5-12-2016

Determining the Impact of Latitude on Parturition Timing in Captive North American River Otters: A Statistical Analysis of AZA Studbook Records

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Recommended Citation

Bailey, Jordan I., "Determining the Impact of Latitude on Parturition Timing in Captive North American River Otters: A Statistical Analysis of AZA Studbook Records" (2016). Thesis. Rochester Institute of Technology. Accessed from

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Determining the Impact of Latitude on Parturition Timing in Captive North American River Otters: A Statistical Analysis of AZA Studbook Records

by

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of
Science in Environmental Science

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May 12, 2016

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Acknowledgements

I would like to express my sincere thanks to each of my committee members who have provided invaluable guidance and support throughout this process. I cannot imagine how this might have come together without each of your input. This would not have been possible without Mr. David Hamilton, who first inspired me to pursue this topic of research during my internship at the Seneca Park Zoo in 2013. My advisor, Dr. Larry Buckley, has truly been a mentor since I first began my studies at RIT. Your comments and time have done so much to shape the scientist I am today. I also wish to thank Dr. Susan Pagano for her thoughtful comments on my approach and analysis to this research.

I extend my thanks to Dr. Michael Osier, associate professor at RIT and Bioinformatics Graduate Program Coordinator. While not a member of my committee, you have also provided incredible support in extracting and organizing my data. Thank you for remembering me from Freshman Symposium back in 2011.

My acknowledgments would not be complete without recognizing my classmates and friends for their help and commiseration over my five years at RIT. Thank you all so much for your empathy, encouragement, and humor.

Lastly, a great deal of credit and my deepest gratitude is owed to my parents, Mark and Rochelle Bailey. You taught me to dream big and to not stop until I had achieved all I set out to do. Your love and support has empowered me to become the woman I am today. Thank you for all the years of listening to discussions of my research, my plans for my research, and my abundant coursework. Thank you for always giving me a shoulder to lean on when needed, and a nudge forward when that was needed more. I love you both and could not have done this without you.

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Abstract

Historically, North American river otters (*Lontra canadensis*) were widely dispersed throughout the North American continent. Trapping pressures and urbanization have led to regional exclusion of North American river otter populations from historic habitats, leading the Association of Zoos and Aquariums to spearhead conservation and captive breeding efforts to maintain genetic diversity of the aquatic mustelid. Difficulties in consistently breeding captive North American river otters have spotlighted a need to understand how geography and life history of adult individuals influence reproductive events. This study analyzed the AZA studbook records for all litters born in captivity from 2008 to 2014 (N = 47) to assess whether any correlations existed between historical data and timing of parturition events. ANOVA tests found significant differences in mean parturition date between litters by dam origin region (F = 6.09, p-value = 0.018) and by parturition location (F = 12.73, p-value = 0.001). A Mann-Whitney u test found a difference (p-value = 0.0365) between median parturition dates of litters born in the north and those born in the south regions. PCA testing showed that the data form independent groups by both dam and sire origin latitudes, confirming the existence of a significant relationship between latitude and the timing of reproductive events. However, this study did not conclusively determine which latitude (the origin of the dam, origin of the sire, or latitude at the time of breeding) has the most influence on reproductive events among the captive North American river otter population. Future work should strive to identify other variables related to physiological condition and/or genetic variation between North American river otter subspecies. These factors may lead to more discreet groups and will likely aid in the development of a predictive model of parturition timing among the captive population using known life history data.

Introduction

Natural history of North American river otters

North American river otters (*Lontra canadensis*) are members of the Mustelidae family in the Carnivora order and belong to the suborder Caniforma. The Mustelidae family consists of 22 genera and over 50 species and includes weasels, badgers, and otters. Fossils of the earliest known mustelids date back to the early Oligocene and were found in Eurasia. Mustelids are characterized by their prominent anal scent glands, as well as by their elongate body shape and short legs. Mustelids have well-developed carnassial teeth and all extant species have no more than one molar after the carnassial teeth. Most genera exhibit induced ovulation and delayed implantation during reproduction (Feldhamer et al., 2015b). Of the over 53 mammalian species known to exhibit delayed implantation, nearly half are mustelid species (Renfree & Shaw, 2000; Sandell, 1990). The Lutrinae is a recognized subfamily of the Mustelidae and encompasses the seven genera and twelve defined otter species (Feldhamer et al., 2015b). There are seven recognized subspecies of *L. canadensis*, although definitions of each vary (AZA Small Carnivore TAG, 2009).

Morphology, behavior, and diet

North American river otters are streamlined, yet stocky, mustelids with a muscular form. The body shape is generally like that of a weasel, although the neck and head are of similar widths and the hips are the broadest point of the otter's body (Larivière & Walton, 1998; Melquist et al., 2003). River otters have five toes with nonretractile claws on each foot and are highly dexterous with sensitive paws (Park, 1971). Their tapered tails are long, forming a third of an adult's total body length, which may be between 98 and 113 cm (Lowery, 1974). Adult North

American river otters weigh between five and fourteen kilograms and males are up to 17% larger than females (Jackson, 1961; Melquist & Hornocker, 1983). The size of both sexes appears to vary among regional populations (Boyle, 2006).

The river otter is built for aquatic strength and speed, with short and muscular legs and fully webbed toes. The long tail acts as a source of both power and maneuverability in the water (Tarasoff et al., 1972). Their maximum reported diving depth is 20 meters, and swimming speeds of up to 11 kilometers per hour have been observed (Hamilton, 1943; Jackson, 1961). River otters have shortened tracheas and decreased lobulation in the lungs, most likely as adaptations for air exchange while diving (Tarasoff & Kooyman, 1973a; Tarasoff & Kooyman, 1973b). North American river otters can remain submerged for up to 4 minutes at a time while traveling underwater (Harris, 1968; Jackson, 1961).

River otters are typically considered top-order carnivores with no aquatic predators and little competition (Boyle, 2006). However, terrestrial predation upon river otters is somewhat common. Felids, canids, and birds of prey have all been observed consuming river otters (Mach, 1985; Melquist & Dronkert, 1987; Melquist et al., 2003; Melquist & Hornocker, 1983; Route & Peterson, 1991).

North American river otters are primarily ambush predators, but have been known to occasionally pursue prey (Park, 1971). Prey is consumed both at the surface of the water and on land, with individuals typically taking catches of larger fish onto land prior to consumption (Chanin, 1985; Park, 1971). Cooperative foraging has been observed, although this does not appear to fully replace solitary foraging behavior despite the resulting increased forage efficiency (Beckel, 1990; Blundell et al., 2002a; Serfass, 1995).

Fish make up the bulk of the North American river otter diet, although river otters are opportunistic foragers and will consume nearly anything available to them (Boyle, 2006; Greer 1955; Melquist et al., 1981; Toweill, 1974). These supplemental prey sources include mollusks, insects, birds, fruits, crustaceans, amphibians and small mammals such as muskrats (Gilbert & Nancekivell, 1982; Hamilton, 1961; Knudsen & Hale 1968; Melquist et al., 2003; Melquist & Hornocker, 1983; Morejohn, 1969; Reid et al., 1994a; Verbeek & Morgan, 1978; Wilson, 1954). Crayfish replace fish as the mainstay of the North American river otter diet in areas where they are more abundant or more readily available (Grenfell, 1974; Malville, 1990; DePue, 2002). Preference for particular fish species appears to be related foremost to availability, followed by ease of capture (Toweill & Tabor 1982; Melquist & Hornocker, 1983). This has led to some observed selectivity in predation upon adult fish; due to their size, adult fish are less able to escape pursuant predators (Erlinge, 1968). In some cases, North American river otter individuals have been observed moving to streams or lakes during trout and salmon spawning runs, presumably to take advantage of the abundance of adult fish (Melquist & Hornocker, 1983; Reid et al., 1994b).

Compared to the majority of the mustelid taxa, river otters are fairly social animals. While individuals are often solitary in the wild, North American river otters do show social plasticity and will form cooperative groups for purposes of reproduction or improved foraging (Blundell et al., 2002b). These groups forage and travel together, sharing dens and latrine sites (Beckel, 1990; Shannon, 1989; Reid et al., 1994b). Typically, a cooperative unit consists of an adult female and her offspring born within the last year (Melquist & Hornocker, 1983). Males sometimes aggregate in groups of up to 21 individuals in coastal systems for the purposes of cooperative foraging on schooling fish (Blundell et al., 2002a; Shannon, 1989; Shannon, 1991).

Instances of males forming social groups in inland environments, as well as mixed-parentage groups of juveniles, have been reported (Larivière & Walton, 1998; Melquist & Hornocker, 1983; Shannon, 1989).

Range and habitat

Historical records from trappers and naturalists indicate that the North American river otter once inhabited nearly every major watershed in Canada and the continental United States (Hall, 1981; Melquist et al., 2003). In particular, the species was abundant along the coast of the Pacific Northwest, in the marshes along the Atlantic coast, in the Great Lakes basin, and throughout New England (Melquist & Dronkert, 1987; Melquist et al., 2003). River otters also occupied most suitable habitats within the continental interior (Boyle, 2006).

