UNIVERSITY OF CALIFORNIA
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# DIETARY ECOLOGY OF COASTAL COYOTES (*CANIS LATRANS*): MARINE-TERRESTRIAL LINKAGES FROM THE HOLOCENE TO PRESENT

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EARTH SCIENCES

by

**Rachel Elizabeth Brown Reid**

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The dissertation of Rachel Elizabeth Brown Reid is approved:

_____
Professor Paul Koch, chair

_____
Professor Diane Gifford-Gonzalez

_____
Professor Daniel Costa

_____
Associate professor Matthew Clapham

_____
Tyrus Miller
Vice Provost and Dean of Graduate Studies

# Table Of Contents

# List Of Figures

# List of Tables

**ABSTRACT**

Dietary Ecology of Coastal Coyotes (*Canis latrans*): Marine-Terrestrial Linkages from the Holocene to Present

by

Rachel Elizabeth Brown Reid

Coyotes (*Canis latrans*) have an expanding North and Central American range and have also been shown to benefit from marine subsidies. Identifying the past and present role coyotes play in linking land and sea, and whether those links are lost or gained through time, will have important implications for the future management of this expanding species. The goals of my dissertation were to: (1) characterize the extent, magnitude and importance of a marine subsidy to modern coyotes on the central California coast; (2) determine whether Holocene coyotes on the central California Coast had an equivalent dietary niche; and (3) begin to evaluate (via predation and competition) the impact of this modern marine subsidy (where it occurs) on key terrestrial species.

To address these goals, I seasonally collected scats along coast-to-inland transects at three modern coastal sites around Monterey Bay over a span of two years. I used discriminant function analysis on a set of scat samples DNA-verified to species to show that morphological traits of gray fox, bobcat and coyote scats are not diagnostic of species; predictive models based on morphology have misclassification rates of ~35%. These results suggest that DNA-verification of scats is required in studies using scat to make claims about diet, abundance or habitat use by any of these

animals in localities where they are sympatric. I characterized modern coyote diets using traditional scat analysis techniques in tandem with stable isotope analyses of scats themselves. I established a diet-to-feces discrimination factor for coyotes by analyzing multiple tissues from road kill carcasses and validated scat stable isotope dietary predictions by carefully comparing scat stable isotope values with isotopes measured in prey remains sourced from the scats.

For the investigation of Holocene coyote dietary ecology I selected six archaeological sites around Monterey Bay with a range of occupation periods spanning from ~3000 – 700 BP. This allowed for establishment of a Holocene baseline with which to compare modern coyote ecology over millennial timescales. My data point to both the existence of a marine subsidy to modern coyotes, and to a positive impact on coyote abundance. Sub-fossil isotope data suggest that Holocene coyotes did not consume marine-derived foods, despite the nearby presence of a mainland seal rookery. These data suggest that the use of marine resources by contemporary coyotes is a new behavior relative to their recent ancestors, perhaps enabled by reduced competition with either humans or other, now-absent consumers (e.g., grizzly bears, *Ursus arctos californicus*).

This dissertation is dedicated to my parents,

David and Martha Brown,

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# Chapter 1

## Introduction

Coastal marine ecosystems are often much more productive than adjacent terrestrial habitats (Polis 1996) and the transfer of energy and resources from the sea can have profound consequences for coastal terrestrial ecosystems (Rose and Polis 1998; Spiller et al. 2010). Many terrestrial animals consume marine foods; in their 2003 review, Carlton and Hodder documented 135 records of marine resource use by 45 different terrestrial mammal species. In many cases, marine resource use is sporadic and opportunistic, however, marine subsidies can be important for the maintenance of a predator population (e.g. Roth 2003), and may facilitate predator expansion (Killengreen et al. 2011). The recent population increase and expansion of medium-sized predators (mesopredators) has largely been attributed to the top-down, releasing effect of widespread apex predator loss (Prugh et al. 2009; Ritchie and Johnson 2009; Ripple et al. 2013), but bottom-up effects, including both anthropogenic and marine subsidies, can also be involved (Killengreen et al. 2011). These explanations are not mutually exclusive; Elmhagen and Rushton (2007) observed a top-down mesopredator release effect for red foxes in Sweden, but found that ecosystem productivity limited its strength. Additionally, access to a particular subsidy may only be gained following the removal of a competitor (Darimont et al. 2009).

Coyotes (*Canis latrans*) are versatile, opportunistic omnivores with both an expanding North and Central American range and a previous record of benefitting from marine (Rose and Polis 1998) and anthropogenic (Fedriani et al. 2001; Kamler et al. 2004) subsidies. A significant body of research now attributes coyote expansion predominantly to wolf extirpation (Berger and Gese 2007; Levi et al. 2012; Ripple et al. 2013), but the role marine subsidies may play in contributing to their expansion has not been investigated. If marine subsidies to coyotes are relatively new, apex predator loss may be facilitating coyote use of marine resources, and depending on the effects the marine subsidy has on coyote populations, a new subsidy could significantly amplify the effects of mesopredator release. I conducted this dissertation research in an effort to better understand the spatial and temporal dynamics of a marine resource subsidy to coyotes in coastal California. I set out to quantify the degree to which modern coastal California coyotes rely on marine resources and to establish the age of the subsidy by comparing modern coyotes with individuals from the Holocene and historical periods.

To quantify modern coyote diets, I collected and analyzed hundreds of mesopredator scats from three coastal California sites. Conventional wisdom suggests that scats are identifiable to species based on their morphology, however I did not find this to be the case. In Chapter Two, I use a subset of carefully measured, DNA-verified scats to show that bobcat, coyote, and gray fox scat can't be distinguished by morphology alone. I built two types of predictive models, neither of which achieved correct classification rates higher than 75% and success rates of that magnitude

required the inclusion of a non-morphological variable (C:N ratio). I did, however, still observe evidence for different "end-member" morphologies among these groups and propose that the predictive models can be used as a first pass scat identification tool in tandem with more accurate methods, such as scat-detecting dogs or DNA.

In Chapter Three, I ground-truth the idea that scat carbon and nitrogen isotope values can serve as proxies for coyote diet. For a set of DNA-verified scats from Año Nuevo, I measured scat stable isotope values and isotope values in the food sources that I found in the scats. I used a Bayesian stable isotope mixing model to predict the composition of coyote diets based on their isotope values and compared the results with traditional scat analysis methods. I found that the details differed, but overall, the methods were largely in agreement. To make scat stable isotope measurements more useful, I also derived a diet-to-feces isotope discrimination factor by dissecting road-kill coyotes and analyzing the isotope values of different tissue types, including scat, hair, muscle and bone collagen.

In Chapter Four, armed with the tools I developed in the previous chapters, I use stable isotopes measured in scat to quantify the importance of marine resources in modern coyote diets. I compared the diets of modern coyotes with Holocene coyote diets derived from bone collagen stable isotope values. I focused on six coastal archaeological sites with occupation times spanning the last ~3000 years and included historical samples from the late 1800's through the 1990's. I found evidence for modern marine resource use by coyotes only at Año Nuevo, which has supported a northern elephant seal breeding colony since the late 1960's. In contrast, I found no

evidence for past marine resource use by coyotes, suggesting that their consumption of seals and sea lions at Año Nuevo today is a new behavior relative to the Holocene. This change in behavior is likely a result of relaxed competition with humans as well as grizzly bears, which were extirpated from California in the early 1900's, and is linked to the constancy of the subsidy. The marine subsidy to coyotes at Año Nuevo appears to be positively impacting their population size, though continued work is required to establish the full effects of the subsidy on the terrestrial coastal ecosystem. Finally, this newly gained access to marine resources has implications for coyote range expansion – it may be that coastal routes absent of apex predators provide coyotes with relatively easy pathways by which to extend their territory.

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# Chapter 2

## Bobcat, coyote and gray fox scats cannot be reliably distinguished by morphology alone

**Keywords:** canid, fecal DNA, felid, mitochondrial DNA, morphology, North America, scat, scat identification

**ABSTRACT**

Coyotes (*Canis latrans*), bobcats (*Lynx rufus*) and gray foxes (*Urocyon cineroargenteus*) are all common mammalian mesopredators in coastal California and are found sympatrically in much of North America. Scats produced by these three animals are quite similar, but have historically been differentiated largely by morphology. I tested the efficacy of morphological classification of scat to species by building two types of predictive models for species identification with a set of well-described, DNA-verified scats ($n$ = 135). I found significant differences among species in only 3 (diameter, mass and C:N ratio) of the 13 variables I considered. Linear discriminant analysis is only 71% predictive with the inclusion of a non-morphological variable in addition to morphological traits. Random forests similarly have only a 62% correct classification rate. Still, the linear discriminant model is able to identify scats with certain traits to species with a high degree of confidence,

lending credence to the idea of "end-member morphologies" for scats produced by these different animals. Alternate methods of scat identification such as mitochondrial (mt) DNA and scat detecting dogs can be expensive, therefore I suggest that predictive morphology based models be used as first order tools to more objectively select scats that require species verification by these other more reliable but costly methods. These results also suggest that previous studies using morphology-based scat identifications may have misrepresented or misinterpreted diets and space use by these sympatric mammals.

