DIETARY ECOLOGY OF ALASKAN GRAY WOLVES:

VARIATION IN SEASONAL FORAGING STRATEGIES

IN A SALMON SUBSIDIZED ECOSYSTEM

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DIETARY ECOLOGY OF ALASKAN GRAY WOLVES: VARIATION IN SEASONAL FORAGING STRATEGIES IN A SALMON SUBSIDIZED ECOSYSTEM

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Abstract

While wolves (*Canis lupus*) are traditionally considered to rely on terrestrial prey, primarily ungulates, they are opportunistic feeders and have been observed to use alternative prey, including marine resources, such as salmon (*Oncorhynchus* spp.). Despite these observations of alternative resource use, the seasonal and inter-annual variation and the relative importance of different dietary components have not been studied at this scale in individual wolves. Over the course of four years, we examined whether, and how extensively, wolves in Lake Clark National Park and Preserve in southwestern Alaska used salmon as a food resource on a seasonal basis using stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$) of wolf guard hair and blood components.

Lake Clark National Park and Preserve in southwestern Alaska is an ideal location for such an examination as it provides wolves with multiple ungulate species and salmon as potential prey resources. The results demonstrate that wolves in the Lake Clark region differ in their use of marine resources (salmon) both spatially and temporally. During the summer, half of the diet of some wolves consisted of salmon while other wolves consumed primarily terrestrial prey. In each of three years, one group of wolves consistently consumed salmon in summer and switched to terrestrial prey in winter. Salmon may be an important food source for wolves during periods when the availability of ungulates is reduced. Diets were similar between individuals within social groups. However, the degree to which wolves consumed salmon was highly variable. Factors that potentially contribute to this variation are discussed. The use of salmon exhibited by wolves in Lake Clark is likely widespread in regions where salmon are abundant and should be taken into consideration in the management of wolves and their prey.

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Chapter 1 An introduction to wolf foraging ecology in Alaska

1.1 Study background

Gray wolves (*Canis lupus*) are among the top predators in the ecosystems of Southwest Alaska, and it is generally assumed that they prey primarily on large ungulates, including caribou (*Rangifer tarandus*), Dall's sheep (*Ovis dalli*), and moose (*Alces alces*) (Bennett et al. 2006; Woolington 2009). These ungulates are also important resources for humans, both economically through sport hunting and as a food resource through subsistence harvest. In the region, it is likely common for wolves and ungulates make use of both state and federal lands, including Lake Clark National Park and Preserve (LACL). However, management directives for these lands are currently in conflict. As part of its designation under the Alaska National Interest Lands Conservation Act (ANILCA) in 1980, LACL is mandated to conserve natural and healthy populations of wildlife. In contrast, the State of Alaska's mandate is to manage for maximum yield on state lands. Consequently, wolves which use federal and adjoining state lands may be subjected to intensive management by the State, which may in turn influence the ecology of LACL.

In addition, the caribou population that has historically used areas of LACL has declined drastically in recent years (Woolington 2011). The cause of the decline is not fully understood (Woolington 2011), but may be a result of changes in habitat conditions or as a result of predation. While there is considerable debate regarding in the exact effects of wolves on prey populations, there is consensus that, as part of a multi-predator ecosystem wolves can be central to sustaining healthy prey populations (Miller et al. 2001; Mech and Peterson 2003). As a result, NPS resource managers are seeking to gain insight into wolf-ungulate relationships in the region. Given that studies of the park's wolf population have not yet been conducted, it is in this context that in 2008 LACL began a baseline study of the wolf population that inhabits the region. One component of this baseline study aims to assess wolf foraging ecology, including the relative contribution of different prey to their diets.

In addition to the need for understanding large mammal ecology of a national park and preserve, studies in the LACL region can provide broader insight into predator foraging ecology. The region is an ideal location to study how wolves potentially make use of alternative (non-ungulate) resources. One alternative resource that wolves may benefit from in Southwest Alaska is salmon (*Oncorhynchus* spp.). The region supports the largest wild salmon fisheries in the world and sockeye salmon (*O. nerka*) are considered a keystone species that influences both the marine and terrestrial environments (Bennett et al. 2006). Salmon may be a valuable resource to wolves at times when the availability of ungulates is reduced, in part because of their predictable abundance and broad spatial availability.

Changing prey availability, as occurs with the summer migration of salmon, has been shown to drive other predators to shift to this valuable and easily acquired food (van Baalen et al. 2001). In Southwest Alaska, the relatively low densities, and changing population sizes and distributions of ungulates may also contribute to wolves consuming non-ungulate prey. In part from the assumed dependence on ungulates, the relative use of alternative prey by wolves is rarely addressed, and is assumed to be relatively low. However, the potential for salmon to be a substantial part of wolf diets may be much greater compared to other potential alternative prey, such as small mammals.

If wolves are consuming salmon, other wolf-prey dynamics may be affected. Salmon could support or enhance wolf populations and thus increase predation pressure on primary prey. Alternatively, salmon could be a resource that wolves use in place of other prey. Thus, the use of salmon may have broad implications on the ecology of Southwest Alaska and particularly to the resources managed in LACL. The first step in assessing whether the use of salmon may influence wolf ecology is assessing the degree to which wolves are consuming salmon.

1.2 Traditional diet analyses

Two traditional methods by which wolf foraging is assessed include kill site and scat analyses (Peterson and Ciucci 2003). Kill site analysis is based on direct observations of kills or prey remains and is an important technique in assessing predation patterns of large ungulates, particularly in winter. Although GPS collar technologies are improving the ability to detect kill sites in summer, sites are more easily observed or detected once snow has covered the ground. Especially in Alaska, kill site observations are frequently conducted from aircraft and thus are most successful at detecting large prey. This likely leads to the use of smaller prey being underestimated. Examining the undigested components through scat analysis however, can improve our ability to detect small animals and is also often used to assess diets during summer (Jędrzejewski et al. 2002).

Selection of prey by wolves is usually associated with the vulnerability of individual prey. Wolf predation is often studied in the context of the impact it has on ungulates, since ungulate populations are often managed for human harvest, and predation can influence ungulate population sizes and demographics. Consequently, many studies assess predation during seasons in which ungulates are most vulnerable (Peterson and Ciucci 2003; van Ballenberghe 2006). Vulnerability increases as winter progresses, snow deepens, and forage for ungulates becomes limiting. Also, during the spring calving season, neonates are especially vulnerable to predation. Though winter and spring are important for wolves, times when ungulates are least vulnerable may be especially critical. The reduced vulnerability of ungulates in the summer and fall may lead to an increased use of alternative prey.

While the seasonality in ungulate vulnerability likely drives much of the seasonality in wolf diets, other changes in diet may occur between years as ungulate availability changes. The Mulchatna caribou herd has historically calved on the western edge of LACL and the herd was thought to be an important resource for wolves in the region (Bennett et al. 2006; Woolington 2009). Significant declines in the herd's

population have occurred over the past decade (Woolington 2011). This decline may be one factor leading wolves to make use of other ungulates or other prey types including salmon.

1.3 Stable isotope analysis background

Stable isotope analysis has become an important tool in studying animal foraging ecology (Dalerum and Angerbjörn 2005; Crawford et al. 2008; Newsome et al. 2009). This technique takes advantage of two critical phenomena. First, animals' bodies are built and maintained using the dietary resources individuals consume (DeNiro and Epstein 1978; 1981). Second, there is natural variation in the abundance of stable isotopes within and between ecosystems (Fry and Sherr 1984; Tamelander et al. 2009; Marshall et al. 2007). This natural variation can be incorporated into the bodies of consumers, from which we can infer the use of different resources (Fry et al. 1978).

Stable isotope analysis is a relatively low cost, and potentially high-resolution, method by which we can examine the dietary ecology of wolves (Szepanski et al. 1999; Darimont and Reimchen 2002; Darimont et al. 2009; Adams et al. 2010; Milakovic and Parker 2011). Stable isotope values are calculated by measuring the ratio of heavy (i.e., ^{13}C or ^{15}N) to light (i.e., ^{12}C or ^{14}N) isotopes in a sample, relative to a standard. This ratio is expressed using delta notation (δ), and the units are in parts per thousand (permil, ‰).

Given that the stable carbon (^{13}C) and nitrogen (^{15}N) isotope values of dietary items are incorporated into consumer tissues in a relatively predictable manner (DeNiro and Epstein 1978; 1981), we can use ^{13}C and ^{15}N values of wolf tissues to estimate the relative contributions of isotopically distinct prey to wolf diets. In addition, animal tissues differ in the rates at which they incorporate the stable isotope values of dietary resources (Tieszen et al. 1983), so an isotopic examination of different tissues from the same animal can provide a temporal component to diet (Appendix A) (Dalerum and Angerbjörn 2005). These tissue-specific incorporation rates are primarily governed by

protein turnover (Carleton and Martínez del Rio 2005). Thus, structural tissues tend to have longer incorporation times than splanchnic tissues, which turn over more rapidly. Metabolically inert tissues, such as hair and claws, incorporate dietary stable isotope values during growth, and once grown, the stable isotope value of these tissues remains unchanged (Dalerum and Angerbjörn 2005).

Differences in isotope values at the base of food webs, and relatively predictable changes in values between trophic levels can create variation in isotope values of organisms within a food web and consequently, of potential prey (Ben-David and Flaherty 2012). For example, differences occur between marine and terrestrial systems (Schoeninger and DeNiro 1984), or within terrestrial systems such as between ungulates that forage on different foods (Ben-David et al. 2001). If wolves are consuming dietary resources that are isotopically distinct, their tissues may reflect this mixture of stable isotope values. By measuring the isotope values of potential prey and wolf tissues we can use mixing models to estimate the relative proportion of isotopically distinct prey consumed during the time period represented by a particular wolf tissue (Adams et al. 2010; Milakovic and Parker 2011; Phillips 2012).

Stable isotope analysis can complement traditional techniques of studying wolf diets and provide insight into aspects of wolf ecology which are less well understood, including the foraging ecology of individuals versus social groups, and temporal changes in diet (Darimont and Reimchen 2002; Urton and Hobson 2005). A tissue sample reflects the diet of one individual while kill site or scat analyses usually infer the diet of groups of wolves inhabiting the area where the kill site or scat was found.

Additionally, the rate at which elements (and their stable isotopes) from dietary resources are incorporated into, and lost from, an organism relates to the rate of protein synthesis and catabolism (Carleton and Martínez del Rio 2005). As a result, tissues that are maintained at different rates can reflect resource use over different time periods (Dalerum and Angerbjörn 2005). Collecting tissues that incorporate the stable isotope values of their diet at different rates at one sampling event can provide a temporal

component to diet analysis. This is in contrast to scat or kill site analyses that require regular assessment to examine dietary changes over time. Because stable isotope analysis relies on assimilated diet, it may also overcome the detection bias inherent in such methods kill site analysis, where small prey may be consumed completely, or scat analysis where there may be differences in the digestibility of prey.

1.4 Previous studies examining the use of salmon by wolves

One example of stable isotope analysis providing insight into the use of a previously unrecognized resource is in the use of salmon by wolves. It can be challenging to estimate the degree to which wolves feed on salmon with traditional methods for a few reasons. Wolves likely feed on salmon most heavily in the summer when tracking is difficult without snow. Salmon consumption could be difficult to observe as individual wolves may be unlikely to stay in one location for an extended time as they would when feeding on a large ungulate. Also, salmon are more thoroughly digested than other prey so can go undetected in scat.

Though previously acknowledged (Young and Goldman 1944), the use of salmon by wolves had not been addressed quantitatively prior to the use stable isotope analysis. This technique has been used to study how wolves make use of marine resources throughout Alaska (Szepanski et al. 1999; Adams et al. 2010) and British Columbia (Darimont and Reimchen 2002; Darimont et al. 2009). Both Adams et al. (2010) and Szepanski et al. (1999) used ^{13}C and ^{15}N of bone collagen to estimate the diet assimilated over multiple years and found wolves at varying distances from the ocean (inland and coastal, respectively) consumed very similar amounts of salmon. In Denali National Park where wolves had access to salmon, but where ungulate densities were relatively low, the diet of wolves was estimated to be approximately 17% salmon (Adams et al. 2010) while three groups of coastal wolves in southeast Alaska consumed 18% salmon on average (Szepanski et al. 1999).

By using the ^{13}C and ^{15}N values of bone collagen to examine diet, Adams et al. (2010) and Szepanski et al. (1999) estimated the use of salmon over multiple years. However, prey selection by wolves can vary seasonally. Thus, salmon have the potential to influence these systems on a much finer time scale than over multiple years. Additionally, because salmon are seasonally abundant there is a high likelihood that they are consumed on a seasonal basis by wolves. This cannot be inferred when examining the diet from bone collagen as it incorporates dietary isotope values at a rate such that it reflects diet over multiple years. Darimont and Reimchen (2002) showed that the use of salmon varied between early and late summer by looking at the enrichment of distal and proximal segments of wolf hair, but did not estimate the proportion of salmon consumed in either of these times. There is also the potential for salmon to be used in the fall or winter as carcasses can remain frozen at shorelines into winter. Thus, a much finer-scale analysis that reveals greater insight into the seasonal and inter-annual dynamics of wolf diets and the extent to which wolves from same region employ similar foraging strategies is required if we are to more thoroughly understand predator-prey relations in Southwest Alaska.

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Chapter 2 Variation in seasonal foraging strategies of gray wolves in a salmon subsidized ecosystem[1]

2.1 Abstract

While wolves (*Canis lupus*) are traditionally considered to rely on terrestrial prey, primarily ungulates, they are opportunistic feeders and have been observed to use alternative prey, including marine resources, such as salmon (*Oncorhynchus* spp.). Despite these observations of alternative resource use, the seasonal and inter-annual variation and the relative importance of different dietary components have not been studied at this scale in individual wolves. Over the course of four years, we examined whether, and how extensively, wolves in Lake Clark National Park and Preserve in southwestern Alaska used salmon as a food resource on a seasonal basis using stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$) of wolf guard hair and blood components.

Lake Clark National Park and Preserve in southwestern Alaska is an ideal location for such an examination as it provides wolves with multiple ungulate species and salmon as potential prey resources. The results demonstrate that wolves in the Lake Clark region differ in their use of marine resources (salmon) both spatially and temporally. During the summer, half of the diet of some wolves consisted of salmon while other wolves consumed primarily terrestrial prey. In each of three years, one group of wolves consistently consumed salmon in summer and switched to terrestrial prey in winter. Salmon may be an important food source for wolves during periods when the availability of ungulates is reduced. Diets were similar between individuals within social groups. However, the degree to which wolves consumed salmon was highly variable. Factors that potentially contribute to this variation are discussed. The use of salmon exhibited by wolves in Lake Clark is likely widespread in regions where salmon are abundant and should be taken into consideration in the management of wolves and their prey.

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2.2 Introduction

Intra-population variation is increasingly being recognized as a central aspect of the foraging ecology of animal populations (Bolnick et al. 2003). This variation is most frequently attributed to dietary differences between sexes or ontogenic stages (Polis 1984; Bolnick et al. 2003; Bryan et al. 2006; Kim et al. 2012). Variations in population-level foraging ecology resulting from differences in the foraging strategies of individuals have received far less attention (Tinker et al. 2008). Despite this lack of attention, variation in the foraging strategies of individuals may be highly influential in shaping the foraging ecology of populations (Araujo et al. 2011).