The North American river otter appears to be capable of occupying all aquatic habitats with permanent access to freshwater and fish or crustacean prey stocks. Today, river otters are found throughout the North American continent, including in marine coastal areas, marshes, and streams spanning a range of ecosystems, from arid scrubland to subalpine forests (Toweill & Tabor 1982, Larivière & Walton, 1998). Regardless of habitat location or type, North American river otter populations appear heavily reliant on high water quality (Boyle, 2006). A survey of wild populations in west central Idaho defined a statistical preference for valley and stream habitats, rather than mountainous or pond-like areas (Melquist & Hornocker, 1983). In general, river otter populations are at their most dense in coastal habitats and low streams with little human disturbance and an abundance of food (Melquist & Dronkert, 1987; Melquist et al., 2003). Inland areas tend be more densely populated in lowlands and valleys with complexes of interconnected waterbodies (Melquist & Hornocker, 1983; Reid et al., 1994b). Urbanization and human activity have a strong dampening effect on river otter density in otherwise desirable habitats (Melquist et al., 2003).

North American river otters also require riparian vegetation and structures such as rocks or fallen trees as part of their habitat. These items provide protective cover and shelter while also increasing bank stability, aquatic nutrients, and prey populations (Boyle, 2006; Melquist & Dronkert, 1987). These complexities in the surrounding terrestrial environment are also critical for the establishment of latrine sites, which are used for scent marking and communication among individuals occupying overlapping ranges (Melquist & Hornocker, 1983; Newman & Griffin, 1994). In addition, wild otters rely on existing dens or natural shelters for breeding (Melquist & Hornocker, 1983). Beavers, through their own activities, provide many of the structures required for river otters to successfully occupy an area and, where the ranges of the two species overlap, river otters prefer habitats with beaver populations over those without (Liers, 1951; Malville, 1990; Melquist & Hornocker, 1983; Reid et al., 1994b). In regions where inland water bodies freeze, winter ice leads to severe restriction of North American river otter home ranges and increased dependence on beaver-constructed dams and dens (Hamilton, 1943; Reid et al., 1994b).

River otters are non-migratory, but can disperse for wide-ranging distances in search of food or more desirable habitat (Larivière & Walton, 1998; Jackson, 1961). Dispersal patterns for the species have not been related to population density (Melquist & Hornocker, 1983). On a daily basis, river otters may travel up to five kilometers, although in some cases individuals have covered distances of up to 42 km in a single day (Melquist & Hornocker, 1983; Reid et al., 1994b). Individuals establish non-discrete home ranges on a yearly basis, with males claiming larger ranges than females. Adult females may occupy 30 to 58 km of waterway compared to home ranges of 50 to 80 km of waterway claimed by each adult male (Melquist & Hornocker, 1983). Average home range size appears to be highly variable by region and season, with some individuals occupying ranges as small as just five km (Mack, 1985; Malville, 1990). In addition, less overlap in territory is observed through the winter months (Bowyer et al., 1995; Mack, 1985; Reid et al., 1994b).

North American river otter individuals typically are most active at night, although they have been shown to exhibit more diurnal behavioral patterns in the winter months, regardless of prey availability (Larivière & Walton, 1998; Mack, 1985; Melquist & Hornocker, 1983). However, this shift in activity level may be limited to populations in the Rocky Mountains, which in some cases are considered a subspecies (Hamilton, 2013; Mack, 1985; Melquist & Hornocker, 1983).

Reproduction

Female North American river otters typically become sexually mature at around fifteen months of age, although most do not breed until two years of age. Males also reach breeding maturity at two years, but often do not breed until older. (AZA Small Carnivore TAG, 2009; Hamilton & Eadie, 1964). At a range-wide level, river otters breed from December to April (Larivière & Walton, 1998). However, breeding of any individual population typically peaks over the course of 2 to 3 months within that timeframe (Fitzgerald et al., 1994; Melquist & Hornocker, 1983). River otters are polygynous, with each male tracking the scent trails of females in heat and attempting to mate with several throughout the breeding season (Fitzgerald et al., 1994). The estrus cycle lasts for 42 to 46 days, but females may have several days of reduced receptivity to copulation within that timeframe (AZA Small Carnivore TAG, 2009; Hamilton & Eadie, 1964; Hamilton and Sullivan, 2015).

To mate, male otters approach females and attempt to mate while holding the female by the scruff of the neck and trying to pin her (Liers, 1951; Towell & Tabor, 1982). Copulation may take anywhere from 16 to 73 minutes and has been reported to occur both in water and on land (Liers, 1951; Shannon, 1991). An observational survey of wild Eurasian river otters (*Lutra lutra*) revealed a marked preference for copulation in the water, suggesting to Kruuk (2006) that, while otters are capable of copulating on land as they frequently do in captivity, they may only do so when there is insufficient aquatic space. Kruuk (2006) also observed an instance of courtship feeding, a behavior that may merit further assessment in North American river otters as such behavior may contribute to increased familiarity between individuals and increased female receptivity of a potential mate.

Implantation of an embryo is not immediate in North American river otters, as embryonic diapause is typical and generally considered a key element of their reproductive cycle (Boyle, 2006; Hamilton & Sullivan, 2015). However, some southern populations have been reported to breed without delayed implantation (Melquist & Dronkert, 1987). Gestation lasts around 60 days after implantation; delayed implantation results in parturition taking place 10 to 12 months after copulation (Hamilton & Eadie, 1964; Liers, 1951). Females typically seek out isolated and cryptic locations within their home range to give birth, often choosing dens made by other animals within a few hundred feet of a body of water (Boyle, 2006; Melquist & Dronkert, 1987; Melquist & Hornocker, 1983).

Litters typically consist of one to three pups in late winter or early spring, although litters of up to five pups have been recorded (Docktor et al., 1987; Hamilton & Eadie, 1964; Hamilton and Sullivan, 2015; Park, 1971; Serfass & Polechla, 2008; Tabor & Wight, 1977). Pups are born blind and toothless, although they have full pelts, and are nursed for the first 12 weeks (Boyle, 2006; Larivière & Walton, 1998; Shannon, 1989; Liers, 1951). Females are the sole caregivers and will care for their offspring until the pups are approximately 38 weeks of age, during which time the pups are taught how to forage and survive on their own (Shannon, 1989; Shannon, 1991). Pups may remain with their family groups for the first 12 to 13 months before dispersing up to 200 km, and as little as 15 km, from their birth dens over the next three months (Blundell et al., 2002b; Melquist & Hornocker, 1983).

Delayed implantation

Delayed implantation is a variation of reproduction in which the development of the offspring is halted after the zygote cleaves into a blastocyst. The blastocyst remains suspended in the reproductive tract until conditions become favorable for implantation, at which point development continues as normal. This process is obligate in some species and facultative in others and occurs in a wide variety of taxa (Feldhamer et al., 2015a). Delayed implantation increases the time between mating and parturition and is typically seasonal, resulting in implantation occurring at approximately the same time in all females of a population (Mead, 1989; Sandell, 1990). Photoperiod is thought to be the primary environmental factor cuing implantation among the majority of species that exhibit this phenomenon (Mead, 1989).

The adaptive function of delayed implantation is poorly understood and may vary among taxa. Five main hypotheses have been proposed: mating is constrained to a specific season due to food resources and a normal gestation period would result in offspring being born in a season with poor food resources such that offspring survival would be nearly impossible (Fries, 1880);

parturition timing is fixed to allow offspring maximum developmental time prior to overwintering, leading to mating taking place in winter and reducing reproductive success due to reduced physiological condition of the parents (Prell, 1930); delayed implantation has no adaptive function (Hamlett, 1935); delayed implantation evolved to limit population size by reducing the number of potential litters born annually in species that otherwise might deliver multiple litters in a year (Heidt, 1970) (although this hypothesis is largely discarded as a possible explanation as it has not held up to deeper research (Williams, 1966)); and delayed implantation evolved in carnivores such that mating occurs when animals are at their physiological prime and parturition occurs when resources for rearing offspring are at maximum (Mead, 1989; Sandell, 1990). Understanding the role of delayed implantation is further complicated because of its patchy occurrence within taxa. Even closely-related, ecologically-similar species may not both exhibit delayed implantation (King, 1984; Mead, 1981; Sandell, 1984). Studies of evolution of delayed implantation in caniform carnivores generally support a basal position of the trait and subsequent losses as fecundity costs for individual species became too much to maintain delayed implantation (Lindenfors et al., 2003). Phenotypic plasticity of the trait has also been indicated as some mustelid fertilized eggs have been able to survive lab-induced delayed implantation (Foresman & Mead, 1978).

Development and maintenance in mustelids

Work assessing the evolution of delayed implantation within the Mustelidae supports the idea that, among closely related species, delayed implantation is more common in seasonal climates and long-lived species (Thom et al., 2004). Interestingly, the results of Thom et al.’s study (2004) support multiple instances of evolution for delayed implantation, conflicting with

Lindenfor et al.’s study (2003). However, Thom et al.’s (2004) data utilized only the Mustelidae, while Lindenfor et al. (2003) looked at the entirety of the caniforms.

In general, the proximate benefits of delayed implantation are thought to be species-specific while the overall adaptive value is likely linked to the resulting time separation in mating and parturition such that food resources are at peak availability for both events (Thom et al., 2004).