## INTRODUCTION

Feces are rich sources of information: they are ubiquitous, easily located in the field, and their collection generally causes little disturbance. Scat is therefore used frequently in wildlife ecology to evaluate the presence/absence of elusive animals (e.g. Palomares et al. 2002), estimate animal abundances (Kohn et al. 1999; Prugh et al. 2008), characterize diets (Reynolds and Aebischer 1991; Rose and Polis 1998; Symondson 2002; Deagle et al. 2005; Casper et al. 2007), and investigate animal health or disease ecology (e.g. Gompper et al. 2003; Liccioli et al. 2012). Historically, scats have been identified to species by morphology, but the morphological distinctions between scats produced by mammalian mesopredators can be difficult to discern and improperly identified specimens can confound and even invalidate research (Harrington et al. 2009). Despite some successes with field based scat identifications (Zuercher et al. 2003; Prugh and Ritland 2005), which are more

reliable when made in conjunction with additional natural sign (e.g. tracks), a number of studies have now documented the pitfalls inherent in relying on morphology alone (Bulinski and McArthur 2000; Davison et al. 2002; Reed et al. 2004; Harrison 2006). Molecular scatology can be employed to more reliably identify scat to species (Foran et al. 1997; Kohn et al. 1999; Bidlack et al. 2007), but it can be a prohibitively expensive tool when large numbers of scats need to be identified and processed. If scat morphology is distinctive, then careful documentation of the morphology of mitochondrial (mt) DNA-verified scats will make it possible for researchers to take measurements and predict with confidence to which species a scat belongs.

The goal of this paper is to evaluate whether a morphometric approach to scat identification is sufficient for distinguishing among scats produced by three of the most common mammalian mesopredators in coastal California: coyotes (*Canis latrans*), bobcats (*Lynx rufus*) and gray foxes (*Urocyon cineroargenteus*). To that end, I have compiled a database of morphological, biogeochemical and contextual traits for a set of scats DNA-verified to species and built predictive models for species identification using two different methods: discriminant function analysis, and random forests, a composite tree-based modeling approach. If morphology is truly indicative of species, then scats produced by different canids and felids should be statistically separable into groups according to differences in their morphologies (and biogeochemistry) and the predictive models should have low misclassification rates.

## STUDY AREA

I collected scats along two ~5 km coast-to-inland transects along roads and

trails at two different sites in the central coast region of California: Año Nuevo State Park and Reserve in San Mateo County, California, and Younger Lagoon Natural Reserve and Moore Creek Preserve in Santa Cruz County, California. Año Nuevo State Park is located about 30 kilometers north of Santa Cruz, California. A number of different habitats occur in the park, including native dunes, coastal terrace prairie and mixed evergreen forest. It is also home to a breeding colony of Northern elephant seals (*Mirounga angustirostris*), which established on the mainland in 1975 (Le Boeuf and Panken 1977). Younger Lagoon Natural Reserve is part of the University of California Natural Reserve System. It protects a remnant y-shaped lagoon on the north side of the town of Santa Cruz, California. Dense coastal shrub, willow thickets and coastal prairie occur within the reserve along with salt and freshwater marsh. Moore Creek Preserve is situated directly inland from Younger Lagoon on the opposite side of Highway 1. A city park since 1998, the Preserve primarily protects managed coastal prairie habitat, which is periodically grazed. Dogs are not allowed in any of these parks and preserves.

## MATERIALS AND METHODS

### Data Collection

I collected mesopredator scats quarterly in 2011 - 2013 to capture changes in scat morphology due to seasonal dietary and weather differences. I collected scat on the same transects each time, initially clearing transects of all scats and then returning a week later to collect the scats deposited during the intervening week. At the time of

collection, I recorded scat locations with a GPS and placed scats in individually marked Ziploc bags with a desiccant to reduce moisture and enhance DNA preservation. In the field I also measured and recorded scat diameter, scat length, number of pieces, length of taper, degree of taper (ratio of taper length to scat diameter), and classified scats as segmented and/or ropey (or neither). In the lab, I recorded scat dry weight. A detailed summary of each of these specific measurements is listed in Table 2.1. I assigned scats to a probable species before sending a subset of samples ($n$ = 135) to Wildlife Genetics International for mtDNA-based species identification. I collected scats under California Fish and Game permit SC-11995 and with the approval of the UC Santa Cruz IACUC (protocol Kochp1105).

**Table 2.1.** Morphological traits considered in the models.

| Measurement | Units | Description |
|---|---|---|
| **Scat diameter** | mm | Measurement at widest point to the nearest $10^{th}$ of a millimeter |
| **Scat length** | cm | Length of longest piece to the nearest 0.5 cm |
| **Taper length** | mm | Length of longest taper down the axis of the scat |
| **Degree of taper** | unitless | Ratio of taper length to scat diameter |
| **Number of pieces** | integer | Number of separate scat pieces |
| **Scat mass** | grams | Total dry weight after freeze drying and baking |
| **Segmented?** | na | Does the scat show segmentation? 1 = yes, 0 = no |
| **Ropey?** | na | Does the scat appear ropey/twisted/woven? 1 = yes, 0 = no |
| **Flat?** | na | Is the scat a flat puddle that lacks other morphological traits? 1= yes, 0= no |

In addition to recording the morphological traits of the scats, I also considered a few non-morphological variables, including the location of the scats on the trail or road, presence of other sign (e.g. tracks or scrape – a scratch mark left on the ground, Appendix 2A: Figure 2.A1), and finally the C:N ratio of the scat (Table 2.2). C:N

**Table 2.2.** Additional, non-morphological variables considered in the models.

| Variable | Units | Description |
|---|---|---|
| **Location** | 3 point scale | Categorical variable describing scat location on the trail/road – middle, edge, or off edge. |
| **Scrape?** | na | Is there a scrape mark near the scat? 1 = yes, 0 = no |
| **C:N Ratio** | unitless | Ratio of carbon to nitrogen atoms in the scat, which is a proxy for the degree of carnivory of the animal. |

ratios vary across ecosystems and through food webs, reflecting underlying organismal allocations to major molecules and chemical structures; terrestrial vascular plants tend to have high C:N ratios (Meyers 1994; Prahl et al. 1994) while animals tend to be much more nutrient rich and therefore have much lower C:N ratios (Sterner and Elser 2002). The C:N ratio of scat, then, should serve as a proxy for an animal's degree of carnivory: animals consuming a largely plant-based diet will produce scats with high C:N ratios and those consuming other animals will produce scats with low C:N ratios. I obtained C:N ratios of the samples as a by-product of stable carbon and nitrogen isotope analyses conducted as part of a different project. To prepare the samples for analysis, I extracted matrix samples from scats by gently breaking apart oven-dried scats over a fine mesh sieve. Matrix material is allowed to fall through the sieve while other scat components, such as fur, feathers or bone are captured above. I then cleaned the powdery matrix material for stable isotope analysis by placing it into filter paper cones and rinsing it first with MilliQ water and then with 0.1N HCl to remove possible $CaCO_3$ contaminants. After the sample is fully dry and homogenized, I weighed approximately 5 mg of scat matrix material into Sn boats. The samples were then combusted via Dumas combustion using a Carlo Erba 1108 elemental analyzer and analyzed on a ThermoFinnigan Delta Plus XP

continuous flow isotope ratio mass spectrometer at the UCSC Stable Isotope Laboratory. I calculated the average analytical precision for the scat data as the *SD* of the C:N ratio of 41 replicates of an internationally calibrated in-house standard (PUGel); precision was 0.2.

**Fecal Genotyping**

I conducted fecal genotyping in collaboration with Wildlife Genetics International. As per their recommendations, prior to being dried, I swabbed the scats with Q-tips, which were then stored dried in unwaxed coin envelopes. DNA is extracted by clipping a small (~3 mm x 3 mm) piece of each swab and processing the clippings as tissue samples using QIAGEN DNeasy Blood and Tissue Kits. The species test is a sequence-based analysis of the mitochondrial 16S rRNA gene (Johnson and O'Brien 1997). The specific primers and analytic conditions that Wildlife Genetics uses are not published, but their results could be reproduced using published methodology. Two variants of this analysis are employed using either primers that amplify across all mammals or primers designed to amplify Carnivora sequences in preference to other mammals. They compare the results to reference data from over 125 species of mammals.

**Statistical Analyses and Predictive Model Construction**

I performed one-way analysis of variance (ANOVA) to identify possible significant differences in the means of the traits of scats produced by the three

different species. I first tested the assumption of normality with the Shapiro-Wilk test and that of homogeneity of variance with the Bartlett test. Some variables required log transformation to meet one or both of these assumptions. When I observed statistically significant differences among groups with ANOVA, I then used the post hoc Tukey test to determine which of the three species contributed to the differences. Means are reported ± one standard deviation (SD) and I tested significance at the $p = 0.05$ level. Some scats lacked diameter, length, taper length and taper index measurements not because the measurements were neglected, but because the scats had irregular morphologies, rendering them irrelevant. These samples were coded as "flat" and I excluded them from the calculation of trait summary statistics, but not from the morphology models. All statistics were performed in R (R Development Core Team, 2013).