In a general sense, the foraging ecology of a population can be described by dietary niche width. A population with a broad dietary niche can be composed of individuals with similar generalist foraging strategies or by individuals with different specialized foraging strategies (Newsome et al. 2009; Matich et al. 2011). Conversely, a population with a narrow dietary niche can only be composed of individuals with similar specialized foraging strategies. This discrepancy highlights the importance of intra-population variation in individual foraging strategy as a primary component determining the total population niche width (Van Valen 1965), and as a result, the foraging ecology of a population. Specialization by individuals has been proposed as a mechanism by which populations can adjust to changes in environmental conditions, including changes in resource availability (Bolnick et al. 2003; Tinker et al. 2008). The degree to which individuals develop foraging specialization is driven primarily by the degree of resource competition (Araujo et al. 2011), either through changes in food availability (Tinker et al. 2008) or consumer densities (Bolnick et al. 2010). Foraging specialization by a subset of individuals may potentially reduce competition (Bolnick et al. 2010) and increase individual foraging efficiency (Bolnick et al. 2003). Plasticity in individual foraging behavior may also allow for increased flexibility in selection among primary and alternative prey sources. This flexibility is an additional mechanism by which

individuals or populations may more readily adjust to temporal or spatial changes in resource availability (van Baalen et al. 2001).

While the availability of primary prey has been proposed as the main mechanism influencing the degree to which individuals shift between prey sources (van Baalen et al. 2001), the proximate causes of prey switching can be complex, especially in multiple-prey and multiple-predator ecosystems. For example, the susceptibility of a prey species to predation can be related to an individuals' health and strength, the availability of suitable habitat for prey to evade predators, and the hunting efficiency of an individual predator (Owen-Smith and Mills 2008). Additionally, the density of prey and of predators, and the extent that their habitats overlap, can also influence the availability of prey.

Individual variation in prey selection may strongly influence population-level predator-prey interactions, for example, in wolf-ungulate systems in Alaska. The foraging ecology of wolf packs is relatively well understood. However, the foraging ecology of individual pack members and the influence that this variation has on niche width of wolf populations remains relatively unstudied (Metz et al. 2011). In most cases, the primary focus of wolf foraging studies has been on the kill rate and species composition of wolves' ungulate prey during the winter (Mech and Peterson 2003). This is in part a result of the dependence on snow tracking to investigate wolf foraging ecology. The few studies that have examined summer wolf diets, however, have noted the frequency with which wolves feed on alternative prey. Depending on availability, alternative prey species can include mammals such as beavers (*Castor canadensis*), microtine rodents, ground squirrels, hares (*Lepus* spp.) (Peterson and Ciucci 2003), migratory geese (Wiebe et al. 2009) and ptarmigan (*Lagopus* spp.) (Spaulding et al. 2000). Fish, including salmon (*Oncorhynchus* spp.), have also been shown to be important alternative prey resources for wolves (Szepanski et al. 1999; Darimont et al. 2003).

Traditionally, wolf diets and foraging ecology have been studied using kill site and scat analyses (Peterson and Ciucci 2003). Kill site analysis uses direct observation of wolf kills to provide information on the demographics of large prey. Unfortunately, kill sites of small ungulates (including calves) and other small prey frequently go undetected as few identifiable prey remains are left at kill sites after wolves finish feeding (Jędrzejewski et al. 2002). Where kill site analysis lacks in its ability to detect small prey, scat analysis can prove valuable to unraveling diet composition. Although many species are frequently detected in scat, the ability to quantify diet composition is difficult. Determining the biomass of small prey consumed can be complicated by differences in the digestibility of different tissues and prey types (Peterson and Ciucci 2003). While kill site and scat analyses are useful for estimating the diet of groups of wolves inhabiting a particular area, these techniques lack in their ability to provide estimates of diet for individuals without significant efforts to track and assign kill sites and scats to individual wolves. As a result, an inherent assumption in diet investigations conducted using kill site and scat analyses is that wolf packs are a homogeneous foraging unit and that individuals within a pack have the same (or similar) diets. Individual wolves are adept predators (Thurber and Peterson 1993; Mech and Boitani 2003) and, like other predators such as sea otters (*Enhydra lutris nereis*, Newsome et al. 2009), grizzly bears (*Ursus arctos*, Edwards et al. 2011), arctic foxes (*Vulpes lagopus*, Giroux et al. 2012), and white sharks (*Carcharodon carcharias*, Kim et al. 2012), have the potential to exhibit different foraging strategies (Urton and Hobson 2005). In addition, without significant labor-intensive tracking efforts, kill site and scat analyses cannot be used to assign a temporal component to dietary resource use.

Though kill site and scat analyses lack in their ability to capture variations in the dietary ecology of individuals, stable isotope analysis is an ideal tool to study diet composition of individuals, and how diet may change over time (Dalerum and Angerbjörn 2005; Martínez del Rio et al. 2009a, b; Newsome et al. 2012). This is possible because carbon (^{13}C) and nitrogen (^{15}N) stable isotope values of dietary

resources are incorporated into consumer tissues in a relatively predictable manner (DeNiro and Epstein 1978; 1981). In addition, consumer tissues differ in the rates at which they incorporate the stable isotope values of dietary resources. Consequently, the stable isotope values of these tissues reflect diet over different time periods (Martínez del Rio and Carleton 2012). By determining the carbon and nitrogen stable isotope values of multiple tissues from an individual wolf, changes in an animal's diet over time can be estimated (Dalerum and Angerbjörn 2005). The rate at which tissues incorporate carbon and nitrogen stable isotopes of dietary resources is primarily governed by protein turnover (Carleton and Martínez del Rio 2005). Thus, structural tissues tend to have a slower incorporation rate and represent diet over a longer time period, relative to splanchnic tissues. Metabolically inert tissues, such as hair and claws, incorporate dietary stable isotope values as they are grown and remain unchanged isotopically afterwards (Dalerum and Angerbjörn 2005). For example, wolves have one annual molt in the early summer (Young and Goldman 1944) so wolf hair incorporates isotope values of prey throughout summer and fall while new hair is growing (Darimont et al. 2003). In contrast, blood components (such as clot and serum) continuously incorporate the stable isotope values of dietary items as they are resynthesized. As a result, these tissues can be used to estimate diet during a period of weeks to months preceding sampling (Milakovic and Parker 2011).

Previous studies that have used stable isotope analysis to study wolf foraging ecology have primarily focused on the diet and trophic relationships of wolves in inland systems (Urton and Hobson 2005; Fox-Dobbs et al. 2007; Derbridge et al. 2012). Additional studies have noted the importance of salmon as an alternative prey source for wolves throughout Alaska (Szepanski et al. 1999; Adams et al. 2010) and British Columbia (Darimont and Reimchen 2002). While the use of salmon by wolves had been previously observed by Young and Goldman (1944) and Mech et al. (1998), and was recently described by Darimont et al. (2003), the extent to which wolves use salmon was not addressed in a quantitative fashion prior to the use of stable isotopes (Szepanski et al.

1999). From these studies, we can conclude that salmon is an important alternative prey resource in both coastal (Szepanski et al. 1999; Darimont et al. 2003) and interior (Adams et al. 2010) systems. In some areas of these systems, salmon make up 18% of wolf diets. Non-ungulate prey is generally considered to be a relatively small component of the overall biomass consumed by wolves; however, Adams et al. (2010) and Szepanski et al. (1999) demonstrate that the amount of salmon consumed by some wolves may not be inconsequential.

Changes in the abundance of salmon, as occurs annually with salmon migrations, have the potential to perturb seasonal relationships between wolves and primary prey resources, namely, ungulates (Darimont et al. 2008). This influence may be seen either in increases in wolf populations due to the seasonal supplementation of prey resources represented by salmon, or increases in ungulate populations due to the removal of predation pressure provided by the introduction of an alternative prey resource.

Although the potential influence of salmon on wolf and ungulate populations has been demonstrated by Adams et al. (2010) and Szepanski et al. (1999), both of these studies used bone collagen to describe the use of salmon over several years. Bone collagen incorporates dietary stable isotope values at a relatively slow rate and reflects diet over a span of multiple years to the lifetime of the individual (Tieszen et al. 1983; Hobson and Clark 1992). As a result, bone collagen is not appropriate to assess short term dietary changes, as may occur between seasons. The use of salmon by wolves most likely occurs on a seasonal basis as observed by Darimont et al. (2002). However, the degree to which the proportion of salmon consumed by wolves changes throughout a year has not yet been examined quantitatively.

Here we report the results of an investigation using ^{13}C and ^{15}N analyses to develop a quantitative estimate of the degree and timing with which individual wolves in Lake Clark National Park and Preserve in Southwest Alaska used salmon as an alternative prey resource. Our study asked the following four questions: A) Do wolves in the Lake Clark region exhibit a generalist foraging strategy or do some individuals

specialize on certain prey? B) Are foraging strategies similar between individuals from the same social group? C) Are there differences in foraging strategies between seasons? D) Do changes in foraging strategies correspond with an increased use of salmon by wolves? Not only did our study allow us to gain novel insight on the intra-annual use of terrestrial and marine resources by individual wolves, but it also allowed us to provide baseline information on the dietary habits of a previously unstudied population of wolves.

2.3 Methods

2.3.1 Study area

Lake Clark National Park and Preserve (LACL) encompasses 16,309 km^2 of Southwest Alaska, at the intersection of the Alaska and Aleutian Mountain Ranges (Figure 2.1). The Chigmit Mountains and Alaska Range bisect LACL into coastal (east) and inland (west) regions. This study focuses on wolves inhabiting the inland portion of the park. Inland monthly mean air temperature ranges from −11°C (January) to 13°C (July) with an annual mean precipitation of 43 cm (Bennett et al. 2006).

The Lake Clark region supports multiple ungulate species including caribou (*Rangifer tarandus*), Dall's sheep (*Ovis dalli*), and moose (*Alces alces*), each at relatively low densities. The eastern edge of the Mulchatna caribou herd range has traditionally reached the western edge of LACL (Woolington 2011). In recent history, the herd used wintering and calving grounds near LACL and was potentially an important prey resource for wolves in the region. Considerable declines in the Mulchatna herd population (peak of ~200,000 individuals in 1996 to a low of ~30,000 individuals in 2008) and changes in their range away from LACL (Woolington 2011) has likely altered the availability of caribou to wolves in the Lake Clark region. If caribou have historically been an important resource for wolves, changes in the population size and range of the Mulchatna herd may influence the degree to which wolves are dependent on other prey. In addition to gray wolves, other predators in the region include brown bears (*Ursus*

arctos), black bears (*Ursus americanus*), lynx (*Lynx canadensis*), coyotes (*Canis latrans*) and wolverines (*Gulo gulo*) (Bennett et al. 2006).

A series of large lakes abutting the Alaska Range serve as headwaters for three major river drainages of Southwest Alaska, the Kvichak, Nushigak, and Kuskokwim. These rivers and their tributaries support the Bristol Bay sockeye salmon (*Oncorhynchus nerka*) fisheries (the largest in the world) and are defining features of the landscape. The 20 year (1991-2011) escapement averages into the Nushigak and Kvichak rivers were 257,572 and 3,552,097 sockeye salmon, respectively (Jones et al. 2012). At 756 river km from the Kuskokwim River mouth, surveys estimated sockeye salmon escapement at 72,021 in 2010 and 35,105 in 2011 (Brazil et al. 2013) into Telaquana Lake. These salmon runs are an important nutrient resource for the region both as live fish and carcasses, and as decomposed nutrients at the base of the food-web (Kline et al. 1993).

2.3.2 Sample collection

To examine gray wolf foraging strategies and estimate diet composition, we analyzed the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of three wolf tissues that represent diet over different time periods: guard hair, blood clot, and blood serum. Twenty-two wolves from nine social groups were sampled during five capture events over four winters (2009-2012) (Table 2.1). Capture events occurred in December 2008, February 2009, February 2010, February-March 2011 and February 2012. Wolves captured in December 2008 and February 2009 were considered to be captured in the same season (winter 2009). Wolves that were captured or observed together were considered members of the same social group.

Wolves were anesthetized with Telazol (500mg, Tiletamine-Zolazepam, Fort Dodge Animal Health, Fort Dodge, IA) by aerial darting from a helicopter. Individuals were fitted with GPS collars (Telonics Inc., Mesa, AZ) that were programed to record locations every 11 or 15 hours. To determine whether variation in foraging strategies

was potentially related to changes in location we used GPS data from recaptured individuals to compare the general areas occupied by each wolf.

At the time of capture, we collected hair and blood samples from each wolf. These tissues were selected because their isotopic characteristics reflect diet over different time frames and collecting them is minimally invasive. Wolf guard hair incorporates dietary stable isotope values as it grows throughout the summer and fall (Young and Goldman 1944; Darimont and Reimchen 2002). Since hair remains metabolically inert after growth, hair collected in the winter represents diet during the preceding summer (Dalerum and Angerbjörn 2005). Blood clot and serum continuously incorporate dietary stable isotope values and thus represent diet over a period up to the time of collection (Hobson and Clark 1993). To our knowledge, the duration represented by blood clot and serum have not previously been measured in wolves. We measured average residence times (Martínez del Rio and Anderson-Sprecher 2008) of ^{13}C and ^{15}N in a captive wolf population fed a marine diet (100% salmon, Appendix A). Serum ^{13}C and ^{15}N mean average residence time was 19.5 days, allowing us to infer diet over the previous 3-4 weeks. Blood clot did not fully incorporate the marine diet within the 70 day study so it likely reflects diet over at least the previous 3-4 months.

Guard hairs were plucked from the base of the dorsal side of the neck and stored in paper envelopes. Blood samples were drawn (18 gauge, 4 cm needles) from the cephalic vein into 10 ml red top serum tubes (BD Vacutainers, BD Diagnostics, Franklin Lakes, NJ). Whole blood was centrifuged to separate serum and clot components which were then stored separately at −80°C. Animal handling protocols were approved by US Fish and Wildlife Service and University of Alaska Anchorage Institution Animal Care and Use committees (protocols #2008023 and #243626-1, respectively).

To determine the stable isotope values of locally available dietary resources, muscle samples of potential wolf prey were collected from the Lake Clark region (caribou [n=2], Dall's sheep [n=3], moose [n=10], and salmon [n=12]). Muscle samples

were stored frozen at −20°C prior to analysis. Prey samples were collected by NPS staff and donated by local hunters.

Blood components were freeze-dried for at least 48 hours and ground to a fine powder with a bead beater (BioSpec Products, Inc., Bartlesville, OK). Prey muscle samples were also freeze dried, then ground to a fine powder with a mortar and pestle. To remove surface oils and debris, whole guard hairs were cleaned in a 2:1 chloroform:methanol solution for 24 hours and rinsed with nanopure water (Darimont et al. 2007). Cleaned and dried guard hairs were ground to a fine powder using a freezer-mill (SPEX SamplePrep, Metuchen, NJ).

