of Non-breeding Period (days)	Standard Deviation (days)
Breeder	70	29	109	29
Non-Breeder	22	8	133	28
Paired	21	8	126	23

## Migration phenology model output

### Linear mixed models

Table S10. Model summaries from 3 linear mixed models fit using the 3 migration metrics (autumn departure, spring arrival, and duration of non-breeding period) as the response. 95% confidence intervals for each coefficient are shown in brackets.

	Autumn Departure	Spring Arrival	Duration of Non-breeding Period
(Intercept)	323.701*** [219.816, 427.586]	84.549* [15.781, 153.317]	-153.650* [-303.982, -3.317]
Sex	3.993 [-5.440, 13.427]	3.389 [-3.097, 9.874]	-3.260 [-16.994, 10.474]
Breeder – Non-Breeder	-18.404** [-31.058, -5.751]	1.211 [-8.372, 10.794]	14.446 [-6.395, 35.288]
Breeder – Paired	-6.083 [-18.281, 6.115]	1.063 [-7.250, 9.375]	13.308 [-4.602, 31.217]
Breeding/Capture Latitude	-4.082*** [-6.333, -1.830]	3.545*** [2.045, 5.045]	5.802*** [2.530, 9.074]
SD (Intercept swan_ID)	7.240	7.432	6.137
SD (Observations)	21.615	7.981	24.752
Num.Obs.	107	75	72
R2 Marg.	0.228	0.338	0.265
R2 Cond.	0.306	0.645	0.308
ICC	0.1	0.5	0.1

+ p < 0.1, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

**Pairwise contrasts of migration timing by breeding status, adjusted using Tukey's HSD**

Table S11. Pairwise contrasts of autumn departure dates by long-distance migrants considered by breeding status.

Contrast	Estimate	SE	Degrees of freedom	Lower 95% CL	Upper 95% CL	t ratio	p value
Breeder - Non-Breeder	18.40	6.43	56.76	2.93	33.88	2.86	0.02
Breeder - Paired	6.08	6.18	65.44	-8.74	20.90	0.98	0.59
Non-Breeder - Paired	-12.32	7.72	65.81	-30.83	6.19	-1.60	0.25

Table S12. Pairwise contrasts of spring arrival dates by long distance migrants considered by breeding status.

Contrast	Estimate	SE	Degrees of freedom	Lower 95% CL	Upper 95% CL	t ratio	p value
Breeder - Non-Breeder	-1.21	4.82	45.60	-12.88	10.45	-0.25	0.97
Breeder - Paired	-1.06	4.18	43.80	-11.20	9.07	-0.25	0.96
Non-Breeder - Paired	0.15	5.39	45.13	-12.90	13.20	0.03	1.00

Table S13. Pairwise contrasts of duration of non-breeding period by long-distance migrants considered by breeding status.

Contrast	Estimate	SE	Degrees of freedom	Lower 95% CL	Upper 95% CL	t ratio	p value
Breeder - Non-Breeder	-14.45	10.56	46.78	-40.01	11.11	-1.37	0.37
Breeder - Paired	-13.31	9.06	44.53	-35.28	8.67	-1.47	0.32
Non-Breeder - Paired	1.14	11.76	48.12	-27.29	29.57	0.10	0.99