In an effort to build the most predictive model for identifying scats to species, I compared the results from two different approaches, multiple discriminant function analysis (DFA) and random forests, each of which has different strengths and limitations. DFA uses linear combinations of numerical predictor variables to optimally separate a categorical response variable – in this case "species" – and subsequently predict group membership for samples of unknown origin. To achieve this, it identifies gradients of variation among groups such that differences between groups are maximized, while within group variation is minimized (McGarigal et al. 2000). The assumptions for DFA include multivariate normality, equality of variance-covariance matrices across groups, and independent observations (McGarigal et al.

2000). DFA is generally robust to violations of these assumptions, at least when sample sizes are large (Williams and Titus 1988; McGarigal et al. 2000). When the covariance matrices among classes are different, then quadratic discriminant function analysis (QDA) may be a more appropriate alternative; the quadratic discriminant function is very similar to linear discriminant function analysis (LDA), but accommodates a class-specific covariance structure (Kuhn 2013). Williams and Titus (1988) proposed a general rule of thumb for sample sizes, specifying that each group should have $N \geq 3P$, where N is the number of occurrences in a group and P is the number of discriminating variables. My sample sizes are relatively small, so I limited the number of predictor variables in the final models to five or less. I standardized the variables and examined the covariance matrices for each group to check for their equality and inspected correlation matrices to check for possible collinearity among variables. The coyote, bobcat, and gray fox covariance matrices are not equivalent, so I performed QDA in addition to LDA. I separated my data into distinct training and test sets and evaluated classification error in the test set. I also assessed classification error on the full data set using a jackknife classification method, which is a leave-one-out cross-validation procedure. I took a stepwise approach to DFA, beginning with an over-fitted model that included all possible variables, and removed the least predictive variables one at a time. I performed LDA and QDA first with the morphological variables alone and again with the inclusion of the three additional non-morphological variables (C:N ratios, presence or absence of a scrape, and location on the trail). Here I present the purely morphological and combined models

with the lowest misclassification rates. I performed DFA in R using the "MASS" package (Venables and Ripley 2002).

The random forest (RF) approach to classification and regression is a non-parametric method developed in machine learning (Breiman 2001). It fits an ensemble of hundreds or thousands of classification trees to a dataset using recursive partitioning, a method that is particularly well-suited to small datasets with many explanatory variables (Strobl et al. 2009*a*). There are no distributional assumptions for either the predictor or response variables in RF and it is capable of handling missing data, making it potentially better suited for ecological datasets (Cutler et al. 2007; Strobl et al. 2009a). Because I have different types of predictor variables, I used cforest in the "party" package in R (Hothorn et al. 2006b; Strobl et al. 2007; 2008) with the default option controls=cforest_unbiased() and I used varimp() to evaluate variable importance (Strobl et al. 2007; 2009a; b). I evaluated model fit with the built in out-of-bag classification estimation. To aid in visualization, I also created a single classification tree using the ctree() command in the "party" package (Hothorn et al. 2006*a*) using a stop criterion based on the univariate *p*-values.

## RESULTS

### Scat Characteristics

I submitted 135 mammalian mesopredator scats to Wildlife Genetics for identification to species. Of those 135 scats, 10 failed identification and 2 contained mixed DNA. The remaining scats include 64 bobcat, 30 coyote, 28 gray fox and 1

spotted skunk. I excluded the failed, mixed and non-target scats from all subsequent analyses, leaving a dataset of 122 positively identified bobcat, coyote and gray fox scats. 33 scat samples are missing values for one or more variable and were therefore excluded from calculations of summary statistics and from discriminant function analysis; I did, however, include them in the random forest model.

Some of the variables I considered (mass and C:N ratio) did not meet the basic assumptions of ANOVA, which I addressed with a log transformation. Of the morphologic traits I considered, only scat diameter and mass are significantly different among groups ($F_{2,99} = 6.90$, $p = 0.0016$; $F_{2,103} = 4.82$, $p < 0.001$; Figure 2.1). Results from the post hoc Tukey tests suggest that coyote scats (16.45 ± 6.4 mm) have different diameters from both gray fox scats (11.5 ± 4.1 mm) and bobcat scats (13.3 ± 5.6 mm), but gray fox and bobcat scats do not have significantly different diameters from one another. Gray fox scats also have significantly different masses (6.6 ± 2.8 g) from both coyote scats (17.9 ± 13.9 g) and bobcat scats (12.4 ± 6.6 g), though bobcat and coyote scats have indistinguishable masses. Of the additional, non-morphologic variables I considered, scat C:N ratio is also significantly different ($F_{2,105} = 29.2$, $p < 0.001$) and the post hoc Tukey test reveals that all three species have statistically significant scat C:N ratios (coyote = 8.6 ± 2.3, bobcat = 6.7 ± 1.1, gray fox = 12.1 ± 5.6).

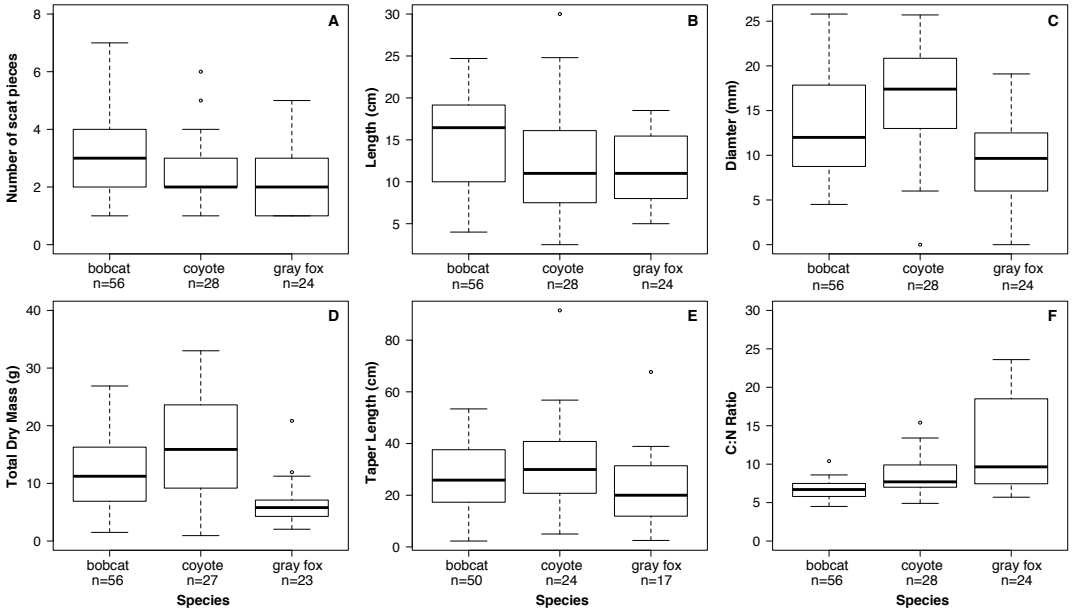


**Figure 2.1.** Boxplots comparing differences in 6 different coyote, bobcat and gray fox scat traits, including, (A) the number of scat pieces, (B) greatest length, (C) greatest diameter, (D) total dry mass, (E) greatest along axis taper length, and (F) scat C:N ratio.

## Discriminant Function Analysis

Separation among scats produced by coyotes, bobcats and gray foxes was not well achieved with the traits considered (Figure 2.2). Neither the LDA model based on morphology alone nor the model that included additional, non-morphological characteristics was very predictive. I included four variables in the morphology alone model: number of scat pieces, diameter, taper length, and log mass. Of these, diameter and log mass contributed most strongly to both the first and second linear discriminant (Table 2.3). There is a statistically significant difference in centroids on the first linear discriminant ($F_{2,57} = 47.99$, $p < 0.001$); the post hoc Tukey test indicates that all three species are significantly different from one another. The

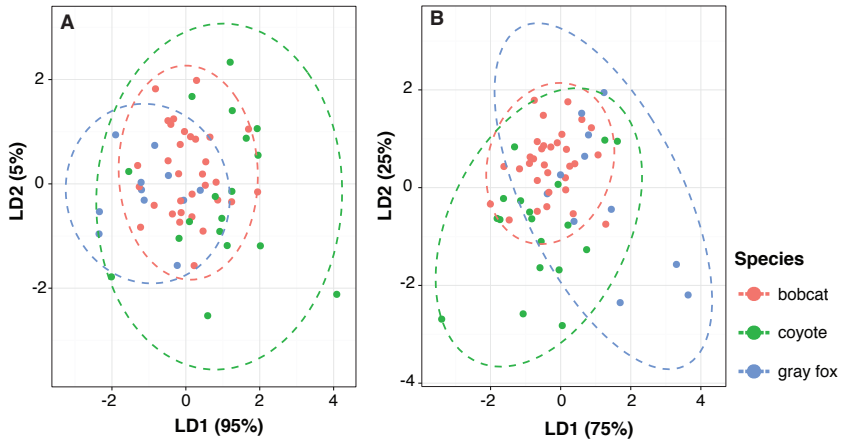


**Figure 2.2.** Linear discriminant plots demonstrating a lack of differentiation between coyote, bobcat and gray fox scats when modeled with (A) purely morphological characteristics and (B) with the inclusion of C:N ratio. The proportion of the trace accounted for by each linear discriminant is quoted in parentheses.