Approximately 1.0 mg of each ground tissue was weighed into tin cups (Costech Analytical Technologies, Inc., Valencia, CA) for analysis. Analysis was performed using a Costech elemental analyzer (Valencia, CA., USA) coupled to a Delta Plus XP continuous-flow isotope ratio mass spectrometer (Thermo Scientific, Waltham, MA., USA) at the University of Alaska Anchorage Environment and Natural Resources Institute Stable Isotope Laboratory. Stable isotope values are reported in delta (δ) notation, which is calculated as: $\delta X = (R_{sample}-R_{standard})/R_{standard} \times 1000$, where R is the ratio of heavy to light isotopes, and relative to international standards (atmospheric nitrogen for $\delta^{15}N$, and Vienna Peedee Belemnite for $\delta^{13}C$). Internal standards (NIST 1547, bowhead whale baleen, Acetanalide, and chicken feathers) were used to determine an accuracy of ±0.1‰ for carbon and ±0.2‰ for nitrogen.

2.3.3 Statistical analyses

We conducted an outlier analysis on stable isotope values of each tissue collected in each of the four winters to test whether Lake Clark wolves exhibited different foraging strategies within each season. When wolves are consuming the same suite of prey we would expect tissue $\delta^{13}C$ and $\delta^{15}N$ to be normally distributed. Outlier analysis can be used to determine whether or not particular data points in a series fall within this distribution (Barnett and Lewis 1978). Although outlier analysis is often used to justify

discarding data points, outliers are not always erroneous and may indicate that normality or variance equality should not be assumed (Zar 1996). In this study, any outlying values likely result from the presence of separate distributions of stable isotope values, and could be indicative of differences in resource use. This is a common technique in physical sciences and increasingly being used in ecological research (Greenacre 2013). We tested for the presence of significantly enriched outliers using a one-tailed Dixon's *Q* test (Dean and Dixon 1951) to determine the likelihood that certain stable isotope values come from separate distributions. A one-tailed test was selected because we are aiming to detect the use of salmon, which is enriched relative to terrestrial resources. We selected the Dixon's *Q* test as it is designed to detect outliers in datasets of small sample sizes from a population with an unknown mean or standard deviation (Rorabacher 1991). Additionally, this test is the most conservative of a multitude of outlier tests (Barnett and Lewis 1978).

The Dixon's *Q* test calculates the likelihood of data coming from separate distributions by determining whether a gap between ordered values is larger than would be expected if data are from a normal distribution relative to the range of the values and the sample size. We calculated the minimum significant gap that would be present if data were from separate distributions at the 90% and 95% confidence levels (Rorabacher 1991) and examined data for these gaps. We tested for significantly enriched outliers in $\delta^{13}C$ and $\delta^{15}N$ of each tissue and each year separately. Although ^{13}C and ^{15}N are often assumed to co-incorporate from diet, we assumed them to be independent variables. Note that values that are statistical outliers may not necessarily be 'biological outliers'. A significant enrichment is strictly an indication that stable isotope values are different between the two groups. We infer that any differences are related to differences in foraging strategy.

When using $\delta^{13}C$ and $\delta^{15}N$ of consumers to estimate the composition of their diet, we must first account for the difference between the stable isotope values of diet and consumer tissues (Martínez del Rio et al. 2009b). This difference, or diet-to-tissue

discrimination, can vary between species, tissues, and diets (Dalerum and Angerbjörn 2005; Lecomte et al. 2011). Preliminary analyses of captive wolf stable isotope data also suggest there are differences between discrimination values of tissues grown while consuming marine or terrestrial diets. When measuring the average residence times of ^{13}C and ^{15}N of captive wolves, we also measured the discrimination between a marine diet (100% salmon) and wolf hair and serum (Table 2.2) and applied these to values of salmon for diet estimates using hair and serum. We supplemented these measured values with values from the literature for other canid species (Table 2.2). The discrimination values between a marine diet to blood clot of arctic fox (Lecomte et al. 2011) were applied to salmon for wolf blood clot. For terrestrial prey, we applied discrimination values for hair, blood cells, and serum from red foxes (*Vulpes vulpes*) fed a terrestrial diet (Roth and Hobson 2000) to wolf hair, clot, and serum.

We used the Bayesian mixing model SIAR (Stable Isotope Analysis in R, Parnell et al. 2010) to estimate the proportion of each prey species in the diet of wolves from the Lake Clark region. The contribution of each prey to individual wolf diets was estimated using the SIARSolo function. SIAR uses a Bayesian framework to estimate the contribution of resources to consumer tissues. This model incorporates variation in prey ^{13}C and ^{15}N values and in diet-to-tissue discrimination values to generate a probability distribution of the contribution of each resource to the diet of an individual during the time period represented by each tissue. We present the most likely proportion (mode) of prey in the diet of each wolf as calculated from each tissue.

2.4 Results

The prey resources we measured included each of the ungulate species potentially available to wolves (caribou, Dall's sheep, and moose) and salmon. Salmon were markedly enriched in ^{13}C and ^{15}N relative to terrestrial prey (Table 2.3). Moose were the most depleted in carbon, Dall's sheep were the most depleted in nitrogen, and caribou were enriched in both carbon and nitrogen relative to moose and Dall's sheep (Table 2.3).

We could not adequately distinguish between terrestrial prey because sample sizes were low for caribou and Dall's sheep; thus, we group these species into 'terrestrial prey' for the remaining results and discussion. Terrestrial prey (n=15) values ranged from -26.77 to -22.65‰ for $\delta^{13}C$ (mean ± SD: -25.19 ± 0.95 ‰) and 0.81 to 4.46‰ for $\delta^{15}N$ (mean ± SD: 2.10 ± 1.02 ‰). Salmon (n=12) were enriched in both ^{13}C (Welch t-test: t=16.54, p<0.001) and ^{15}N (t=36.56, p<0.001) relative to terrestrial prey. Salmon $\delta^{13}C$ values ranged from -21.50 to −20.15‰ (mean ± SD: −20.67 ± 0.12‰) and $\delta^{15}N$ from 11.98 to 13.06‰ (mean ± SD: 12.43 ± 0.10‰). While terrestrial prey could not be clearly distinguished from one another as unique dietary items, marine (salmon) and terrestrial resources were distinct.

Stable isotope values of wolf tissues varied within and between years (Figure 2.2). Eight of the 12 samples analyzed (3 tissues x 4 years) included significantly enriched outliers (Figure 2.3). Samples that included significantly enriched outliers in $\delta^{13}C$ and $\delta^{15}N$ (p<0.05) were 2009 serum, 2011 hair, and 2012 hair. The same individuals were enriched in both isotopes in these cases. Five tissues included outliers for either $\delta^{13}C$ or $\delta^{15}N$. Values of 2009 hair (p<0.05), 2009 blood clot (p<0.10), and 2012 blood clot (p<0.10) included outliers that were significantly enriched in ^{13}C. Values of $\delta^{15}N$ for 2010 serum (p<0.05) and 2011 blood clot (p<0.10) also included outliers.

Hair, blood clot, or serum from eleven individuals in four social groups (Chekok, Nikabuna, Telaquana, and Tela2) were significantly enriched in ^{13}C or ^{15}N (p<0.10) (Figure 2.3). From 2009 through 2011, social group members were in the same outlier group. In 2012 however, hair $\delta^{13}C$ and $\delta^{15}N$ from two individuals (LC1119 and LC1226) in two social groups (Tela2 and Nikabuna, respectively) were significantly enriched (p<0.05) relative to their group members (LC1118 and LC1225).

The most likely (mode) proportion of salmon consumed varied greatly between individual wolves and between tissues. Estimates ranged from 1% to 89% among samples (Table 2.4). The proportion of salmon in an individuals' diets during each summer consistently ranged from 1% to over 50% (Table 2.4). In winters 2010 through

2012 salmon was a smaller component of diet (less than 40%). However, estimates made using blood clot and serum from winter 2009 indicated diets of the Telaquana wolves (LC0801 and LC0802) consisted primarily of salmon. The proportion of salmon ranged from 64% to 89% using values of blood clot and serum from both individuals.

Substantial contributions of salmon to wolf diets were most often associated with the presence of outliers when samples included outliers enriched in both isotopes (2009 serum, 2011 hair, 2012 hair). Individuals with tissues significantly enriched in ^{13}C and ^{15}N consumed at least 38% more salmon than individuals whose tissues were relatively depleted (Table 2.4). Hair and blood clot from 2009 were enriched in ^{13}C only and were also indicative of salmon being a large component of some individuals' diets. The difference between the enriched and depleted individuals however was not as great ($\leq$28% difference) compared to samples with enriched outliers in both isotopes. Additional differences in foraging strategies as represented by significantly enriched values, such as in 2010 serum, 2011 clot and 2012 clot, do not appear related to salmon consumption. These are potentially representative of different terrestrial diet patterns. The difference in isotope values within these samples represents minimal differences ($\leq$8%) in the proportion of salmon consumed.

2.5 Discussion

The range in isotope values of each wolf tissue indicates significant heterogeneity in diet composition within and among wolves in the Lake Clark region. When we examine the distribution of stable isotope values of each tissue from each year (Figures 2.2 and 2.3), it is clear that not all wolves consume the same mixture of prey within a season or year (Table 2.4). Although the diets of some wolves were rich in salmon (Figure 2.2, Table 2.4), their diets shifted between seasons and years (Figure 2.3). We suggest that these dietary patterns are a function of social interactions as well as the seasonal dynamics of prey availability, including salmon.

2.5.1 Variation in foraging strategies within social groups

Stable isotope values of individuals from the same social group were in the same outlier group from 2009 through 2011 (Figure 2.3). This reflects shared strategies within groups. In 2012 however, ^{13}C and ^{15}N values of individuals from the Tela2 and Nikabuna social groups were in separate outlier groups. In both of these social groups, stable isotope values of hair revealed that one individual made considerable use of salmon during the summer (52% and 44%) while the other individual consumed primarily terrestrial prey (1% and 1% salmon).

Isotope values of tissues collected when the Tela2 wolves were initially captured in winter 2011 at that time showed each wolf had the same terrestrial diet (Figure 2.4). Both individuals received a GPS collar in winter 2011 that then recorded their locations for the following year. These locations correspond to the time periods represented by tissues collected in 2012. A geographical separation between the two individuals occurred during the summer (Apr-2011 to Oct-2011; Figure 2.5), and this coincides with a separation in hair stable isotope values (Figure 2.4). Following this separation, both wolves began traveling together throughout the fall and winter (Oct-2011 through Feb-2012; Figure 2.5). Stable isotope values corresponding to this later period (blood clot and serum) indicate that their diets became increasingly similar over the fall and winter (Figure 2.4). Hair values of wolf LC1119 were significantly enriched relative to wolf LC1118 at the 95% CI, blood clot at 90%, and serum values were not different from each other (Figure 2.3). Hence, their isotope values indicated that when these wolves were together, they were consuming similar prey and when they were apart they used different foraging strategies. Data from the Nikabuna wolves indicate a similar pattern of behavior. Metz et al. (2012) recently showed how the cohesiveness with which wolf social groups forage can vary between seasons, based on the age structure of the group, and sometimes depending on the size of prey.

Generally, kill rate and diet is defined for the social group, often from the interpretation of the behavior of select few individuals within a group (Peterson and

Ciucci 2003). Variation between individuals within groups is rarely addressed and is likely assumed to be small. The isotopic data from individuals within social groups in the first three years of our study support this idea. However, the differences in summer diet by wolves in the Tela2 and Nikabuna groups suggests that variation within groups can be significant. These differences in foraging behavior may not have been detected or incorporated into our assessment of predation patterns had we only assessed diet using traditional techniques. Assuming these individuals were consuming the same resources during the summer, or by only tracking one of the individuals in a group, it is likely we would have missed an important facet of their behavior. In areas similar to the Lake Clark region, where there appears to be relatively few wolves and low ungulate densities, the prey selected by individual wolves has the potential to be relatively influential in the dynamics of wolf-ungulate relationships. Consequently, understanding and quantifying the variation between individuals may be critical in understanding the ecology of this system.

2.5.2 Seasonal patterns in foraging strategies

Wolves often change their predation patterns based on seasonal or inter-annual changes in the vulnerability (and or availability) of their primary prey (Ballard et al. 1987; Jędrzejewski et al. 2002; Owen-Smith and Mills 2008; Wiebe et al. 2009; Metz et al. 2012). There are notable differences in the use of marine versus terrestrial resources especially within summers and also between summers and between winters. Predation by wolves on ungulates is often parsed into two seasons, winter and summer, because the dynamics of prey and predators differ significantly between these two seasons. In spring or early summer, large predators often focus on neonatal ungulates, as they are especially vulnerable at this time. Likewise, vulnerability of ungulates (of many age classes) varies significantly upon winter conditions. As a result, predation is most often studied at the time of calving in the spring and throughout the winter (Dale et al. 1994; Mech and Peterson 2003). Calves born during spring or early summer are especially vulnerable and

provide wolves a relatively large resource pulse that often corresponds with the birth of wolf pups (Packard 2003). Winter conditions and snowpack affect ungulate vulnerability (Mech et al. 1987) and thus the likelihood of wolf predation (Ballard et al. 1987; Mech and Peterson 2003; Wilmers and Getz 2005).

Ungulates are far less susceptible to wolf predation during the mid to late summer, and as a result, wolves may increase their use of alternative prey (Spaulding et al. 1998). In our study, the stable isotope values of hair grown during this period are enriched thus indicating that salmon was an important resource to several individuals at this time (Figure 2.2). For example, on average, over half (55%) of the diet of wolves in the Chekok group consisted of salmon in each of three summers. In contrast, blood clot and serum values indicated they consumed primarily terrestrial diets throughout the winter (26% salmon).

Within this social group, wolf LC0906 was captured in three years (2009, 2010, and 2011). The consistency in stable isotope values of each tissue among years indicates a consistent diet within each season (Figure 2.6). Between seasons, however, this individual shifted from a diet rich in salmon during the summer to a primarily terrestrial diet during the winter (Figure 2.6). This consistency in overall diet is mirrored by a consistency in locations throughout the year (Figure 2.7).

Seasonal shifts in diets may result from seasonal shifts in resource availability. In winter 2009, we detected the use of salmon by wolves in the Telaquana group, up to 89%, which was in contrast to subsequent years. One notable difference, however, is that tissue samples were collected from the Telaquana wolves in December (2008) while those from other individuals that same winter (and all other winters) were collected later in the season (February). Consequently, values of blood clot and serum from Telaquana wolves represent diet over time periods earlier in the fall and winter compared to tissues collected later in the winter. This provides some initial insight into potential differences in prey use within a winter.

As snow depths increase throughout winter, ungulates become more vulnerable to predation. Salmon, however, may become less available as winter progresses as well. After spawning, some carcasses can remain along lake shores throughout the fall and winter. Wolves may continue to consume salmon long after spawning has ended if carcasses remain available. If availability of salmon is influenced by snowfall or the extent of ice buildup, wolves may be more likely to consume salmon during the winter, especially in years when snowfall is poor or in areas where lakes and rivers remain clear of ice. Adams et al. (2010) noted that wolves in Denali National Park likely fed on salmon which spawn late into winter. In the Lake Clark region, there are no late winter runs of salmon, although the Telaquana wolves were observed feeding on salmon carcasses frozen at lake shores throughout the fall and at the time of their capture in December.

2.5.3 Salmon as an alternate food resource

The contribution of salmon to the diets of individual wolves was highly variable over the seasons represented by each sample. In some seasons, salmon contributed minimally (1%) to the diet of some individuals and substantially (89%) to others (Table 2.4). Wolves employed distinct marine and terrestrial foraging strategies in winter 2009 (serum) and summers of 2011 and 2012.