AZA husbandry guidelines for captive North American river otters

The AZA Otter Care Manual is used as a guide for standard care among all AZA-affiliated institution and was formed through the review of research of *in situ* and *ex situ* animals (AZA Small Carnivore TAG, 2009). Thus, any study of the AZA captive river otter population can assume that all study individuals were kept under the same range of conditions outlined here.

Habitat and diet

Outdoor North American river otter habitats should include covered areas to protect animals from the sun and indoor habitats should be kept between 10 and 24°C (AZA Small Carnivore TAG, 2009; Reed-Smith, 2004a; Wallach & Boever, 1983). Dry land and well-ventilated nest sites are also an important part of creating a healthy captive habitat (AZA Small Carnivore TAG, 2009). AZA-approved habitats consist of a variety of substrates, primarily natural, that allow captive otters to engage in natural activities such as digging and grooming (AZA Small Carnivore TAG, 2009). Indoor areas should utilize an artificial light-cycle that mimics the natural photoperiod of the area where each holding institution is set to preserve natural behaviors (AZA Small Carnivore TAG, 2009; Bateman et al., 2009).

Space allotment for captive individuals should consist of a ratio of 4:1 land/water area, although a 3:1 ratio is adequate if the exhibit is large with structures of varying heights and hardness (AZA Small Carnivore TAG, 2009; Reed-Smith, 2001; Reed-Smith, 2004a). The minimum area allotment is 150 square meters for a pair of otters, with 25 square meters of land and 10 square meters of water needed per additional animal (Duplaix-Hall, 1975; Reed-Smith, 2004a).

Captive river otters should be provided with meals at least three times a day and up to five times daily. Some portion of the food should be placed throughout the exhibit to encourage natural foraging behaviors. A mix of freshwater fish species, prepared feline diet, carrot, and animal bones are some of the recommended elements of a complete otter diet (AZA Small Carnivore TAG, 2009).

Breeding

All births of North American river otter individuals in AZA-institutions are reported to the studbook keeper, currently David Hamilton, General Curator at the Seneca Park Zoo. The document also contains all known information regarding pedigree, origin, transfers, and health of each captive individual. This data is used to monitor the genetic diversity of the captive population and to make breeding and transfer recommendations to institutions that currently have, or are looking to have, a North American river otter exhibit (AZA Small Carnivore TAG, 2009). If a breeding recommendation requires the movement of individuals between institutions, it is suggested that transfers occur one year prior to breeding efforts. This is meant to address the possibility that latitude impacts the timing of reproductive events (AZA Small Carnivore TAG, 2009; Bateman et al., 2009).

In mixed-sex captive groups, contraception is recommended when breeding is not desired. When breeding is desired, the pair are to be kept together after successful introduction and provided with adequate cover and time without disturbance. The Otter Care Manual notes that copulation may take up to 60 minutes and will most frequently take place in the water. Copulation should not be interrupted to increase the likelihood of successful fertilization (AZA Small Carnivore TAG, 2009). A pair may attempt copulation multiple times while the female is in estrus (Reed-Smith, 2004b).

Due to delayed implantation, female behavioral changes and differences in appetite may be the only indicators of an approaching parturition event. In the days prior to parturition, females often show increased aggression towards any males in their enclosure. This aggression typically continues for a short time after the pups are born. The AZA recommends that pairs be separated and that any transfer between indoor and outdoor exhibit areas occur such that neither individual can see the other (AZA Small Carnivore TAG, 2009). It is considered valuable to understand when females may be approaching their respective parturition dates such that husbandry practices may be adapted to minimize stress for all otters in shared exhibit spaces.

Research of reproductive events

Captive research studies on North American river otters have found that geographic latitude influences progesterone levels in females and may have an impact on testosterone concentrations in males (Bateman et al., 2008). Later studies have indicated that no correlation exists between the timing of progesterone increases in North American river otter females and the latitude during the pregnancy, but a slight correlation exists with the latitude at which the female was born. Male testosterone spikes peak based on the latitude of their current location, but seem more directly related to photoperiod (AZA Small Carnivore TAG, 2009). This may impact translocations and reintroductions among wild populations as a genetic component to reproductive timing may cause females to have reduced fitness if they are delivering pups at a disadvantageous point in the season for resource support. To date, no overarching study assessing reproductive success in captive or reintroduced populations has been published.

North American river otter conservation

North American river otters have historically been a species of concern due to significant population declines and regional disappearances from a combination of heavy trapping pressures, habitat destruction, and poor water quality (Serfass & Polechla, 2008). By the early 20th century, extirpations had occurred throughout much of the historical North American river otter range (Lariviere & Walton, 1998; Melquist & Dronkert, 1987; Melquist et al., 2003; Nilsson, 1980; Raesly, 2001; Stevens et al., 2011).

Protection and management of wild populations

The Clean Water Act of 1972 and the establishment of trapping limits and bans have led to widespread wild recovery of wild populations (Melquist et al., 2003 Raesly, 2001;). In 2008, North American river otters (*Lontra canadensis*) were declared to be of least conservation concern by the International Union for Conservation of Nature (IUCN). Modern management practices as well as reintroductions and habitat restoration had increased the overall population to stable levels (Melquist et al., 2003; Polechla, 1990; Serfass & Polechla, 2008). The total wild population in North America likely is in excess of 100,000 individuals based on the reported size of trapping harvests in the last few decades (Boyle, 2006; Melquist et al., 2003). Still, some state- and municipal-level populations of North American river otter are of interest to conservation programs and the wisdom of reintroductions in light of the currently stable genetic structures in many populations has been questioned (AZA Small Carnivore TAG, 2009; Serfass & Polechla, 2008).

To date, reintroduction efforts have been undertaken in 22 states and one Canadian province, with general success (Melquist et al., 2003; Raesly, 2001; Spinola et al., 2008). Of the 22 participating states, 14 used North American river otters captured from Louisiana populations due to the high population density at the time the programs began (Melquist et al., 2003; Raesly, 2001). However, recent research using multiple genetic markers has identified three subpopulations within Louisiana that group by occupied region (Latch et al., 2008). Latch et al. noted that breeding seasons differed between the subpopulations, which may be a result of differences in diet by proximity to the coast or strictly by population access to freshwater (2008). It has been suggested that these differences, having not been considered originally, may impact the success of reintroduction and captive breeding programs, as well as species-level genetic diversity, over generations (Brandt et al., 2014).

There is evidence that among carnivores, such as the North American river otter, the reintroduction of wild-caught individuals has significantly higher success in terms of survival rate than the reintroduction of captive-born individuals (Jule, Leaver, & Lea, 2008). Currently, the AZA supplements the captive breeding population with wild-caught individuals, particularly rescued pups from rehabilitators and nuisance animals, to maintain healthy diversity as the captive population reproduces unpredictably (Hamilton, 2013). However, if improvements to captive breeding programs could be made to increase and maintain a predictable level of reproductive success, the captive population may be able to maintain genetic diversity without relying on introduction of wild-born individuals. These wild-born individuals may then better serve conservation goals through carefully considered rehabilitation and translocation to support threatened wild populations.

AZA Species Survival Plan

In February of 2000, the Association of Zoos and Aquariums (AZA) established a Population Management Plan (PMP) and began keeping a studbook for North American river otters. This program later developed into a Species Survival Plan (SSP), including breeding recommendations for maintenance of the captive population. The North American river otter population is currently managed as a Green-level SSP, meaning the population is demographically sustainable for more than 100 years or 10 generations. The captive population reached green status in 2011. As of 2015, the SSP included 282 individual otters at 111 participating AZA-member or affiliated institutions. In the first fourteen years after the studbook was begun, just 47 individuals, less than one-fifth of the captive population, had been successfully bred (Hamilton and Sullivan, 2015).

Recent research indicates that geographic latitude may influence the timing of breeding behaviors in North American river otters, with more southern populations breeding in winter while northern populations breed later in the spring season (AZA Small Carnivore TAG, 2009; Bateman *et al.*, 2008). Differences among populations as a result of geography may occur at a very small scale, as three unique populations were observed to have distinctive breeding seasons just within the state of Louisiana (Latch *et al.*, 2008). Current attempts to account for these variations in breeding practices sometimes involve transferring individual otters from different latitudes one season prior to any breeding attempts (AZA Small Carnivore TAG, 2009).

Research goals

In developing plans for a healthy and stable captive population, as well as management of wild populations of North American river otters, geographical differences and potential subspecies classifications need to be carefully considered. It is critical that any variables with significant influence on reproductive fitness and timing be understood and accounted for in making breeding, transfer, and relocation recommendations. Knowledge of these variables and their relative impacts upon the reproductive cycles of river otter populations can then be used to improve upon current husbandry and wildlife management practices.

This study focused on a statistical analysis of historical birth records to assess whether or not latitude has a significant influence on parturition timing or litter demographics in the AZA's captive population of North American river otters. The latitudes of record examined included the latitude of origin for each dam and sire, as well as the latitude at which each parturition event occurred during the study period. Other factors thought to relate to reproductive fitness, as described in Table 1, were studied to identify any potential correlations within the studbook. These included the sex ratio of resulting pups and survivorship of pups to sexual maturity. Had any of these additional variables been found to correlate to each other or to the timing of reproductive events, valuable research questions would have been identified for future work. Based upon previous research, I hypothesized that the birth latitude of the dams would be the most significant factor influencing parturition date and predicted that parturition date would change along a latitudinal gradient of the dams' birth latitudes, with more southern-born dams having earlier parturition dates than their northern counterparts.