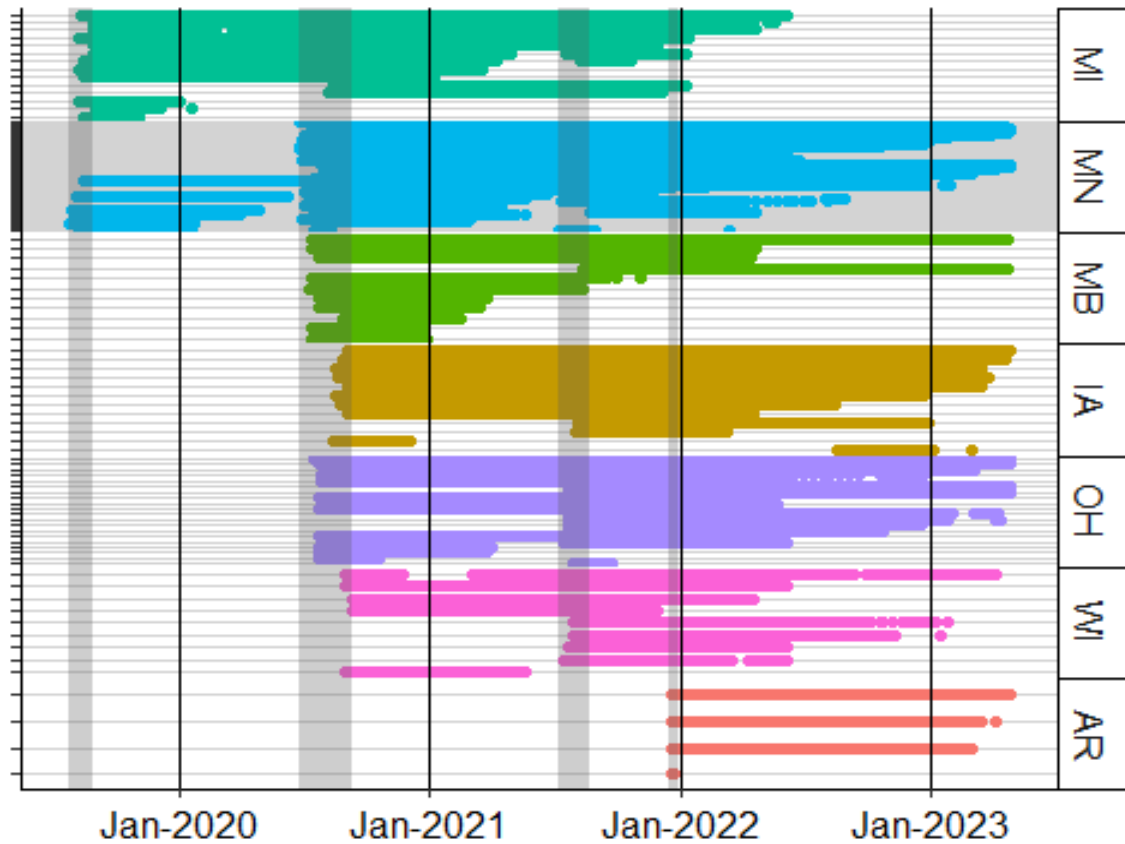


Figure S1. An overview of GPS telemetry data received from all collared IP trumpeter swans. Each line represents the period of data collection from a single collared swan. The grey regions indicate periods of collar deployment. The black lines are 1 January of each year. Number of deployments (including redeployments) by state/province are: Michigan ( $n=14$ ), Minnesota ( $n=56$ ), Manitoba ( $n=11$ ), Iowa ( $n=12$ ), Ohio ( $n=20$ ), Wisconsin ( $n=9$ ), and Arkansas ( $n=4$ ).

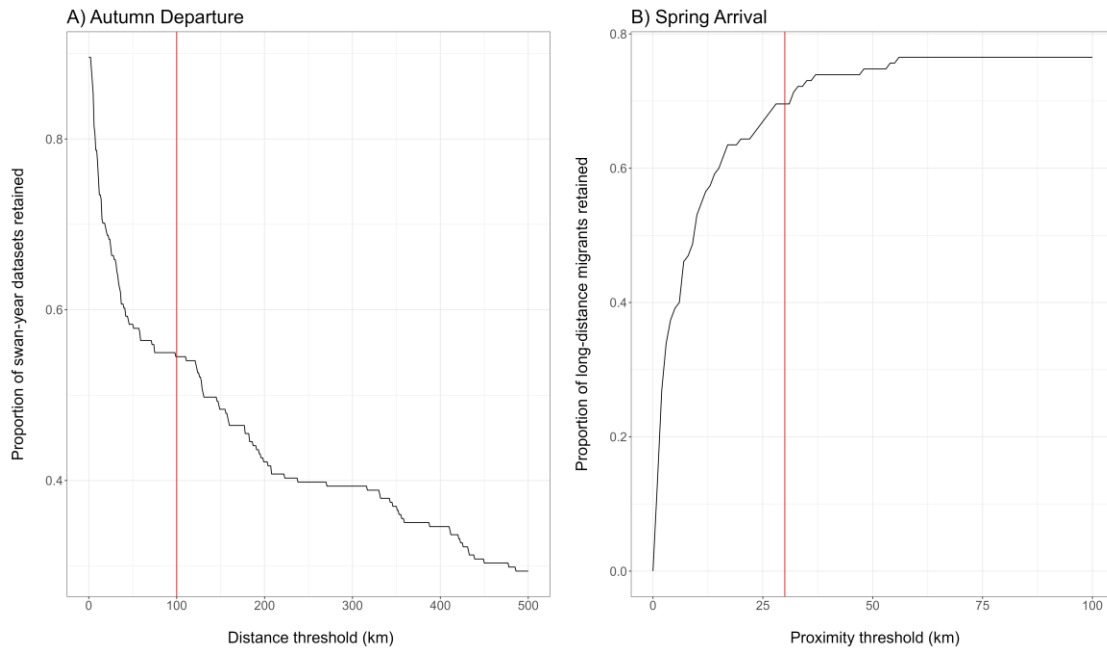


Figure S2. Sensitivity analyses for the thresholds used as cutoffs for whether to estimate migration timing (autumn departure and spring arrival). (A) The proportion of swan-year datasets that satisfy distance threshold cutoffs (0–500 km) as well as other rules (e.g., ignoring changepoints <2 days and segments <2 km from each other, potential departure before 31 Dec) for being considered a ‘long-distance’ migrant, and estimating an autumn departure date. The red line indicates the threshold of 100 km used in the analysis. (B) The proportion of swan-year datasets that satisfy a proximity threshold cutoff (0-100 km), defined as the required proximity to the previous year’s breeding/capture territory (i.e., the distance from the previous year territory that the swan must be within the following year), as well as other rules (e.g., previously satisfied criteria for estimating an autumn departure, potential arrival after 31 Dec) used to estimate a spring arrival date. The red line indicates the threshold of 30 km used in the analysis.