The contribution of salmon to wolf diets has been examined in coastal British Columbia (Darimont et al. 2009), Southeast Alaska (Szepanski et al. 1999), and interior Alaska (Adams et al. 2010). In each of these studies, the relative use of salmon was partially attributed to geographical differences in ungulate and/or salmon availability. There are likely differences in ungulate abundance across the Lake Clark region; however, neither the spatial patterns of ungulate distribution nor their densities are known. We assumed that all wolves living in the Lake Clark region would have access to salmon. As most wolves dispersed or died we could not adequately assess the areas in which they likely lived. This makes it particularly difficult to assess the potentially

available resources, or areas of particular importance, to these individuals and social groups. Social-groups that remained in the study area with active collars (Chekok, Telaquana, and Tela2), and whose general territories could be assessed, appeared to make greater use of salmon than those who did not remain in the study area.

The range in the relative contribution of salmon to the diets of Lake Clark wolves appears greater than has been reported for other regions (Szepanski et al. 1999; Darimont et al. 2009; Adams et al. 2010). One reason for this may be the relatively short time periods for which we estimate diet. Salmon may be available to wolves in a particular season or location and may only be a primary component of diet during a short window of time. On an annual basis, salmon may be less important than terrestrial prey, yet this work shows that salmon are likely an important or critical resource over short time periods or at times when availability of ungulates is potentially low.

Salmon was consistently a large component of some individual's diets during the summer (including individuals in the Chekok and Telaquana groups), though the percent salmon consumed was widely variable between wolves (range: 1-66%, Table 2.4). The range in the contribution of salmon was similar among summers. In addition to being predictable and plentiful, salmon may also be an important source of lipids for young wolves. Accumulating a large lipid store early in life may increase the chances of survival for young wolves (Robbins 1993). This was noted by Bryan et al. (2006) who found that when harbor seals were a moderate component of adult wolf diets (23.9%), they were an even greater component of the diets of young wolves (45.7%). The authors suggested that when prey with a high-fat content are abundant, adults may selectively provision young with prey of higher-fat content (Bryan et al. 2006). The relatively high lipid content of salmon compared to terrestrial prey sources may lead to social groups (packs) of wolves that are provisioning young to be more likely to seek out a diet rich in salmon.

Darimont et al. (2009) found differences in total isotopic niche width and degree of specialization among a population of wolves living on outer islands, inner islands, and

the mainland of coastal British Columbia. They suggested that niche width and specialization were primarily related to differences in availability of marine and terrestrial prey, and inter- and intra-specific competition within each sub-region. Although we lack information regarding the distribution/population of potential prey species and competitors throughout our study area, variation between years could drive wolves to exhibit different strategies over time.

2.6 Conclusions

It is not likely that the heterogeneity and foraging strategies we have observed in the Lake Clark wolves is unique. Our study however, does provide an initial glimpse into phenomenon that may be more widespread than previously thought. Given the ubiquity of salmon across much of Alaska, wolves throughout the state may be using this non-ungulate resource to a degree that influences wolf-ungulate relationships. Though we have considered salmon to be an alternative resource, they may actually be a primary and critical resource in some regions or during some seasons. A more in-depth examination of the influence of salmon on terrestrial predator-prey systems is clearly warranted.

2.7 Acknowledgments

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2.9 Figures

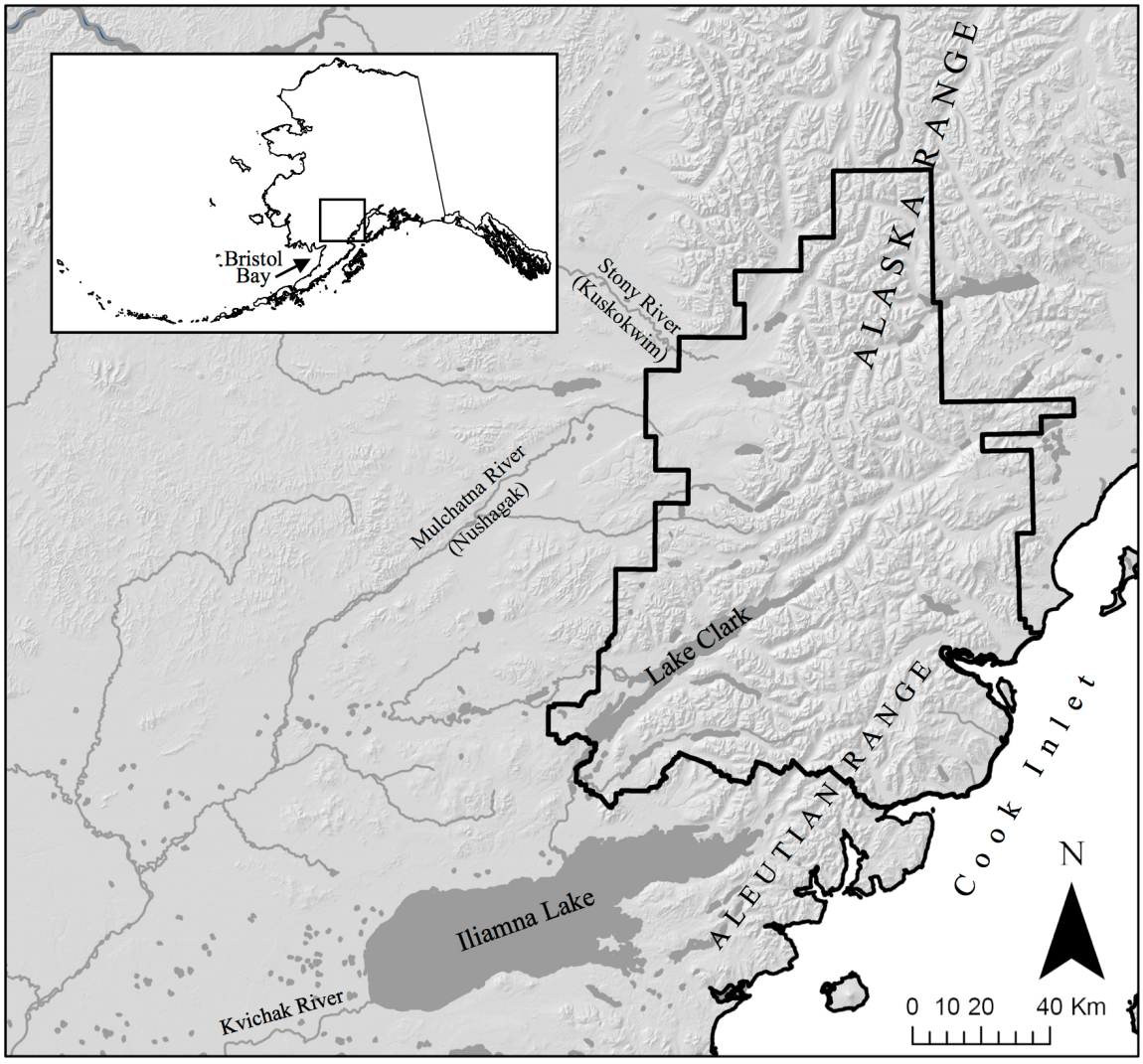

Figure 2.1 The Lake Clark region in Southwest Alaska. Lake Clark National Park and Preserve is outlined in black.

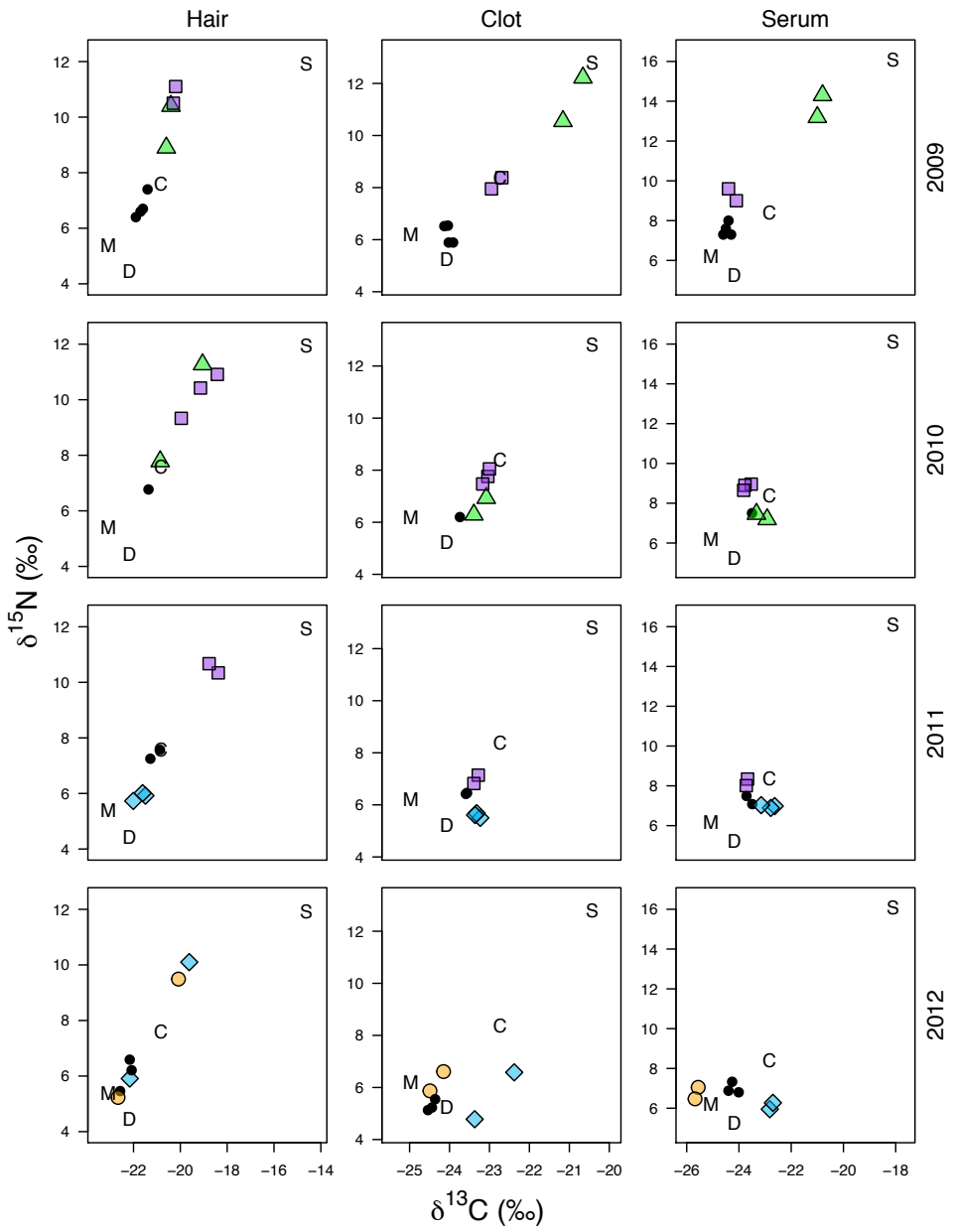

Figure 2.2 $\delta^{13}C$ and $\delta^{15}N$ of wolf tissues collected from 2008 through 2012. Each plot shows a single tissue (columns) collected in each of four winters (rows). Mean values of prey (Table 2.3) are included as reference points (C=Caribou, M=Moose, D=Dall's Sheep, S=Salmon) and have been adjusted for diet to tissue discrimination (Table 2.2). Individuals from the same social group have the same colored symbol (Chekok: purple squares, Nikabuna: orange circles, Telaquana: blue diamonds, Tela2: green triangles) and other individuals are identified by black circles.

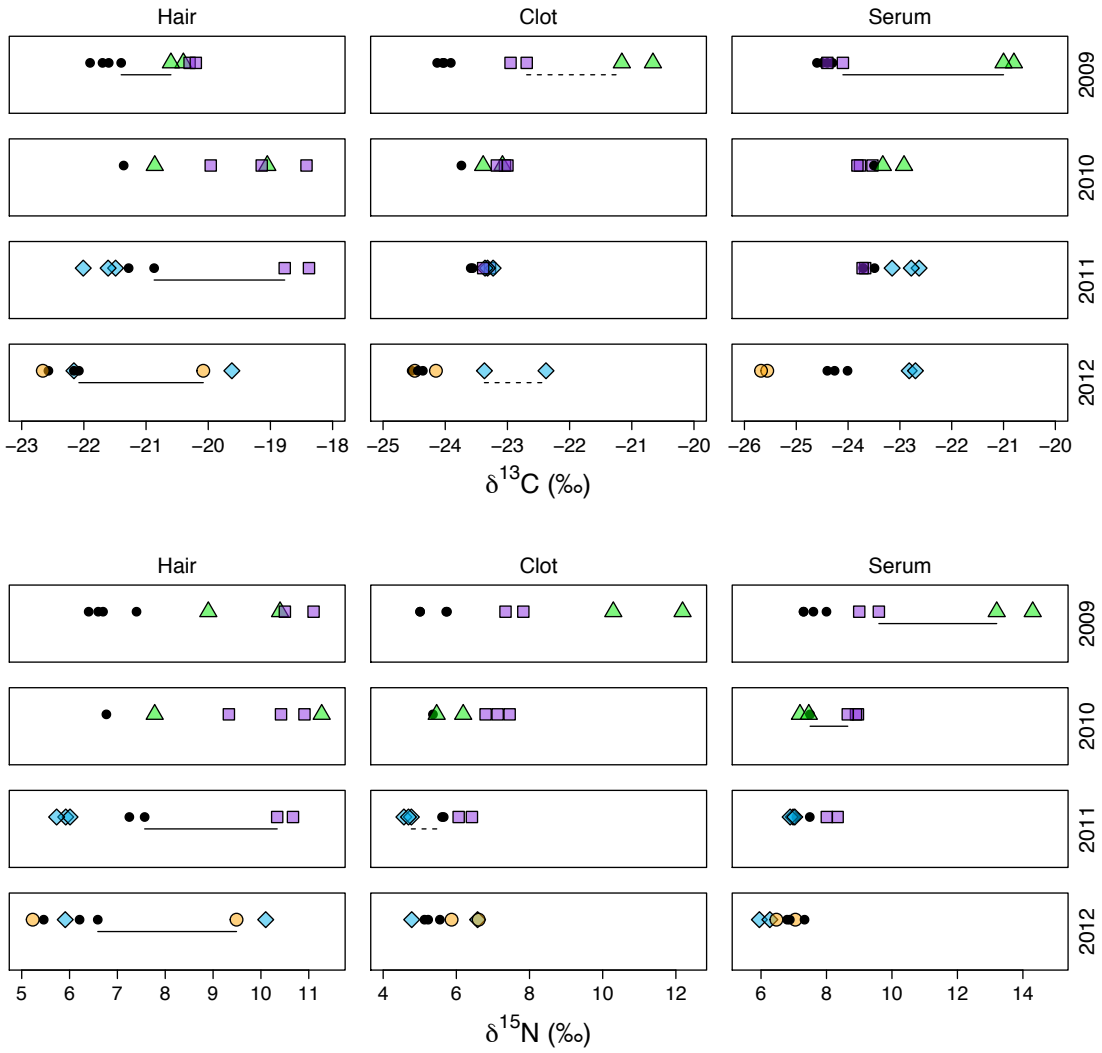

Figure 2.3 Significant enrichment in ^{13}C (top) and ^{15}N (bottom) of wolf tissues collected from 2008 through 2012. Each plot shows a single tissue (columns) collected in each of four winters (rows). Significant gaps (enrichment) between values are signified by solid ($p \leq 0.05$) and dashed ($p \leq 0.10$) lines. Individuals from the same social group have the same colored symbol (Chekok: green squares, Nikabuna: orange circles, Telaquana: green triangles, Tela2: blue diamonds) and other individuals are identified by solid circles.