**Table 2.3.** Coefficients of linear discriminants for (A) the first LDA model with only morphological traits and (B) the second LDA model, which includes scat C:N ratio.

| **A. Model 1** | | | **B. Model 2** | | |
|---|---|---|---|---|---|
| Variable | LD1 | LD2 | Variable | LD1 | LD2 |
| Length | -0.433 | 0.234 | Length | -0.376 | -0.199 |
| Diameter | -0.798 | -0.817 | Diameter | -0.409 | -0.840 |
| Taper Length | -0.442 | -0.194 | Taper Length | -0.275 | -0.376 |
| log Mass | -0.593 | 0.746 | log Mass | -0.482 | -0.226 |
| | | | log C:N ratio | 0.779 | -0.693 |

statistically significant difference between centroids on the second discriminant ($F_{2,57}$ = 16.79, $p < 0.001$) is, however, driven solely by the coyote scat centroid. Only 60% of the scats in the training set were correctly identified to species and performance in the test set increased to 67% correctly classified. For the entire dataset, 62% of the jackknifed predictions were correct. There are species-specific differences in model performance – bobcat scats were consistently classified correctly more often than scats from the other two species.

Predictions improved slightly with the inclusion of scat C:N ratio. The best performance was achieved by a model that included number of scat pieces, diameter, taper length, log mass and log C:N ratio. Log C:N ratio and log mass contribute most strongly to the first linear discriminant and diameter and log C:N ratio contribute most strongly to the second (Table 2.3). Statistically significant differences in centroids are driven by both coyote and gray fox scats along the first linear discriminant ($F_{2,57} = 47.99$, $p < 0.001$) and by coyote scats along the second ($F_{2,57} = 16.79$, $p < 0.001$). 74% of the scats in the training set were correctly classified, followed by 75% in the test set and 71% in the jackknifed full dataset, all of which are comparable to the morphology alone model, though slightly improved. Coyote and gray fox scats were identified correctly more often than bobcat scats in both the test set and jackknifed full dataset. I held the composition of the training and test sets constant across models to facilitate comparison. Because the data set is small, the exact composition of the training set does impact model performance to a certain degree, but overall predictivity in the full jackknifed dataset never exceeds 75%.

Predictions did not improve significantly with the use of the QDA model. The morphology alone model (length, diameter, taper length and log mass) correctly identified 68% of the scats in the training set to species. Performance in the test set increased to 75% correctly classified. With the addition of log C:N ratio as a predictor variable, 75% of the scats in the training set were correctly identified to species and just 71% in the test set. Notably, both QDA models correctly identify coyote scats with greater frequency than the LDA models.

**Random Forests**

Results from the RF model are similar to the DFA model results, despite increasing the size of the dataset with the inclusion of scats with missing variables. The out-of-bag estimate of correct classification is 57% for the morphology alone model, in which mass and diameter are by far the most important variables (Figure 2.3). When all of the variables are included, the out-of-bag estimate of correct classification rate improves only slightly to 62%. Mass remains the most important variable followed by C:N ratio and diameter (Figure 2.3). Other non-morphological variables, including location and scrape, gain greater importance that many of the variables considered in the morphology alone model. Of the misclassified scats, just 2 were bobcat, 21 were coyote and 18 were fox, suggesting that the model is better able to identify bobcat scats than either coyote or gray fox. The single classification tree identified 5 significant splits in the data (Figure 2.4), one in the C:N ratio, two in mass, and one each in diameter and taper length.

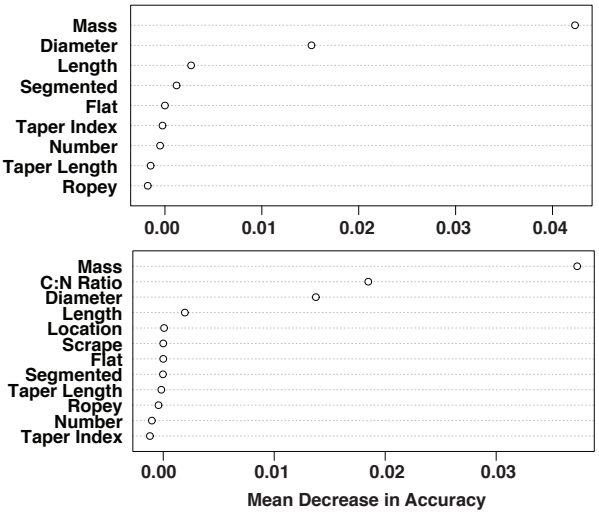


**Figure 2.3.** Variable importance plots for predictor variables from random forests (RF) classifications used for predicting unknown scats to species using (A) purely morphological traits and (B) additional non-morphological predictors.

## DISCUSSION

Both the RF and DFA model results indicate that scats produced by coyotes, bobcats and gray foxes cannot be reliably distinguished by morphology alone, nor even with the inclusion of additional non-morphological variables. Neither modeling method achieves greater than a maximum 75% accuracy, regardless of the variables considered. This should perhaps not be surprising, given that I observed very few significant differences in scat traits between species – only diameter, mass, and C:N ratio are statistically separable by species, and of these, only scat C:N ratios are distinct for all three species. Mostly, there is a lot of morphological overlap between

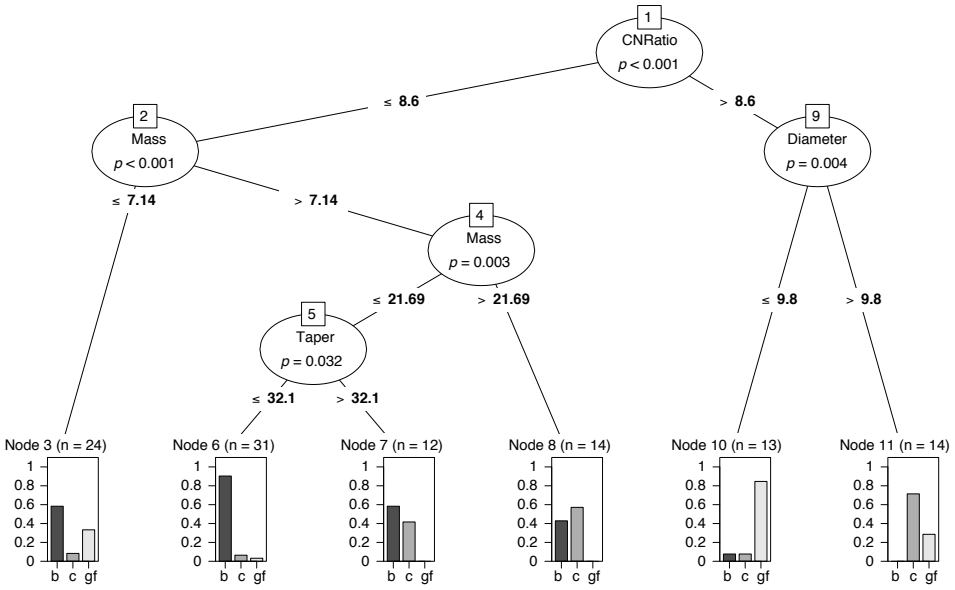


**Figure 2.5.** Conditional inference classification tree for mammalian mesopredator scats showing the scat traits that significantly classified scats to species. Significant traits are circled and ranked (top-most variable has the highest correlation) and shown with univariate p-values. The values of the split cut-offs are listed in the branches. The bar plots illustrate the proportion of total scats classified by the predictor variables (indicated by the n-value) to each of the three possible species: bobcat (b), coyote (c) and gray fox (gf).

species, an observation made previously for scat diameters (Weaver and Fritts 1979; Danner and Dodd 1982; Farrell et al. 2000; Reed et al. 2004).

Despite the significant overlap, there are still some morphological differences between mammalian mesopredator scats. The second LDA model identified 2 gray fox scats correctly with >90% confidence, and these scats have traits in common – they have high C:N ratios (≥ 19.1), they are not segmented, and they weigh very little (≤ 3.4 g). The second QDA model performed even better, identifying 4 fox scats correctly with >90% confidence and the same general traits hold. Misclassified gray fox scats (LDA: n = 8, QDA: n=7), on the other hand, all have lower C:N ratios (8.59 ± 4.0; 7.0 ± 0.8) and tend to be segmented or to have a higher diameter (13.5 ± 4.4 mm; 14.2 ± 4.8 mm) than gray fox scats on average.