The results of this research may better inform management and husbandry practices among AZA institutions by investigating suspected relationships between latitude and the timing of reproductive events in North American river otters (Bateman et al., 2008). Such knowledge would assist the AZA in management of a genetically diverse captive population independent of future additions of wild-caught individuals. Identification of significant factors influencing reproductive success and parturition timing among captive North American river otters will have the potential to change how breeding and transfer recommendations are made and should lead to more successful mate pairings in the future. This research also has the potential to change how future reintroduction efforts are managed. If geographic origin is found to be related to parturition timing, it is likely that wild populations experience differences in survivorship among litters as an effect of when they are born. This may be the result of differences in resources or threats and, regardless of causation, would make the appropriate selection of captive individuals to recolonize depopulated areas critical for ensuring the long-term success of future populations.

Methods

Data collection

The study assessed the official AZA Studbook records for all North American river otter captive-bred births, as well as intakes of wild-born pups, from the 2008 breeding season through the season of 2013, as provided by the AZA Species Survival Plan Coordinator and Studbook Keeper, David Hamilton. Studbook records were accessed through PopLink, a Dbase software, and formatted for analysis using a Pearl script to extract data from the standard PopLink reports. To accurately compare differences in parturition dates across the multi-year study, and to capture early-winter litters in the same breeding year as pups born in spring of the same breeding season, parturition dates were converted to a Julian day count, with October 1 of the previous year as the first day of the breeding season. For example, litters considered part of the 2014 breeding season were all litters with parturition dates from October 1, 2013 to September 30, 2014. The birthdates of both the dams and sires were similarly converted to a day count from October 1 of the previous year.

The dataset consisted of 46 litters, with 32 dams and 30 sires. Origin data was unavailable for six dams and nine sires, including the case of one litter captured as pups. In addition, one female captured during the study period was pregnant at the time of capture. Data regarding transfers was only available for 13 dams and 14 sires. As it is not yet fully understood which variables are relevant to determination of parturition timing, each variable listed in Table 1 was assessed for each parturition event. Survivorship of offspring one and two years after parturition was assessed to confirm the assumption that survivorship would not vary significantly among captive populations due to the use of standardized care across all AZA institutions.

In cases where a parent or litter was wild-caught, the latitude of origin was recorded as the first AZA institution recorded, provided that institution was in the same state where the capture occurred. In cases when the institution of record was outside the state of capture, the latitude for the center point of the state of capture was used as the best approximation available for the latitude of origin.

Statistical Testing

Statistical tests were performed using Minitab 17.0 and followed the conceptual approach as discussed by Pagano and Gauvreau (2000). Principal components analyses were performed using the analytic process explained by Harlow (2014) and were produced through Minitab 17.0.

All variables in Table 1 were tested for normality using the Anderson-Darling normality test. All but three variables were found to follow a non-normal distribution: latitude of the dam's birth site (p-value = 0.349), latitudinal distance from the dam's birth site to the parturition site (p-value = 0.117), and the day count since the sire's last transfer (p-value = 0.064). Due to the non-normal nature of the majority of the dataset, a Spearman's rho matrix was used to determine if any variables had significant monotonic relationships to each other.

A principal components analysis was created using a Varimax orthogonal rotation from a correlation matrix, as described by Harlow (2014). The PCA was run with two principal components after review of the eigenvalues for each component (Table 3) and the scree plot (Figure C-1). While the first three components had eigenvalues greater than 1.0, a leveling off in eigenvalue occurred after the second component (Figure C-1), making it reasonable to exclude the third component from analysis (Harlow, 2014). All variables in the dataset with eigenvectors of at least |0.30| along one or more components were used: season day count of parturition, season day count of dam’s birth, dam’s birth latitude, sire’s birth latitude, season day count of sire’s birth, latitude of parturition, proportion of males born, and litter count at birth (Table 4).

Due to records with missing data for one or multiple studied variables, the sample size for the PCA was 33 litters. All litters were assigned a unique identifier based upon the origin latitude of the dam. These identifiers ranged from “A-Z”, followed by “a-t”, with the litter birthed by the dam from the southernmost latitude assigned “A” and the litter birthed by the dam from the northernmost latitude assigned “t” (see Table A-1 for a complete list of litters by code).

The data were further tested using assigned groups. Parturition events were grouped three unique times as either “North” or “South” by sire’s birth latitude; dam’s birth latitude; and parturition event latitude. The threshold latitude for assigning regions was 37.00°N; all latitudes equal to or greater than 37.001°N were assigned to the North category, all latitudes less than 37.00°N were assigned to the South. The threshold latitude was chosen after results of the first PCA indicated independent grouping of the data followed divisions at or near this value. Cases where the latitudinal origin of at least one parent was unavailable were excluded from analysis in the PCAs. The assigned groups were used in Mann-Whitney U tests for differences in median season day count of parturition, ANOVA tests for differences in mean season day count of parturition when the data followed a normal distribution, and in Kruskal-Wallis H tests for differences in the median survival ratios two years after parturition.

Results

Spearman's Rho Testing

The Spearman's rho test was used to identify significant monotonic relationships (p-value $\leq$ 0.05) in the dataset. Table 2 summarizes the r_s and p-values for significant results related to the season day count of parturition, dam's birth, and sire's birth for each litter. Each of the identified relationships were positive in nature with r_s of at least 0.3. The relationship between the season day count of the sire's birth and the latitude of the sire's birth site was strong ($r_s = 0.667$; p-value = 0.000), while the relationship between the distance from the dam's last transfer site to the season day count of parturition was very strong ($r_s = 0.812$; p-value = 0.001). It is interesting to note that the Spearman's rho matrix did not identify a significant relationship between the season day count of parturition for litters born during the study period and the latitude at which these litters were born (p-value = 0.993).

The proportion of males born in each litter was found to be related ($r_s = 0.554$; p-value = 0.40) to the distance from the sire's last location (when not the sire's origin location) and the parturition event. A weak relationship ($r_s = 0.350$; p-value = 0.027) existed between the proportion of males born in each litter and the distance between the dam's origin and the parturition site. Lastly, the survival ratios of litters one year and two years after parturition were found to have a very strong relationship, with a r_s value of 0.933 and a p-value of 0.000.

For the complete matrix of results, see Tables B-1 through B-6.

Principal Components Analyses

The two principal components assessed in the PCA accounted for 59.7% of the total variance in the dataset. Along the first component, sire birth latitude and parturition latitude were the most influential factors (Figure 1). The second component was primarily driven by the proportion of male pups born in each litter (Figure 1). Grouping among the litters by parental origin is clearly seen in Figure 2, where litters with both parents originating from northern latitudes grouped to the right of the y-axis. Litters with a northern dam and a southern sire grouped below the x-axis and tended to group further to the right than litters with two southern parents. Litters B and D sorted the furthest left out of all the litters with two southern-born parents, although only litter j was an outlier (Figure C-2).

Mann-Whitney U Testing

As the data did not follow a normal distribution but shared a similar negative skew, a Mann-Whitney U test was used to compare the median season day count of parturition between litters with dams originating in the North and South regions, litters sired by males originating in the North and South regions, and litters born in the North and South regions.

The difference in median season day count of parturition was significant between litters with dams from the North and litters with dams from the South ($N_{North} = 27$, $N_{South} = 13$, p-value = 0.01). The point estimate of difference was 22 days, with a 95% confidence interval for the population difference of 5.00 to 39.99 days. There was also a significant difference between litters sired by males from the North and those sired by males from the South ($N_{North} = 18$, $N_{South} = 19$, p-value < 0.00). The point estimate of difference, by sire's region of origin, in median season day count of parturition was 31.50 days. The 95% confidence interval for the estimated population difference in median day count of parturition was 20.00 to 48.01 days.

The difference in median parturition day count was also significant when comparing litters based upon where parturition took place ($N_{North} = 42$, $N_{South} = 4$, p-value = 0.02). The point estimate of the difference in median parturition day count was 57.50 days. While the 95% confidence interval for the population difference in median season day counts of parturition was 17.00 to 86.01 days.

ANOVA Testing

Prior to utilizing the one-way ANOVA test, equal variances were tested using multiple comparison intervals for the season day count of parturition by dam region of origin, sire region of origin, and parturition region, as assigned by this author. The dam region of origin and the parturition region were found to have equal variances (a test of difference in variance resulted in p-value = 0.839 and p-value = 0.658, respectively). However, the season day count of parturition as grouped by the sire's assigned region of origin was found to have unequal variance, with a p-value of 0.028. Thus, differences in median season day count of parturition by sire region of origin was tested using a Kruskal-Wallis H test.

An ANOVA test for differences in mean season day count of parturition between dam's originating in the north or south regions found a significant difference ($F_{1,38} = 6.09$, p-value = 0.018). A second ANOVA test for differences in mean season day count of parturition between parturition regions was also significant ($F_{1,44} = 12.73$, p-value = 0.001). Figure 3 shows the mean season day count of parturition and standard deviation for each group.