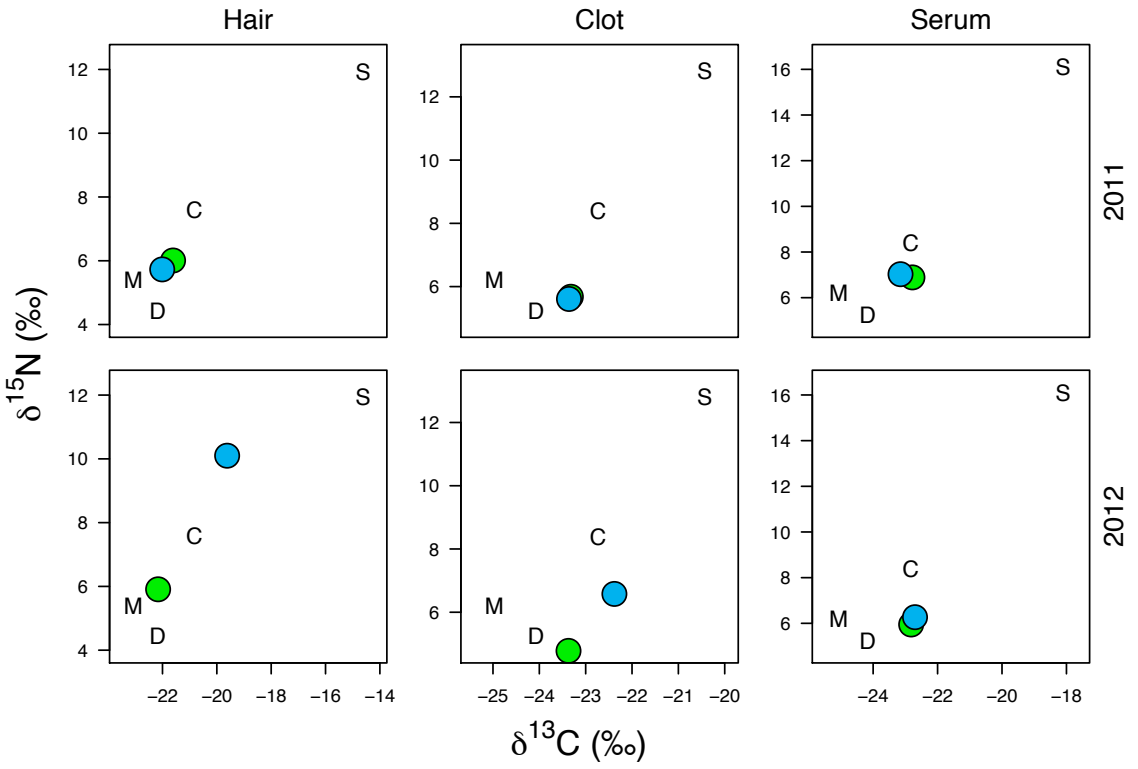

Figure 2.4 $\delta^{13}C$ and $\delta^{15}N$ of Tela2 wolves from 2011 and 2012. $\delta^{13}C$ and $\delta^{15}N$ of each tissue collected from LC1118 (green) and LC1119 (blue) of the Tela2 group in 2011 (top row) and 2012 (bottom row). Mean values of prey (Table 2.3) are included as reference points (C=Caribou, M=Moose, D=Dall's Sheep, S=Salmon) and have been adjusted for diet to tissue discrimination (Table 2.2).

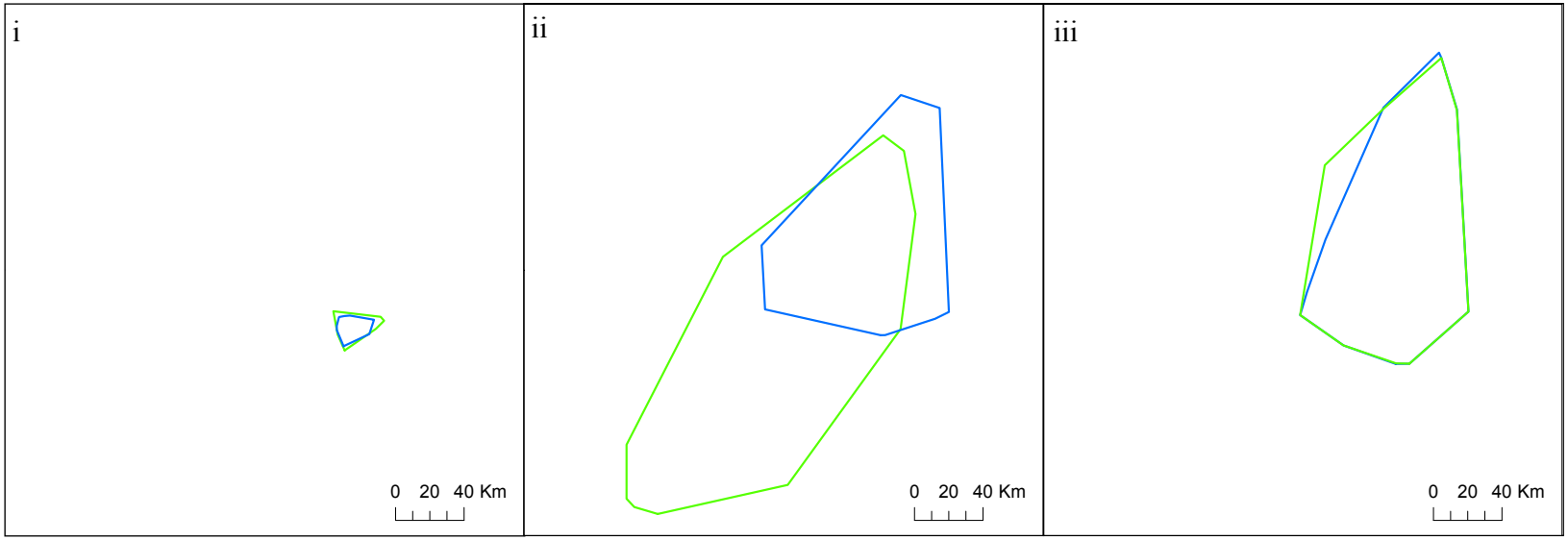

Figure 2.5 Minimum convex polygons (MCP) of GPS locations recorded by collars deployed on Tela2 wolves in 2011. MCPs show relative locations of wolf LC1118 (green) and wolf LC1119 (blue) and indicate when both wolves were together (i and iii) and separate (ii). Each map corresponds to the following dates i: 1-Mar-2011 to 21-Mar-2011, ii: 22-Mar-2011 to 30-Sept-2011, and ii: 1-Oct-2011 to 20-Feb-2012. Locations correspond to stable isotope values hair (ii) and serum (iii) collected in 2012 (Figure 2.4).

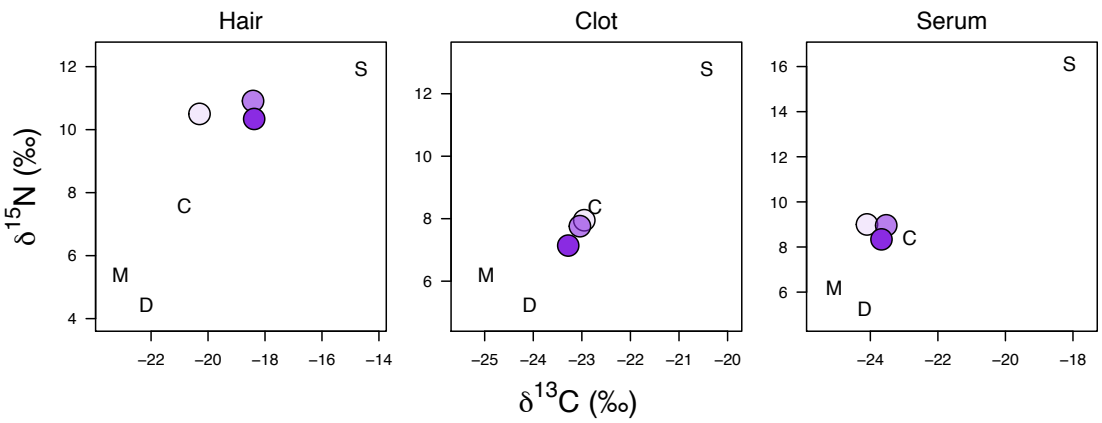

Figure 2.6 $\delta^{13}C$ and $\delta^{15}N$ of wolf LC0906 from 2009 through 2011. Each plot shows $\delta^{13}C$ and $\delta^{15}N$ of tissues collected in 2009 (light purple), 2010 (medium purple), and 2011 (dark purple). Mean values of prey (Table 2.3) are included as reference points (C=Caribou, M=Moose, D=Dall's Sheep, S=Salmon) and have been adjusted for diet to tissue discrimination (Table 2.2).

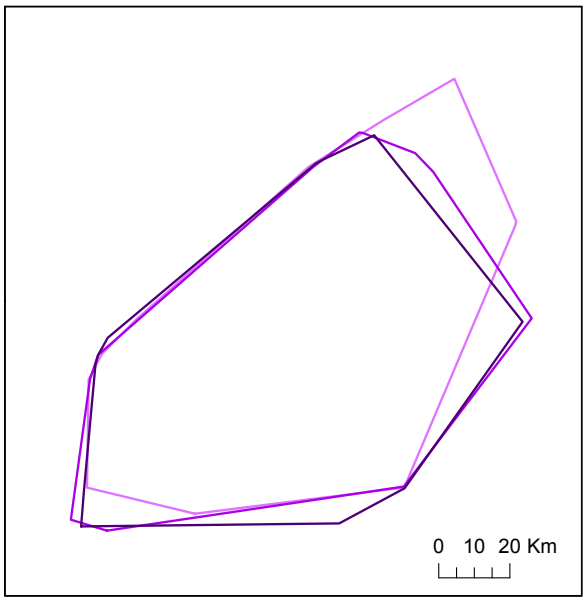

Figure 2.7 Minimum convex polygons (MCPs) of GPS locations recorded by collars deployed on wolf LC0906 from 2009 through 2011. MCPs show relative location of wolf LC0906 in 2009 (light purple), 2010 (medium purple), and 2011 (dark purple) corresponding to locations collected from 7-Feb-2009 to 1-Feb-2010 (n=343), 7-Feb-2010 to 1-Mar-2011 (n=565), and 1-Mar-2011 to 9-Dec-2011 (n=573), respectively. The polygon from 2009 corresponds with isotope values of tissues collected in 2010 and the 2010 polygon corresponds to tissues collected in 2011. Locations from 2011 do not correspond with tissues, but the MCP is shown to establish the consistency of this wolf's range throughout our study.

2.10 Tables

Table 2.1 Individual wolves captured throughout the study between 2008 and 2012. An asterisk (*) indicates that the individual had been captured in a previous year.

Wolf ID	Date Captured		Sex	Social Group
LC0801	4-Dec-08		M	Telaquana
LC0802	5-Dec-08		M	Telaquana
LC0903	6-Feb-09		M	Long Lake
LC0904	6-Feb-09		F	Long Lake
LC0905	7-Feb-09		F	Kijik
LC0906	7-Feb-09		F	Chekok
LC0907	7-Feb-09		F	Chekok
LC0908	8-Feb-09		M	Kijik
LC0906	7-Feb-10	*	F	Chekok
LC0907	7-Feb-10	*	F	Chekok
LC1011	7-Feb-10		M	Chekok
LC0802	8-Feb-10	*	M	Telaquana
LC1013	8-Feb-10		M	Ptarmigan
LC1014	12-Feb-10		M	Telaquana
LC1115	28-Feb-11		M	Lower Twin
LC1116	28-Feb-11		F	Lower Twin
LC1117	1-Mar-11		M	Tela 2
LC1118	1-Mar-11		M	Tela 2
LC1119	1-Mar-11		M	Tela 2
LC0906	2-Mar-11	*	F	Chekok
LC1120	2-Mar-11	*	M	Chekok
LC1118	20-Feb-12	*	M	Tela 2
LC1119	20-Feb-12	*	M	Tela 2
LC1224	21-Feb-12		F	Chulitna
LC1225	23-Feb-12		F	Nikabuna
LC1226	23-Feb-12		M	Nikabuna
LC1227	24-Feb-12		F	Kijik
LC1228	24-Feb-12		M	Kijik

Table 2.2 Diet-to-tissue discrimination values used to estimate wolf diets.

Diet (prey)	Tissue	$\Delta^{13}C \pm SD$ (‰)	$\Delta^{15}N \pm SD$ (‰)	Source	Species
Terrestrial	Hair	2.6 ± 0.2	3.4 ± 0.3	Roth and Hobson 2000	Red fox
	Clot	0.7 ± 0.2	2.6 ± 0.3	Roth and Hobson 2000	Red fox
	Serum	0.6 ± 0.2	4.2 ± 0.3	Roth and Hobson 2000	Red fox
Marine	Hair	6.05 ± 0.40	-0.49 ± 0.40	Stanek unpublished data (Appendix A)	Gray wolf
	Clot	0.24 ± 0.16	0.38 ± 0.16	Lecomte et al. 2011	Arctic fox
	Serum	2.57 ± 0.15	3.68 ± 0.40	Stanek unpublished data (Appendix A)	Gray wolf

Table 2.3 $\delta^{13}C$ and $\delta^{15}N$ of potential wolf prey in the Lake Clark region collected between 2009 and 2012.

Source	*n*	$\delta^{13}C \pm SD$ (‰)	$\delta^{15}N \pm SD$ (‰)
Terrestrial	15	-25.19 ± 0.95	2.10 ± 1.02
Caribou	2	-23.43 ± 0.04	4.19 ± 0.38
Dall's Sheep	3	-24.77 ± 0.29	1.04 ± 0.38
Moose	10	-25.67 ± 0.62	2.00 ± 0.48
Marine (Salmon)	12	-20.67 ± 0.42	12.43 ± 0.34

Table 2.4 Percent salmon in the diets of wolves in Lake Clark as related to outlier analysis results.

			% Salmon		
Year	Tissue	Isotope with outliers	Single group (*n*)	Depleted group (*n*)	Enriched group (*n*)
2009	Hair	^{13}C		3-12 (4)	35-57 (4)
	Clot	^{13}C		10-41 (6)	69-89 (2)
	Serum	^{13}C, ^{15}N		5-25 (6)	64-73 (2)
2010	Hair		10-66 (6)		
	Clot		16-36 (6)		
	Serum	^{15}N		9-12 (3)	19-22 (3)
2011	Hair	^{13}C, ^{15}N		1-18 (5)	56-61 (2)
	Clot	^{15}N		8-10 (3)	18-27 (4)
	Serum		5-17 (7)		
2012	Hair	^{13}C, ^{15}N		1 (5)	44-52 (2)
	Clot	^{13}C		1-21 (6)	25 (1)
	Serum		1-12 (7)		

Chapter 3 General conclusions

Southwest Alaska is a prime location to study how wolves subsist in diverse and dynamic environments. Our findings reveal substantial temporal and individual variability in diet composition and foraging strategies of wolves in the Lake Clark region. The heterogeneity we have observed is likely not restricted to Southwest Alaska. Given the ubiquity of salmon across much of Alaska, wolves throughout the state may be using this non-ungulate resource to a degree that influences wolf-ungulate relationships. Additionally, differences in foraging ecology among individuals and social groups of wolves may be more widespread than previously considered. Consequently, these facets of wolf foraging ecology may not be accounted for when assessing wolf-ungulate dynamics in spite of their potential influence.