These results suggest that when certain scat characteristics are observed together (e.g. high C:N ratio, low mass, and lack of segmentation), identification to species can be made confidently, and there is credence to the idea that there are end member morphologies for scats produced by these different species. Bobcat scats are often described as segmented or constricted (Halfpenny 2008) and indeed, I found that 84% of the bobcat scats in the full dataset are segmented, while just 38% of gray fox scats are segmented (though 70% of coyote scats are also segmented). Because bobcats are obligate carnivores, bobcat scats also tend to have lower C:N ratios, therefore a segmented scat with a low C:N ratio may be attributable to a bobcat with a higher degree of confidence. I also only observed scrapes next to bobcat scats,

suggesting that the presence of a scrape is an indicator for bobcats. Problems arise when scats exhibit mixtures of traits that may be typically "bobcat" or typically "coyote", making the LDA model unable to assign a scat to species with a high degree of confidence (Figure 2.5). In both the second LDA and second QDA models, only a handful of scats were assigned to the incorrect species with a high degree of confidence. Instead, the majority of the misidentifications are made when the balance is tipped only slightly in favor of the incorrect species (posterior probabilities between 50-60%). Because strong false positives (probability of incorrect species

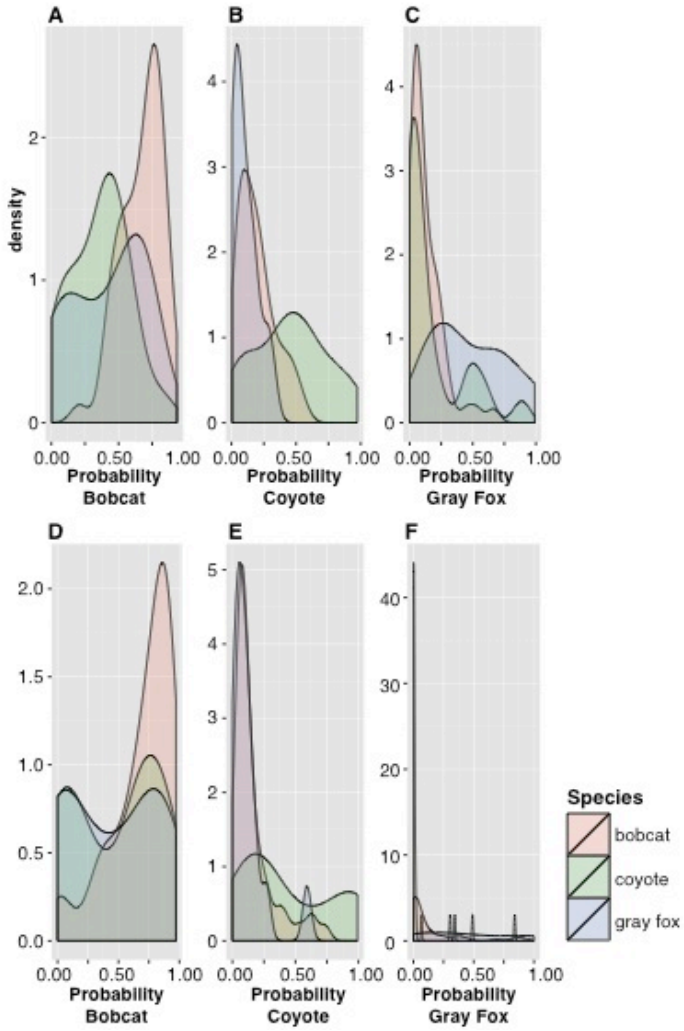


**Figure 2.5.** Probability distributions of the posterior probability assignments of scats to species in the 2nd (non-morphological) LDA model for (A) bobcat, (B) coyote and (C) gray fox, and the same distributions from the 2nd QDA model for (D) bobcat, (E) coyote and (F) gray fox. Misidentifications by the LDA modelare primarily confined to at or below ~0.6.

>80%) are rare (<5%), the LDA or QDA models may still be useful tools for scat identification when used in conjunction with alternate identification methods. Depending on the research question, a posterior probability cut-off of a certain value could be assigned (e.g. 85 or 90%) and scats with predictions to species that fall below that value would then require verification by another method.

## CONCLUSION

These results demonstrate that bobcat, coyote and gray fox scats are morphologically very similar and that contextual information such as location on trail or the presence or absence of a scrape only minimally increases the probability of correct identification. Certainly further information, such as proximity of fresh tracks, could improve identifications, but this information is often lacking, particularly when many scats are collected at once. The LDA and QDA models and classification tree presented here provide researchers with a first-pass tool to predict unknown scats to species with some level of confidence and to then seek verification by other methods for scats predicted to species below a confidence threshold. Either mtDNA-analyses or scat-detecting dogs (e.g. Smith et al. 2003) could be used to provide this check. Using the model to select scats needing further verification allows for at least some reduction in cost. Given the significant overlap in their scat morphology, it becomes critically important for researchers and wildlife mangers investigating space use or possible dietary partitioning by these animals to assess morphology-based identifications with alternate methods, as described here. Previous research on these

animals that relied on scat morphology for species identification (e.g. Neale and Sacks 2001a; b) may benefit from a second glance.

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**APPENDIX 2A.** ILLUSTRATION OF A SCRAPE

**Figure 2.A1.** Scat 012712ANNU03 shown next to scrape (disturbed dirt is visible to the left of the scat). This scat was mtDNA-verified as bobcat.

# Chapter 3

## Do stable carbon and nitrogen isotopes in coyote scats accurately reflect diet?

**Keywords:** Stable isotopes, keratin, collagen, scat, fractionation

**ABSTRACT**

We determined tissue-to-tissue apparent carbon and nitrogen isotope enrichment factors ($\varepsilon^{13}$* and $\varepsilon^{15}$*, respectively) between bone collagen, hair keratin, muscle and scat for 4 coyotes (*Canis latrans*) as well as 2 bobcats (*Lynx rufus*) and 2 gray foxes (*Urocyon cinereoargenteus*). We found the mean ε* values among proteinaceous tissues to be consistently small, while mean $\varepsilon^{13}$* and $\varepsilon^{15}$* values for coyotes between collagen and scat (5.3‰ ± 1.2, 2.0‰ ± 0.9) and between keratin and scat (4.4‰ ± 1.7, 1.4‰ ± 0.8) are considerably higher. $\varepsilon^{13}$* values between scat and other tissues vary along a gradient, with greater $\varepsilon^{13}$* values found in more carnivorous animals (coyote > gray fox > herbivore). To validate the use of carbon and nitrogen isotope values measured in scats themselves, we compared scat stable isotope values directly with diet values measured in the scat contents. We then used a Bayesian stable isotope mixing model to generate estimates of the proportional contributions of various prey components to diet and compared these results with diet predictions

based on traditional scat-analysis techniques (frequency of occurrence and % by volume). All three methods identify terrestrial mammals as the greatest contributors to coyote diets, followed by plants and marine mammals. Traditional scat analysis techniques are known to overestimate the importance of small prey items in carnivore diets. We found diet estimates from scat stable isotope values to diverge from this trend, emphasizing larger prey instead. Relative to traditional scat analysis techniques, stable isotopes are comparatively quick to measure, but they also may generally provide a lower resolution estimate of diet that is dependent on the amount of isotopic variation in the ecosystem (e.g. marine vs. terrestrial prey). A combination of these methods will likely provide the best estimate of actual diet.

## INTRODUCTION

As the number of studies using stable isotopes to infer the diets and foraging behaviors of wild animals grows, the importance of species-specific diet-to-tissue discrimination factors is becoming more apparent. Many authors have now shown that isotopic incorporation can differ among tissues due to isotopic routing, as well as variable rates of protein turnover or tissue synthesis (see reviews by Martínez del Rio et al. 2009; Boecklen et al. 2011). Controlled feeding experiments provide a rigorous approach to determining species- and tissue- specific diet-to-tissue discrimination factors. A downside to this approach is that incorporation may be so slow that the experiments are prone to errors (Perga and Grey 2010), or that experimental diets may sometimes be so unlike natural diets (for example, dramatically differing in

digestibility or with an unrealistic macromolecular composition) that the resulting discrimination factors may be difficult to apply in the wild (Kurle et al. 2014). There are also ethical considerations with regard to tissue sampling and experiment duration. Fox-Dobbs et al. (2007) circumvented these issues for wolves by examining the Isle Royale wolf-ungulate system, arguing that the closed island system with few possible wolf food sources closely approximates a controlled feeding study.

Some mammalian omnivores and carnivores have been studied experimentally (Hilderbrand et al. 1996; Ben-David and Schell 2000; Roth and Hobson 2000; Ben-David et al. 2012; Hobson and Quirk 2014; Parng et al. 2014), but experimental measurements of trophic fractionations are lacking for coyotes (*Canis latrans*), one of the most abundant and ecologically-impactful carnivores in North and Central America. To establish discrimination factors for coyotes (*Canis latrans*), I took a different approach than Fox-Dobbs et al. (2007), using different tissues collected from individual road kill carcasses. I compared the carbon and nitrogen isotope values of hair keratin, muscle protein, bone collagen and scat from coyotes (*Canis latrans*) and two other mammalian mesopredators: bobcats (*Lynx rufus*) and gray foxes (*Urocyon cinereoargenteus*). Analysis of tissue-to-tissue apparent enrichment factors are useful, as they facilitate comparison among studies that rely on different tissues; for example, collagen is often the tissue of choice for studies using historical and archaeological materials, while modern studies tend to default toward more easily sampled tissues, such as hair keratin.

Scat is also an easily sampled tissue and stable isotope analyses of scat have the potential to provide a quick and accurate means of gaining dietary information. Traditional scat analyses, which include scat dissections and identification and quantification of the material they contain, are widely used to document diets of animals. Although extremely effective qualitatively, quantitative estimates of dietary contributions based on traditional scat analyses have a number of known biases. These include differential detectability of different types and size classes of foods (Weaver and Hoffman 1979; Meriwether and Johnson 1980; Reynolds and Aebischer 1991; Kelly and Garton 1997), observer bias (Spaulding et al. 2000), and variation in results stemming from the chosen method of diet quantification (Klare et al. 2011). Traditional scat analyses are also labor intensive and time consuming. Nonetheless, over the past century, scat analyses have been the most often used technique to quantify animal diets (Klare et al. 2011). If stable isotopes in scat predict diet, they could not only offer a way to more rapidly and accurately quantify animal diet from scat, but also provide a noninvasive isotope record in a very high turnover rate tissue. This could allow for the isotopic investigation of seasonal dietary shifts that are otherwise masked by signal attenuation (Sponheimer et al. 2003b; Botha and Stock 2005; Codron et al. 2007; Codron and Codron 2009; Hatch et al. 2011; Blumenthal et al. 2012).