Kruskal-Wallis H Testing

As noted earlier, the difference in median season day count of parturition by sire origin region was tested using the non-parametric Kruskal-Wallis H test. A significant difference in parturition timing was found ($H_1 = 18.77$, p-value = 0.00) by sire origin.

A Kruskal-Wallis H test was also used to compare the median survival ratios of litters two years after parturition by whether litters were born in the assigned regions of North and South, whether the dams were born in the North or South regions, and by whether the sires were born in the North or South regions. When adjusted for ties, the results of each test were insignificant ($H_1 = 0.09$, p-value = 0.769; $H_1 = 0.00$, p-value = 0.976; and $H_1 = 2.59$, p-value = 0.107, respectively).

Discussion

Does parturition timing vary by parental origin or present location?

Despite the suspected relationship between the origin of a North American river otter litter's dam and that litter's parturition date, this study did not find any significant linear relationship between these variables in the studbook records. However, significant monotonic relationships were found to exist among the dataset. It is important to note that in the case of each parent, a stronger relationship was found to exist between their birth latitudes and their own season day counts of birth than was found to exist among those same factors and their offspring. This may have resulted from the uneven latitudinal distribution of AZA facilities that successfully bred North American river otters during the study period. A majority of captive-born litters were born north of 35°N, while the distribution of breeding males and females during the study period were more evenly spread across a latitudinal gradient. Repetition of this study with a larger dataset, such as one with AZA facilities outside of North America (should they exist) or with additional years of studbook records, would likely minimize this problem. At this time, there were insufficient data to develop a useable model for predictions of parturition timing in the captive population.

Efforts to identify distinctive regional differences in parturition dates did reveal some differences in parturition timing. The mean parturition dates of each litter varied significantly by the origin region of the dams, as well as by the region in which parturition occurred, while the median parturition dates were significantly different by sire origin region. Mann-Whitney U tests revealed further differences between median parturition dates by sire origin, dam origin, and parturition location. These results provide further support for the idea that the latitudinal origins of individual female river otters have major impacts on the timing of reproductive events within the species (Bateman et al., 2008). Such a relationship may be explained by a genetic component to the timing of implantation among river otters, which may exist as a result of differential reproductive fitness among females by parturition date in various parts of the species' range (Mead, 1989; Sandell, 1990). It has also been suggested that variations in diet and habitat among wild populations may influence the timing of reproductive events and should be further investigated on a regional scale (Latch et al., 2008).

While this study has contributed to the body of evidence tying North American river otter reproduction to geography, I was unable to conclusively determine which geographical data has the greatest influence on parturition timing. The use of artificially designated categories for comparing regional differences, rather than the use of subspecies classifications made it impossible to determine whether a genetic component is driving the perceived differences.

Do any recorded factors influence litter survivorship?

Survivorship of litters both one and two years after parturition were found to be strongly correlated, indicating that once a litter makes it through the first year, survivorship until sexual maturity is not of further significant concern. As expected, there was no significant difference among mean ranks of survival ratios for litters one year after birth between regions. This supports the idea that consistent husbandry practices across all North American AZA institutions have resulted in equal survivorship of NARO pups, regardless of birthplace.

While survivorship had no influence on the PCA, the second principal component was most strongly driven by the proportion of males born in each litter (Table 4 and Figure 1). It would be interesting to study how the physiological fitness of captive-born offspring may relate to each of these factors tied into the second principal component. Body weight or size, as well as growth rate, of offspring are all valuable factors that may affect parturition timing or be indicative of parental health at the time of breeding. In the absence of such physical data, survivorship and sex ratio were used to approximate these metrics of health. Thus, it can be concluded that survivorship for assessing the quality of captive care is not a concern, but the use of survivorship data in determining breeding success may yet have both academic and practical value.

Do the data form independent groups?

The PCA included in this study was run using unassigned groups and later given symbols to denote the origins of the parental pairings. As shown in Figure 2, litters formed complex groups based upon the interaction of each parent's origin. Litters with two southern-born parents grouped towards the lower end of the first component, while those litters with two northern-born parents grouped towards the higher end of the first component. The eigenvectors of each variable included in the PCA (Table 4) suggest the size of each litter and the timing of each parent's birth may have a greater influence than has been previously speculated on in the current literature.

While the data do form independent groups, the driving force behind the sorting of the data remains unclear as these independent groups are not fully exclusive to each other. Genetic diversity, subspecies classifications, or environmental impacts on reproductive development are all possible explanations for the phenomena and further research into each of these is needed to understand what is likely a complexity of interactions.

Limitations of the data

Limitations of the dataset likely had a large impact on the outcome of statistical testing. Of 46 litters analyzed, only four were born at latitudes below 37.00° N. Out of the 32 dams, 14 gave birth multiple times during the study period. Dam 2054 was the most prolific, giving birth to four litters. Only 11 dams were born south of 37° N, accounting for just over one-third. Of the 30 sires in the study, 14 were born south of 37° N, accounting for slightly less than one-half of all sires. Sires 2147 and 2149 each sired 4 litters during the study period. Origin data were missing from three dams and eight sires, limiting the sample size.

In addition, none of the data included in this study carried a physiological fitness component beyond age. Health of an individual animal would likely have influence over litter size and may have some influence on parturition timing. Weight, size, and even body fat percentage are all variables likely to have some influence on reproductive hormone concentrations, gamete numbers, and the ability of females to successfully carry multiple fetuses to term. Inclusion of such data could only improve the monitoring of captive breeding programs and increase the likelihood of developing useful models to predict the timing of key reproductive events, such as peak sperm production, ovulation, and parturition.

Future research

It would be of great future interest to identify the degree of genetic variance among the subspecies of North American river otter. This may prove to be a more precise and consistent predictor of parturition timing than latitude and could be used to vastly improve analysis of data using assigned categories.

Future work should strive to quantify the differences in reproductive success between breeding pairs based upon where both the male and female were born and have since been kept. While the results of this study indicate that no harm is being done to captive breeding efforts by transporting individuals for recommended pairings in the form of either changes to parturition timing or sex ratios and survivorship of resulting litters, understanding how the recommendations themselves influence reproductive success would be immensely valuable.

Conclusion

Results of this study add to the body of evidence that latitude influences parturition timing, especially the latitude at which the dam was born. Future efforts expanding on this body of work will be critical to developing a more complete understanding of river otter reproduction physiology and behavior. More research will provide insight into how current practices may be adapted to improve the likelihood of successful breeding efforts with the AZA's captive population of North American river otters.

While this study did not succeed in development of a predictive model for parturition timing based on AZA records for North American river otters, several factors have been suggested as having influence on the timing of reproductive events among the captive population. The timing of implantation, and thus parturition, is unlikely to be solely driven by geography. Differences in the health and body composition of each parent, as well as in the diet of breeding females from the breeding season through gestation, likely have some influence on the timing of these events. Tracking these variables is therefore of great value to future researchers and may greatly contribute to the development of a model for improving husbandry practices at AZA institutions participating in North American river otter breeding programs.

Tables

Table 1. Study variables. The table lists all variables contained within the dataset for AZA North American river otter breeding recommendations and resulting litters for the 2008-2014 breeding seasons.

Latitude Variables	**Time Variables**	**Litter Variables**
Latitude of dam’s birth site	Season day count of dam’s birth	Litter size at parturition
Latitude of sire’s birth site	Season day count of sire’s birth	Sex ratio of litter at time of parturition
Latitude of parturition site	Season day count of parturition event	Survivorship of litter one year after parturition
Latitudinal distance from sire’s birth site to parturition site	Day count since sire’s last transfer	Survivorship of litter two years after parturition
Latitudinal distance from dam’s birth site to parturition site	Day count since dam’s last transfer	
Latitudinal distance from sire’s prior location (when not birth site) to parturition site	Age of dam at time of parturition	
Latitudinal distance from dam’s prior location (when not birth site) to parturition site	Age of sire at time of parturition	

Table 2. Spearman's rho results of interest. The table shows all significant monotonic relationships found to exist between season day counts of parturition, dam's birth, and sire's birth for each litter and the variables from Table 1 in white cells. Gray cells contain insignificant results.

		Day Count of Parturition	**Day Count of Dam's Birth**	**Day Count of Sire's Birth**
Latitude of parturition site	r_s	0.001	0.405	0.266
	p-value	0.993	0.006	0.097
Latitude of dam's birth site	r_s	0.400	0.591	0.128
	p-value	0.011	0.000	0.464
Latitude of sire's birth site	r_s	0.564	0.069	0.667
	p-value	0.000	0.685	0.000
Distance from dam's last transfer to parturition site	r_s	0.812	0.588	0.450
	p-value	0.001	0.035	0.123
Dam's age at parturition	r_s	0.331	0.009	-0.117
	p-value	0.026	0.955	0.471

Table 3. Eigenvalues of components in PCA. The table shows the eigenvalues for each of six components assessed by the correlation matrix PCA, as well as the percentage of total variance accounted for in each component. As indicated by the cumulative percentage of variance column, the two-component PCA accounted for 59.7% of the total variance within the dataset.