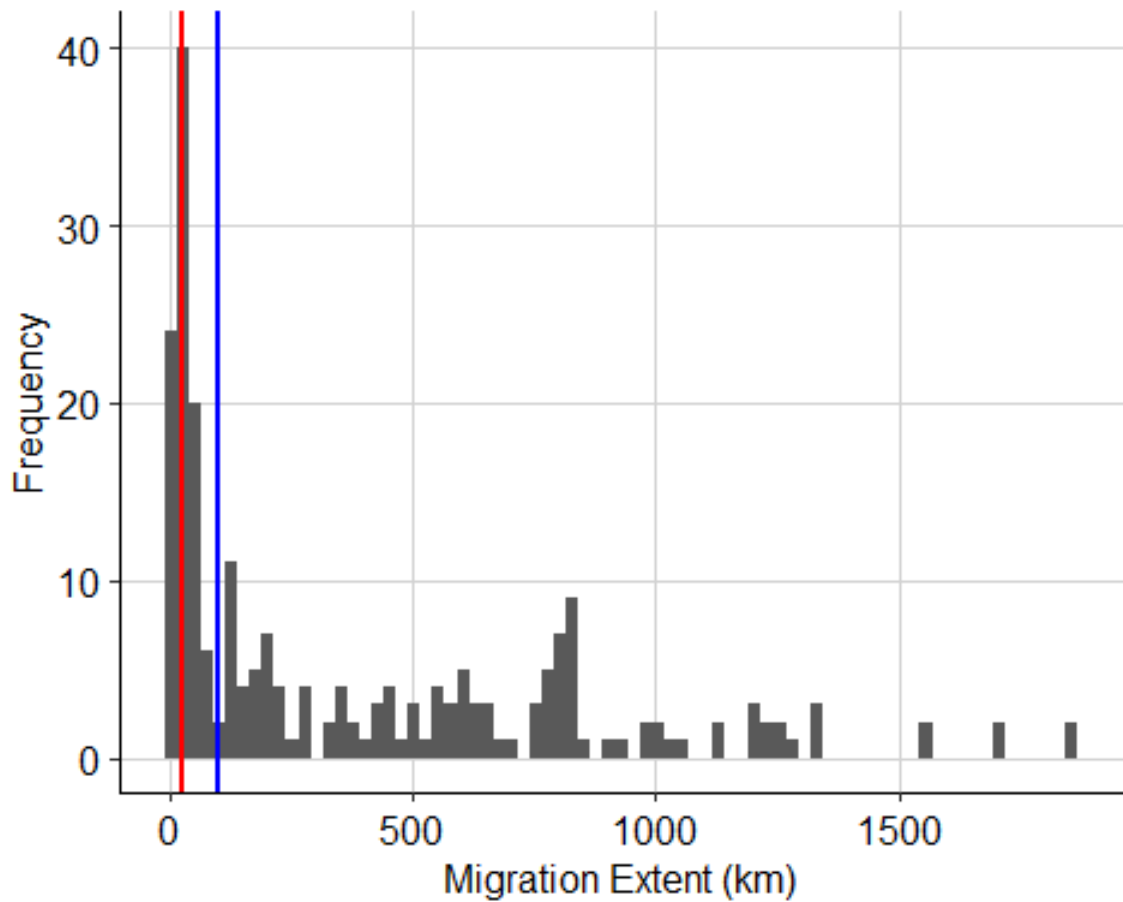


Figure S3. Histogram of the migration extent (farthest distance from breeding territory during the non-breeding period; i.e., maximum annual displacement segment from piecewise regression model) for all 221 swan-year datasets. The distance thresholds used to define categories of migration (local movements = 0-25 km, regional migration = 25-100 km, long-distance migration >100 km) are shown with the red (25 km) and blue (100 km) lines.