Scats are composed of a combination of undigested food, sloughed epithelial tissues and microbiota (Putman 1984; Sponheimer et al. 2003c); depending on the proportional breakdown of these components, there is concern that scat stable isotope

values will not be representative of assimilated diet. Both experimental work (Coates et al. 1991; Sponheimer et al. 2003a; b; Varo and Amat 2008; Wittmer et al. 2010) and field tests (Codron et al. 2005; 2006; Codron and Codron 2009; Hatch et al. 2011; Blumenthal et al. 2012), however, support the notion that scat stable isotope values do reflect ingested diet for a variety of organisms, though tests have not been carried out in coyotes.

This study's research goals were threefold: 1) to determine tissue-to-tissue enrichment factors in coyote tissues, 2) to derive scat-to-diet isotope discrimination factors for coyotes by combining the tissue-to-tissue enrichment factors determined in this study with Roth and Hobson's (2000) experimentally derived fractionation factors for red foxes (*Vulpes vulpes*), and 3) to ground-truth the use of stable isotope analysis of coyote scats by comparing stable isotope values measured in scats themselves with values measured in scat contents. I further compared quantitative estimates of the proportional contributions of prey to coyote diets derived from Bayesian stable isotope mixing models with diet composition estimates derived from traditional scat analysis techniques. We present stable isotope data from multiple tissues sampled from road kill coyote carcasses, as well as those from bobcat and gray fox, and from seasonally collected DNA-verified coyote scats.

## MATERIALS AND METHODS

### Sample acquisition

I obtained tissue samples from road kill coyotes, gray foxes and bobcats collected under California Fish and Game permit SC-11995 to R. Reid and from the University of California, Santa Cruz (UCSC) Natural History Museum collections. Specimens were stored frozen until dissection. I collected fresh mesopredator scats quarterly along an ~6 km coast-to-inland transect in 2011 – 2013 at Año Nuevo State Park and Reserve (San Mateo County, CA). One week prior to collection, we cleared the transect of all scats, such that collected scats would all be no greater than a week old. At the time of collection, we took measurements (diameter, length, etc.), recorded scat locations by GPS, and placed scats in individually marked Ziploc bags with a desiccant to reduce moisture and enhance DNA preservation. Scats were stored in a -4°C freezer until DNA samples could be taken, then freeze dried and oven dried to kill parasites (60°C for 48 hours). Scat collection followed the guidelines of the American Society of Mammalogists (Sikes and Gannon 2011). I also had the approval of both the UCSC Institutional Animal Care and Use Committee and the Office of Environmental Health and Safety for the acquisition and analysis of scat and road kill carcass tissues.

**Sample preparation**

*Carcass Samples*

I took fur, muscle, bone and feces samples from each animal carcass. I clipped a small tuft of fur from the animals' dorsum. Muscle tissue was consistently sampled from the right trapezius. I defleshed and removed a small chip of bone (~50 mg) for collagen extraction; if the carcass did not have easily sampled broken bones, we defaulted to a chip from the mandible. I sampled feces from what remained in the animals' colon. When possible, depending on the condition of the carcass, I also extracted the contents of the animals' stomach to approximate recent diet. I prepped hair, bone and scat samples for isotopic analyses in the same manor as described below. Muscle samples were freeze dried overnight and ground into a powder in an agate mortar and pestle. I lipid extracted both the muscle tissue and bone collagen by repeatedly (2 – 3 times) immersing the samples in 5 mL of petroleum ether (Dobush et al. 1985), sonicating for 15 min and rinsing 5 times in MilliQ water before freezing and freeze drying the samples overnight. I then re-homogenized the muscle samples and, like the collagen and hair samples, weighed ~0.7 mg of material into 5 x 9-mm tin capsules.

*Scat Samples*

I conducted fecal genotyping in collaboration with Wildlife Genetics International. As per their recommendations, prior to being dried, I swabbed the scats with Q-tips, which were then stored dried in unwaxed coin envelopes. DNA is

extracted by clipping a small (~3 mm x 3 mm) piece of each swab and processing the clippings as tissue samples using QIAGEN DNeasy Blood and Tissue Kits. The species test is a sequence-based analysis of the mitochondrial 16S rRNA gene (Johnson and O'Brien 1997). The specific primers and analytic conditions that Wildlife Genetics uses are not published, but their results could be reproduced using published methodology. Two variants of this analysis are employed using either primers that amplify across all mammals or primers designed to amplify Carnivora sequence in preference to other mammals. The results are compared to reference data from over 125 species of mammals.

To prepare scat samples for isotopic analysis, I extracted the matrix material by gently breaking apart oven-dried scats over a fine mesh sieve. The matrix passes through the sieve while other scat components, such as fur, feathers or bone are captured above. I then cleaned the powdery matrix by placing it in filter paper cones and rinsing it first with MilliQ water, then with 0.1N HCl to remove any $CaCO_3$, and again with MilliQ. Because previous authors have conjectured that rinsing with distilled or MilliQ water following acidification may introduce bias in sample $\delta^{15}N$ values (Bosley and Wainright 1999; Jacob et al. 2005), I sought to characterize this possible bias by comparing 7 paired samples that were either a) rinsed following acidification or b) not rinsed following acidification. I found no significant difference between the $\delta^{15}N$ values of the two sample treatment groups (paired t-test: mean sample difference = -0.11‰, df = 6, $p = 0.055$; Appendix 3A:

Figure 3.A1). After the scat samples were fully dry and homogenized, I weighed approximately 5 mg of scat matrix into 5 x 9-mm tin boats for isotopic analysis.

Following matrix extraction, I placed scats in bags made from nylon stockings and washed them in an automatic washing machine (Klare et al. 2011) without detergent to remove any residual matrix and to better separate the remaining components. Once dry, I placed the scat contents on a gridded sorting tray to estimate the percent by volume contributions of mammal, bird, reptile, invertebrate and plant components to the nearest 5% (McDonald and Fuller 2005). While the fur was spread out, I sampled guard hairs from the center of each grid cell until I had examined 40-50 hairs and identified them to the finest taxonomic level possible through comparison with a guard hair reference collection housed at UCSC and with published keys (Mayer 1952; Tumlison 1983; Debelica and Thies 2009). I then grouped identified hairs into four categories: marine mammals, small terrestrial mammals (≤ 1 kg), medium terrestrial mammals (> 1 kg, < 30 kg), and large terrestrial mammals (≥ 30 kg). To facilitate comparison with other studies I also calculated the frequency of occurrence of individual prey species (Fedriani et al. 2001): percentage of occurrence = number of occurrences of prey type x 100/ total number of occurrence.

To enable comparison between scat and diet component isotopic values, I cleaned and analyzed a subset of identified scat components. I rinsed hair and feather samples with MilliQ water, immersed them in petroleum ether and sonicated for 15 minutes, rinsed them again and dried them in a 60°C oven overnight. I decalcified

bone fragments in 0.5N HCl for ~72 hours. This was followed with a 0.1N NaOH treatment for 24 hours to remove humic acids. I then rinsed the collagen samples five times in MilliQ water, and lipid extracted them in the same manner as the muscle tissue, rinsed them 5x with MilliQ and froze and freeze dried them overnight. Arthropod, vegetation and seed samples were repeatedly rinsed and sonicated in MilliQ water (4x for 15 min), dried (60°C oven) and then crushed with an agate mortar and pestle. I weighed ~0.7 mg of hair, collagen and arthropod samples into 5 x 9-mm tin boats. I divided vegetation samples into aliquots of ~0.4 mg for carbon isotopes and ~3 mg for nitrogen isotopes, and sealed them in 5 x 9-mm tin boats.

**Isotopic analysis**

All samples were combusted via Dumas combustion using a Carlo Erba 1108 elemental analyzer and analyzed for $\delta^{13}C$ and $\delta^{15}N$ values on a ThermoFinnigan Delta Plus XP continuous flow isotope ratio mass spectrometer at the UCSC Stable Isotope Laboratory. I report our results using δ notation, in which

$$\delta^{H}X = ((R_{sample}/R_{standard}) - 1) \times 1{,}000 \qquad (3.1)$$

where R is the ratio of the heavy isotope to light isotope for element X (e.g. Sulzman et al. 2007). Carbon isotope values are reported relative to Vienna Pee Dee Belemnite (a marine carbonate) and nitrogen isotope values are reported relative to air, and the resulting value is expressed in parts per thousand (i.e., per mil, ‰). Often the offset, or fractionation, between two substances or tissues is expressed by Δ notation (Martínez Del Rio et al. 2009), in which

$$\Delta^{H}X_{a-b} = \delta^{H}X_{a} - \delta^{H}X_{b} \qquad (3.2)$$

Though Δ values are relatively simple to calculate, they become less accurate with increasing differences between the δ values of the substances of interest (Cerling and Harris 1999; Crowley et al. 2010). Because scat isotope values have the potential to be quite different from other tissues, following the logic in Passey et al. (2005), I instead report the fractionation factor (α) and isotope enrichment values (ε):

$$\alpha_{a-b} = (\delta^{H}X_{a} + 1{,}000)/(\delta^{H}X_{b} + 1{,}000) \qquad (3.3)$$

$$\varepsilon_{a-b} = (\alpha_{a-b} - 1) \times 1{,}000 \qquad (3.4)$$

Furthermore, I use the notation ε*, the apparent enrichment value, to denote that this is a non-equilibrium fractionation factor. Sample isotopic values are corrected for size, drift and source stretching effects. I calculated the average analytical precision for the scat data as the *SD* of the $\delta^{13}C$ and $\delta^{15}N$ values of 41 replicates of an internationally calibrated in-house standard (PUGel); precision was 0.2‰ for carbon and 0.1‰ for nitrogen. I similarly calculated the average analytical precision for the muscle, collagen, keratin and arthropod samples as the *SD* of the $\delta^{13}C$ and $\delta^{15}N$ values of 20 replicates of a different internationally calibrated in-house standard (Acetanilide); precision was also 0.2‰ for carbon and 0.1‰ for nitrogen.