Component	Eigenvalue	% of Total Variance	Cumulative % Variance
1	3.1170	39.0	39.0
2	1.6557	20.7	59.7
3	1.0591	13.2	72.9
4	0.7875	9.8	82.7
5	0.6813	8.5	91.3
6	0.4398	5.5	96.8
7	0.1635	2.0	98.8
8	0.0960	1.2	100.0

Table 4. Eigenvectors of variables included in PCA. The table shows the eigenvectors for each factor in the two principal components assessed as part of the PCA. The first component is associated with the x-axis, while the second component is associated with the y-axis. These relationships are visualized in Figure 1 as the loading plot.

Variable	**Component 1**	**Component 2**
Season Day Count of Parturition	0.337	0.230
Season Day Count of Dam's Birth	0.391	-0.412
Dam Birth Latitude	0.406	-0.350
Sire Birth Latitude	0.441	0.266
Season Day Count of Sire's Birth	0.405	0.292
Latitude of Parturition	0.441	-0.146
Proportion of Males Born	-0.013	-0.590
Litter Count at Birth	0.129	0.360

Figures

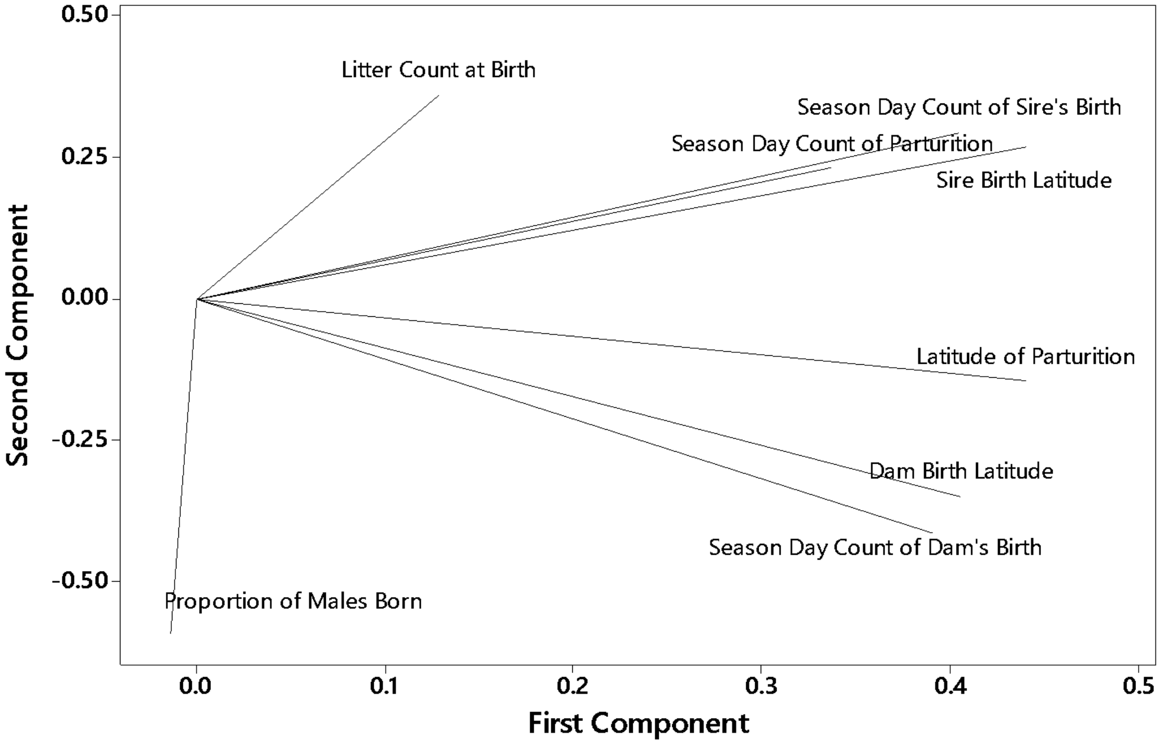

Figure 1. Loading plot of two-component PCA. The above figure shows the loadings for the factors influencing each of the two components in a Varimax orthogonal rotation. For a quantitative assessment of the influence of each factor on each component, in the form of the eigenvectors, see Table 4.

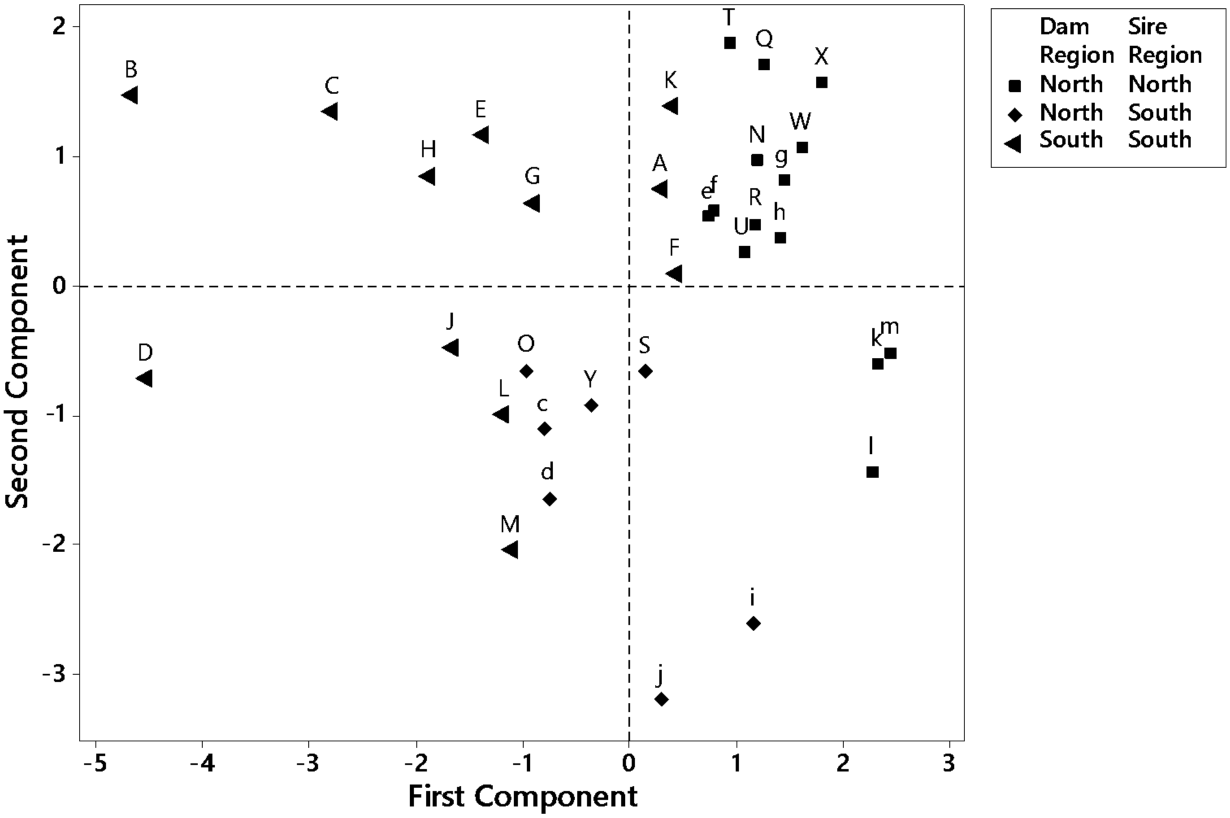

Figure 2. Score plot of two-component PCA. The above figure shows the score plot for the PCA, with each litter record (n = 33) coded according to Table A-1. Categorical groupings were assigned to each parents' origin region using a threshold value of 37.0°N. Parents originating from latitudes less than 37.0°N were considered to be from the "South" region and those from 37.0°N and above were considered to be from the "North" region. The litters were then each assigned a symbol corresponding to the origin of both parents, as explained in the legend.