## Chapter 4

### Appendix A

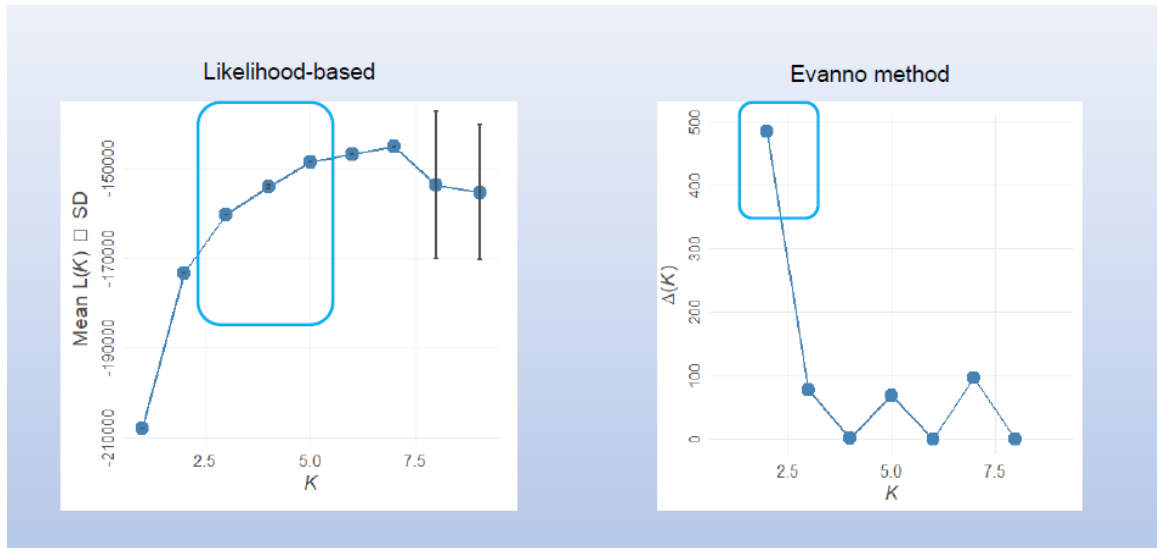


Figure S1. CLUMPP output for North American trumpeter swans (*Cygnus buccinator*) using the  $\ln(\text{Pr}(X|K))$  ‘Likelihood’ method of Pritchard (2009) and the ‘Evanno’ method (Evanno et al. 2005). The blue box superimposed over the results indicates the range of values chosen by each method at the optimal K value for program STRUCTURE.

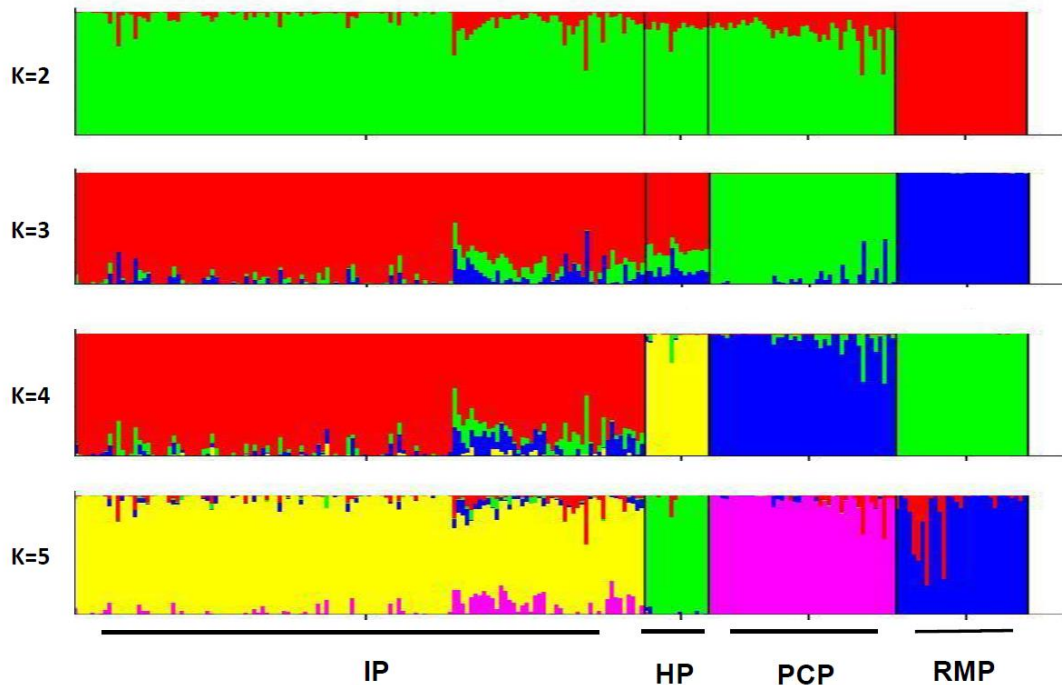


Figure S2. Results of the STRUCTURE analysis of North American trumpeter swans (*Cygnus buccinator*) based on 1,000 SNPs for K values 2 through 5. The optimal range of K=3–5 from the Pritchard (2009) method matched our *a priori* expectations (based on geographic patterns) of K=4. Higher values above 4 resulted in over-splitting within groups.