**Data analysis**

I used Stable Isotope Analysis in R (SIAR) (Parnell et al. 2010), a Bayesian stable isotope mixing model, to estimate the proportional contributions of various scat components to coyote diets. SIAR is capable of accounting for error in estimates of

trophic enrichment factors as well as for variations in the elemental concentrations of C and N in the food sources, which could otherwise bias model output (Phillips and Koch 2002). Scat components, which largely represent the indigestible portion of animal diet, are proxies for assimilated diet. To convert stable isotope values measured in these indigestible components, such as hair or feathers, to assimilated diet (i.e. the muscle tissue that is digested and absorbed) I applied published organism and tissue specific correction factors (Table 3.1). Using these transformed values, I ran the mixing models for each scat individually as well as for all of the scat samples collectively. Based on the results of the animal dissections, I also corrected scat isotope values to diet by adding 1.8‰ ± 1‰ for $\delta^{13}C$ values and subtracting 1.8‰ ± 2‰ for $\delta^{15}N$ values; details regarding our arrival at these specific scat-to-diet discrimination factors are further explained in the Results and Discussion. The organism based coyote dietary categories outlined above (bird, invertebrate, etc.) did not necessarily coincide with distinct isotopic categories. For example, our subset of scat samples contained feathers from both a bird feeding primarily on marine resources and from another feeding primarily on terrestrial resources; while both are one possible dietary source in the isotope mixing model. For the purposes of the mixing model, I therefore kept these birds as separate source inputs. To enable

**Table 3.1.** Organism and tissue specific corrections applied to coyote food source $\delta^{13}C$ and $\delta^{15}N$ values for input into the SIAR mixing model.

| Organism | $\delta^{13}C_{keratin\text{ - }muscle}$ | $\delta^{13}C_{collagen\text{ - }muscle}$ | $\delta^{13}C_{skin\text{ - }muscle}$ | $\delta^{15}N_{keratin\text{ - }muscle}$ | $\delta^{15}N_{collagen\text{- }muscle}$ | $\delta^{15}N_{skin\text{- }muscle}$ | Citation |
|---|---|---|---|---|---|---|---|
| *Zalophus californianus* | -1.9 ± 0.5 | - | - | -0.6 ± 0.4 | - | - | C correction - Hobson et al. 1997, N correction - Hobson et al. 1996 |
| *Mirounga angustirostris* | -1.4 ± 0.5 | - | | -0.6 ± 0.4 | | | Hobson et al. 1996 |
| Unknown reptile | - | - | -1.3 ± 0.7 | - | - | -1.0 ± 0.4 | C correction - Warne et al. 2010; N correction - Seminoff 2009 |
| *Urocyon cineroargenteus* | -1.5 ± 0.5 | - | - | no change | - | - | Roth and Hobson 2000 |
| Unknown bird | -0.3 ± 0.5 | - | - | -0.6 ± 0.3 | - | - | Hobson and Clark 1992 |
| Rodent | -1 ± 0.2 | -2.7 ± 1 | - | -0.8 ± 0.4 | no change | - | keratin correction - Miller et al. 2008; collagen correction - Tieszen 1983 |
| Rabbit | no change | - | - | no change | - | - | Hildebrand 1996 |
| *Odocoileus hemionus* | -1.6 ± 1 | -4 ± 1 | - | no change | no change | - | keratin correction - Codron et al. 2007; collagen correction - Ambrose 1993; Newsome 2004 |
| *Sus scrofa* | -1.9 ± 0.5 | - | - | no change | - | - | Nardoto et al. 2006 |

comparison between the mixing model predictions and those derived from the two other scat analysis techniques, I recast the more numerous mixing model source categories into the eight organism-based dietary categories described above by combining the model predicted modal values. I performed all statistical analyses in R (R Development Core Team, 2013).

## RESULTS

### Apparent isotope enrichment factors

I examined 4 coyote, 2 bobcat and 2 gray fox carcasses, though not all of the target materials were available from each carcass (Table 3.2). Carbon isotope values in coyote, bobcat and gray fox tissues increase consistently from scat, to muscle, to hair keratin, to bone collagen (Figure 3.1). Nitrogen isotope values follow roughly the same trend, though the magnitude of change is much less, and muscle values are equal to or slightly higher than hair keratin values. Mean $\varepsilon^{13}$* and $\varepsilon^{15}$* values between proteinaceous tissues (keratin and muscle) are consistently small (Table 3.3). Mean $\varepsilon^{13}$* and $\varepsilon^{15}$* values between collagen and scat (5.3‰ ± 1.2, 2.0‰ ± 0.9, respectively) and between keratin and scat (4.4‰ ± 1.7, 1.4‰ ± 0.8, respectively) are, however, quite high for coyotes. $\varepsilon^{13}$* values between both collagen and scat and keratin and scat from an individual gray fox (Fox#1 - *Urocyon cinereoargenteus*) are only half as large (2.4‰ and 2.0‰, respectively) and gray fox $\varepsilon^{15}$* values are slightly higher than observed in coyote tissues (3.2‰ and 2.9‰, respectively; see Table 3.3). Given that I chose to work with road kill carcasses, I was not able to directly measure the diets of

**Table 3.2**. List of mesopredator carcasses examined and tissues sampled.

| ID | Family | Genus | Species | Sex | Mass (kg) | Age | Date collected | Hair | Bone collagen | Muscle | Feces |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Coy1 | Canidae | *Canis* | *latrans* | male | 11.3 | adult | 2012 | x | x | - | x |
| Coy2 | Canidae | *Canis* | *latrans* | female | 11.5 | adult | 2012 | x | x | x | x |
| Coy3 | Canidae | *Canis* | *latrans* | male | 4.2 | juvenile | 13-Oct-12 | x | x | x | x |
| Coy4 | Canidae | *Canis* | *latrans* | male | 15.4 | adult | 2013 | x | x | x | x |
| Fox1 | Canidae | *Urocyon* | *cinereoargenteus* | female | 3.1 | adult | 13-Oct-12 | x | x | x | x |
| Fox2 | Canidae | *Urocyon* | *cinereoargenteus* | male | 2.6 | adult | 3-Jan-10 | x | x | x | - |
| Bob1 | Felidae | *Lynx* | *rufus* | unknown | 9.3 | unknown | unknown | - | x | x | - |
| Bob2 | Felidae | *Lynx* | *rufus* | male | 12.1 | subadult | 2009 | x | x | x | x |



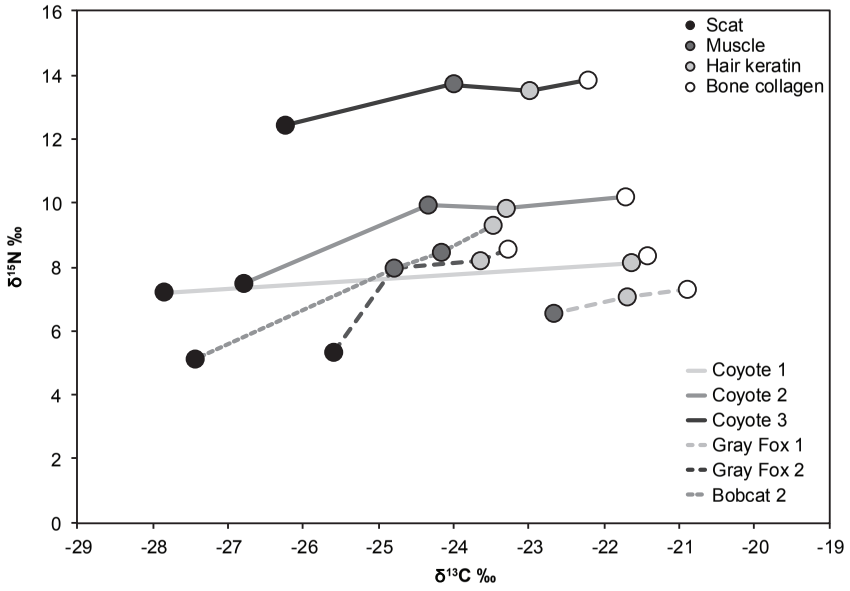


**Figure 3.1.** $\delta^{13}C$ and $\delta^{15}N$ values measured in tissues of road kill coyotes, gray foxes and bobcats. Symbol colors denote type of tissue sampled: black = scat, dark gray = muscle, light gray = hair keratin, and white = bone collagen. Tissues from the same individual are connected by lines with different dash patterns and shades to distinguish it by species and by individual.

the individual animals I examined, however I was still able to derive $\varepsilon^{13}*_{scat\text{-}diet}$ and $\varepsilon^{15}*_{scat\text{-}diet}$ by combining our results with diet-to-hair fractionation factors determined for red foxes by Roth and Hobson (2000); for coyotes, I calculated a $\varepsilon^{13}*_{scat\text{-}diet}$ value of +1.8‰ ± 1‰ for $\delta^{13}C$ and $\varepsilon^{15}*_{scat\text{-}diet}$ value of -1.8‰ ± 2‰ for $\delta^{15}N$ (Figure 3.2). A combination with Roth and Hobson's (2000) diet-to-muscle fractionation factors results in the same scat to diet enrichment factors within error.