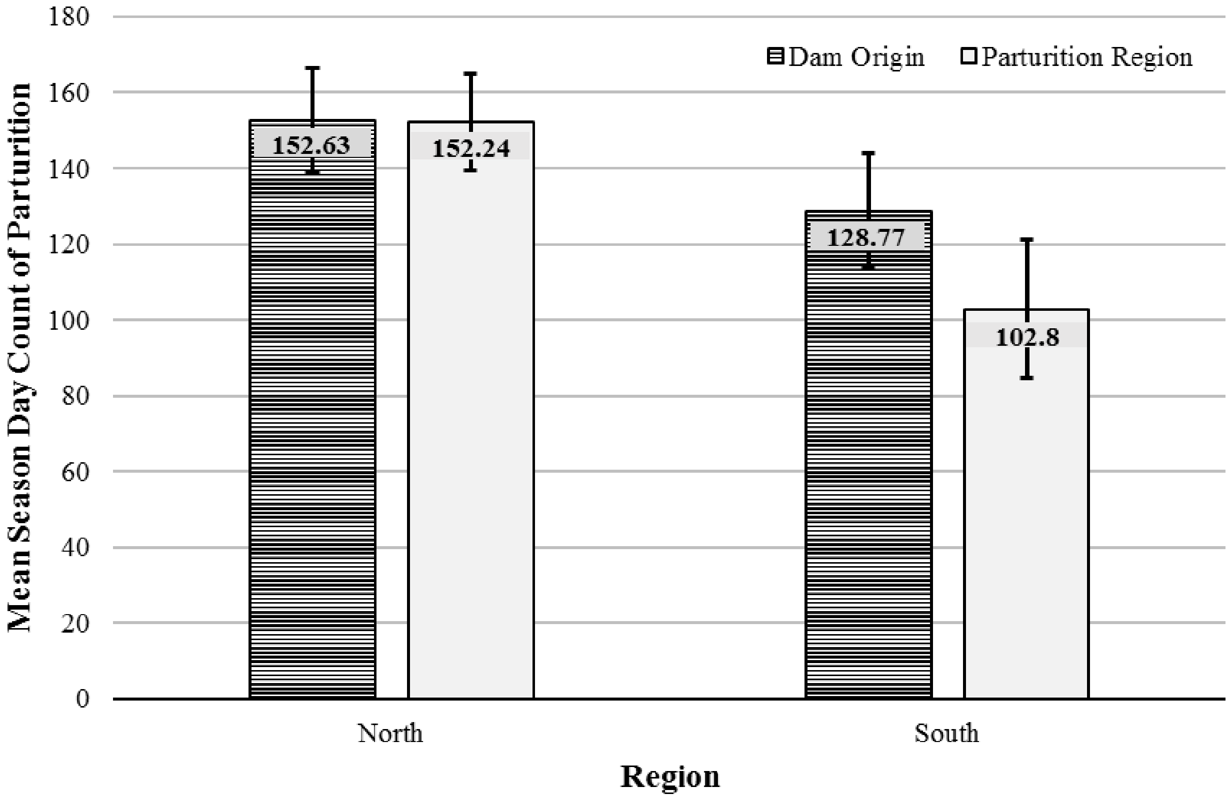

Figure 3. Mean season day count of parturition by assigned groups. The chart shows the mean season day count for each of the following groups: litters with dams from the north (n = 27), litters with dams from the south (n = 13), litters born in the north (n = 42), and litters born in the south (n=4). The bars denote the mean ± 1 standard deviation for each group. A significant difference was found to exist in mean season day count of parturition by region for both dam origin and parturition location.

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Appendix A: Coded Birth Records

Table A-1. Coded birth records. The table lists all North American river otters born in AZA-affiliated institutions between 2008 and 2014. Litters were assigned an identification letter based upon the origin latitude of the dam of record where “A” represents the litter with the southernmost dam and “t” represents the litter with the northernmost dam. These identifiers were used in the PCA. The dam and sire IDs listed below are unique codes assigned by the AZA to each river otter within the captive population for management purposes. Notations of “Absent” indicate missing knowledge of one or both parents’ identities.

Litter ID	Parturition Date	Dam ID	Sire ID	Litter ID	Parturition Date	Dam ID	Sire ID
A	2/16/2014	2504	2506	**W**	3/1/2014	2226	2304
B	12/23/2009	1999	2169	**Y**	2/17/2009	2428	2429
D	12/8/2008	2317	2316	**Z**	3/1/2014	2300	1904
C	1/13/2014	2317	2349	**a**	3/25/2010	2300	1904
E	2/25/2014	2267	2071	**d**	2/24/2013	2306	2242
G	3/22/2009	1893	1720	**c**	2/18/2012	2306	2242
F	1/9/2010	2376	2375	**b**	2/15/2011	2306	*Absent*
H	2/11/2012	2370	2071	**f**	3/26/2009	2246	2130
I	3/3/2014	2455	*Absent*	**e**	3/20/2010	2246	2130
J	3/2/2011	2249	2310	**g**	3/5/2013	2416	2257
K	2/26/2012	2503	2506	**h**	2/15/2014	2407	2149
M	2/19/2013	2231	2170	**j**	11/8/2013	2358	2390
L	1/31/2014	2231	2170	**i**	1/28/2013	2358	2390
R	3/9/2010	2216	2149	**m**	4/2/2014	2209	2254
Q	3/5/2009	2216	2149	**l**	3/27/2011	2209	2254
P	3/19/2009	1940	2230	**k**	3/20/2012	2209	2254
O	2/9/2013	2120	2386	**n**	3/18/2014	2178	*Absent*
N	2/28/2011	2216	2149	**t**	3/30/2012	2054	2147
S	3/2/2010	2155	2011	**s**	3/26/2009	2054	2147
T	2/26/2014	2431	2239	**r**	3/21/2010	2054	2147
U	3/19/2010	2131	1766	**q**	3/20/2011	2054	2147
V	3/19/2014	2441	*Absent*	**p**	3/11/2013	2384	2385
X	3/9/2014	2225	2304	**o**	2/23/2009	*Absent*	*Absent*

Appendix B: Complete Spearman’s Rho Correlation Matrix

Table B-1. Spearman’s rho matrix, part one. The table below contains the first of six tables containing the complete results of the Spearman’s rho matrix. Gray cells indicate blank cells in the matrix.

		Day Count of Parturition	**Dam’s Age at Parturition**	**Latitude of Dam’s Birth Site**	**Day Count of Dam’s Birth**
Dam’s age at parturition	r_s	0.331			
	p-value	0.026			
Latitude of dam’s birth site	r_s	0.400	-0.036		
	p-value	0.011	0.823		
Day count of dam’s birth	r_s	0.070	0.009	0.591	
	p-value	0.646	0.955	0.000	
Distance from dam’s birth site to parturition site	r_s	0.212	0.102	0.710	0.244
	p-value	0.189	0.532	0.000	0.130
Day count since dam’s last transfer	r_s	0.093	0.637	-0.074	-0.102
	p-value	0.557	0.000	0.662	0.520
Distance from dam’s last location to parturition site	r_s	0.812	0.017	0.741	0.588
	p-value	0.001	0.957	0.022	0.035
Latitude of sire’s birth	r_s	0.564	0.109	0.452	0.069
	p-value	0.000	0.520	0.008	0.685
Day count of sire’s birth	r_s	0.256	-0.117	0.128	0.115
	p-value	0.110	0.471	0.464	0.478
Sire’s age at parturition	r_s	0.160	0.181	-0.045	-0.135
	p-value	0.325	0.263	0.798	0.405
Distance from sire’s birth site to parturition site	r_s	-0.041	-0.019	-0.235	-0.127
	p-value	0.819	0.914	0.212	0.473
Day count since sire’s last transfer	r_s	0.172	0.043	-0.003	-0.244
	p-value	0.338	0.810	0.989	0.171
Distance from sire’s last location to parturition site	r_s	-0.308	0.233	0.083	0.382
	p-value	0.284	0.423	0.779	0.178

Table B-2. Spearman's rho matrix, part two. The table below contains the second of six tables containing the complete results of the Spearman's rho matrix.

		Day Count of Parturition	**Dam's Age at Parturition**	**Latitude of Dam's Birth Site**	**Day Count of Dam's Birth**
Latitude of parturition site	r_s	0.001	-0.218	0.400	0.405
	p-value	0.993	0.151	0.011	0.006
Proportion of male pups born	r_s	-0.105	-0.202	0.150	0.077
	p-value	0.488	0.183	0.354	0.614
Litter size at parturition	r_s	0.014	0.022	-0.146	0.015
	p-value	0.927	0.888	0.367	0.921
Survivorship of litter one year after parturition	r_s	-0.215	-0.118	0.034	0.227
	p-value	0.151	0.440	0.837	0.134
Survivorship of litter two years after parturition	r_s	-0.105	-0.104	0.143	0.197
	p-value	0.487	0.498	0.380	0.194

Table B-3. Spearman’s rho matrix, part three. The table below contains the third of six tables containing the complete results of the Spearman’s rho matrix. Gray cells indicate blank cells in the matrix.

		Distance from dam’s birth site to parturition site	**Day count since dam’s last transfer**	**Distance from dam’s last location to parturition site**	**Latitude of sire’s birth**
Day count since dam’s last transfer	r_s	0.099			
	p-value	0.560			
Distance from dam’s last location to parturition site	r_s	0.638	0.076		
	p-value	0.065	0.806		
Latitude of sire’s birth	r_s	0.160	0.111	0.366	
	p-value	0.373	0.532	0.219	
Day count of sire’s birth	r_s	-0.095	-0.263	0.450	0.667
	p-value	0.589	0.116	0.123	0.000
Sire’s age at parturition	r_s	-0.149	0.144	-0.504	-0.174
	p-value	0.393	0.395	0.079	0.303
Distance from sire’s birth site to parturition site	r_s	0.124	0.243	0.426	0.078
	p-value	0.515	0.180	0.167	0.661
Day count since sire’s last transfer	r_s	0.069	0.108	-0.268	0.142
	p-value	0.708	0.570	0.520	0.454

Table B-4. Spearman's rho matrix, part four. The table below contains the fourth of six tables containing the complete results of the Spearman's rho matrix.