Though we have considered salmon to be an alternative-resource, salmon may actually be a primary and critical resource to wolves in some regions or during certain seasons. In this study we documented salmon contributing to over half of an individual wolf's diet during a particular season. If these are common phenomena we may be disregarding a primary food source when assessing wolf predation patterns. Given their potential influence, a more in-depth examination of the influence of salmon on terrestrial predator-prey systems is clearly warranted.

Appendix A

Diet Switch Experiment[1]

A.1 Introduction

Carbon and nitrogen stable isotope analysis has become a frequently used tool in assessing seasonal changes in the dietary ecology of animals (Dalerum and Angerbjörn 2005; Newsome et al. 2009; Ben-David and Flaherty 2012). However, to use this technique effectively, we must understand how quickly and how consistently the isotopic values of dietary resources are incorporated into a consumer's tissues (Martínez del Rio and Carleton 2012). These parameters, referred to as the incorporation rate and the diet-to-tissue discrimination value, can vary between species, tissues and diets (Martínez del Rio et al. 2009b). Consequently, it is essential that we use incorporation rates and diet-to-tissue discrimination values that are appropriate for a given study.

A common technique to estimate dietary changes over time is to examine differences in isotope values within and between tissues (Dalerum and Angerbjörn 2005; Martínez del Rio et al. 2009a). Incorporation rates are required for estimating the time periods over which each tissue represents diet. Tissue-specific diet-to-tissue discrimination values are required so that inherent differences in isotope values between tissues are not incorrectly attributed to dietary variation. Comparing segments of continuously grown tissues, such as vibrissae, can also be used to assess changes in diet over time. However to assign a temporal component to each segment we must know the growth rate of these tissues.

To augment a recent National Park Service study assessing the use of salmon (*Oncorhynchus* spp.) by wolves (*Canis lupus*) in Lake Clark National Park and Preserve, we conducted a diet switch experiment with captive wolves housed at the Alaska Zoo, Anchorage, AK. In this experiment we measured the carbon (^{13}C) and nitrogen (^{15}N) stable isotope incorporation rates and diet-to-tissue discrimination values for plasma, serum, red blood cells, blood clots, hair and vibrissae of wolves consuming a marine diet.

[1] Prepared for submission as Stanek AE, Wolf N, Welker JM and Jensen S.

Incorporation rates and discrimination values for wolves on a marine diet have not previously been measured. Previous studies examining the diets of wolves in natural settings have applied values derived from red foxes (*Vulpes vulpes*) on a terrestrial diet (Roth and Hobson 2000).

In this study we address the following questions regarding wolves consuming a marine diet:

1) What time periods are represented by ^{13}C and ^{15}N in wolf serum, plasma, red blood cells, and clot?

2) What is the^{13}C and ^{15}N diet-to-tissue discrimination values for wolf serum, plasma red blood cells, clot, hair, and vibrissae?

3) What is the growth rate of wolf vibrissae?

A.2 Methods

A.2.1 Sample collection

The Alaska Zoo (Anchorage, AK, USA) maintains a captive population of six mature gray wolves (three males and three females). To measure diet-to-tissue discrimination and stable isotope incorporation in wolves we conducted a diet switch experiment (Martínez del Rio and Carleton 2012). Normally the wolves receive a consistent diet based on nutritional needs with occasional shifts based on the seasonal availability of animal proteins. To guard against any inconsistencies in diet during our study, we conducted this experiment during a period in which wolves were maintained on consistent diets. Prior to the study, all wolves were maintained on a terrestrial diet consisting of 100% beef chow (Tripple A Brand Meat Company, Burlington, CO) for 35 days (Figure A.1). Following the terrestrial diet, the diet of all wolves was switched (on day 1) to consist of 100% salmon (Table A.1) for 70 days (Figure A.1).

On day 0, a small patch of hair (approximately 3 x 3 cm) was shaved from the right front shoulder and two vibrissae from the right cheek were clipped at the skin surface from each individual. Hair that grew into the shaved patch was resampled on day

70. Stable isotope values of whole hair grown while wolves were only consuming salmon were compared to diet (salmon) ^{13}C and ^{15}N to calculate diet-to-tissue discrimination.

In addition to measuring discrimination in vibrissae, we also measured vibrissae growth rate using two techniques. First, we measured the length each clipped vibrissae grew at the end of 70 days. Second, we administered an oral dose of 50 ml. of water enriched in ^{2}H. Drinking water ^{2}H is incorporated into keratinaceous tissues (Podlesak et al. 2007; Wolf et al. 2011). This method provides an isotopic 'label' that can be used to isotopically mark day 0 along vibrissae. Though the incorporation rate and discrimination value between water and vibrissae is unknown, we are only aiming to detect an enrichment in vibrissae segment ^{2}H, thus, the magnitude of this enrichment is less important. On day 70 we plucked vibrissae which were assumed to have been growing prior to and throughout the study and measured the length along the vibrissae where the ^{2}H pulse was incorporated. Animal handling protocols were approved by The Alaska Zoo and the University of Alaska Anchorage Institution Animal Care and Use Committee (protocol #357268-1).

Diet, hair, and vibrissae samples were prepared using the methods described by Newsome et al. (2009, 2010). Briefly, diet specimens were rinsed clean using distilled water and digestible sections were removed for analysis. Digestible portions were freeze dried for 48 hours and ground to a fine powder using a mortar and pestle. Approximately 1.0 mg of the powdered samples were sealed in tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis. Hair and vibrissae samples were rinsed with a 2:1 chloroform methanol mixture to remove surface contaminants. Following cleaning, vibrissae were subsampled into approximately 0.5-0.6 mg segments for $\delta^{13}C$ and $\delta^{15}N$ analysis and 0.15-0.20 mg segments for $\delta^{2}H$ analysis and sealed in tin or silver capsules, respectively. Hair was ground to a fine powered using a freezer-mill (SPEX Sample Prep, Metuchen, NJ). Approximately 0.5 mg of the ground hair was sealed in a tin capsule for analysis. Blood components were oven dried at 60°C for at least 24 hours and ground to a fine powder

with a mortar and pestle. Approximately 0.8-1.0mg of homogenized blood components were weighed into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis.

Carbon and nitrogen isotope values were determined using a Costech elemental analyzer (Valencia, CA., USA) coupled to a Delta Plus XP continuous-flow isotope ratio mass spectrometer (CFIRMS) (Thermo Scientific, Waltham, MA., USA). Hydrogen isotope values were determined using a Thermo temperature conversion elemental analyzer coupled to a Delta Plus XP CFIRMS. Stable isotope analysis was performed at the Environment and Natural Resources Institute Stable Isotope Lab, University of Alaska Anchorage. Stable isotope values are reported in delta (δ) notation, which is calculated as: $\delta X = (R_{sample}-R_{standard})/R_{standard} \times 1000$, where R is the ratio of heavy to light isotopes, and relative to international standards (atmospheric nitrogen for $\delta^{15}N$, and Vienna Peedee Belemnite for $\delta^{13}C$). Internal standards (NIST 1547, bowhead whale baleen, Acetanalide, and chicken feathers) were used to determine an accuracy of ±0.1‰ for carbon and ±0.2‰ for nitrogen. Internal standards (bowhead whale baleen, turkey feathers, and chicken feathers) were used to determine an accuracy of ±X.X‰ for hydrogen.

A.2.2 Analysis of incorporation and diet-to-tissue discrimination

We measured isotopic incorporation of blood components using the average residence time model from Martínez del Rio and Anderson-Sprecher (2008). Incorporation curves for serum and plasma were calculated for individual wolves. Diet-to-tissue discrimination values were determined by subtracting the $\delta^{13}C$ or $\delta^{15}N$ values of diet from the mean asymptotic values of wolf plasma and serum.

A.3 Results

Serum (Figure A.2) and plasma (Figure A.3) fully incorporated the marine diet during the study, and reflect diet over the previous 2-3 weeks (Table A.2). Red blood

cells (Figure A.4) and blood clot (Figure A.5) did not fully incorporate the marine diet during the study, so reflect diet over a period longer than 70 days prior to collection.

Diet to tissue discrimination ($\Delta_{tissue\text{-}diet}$) values ranged from 1.77‰ in plasma to 6.05‰ in hair for ^{13}C and from -0.49‰ in hair to 3.68‰ in serum for ^{15}N (Table A.2, Figures A.2 and A.3). As red blood cells and clot did not fully incorporate the marine diet (Figures A.4 and A.5), discrimination values could not be calculated.

Vibrissae growth rates were measured using two methods. First, one to two vibrissae that were clipped at the skin's surface on day 0 were clipped again on day 70 and the length each grew in 70 days was measured in mm. If two vibrissae were clipped, the longer vibrissa was also segmented to measure $\delta^{13}C$ and $\delta^{15}N$ (Figure A.6). After 70 days, the mean length of each clipped vibrissae (n=10) was 26 mm (mean growth rate of 0.37 mm per day). Lengths of replicate vibrissae at the end of 70 days were not necessarily similar. Two vibrissae were measured from Denali (37 mm and 14 mm), Lucky (27 mm and 13 mm) and Rohn (26 mm and 20 mm). Mean vibrissae length for each wolf (n=6) after 70 days was 27 mm (0.39 mm per day).

We also measured vibrissae growth rate with a deuterium label. Four vibrissae showed sections clearly depleted and enriched in $\delta^{2}H$ (Figure A.7), representing vibrissae segments grown prior to and after the administration of $\delta^{2}H$, respectively. The first enriched segment of these vibrissae occurred at 14mm (Ruby), 18mm (Rohn), 19mm (Lucky), and 32mm (Windy). All segments of the vibrissae from Denali (35mm) were enriched. The most distal segment of the vibrissae from Nikolai (at 16mm) was depleted relative to more proximal segments however it was relatively enriched compared to depleted segments from other wolves.

A.4 Discussion

Since it is well known that isotopic incorporation rates and diet-to-tissue discrimination values can vary between tissues, diets, and species, the use of appropriate values for these parameters is essential for reliable dietary investigations using stable

isotopes. Previous studies using stable isotope analysis to study the diet of wolves have used discrimination values estimated for red foxes on a terrestrial diet (Roth and Hobson 2000). Our results suggest these values may not be appropriate for wolves consuming marine resources. By providing experimentally-derived ^{13}C and ^{15}N incorporation rates and diet-to-tissue discrimination values from captive wolves, our work will allow for dependable estimates of dietary resource use in wild wolf populations. In addition, our estimates provide a basis for interpreting the data from previous studies designed to examine the dietary ecology of wolves using stable isotope analysis.

A.5 Acknowledgments

The support and dedication of The Alaska Zoo, Dr. Riley Wilson, and the Pet Stop veterinarians has been greatly appreciated.

A.6 References

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A.7 Figures

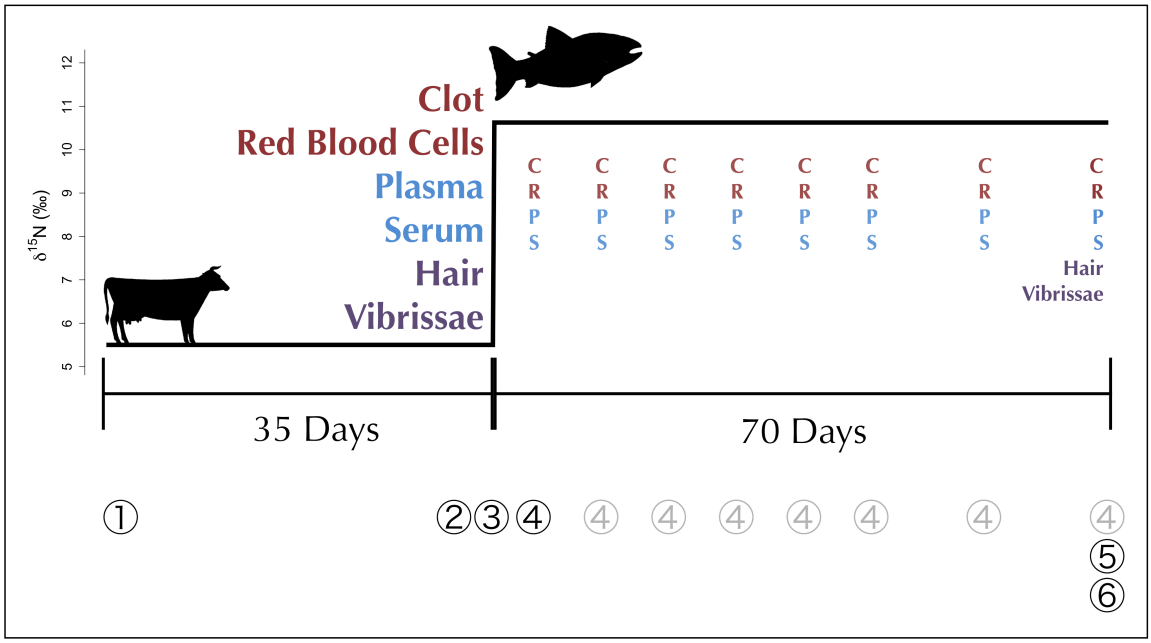

Figure A.1 Schematic describing timeline of events in diet switch experiment. Numbers correspond to the following events:

1) Maintained wolves on terrestrial diet ($\delta^{13}C$ = -18.15‰, $\delta^{15}N$ = 5.84‰) for 35 days
2) Collected tissues for baseline values on day 0 (serum, plasma, red blood cells, clot, and shaved a patch of hair off shoulder)
3) Clipped 3 vibrissae at surface of skin and administered water enriched in ^{2}H
4) Switched wolves to marine diet ($\delta^{13}C$ = -22.13‰, $\delta^{15}N$ = 10.81‰) starting on day 1
5) Collected blood on days 7, 14, 21, 28, 35, 42, 56, and 70
6) Collected hair from shaved patch on day 70
7) Collected previously clipped vibrissae on day 70