		Distance from dam's birth site to parturition site	**Day count since dam's last transfer**	**Distance from dam's last location to parturition site**	**Latitude of sire's birth**
Distance from sire's last location to parturition site	r_s	0.458	0.233	0.355	-0.435
	p-value	0.099	0.423	0.490	0.120
Latitude of parturition site	r_s	-0.203	-0.187	0.137	0.148
	p-value	0.208	0.236	0.655	0.383
Proportion of male pups born	r_s	0.350	-0.022	0.341	-0.224
	p-value	0.027	0.889	0.255	0.182
Litter size at parturition	r_s	-0.085	0.026	-0.141	0.072
	p-value	0.604	0.872	0.646	0.671
Survivorship of litter one year after parturition	r_s	-0.076	-0.061	-0.342	-0.210
	p-value	0.641	0.700	0.253	0.212
Survivorship of litter two years after parturition	r_s	0.029	0.001	-0.266	-0.126
	p-value	0.859	0.995	0.380	0.457

Table B-5. Spearman’s rho matrix, part five. The table below contains the fifth of six tables containing the complete results of the Spearman’s rho matrix. Gray cells indicate blank cells in the matrix.

		Day count of sire’s birth	**Sire’s age at parturition**	**Distance from sire’s birth site to parturition site**	**Day count since sire’s last transfer**
Sire’s age at parturition	r_s	-0.293			
	p-value	0.067			
Distance from sire’s birth site to parturition site	r_s	0.098	-0.102		
	p-value	0.641	0.567		
Day count since sire’s last transfer	r_s	-0.115	0.662	-0.021	
	p-value	0.524	0.000	0.919	
Distance from sire’s last location to parturition site	r_s	-0.190	0.184	0.580	0.244
	p-value	0.516	0.529	0.038	0.401
Latitude of parturition site	r_s	0.266	-0.204	-0.538	-0.529
	p-value	0.097	0.206	0.001	0.002
Proportion of male pups born	r_s	-0.209	-0.250	-0.018	-0.900
	p-value	0.196	0.120	0.920	0.618
Litter size at parturition	r_s	0.054	0.170	0.021	0.245
	p-value	0.741	0.295	0.904	0.170
Survivorship of litter one year after parturition	r_s	-0.110	0.069	0.134	-0.032
	p-value	0.501	0.672	0.450	0.858
Survivorship of litter two years after parturition	r_s	-0.113	0.129	0.086	0.058
	p-value	0.487	0.428	0.628	0.750

Table B-6. Spearman's rho matrix, part six. The table below contains the last of six tables containing the complete results of the Spearman's rho matrix. Gray cells indicate blank cells in the matrix.

		Distance from sire's last location to parturition site	**Latitude of parturition site**	**Proportion of male pups born**	**Litter size at parturition**
Latitude of parturition site	r_s	-0.651			
	p-value	0.012			
Proportion of male pups born	r_s	0.554	-0.037		
	p-value	0.040	0.808		
Litter size at parturition	r_s	-0.438	-0.127	0.026	
	p-value	0.118	0.399	0.863	
Survivorship of litter one year after parturition	r_s	0.427	0.066	0.113	-0.163
	p-value	0.128	0.663	0.455	0.280
Survivorship of litter two years after parturition	r_s	0.370	0.048	0.129	-0.253
	p-value	0.193	0.753	0.394	0.090

Appendix C: Supporting Material for Principal Components Analyses

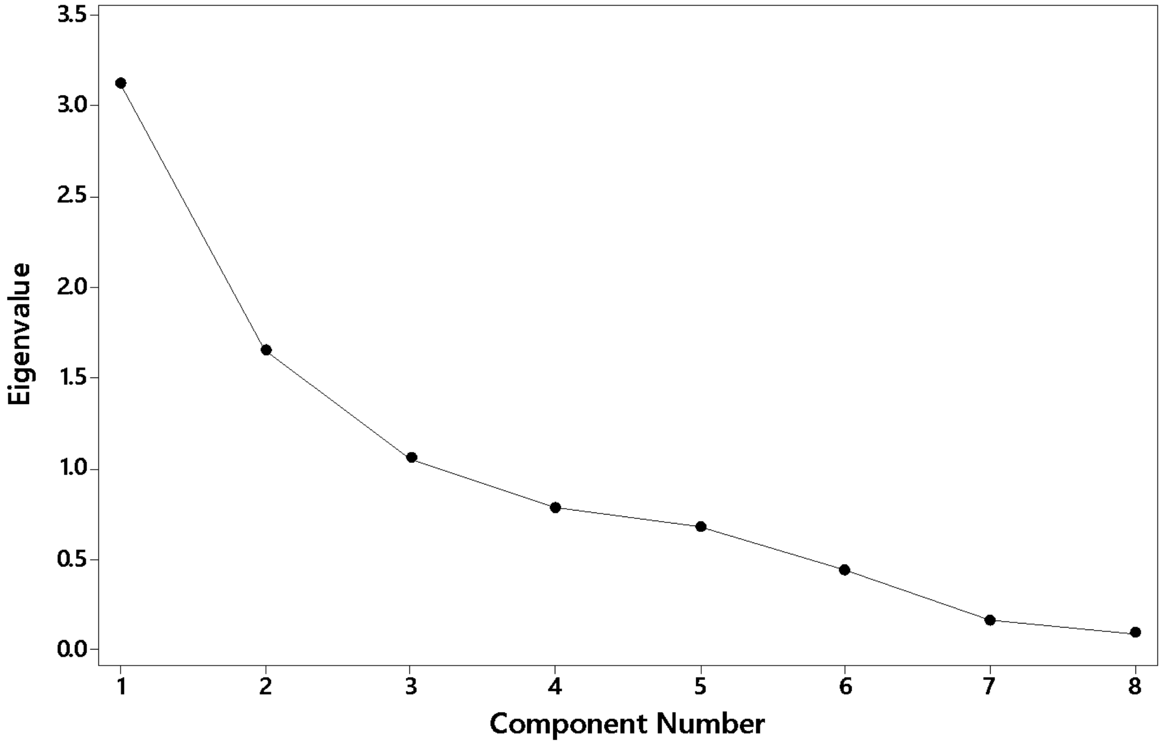

Figure C-1. Scree plot of PCA. The figure shows the resulting scree plot for a correlation matrix PCA using season day count of parturition, season day count of dam's birth, dam birth latitude, season day count of sire's birth, sire birth latitude, latitude of parturition, proportion of males in the litter, and the size of the litter at birth. While components one through three had eigenvalues greater than 1.0, a clear leveling-off occurred after component two.

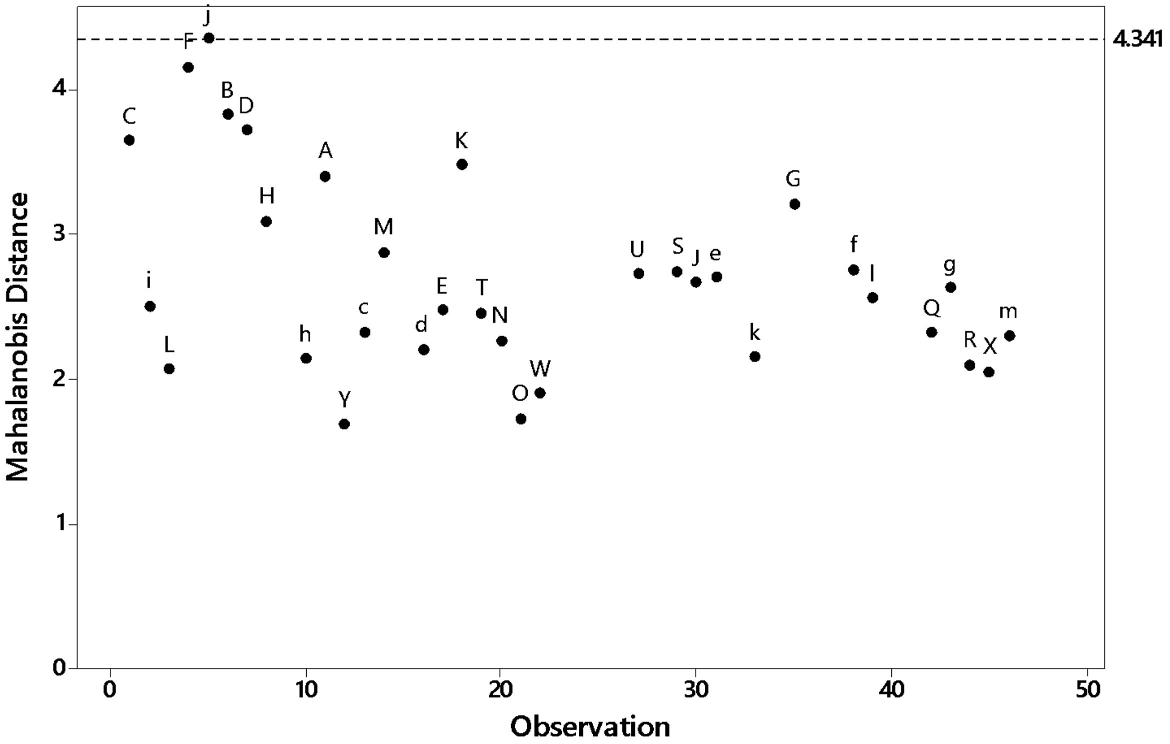

Figure C-2. Outlier plot of PCA. The figure shows each of the litters used in the second correlation matrix PCA (n = 33) and their Mahalanobis distance from the mean of the data distribution. Litter j is the only outlier among the dataset used to create the PCA. The litters are coded according to Table A-1.