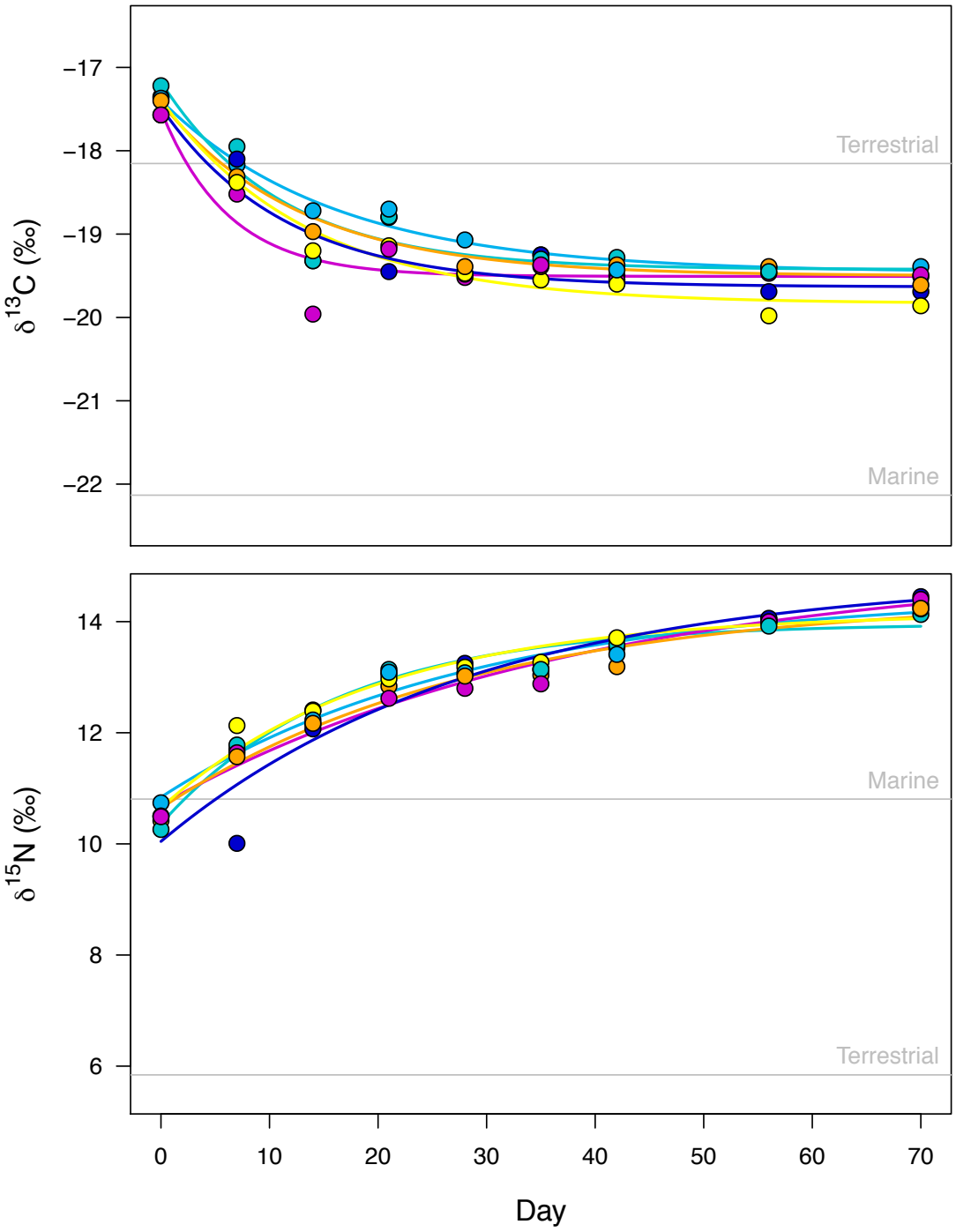

Figure A.2 $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) and incorporation curves of wolf serum after switching from a terrestrial to a marine diet. Colors represent individual wolves (Denali: light blue, Lucky: teal, Nikolai: pink, Rohn: orange, Ruby: yellow, Windy: dark blue).

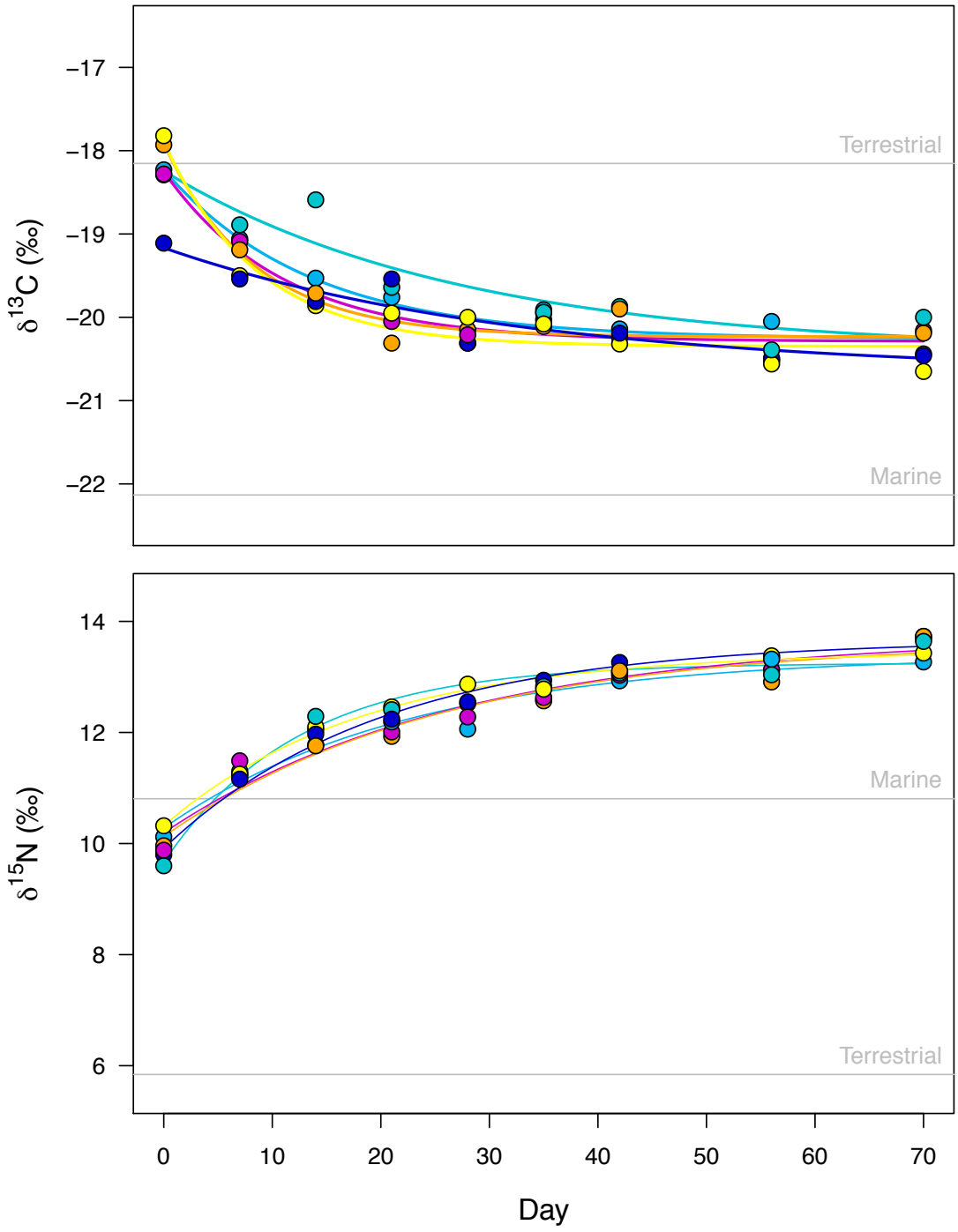

Figure A.3 $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) and incorporation curves of wolf plasma after switching from a terrestrial to a marine diet. Colors represent individual wolves (Denali: light blue, Lucky: teal, Nikolai: pink, Rohn: orange, Ruby: yellow, Windy: dark blue).

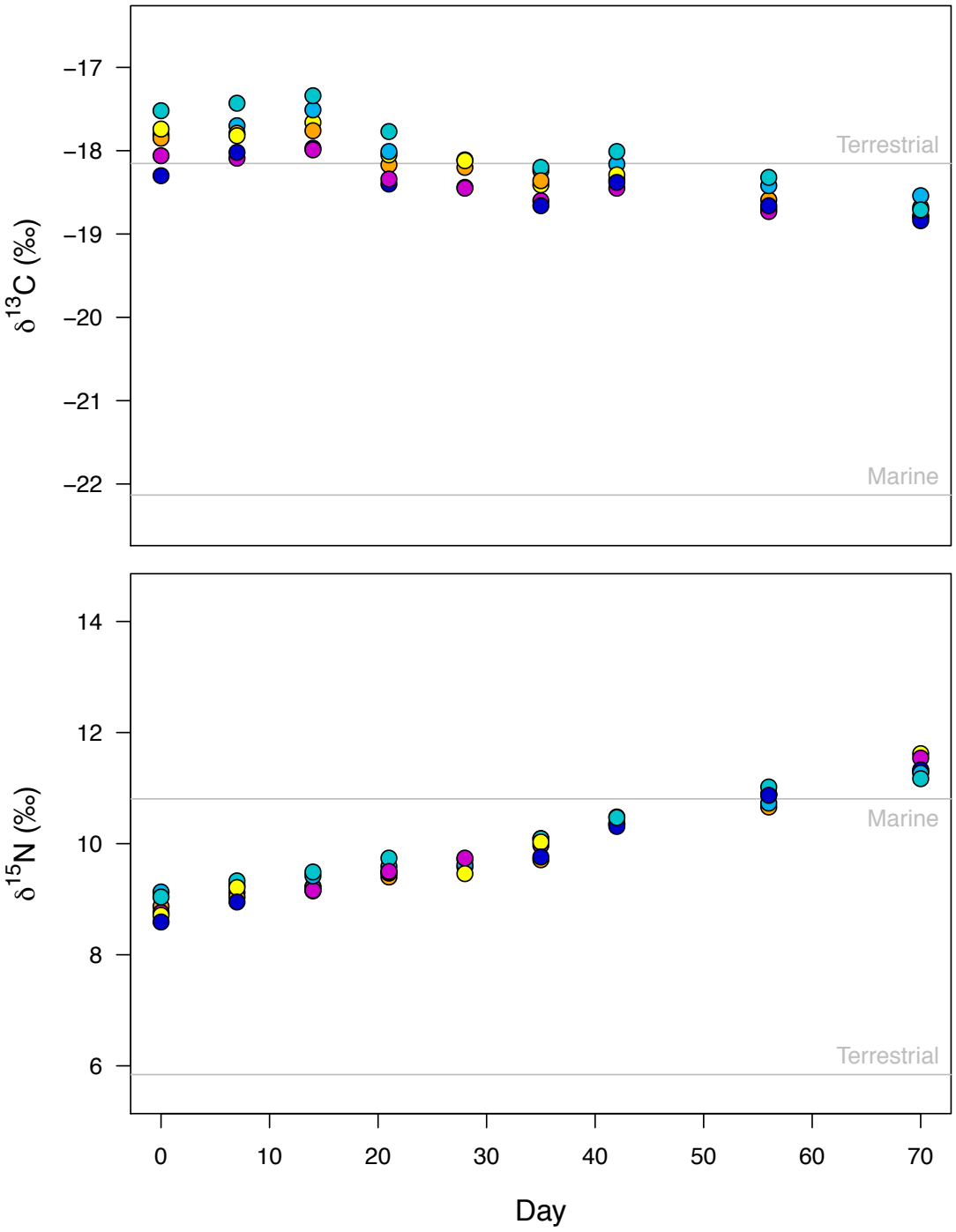

Figure A.4 $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) of wolf red blood cells after switching from a terrestrial to a marine diet. Colors represent individual wolves (Denali: light blue, Lucky: teal, Nikolai: pink, Rohn: orange, Ruby: yellow, Windy: dark blue).

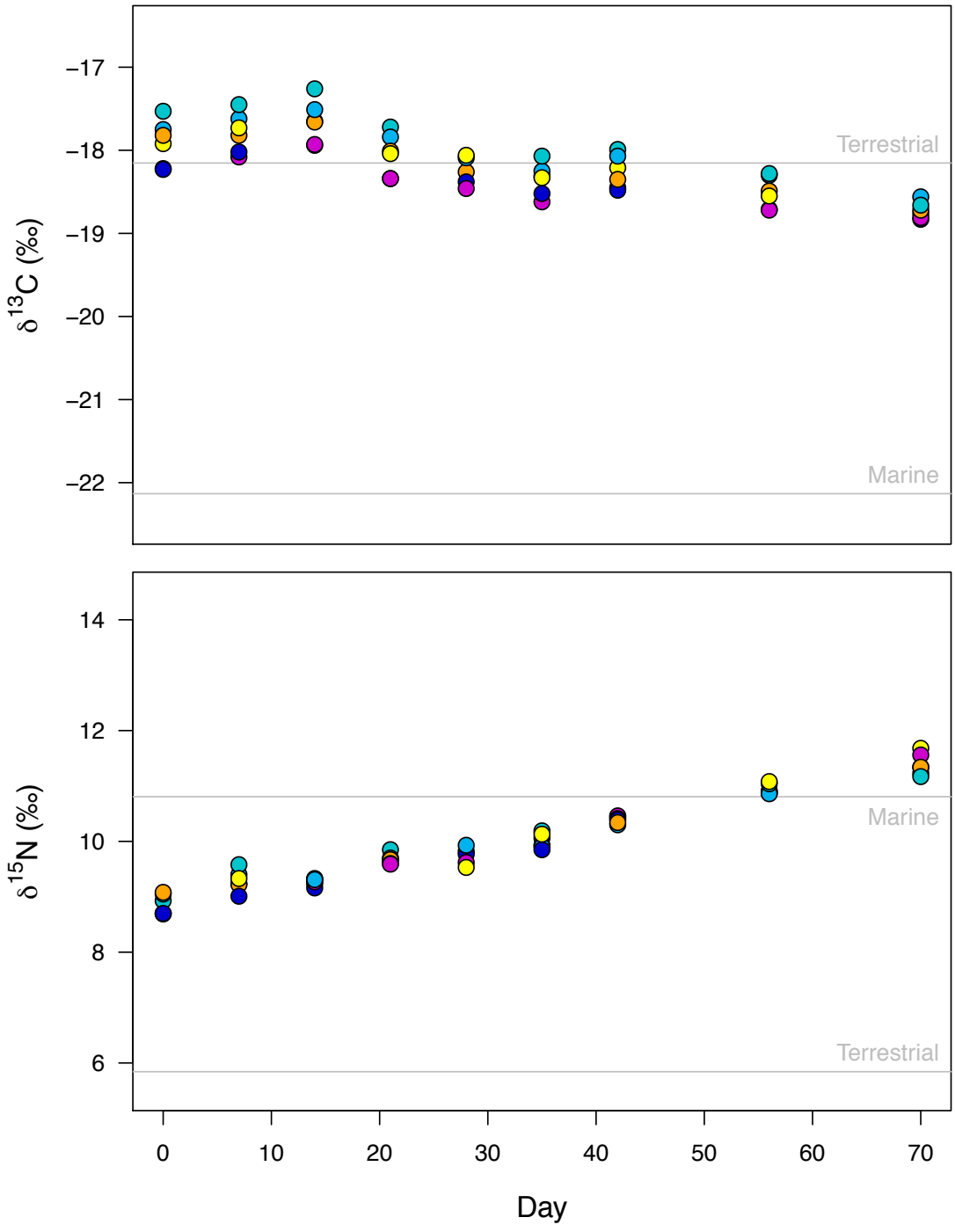

Figure A.5 $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) of wolf blood clot after switching from a terrestrial to a marine diet. Colors represent individual wolves (Denali: light blue, Lucky: teal, Nikolai: pink, Rohn: orange, Ruby: yellow, Windy: dark blue).

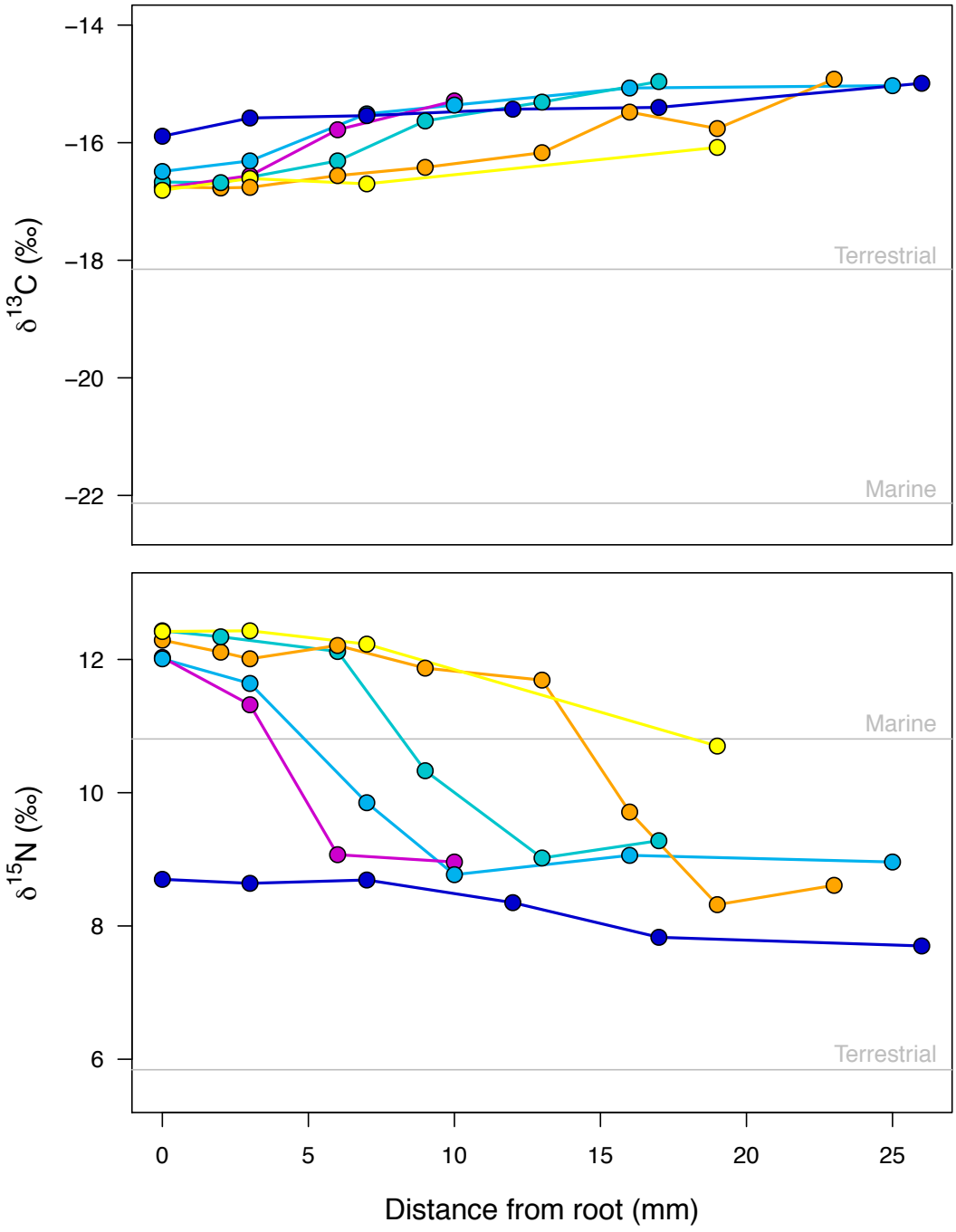

Figure A.6 $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) of wolf vibrissae segments after switching from a terrestrial to a marine diet. Distal ends were cut at the skin's surface on Day 0 and proximal ends (0 mm) were cut on day 70. Colors represent individual wolves (Denali: light blue, Lucky: teal, Nikolai: pink, Rohn: orange, Ruby: yellow, Windy: dark blue).

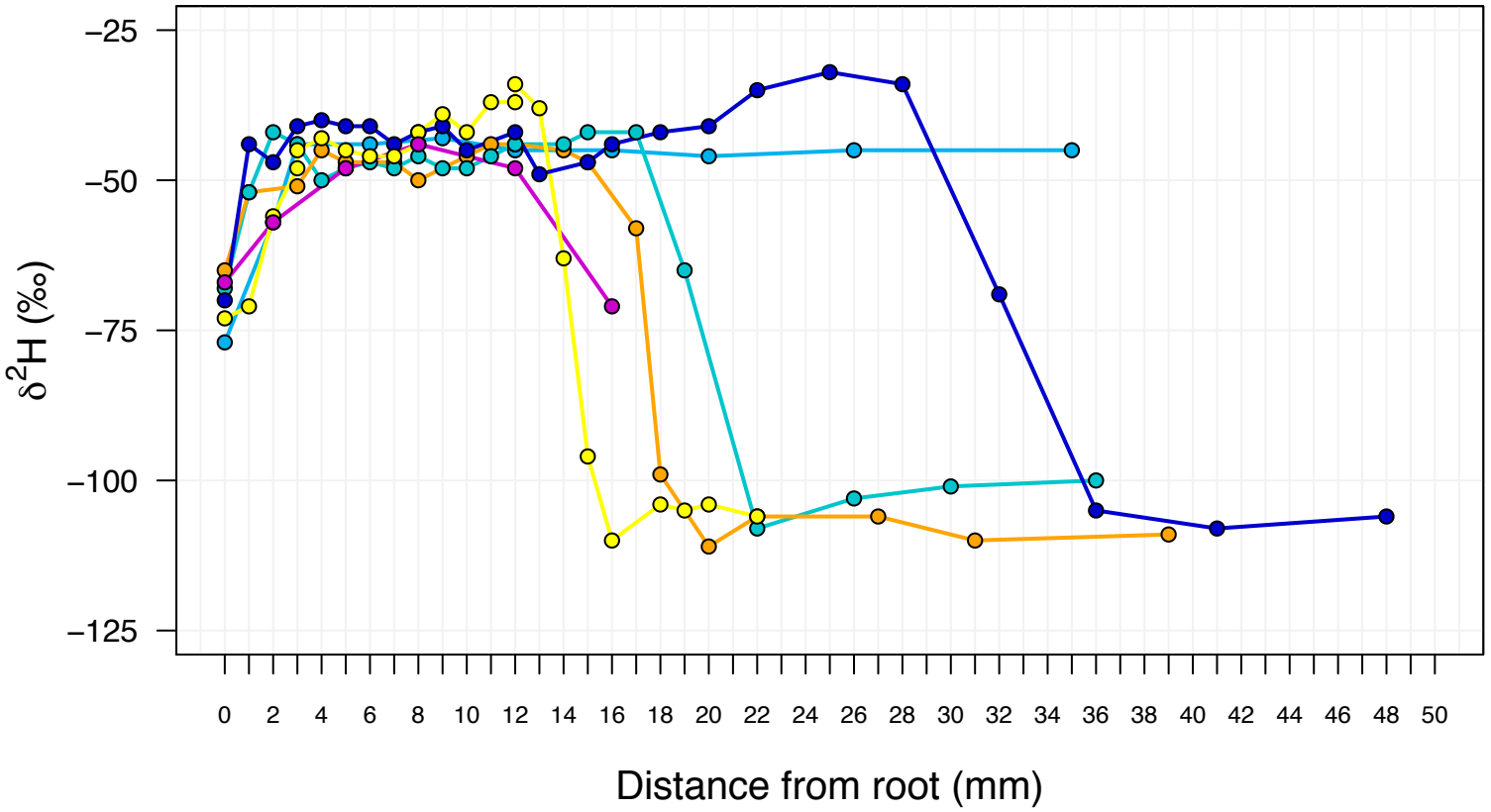

Figure A.7 δ^2H of captive wolf vibrissae (plucked on day 70) segments grown after receiving deuterated water. Colors represent individual wolves (Denali: light blue, Lucky: teal, Nikolai: pink, Rohn: orange, Ruby: yellow, Windy: dark blue).

A.8 Tables

Table A.1 $\delta^{13}C$ and $\delta^{15}N$ of terrestrial (Tripple 'A') and marine (salmon) diets fed to captive wolves.

Diet	*n*	$\delta^{13}C \pm SD$ (‰)	$\delta^{15}N \pm SD$ (‰)
Terrestrial (Tripple 'A')	2	-18.15 ± 0.70	5.84 ± 0.09
Marine (salmon)	6	-22.13 ± 0.61	10.81 ± 0.48

Table A.2 Mean ± SD diet-to-tissue discrimination values and average residence times (ART) of ^{13}C and ^{15}N of captive wolf tissues.

Tissue	$\Delta^{15}N \pm SD$ (‰)	ART ^{15}N (days)	$\Delta^{13}C \pm SD$ (‰)	ART ^{13}C (days)
Plasma	2.72 ± 0.19	21.08	1.77 ± 0.16	16.97
Serum	3.68 ± 0.40	27.04	2.57 ± 0.15	11.86
Whole Hair Day 70	-0.49 ± 0.40	NA	6.05 ± 0.40	NA

Appendix B

Additional Lake Clark Data

Table B.1 $\delta^{13}C$, $\delta^{15}N$, and estimates of contribution of salmon (%) from each tissue sample collected from Lake Clark wolves.

Year	Tissue	WolfID	Social group	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Salmon (%)	95% CI
2009	Hair	LC0905	Kijik	6.40	-21.90	3	0 - 14
		LC0908	Kijik	6.60	-21.70	5	0 - 16
		LC0903	LongLake	6.70	-21.60	6	0 - 17
		LC0904	LongLake	7.40	-21.40	12	0 - 23
		LC0802	Telaquana	8.90	-20.60	35	3 - 44
		LC0801	Telaquana	10.40	-20.40	49	7 - 59
		LC0906	Chekok	10.50	-20.30	51	8 - 60
		LC0907	Chekok	11.10	-20.20	57	15 - 68
2009	Clot	LC0905	Kijik	5.89	-24.02	10	1 - 21
		LC0908	Kijik	5.89	-23.91	10	1 - 21
		LC0904	LongLake	6.52	-24.13	20	6 - 28
		LC0903	LongLake	6.54	-24.04	19	6 - 28
		LC0906	Chekok	7.95	-22.95	36	25 - 45
		LC0907	Chekok	8.38	-22.69	41	30 - 50
		LC0802	Telaquana	10.55	-21.16	69	60 - 78
		LC0801	Telaquana	12.22	-20.66	89	81 - 95
2009	Serum	LC0903	LongLake	7.30	-24.30	5	0 - 13
		LC0908	Kijik	7.30	-24.60	5	0 - 13
		LC0904	LongLake	7.60	-24.50	7	0 - 15
		LC0905	Kijik	8.00	-24.40	13	1 - 19
		LC0906	Chekok	9.00	-24.10	21	12 - 29
		LC0907	Chekok	9.60	-24.40	25	17 - 31
		LC0802	Telaquana	13.20	-21.00	64	55 - 70
		LC0801	Telaquana	14.30	-20.80	73	66 - 79
2010	Hair	LC1013	Ptarmigan	6.77	-21.36	10	1 - 20
		LC1014	Telaquana	7.78	-20.86	18	3 - 31
		LC1011	Chekok	9.33	-19.96	43	15 - 50
		LC0907	Chekok	10.42	-19.14	57	34 - 66

Table B.1 continued

Year	Tissue	WolfID	Social group	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Salmon (%)	95% CI
2010	Hair	LC0906	Chekok	10.91	-18.42	65	50 - 75
		LC0802	Telaquana	11.27	-19.05	66	56 - 76
2010	Clot	LC1013	Ptarmigan	6.20	-23.74	15	3 - 26
		LC1014	Telaquana	6.29	-23.39	18	5 - 28
		LC0802	Telaquana	6.93	-23.08	25	14 - 35
		LC1011	Chekok	7.47	-23.17	30	20 - 40
		LC0906	Chekok	7.76	-23.04	33	23 - 43
		LC0907	Chekok	8.05	-23.00	36	27 - 46
2010	Serum	LC0802	Telaquana	7.19	-22.92	12	3 - 20
		LC1014	Telaquana	7.46	-23.33	10	1 - 18
		LC1013	Ptarmigan	7.50	-23.50	9	1 - 17
		LC1011	Chekok	8.65	-23.82	19	8 - 27
		LC0907	Chekok	8.90	-23.77	22	11 - 29
		LC0906	Chekok	8.96	-23.53	22	12 - 30
2011	Hair	LC1119	Tela2	5.73	-22.01	1	0 - 11
		LC1117	Tela2	5.92	-21.49	6	0 - 15
		LC1118	Tela2	6.01	-21.61	5	0 - 14
		LC1116	LowerTwin	7.25	-21.28	11	1 - 23
		LC1115	LowerTwin	7.57	-20.87	18	5 - 30
		LC0906	Chekok	10.34	-18.38	56	43 - 67
		LC1120	Chekok	10.67	-18.77	61	41 - 70
2011	Clot	LC1117	Tela2	5.50	-23.23	8	0 - 19
		LC1119	Tela2	5.61	-23.36	9	0 - 19
		LC1118	Tela2	5.68	-23.32	10	1 - 20
		LC1115	LowerTwin	6.42	-23.59	18	6 - 28
		LC1116	LowerTwin	6.46	-23.56	19	6 - 29
		LC1120	Chekok	6.82	-23.39	24	12 - 33
		LC0906	Chekok	7.14	-23.28	27	16 - 37

Table B.1 continued

Year	Tissue	WolfID	Social group	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Salmon (%)	95% CI
2011	Serum	LC1118	Tela2	6.89	-22.78	14	4 - 21
		LC1117	Tela2	6.98	-22.63	16	6 - 22
		LC1119	Tela2	7.03	-23.15	8	1 - 17
		LC1115	LowerTwin	7.08	-23.49	5	0 - 14
		LC1116	LowerTwin	7.49	-23.71	8	0 - 16
		LC1120	Chekok	8.01	-23.72	13	3 - 21
		LC0906	Chekok	8.34	-23.67	17	5 - 25
2012	Hair	LC1225	Nikabuna	5.23	-22.66	1	0 - 7
		LC1224	Chulitna	5.46	-22.57	1	0 - 8
		LC1118	Tela2	5.91	-22.16	1	0 - 11
		LC1228	Kijik	6.21	-22.08	1	0 - 12
		LC1227	Kijik	6.59	-22.16	1	0 - 14
		LC1226	Nikabuna	9.49	-20.08	44	11 - 53
		LC1119	Tela2	10.10	-19.62	52	21 - 61
2012	Clot	LC1118	Tela2	4.78	-23.37	1	0 - 13
		LC1224	Chulitna	5.13	-24.54	3	0 - 12
		LC1227	Kijik	5.23	-24.44	4	0 - 13
		LC1228	Kijik	5.55	-24.36	7	0 - 16
		LC1226	Nikabuna	5.87	-24.49	11	0 - 19
		LC1119	Tela2	6.58	-22.38	25	12 - 37
		LC1225	Nikabuna	6.61	-24.15	21	7 - 29
2012	Serum	LC1118	Tela2	5.95	-22.82	10	2 - 16
		LC1119	Tela2	6.27	-22.70	12	3 - 19
		LC1226	Nikabuna	6.47	-25.68	1	0 - 6
		LC1224	Chulitna	6.80	-24.01	1	0 - 11
		LC1228	Kijik	6.88	-24.40	1	0 - 10
		LC1225	Nikabuna	7.05	-25.56	1	0 - 9
		LC1227	Kijik	7.33	-24.26	7	0 - 14