**Coyote Diet Quantification from Scat**

I chose a subset of 12 DNA-verified coyote scats, collected in two different seasons (spring and fall), to fully dissect. I identified 25 different dietary components in this scat subset; unknown grass, black-tailed deer (*Odocoileus hemionus*) and

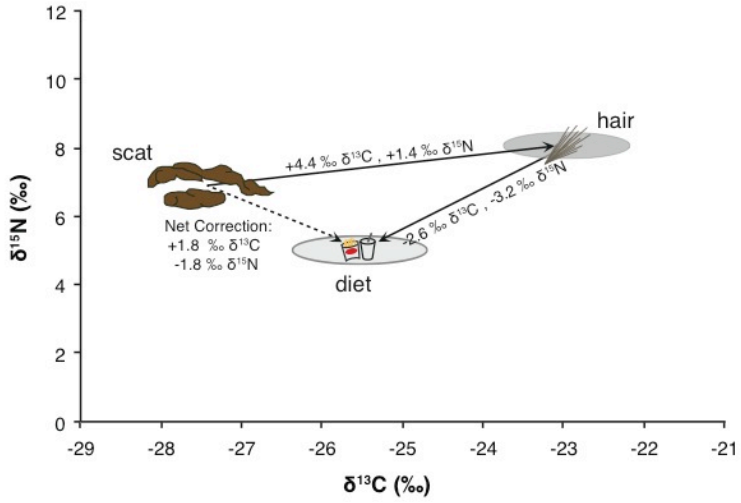


**Figure 3.2.** An illustration of how we derived a scat-to-diet correction for coyotes. Starting with the scat isotope values, we corrected to hair keratin based on the apparent discrimination factors we calculated from the coyote dissections. We then corrected from hair to diet by applying the discrimination factors for red foxes determined by Roth and Hobson (2000). The scat-to-diet correction is the net result.

**Table 3.3.** Apparent carbon and nitrogen isotope enrichment factors between sampled tissues of road kill coyote, bobcat and gray fox carcasses.

| | $\varepsilon^{13}$* | | | | | |
|---|---|---|---|---|---|---|
| **Specimen** | **bone-hair** | **bone-muscle** | **hair-muscle** | **hair-scat** | **bone-scat** | **muscle-scat** |
| Coy1 - *Canis latrans* | 0.2 | - | - | 6.4 | 6.6 | - |
| Coy2 - *Canis latrans* | 1.6 | 2.7 | 1.0 | 3.6 | 5.2 | 2.5 |
| Coy3 - *Canis latrans* | 0.8 | 1.8 | 1.0 | 3.3 | 4.1 | 2.3 |
| Coy4 - *Canis latrans* | - | - | - | - | - | - |
| **Mean** | **0.9** | **2.2** | **1.0** | **4.4** | **5.3** | **2.4** |
| **Standard Deviation** | **0.7** | **0.6** | **0.0** | **0.2** | **0.7** | **0.2** |
| Bob1 - *Lynx rufus* | - | 2.6 | - | - | - | - |
| Bob2 - *Lynx rufus* | - | 0.7 | - | - | 4.1 | 3.4 |
| Fox1 - *Urocyon cineroargenteus* | 0.4 | 1.6 | 1.2 | 2.0 | 2.4 | 0.8 |
| Fox2 - *Urocyon cineroargenteus* | 0.8 | 1.8 | 1.0 | - | - | - |
| | $\varepsilon^{15}$* | | | | | |
| Coy1 - *Canis latrans* | 0.2 | - | - | 0.9 | 1.1 | - |
| Coy2 - *Canis latrans* | 0.4 | 0.3 | -0.1 | 2.3 | 2.7 | 2.4 |
| Coy3 - *Canis latrans* | 0.3 | 0.2 | -0.2 | 1.1 | 1.4 | 1.2 |
| Coy4 - *Canis latrans* | - | - | - | - | - | - |
| **Mean** | **0.3** | **0.2** | **-0.1** | **1.4** | **1.7** | **1.8** |
| **Standard Deviation** | **0.1** | **0.1** | **0.1** | **0.9** | **0.9** | **0.8** |
| Bob1 - *Lynx rufus* | - | -0.5 | - | - | - | - |
| Bob2 - *Lynx rufus* | - | 0.9 | - | - | 4.2 | 3.2 |
| Fox1 - *Urocyon cineroargenteus* | 0.3 | 0.6 | 0.3 | 2.9 | 3.2 | 2.6 |
| Fox2 - *Urocyon cineroargenteus* | 0.3 | 0.7 | 0.5 | - | - | - |

California sea lion (*Zalophus californianus*) were the most frequently identified species followed by rabbits (*Sylvilagus bachmani*). The estimates derived from the different methods for quantifying coyote diet from scat are similar, but as expected, not identical (Figure 3.3). Collectively, terrestrial mammals have the

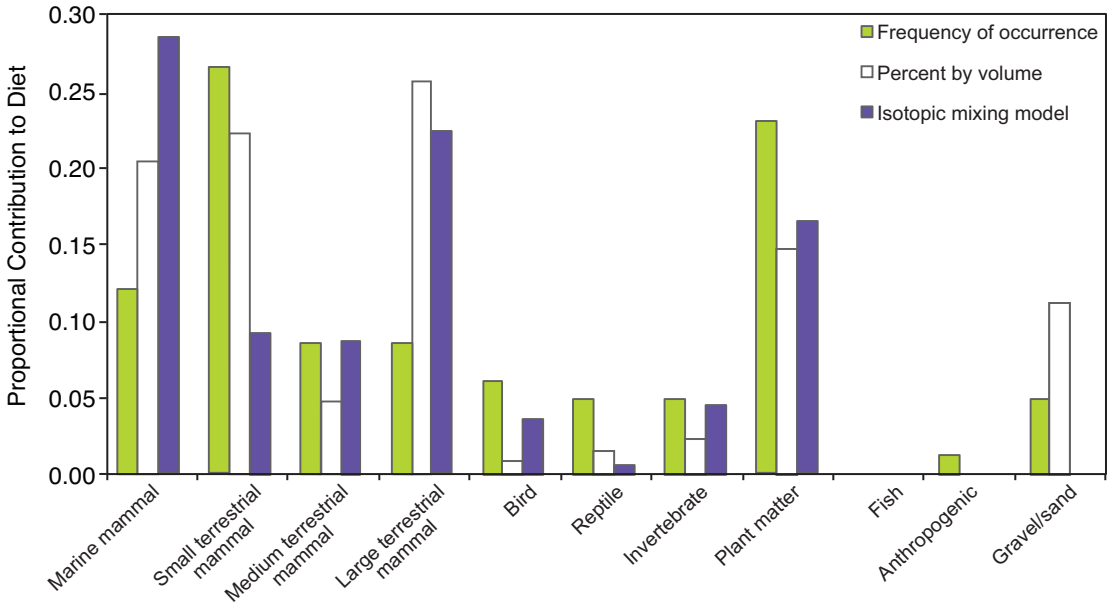


**Figure 3.3.** Comparison of the proportional contributions of marine mammals, terrestrial mammals (small, medium and large), birds, reptiles, invertebrates, plants, fish, anthropogenic material and gravel/sand to 12 DNA-verified coyote scats as identified by three methods: frequency of occurrence (green), percent by volume (white) and isotopic mixing models (purple).

highest frequency of occurrence in the scats (43%), followed by various forms of vegetation (23%) and then marine mammals (12%); birds, sand/gravel, invertebrates and reptiles make up the remaining 22%. The percent by volume method similarly identifies terrestrial mammals – represented in scat by both fur and bone - as the most frequently occurring coyote scat component (54%), followed by marine mammals (20%) and then vegetation (13%); the remaining 13% is comprised of sand/gravel,

invertebrates, birds, and reptiles. Once corrected to diet, scat stable isotope values fall consistently within the isotope space created by the dietary components found in them, with only one exception (sample 091011AN008) (Figure 3.4, Appendix 3B: Table 3.B1). The aggregated mixing model predictions diverge slightly from the two more traditional scat analysis techniques; looking at the mode for model predictions for proportional contributions, we see that terrestrial mammals still contribute the most to coyote diets (41%), but marine mammals account for a higher percentage (30%) than predicted by the other two methods.

## DISCUSSION

### Carbon and nitrogen isotope enrichment factors

While the carbon in animal tissues is sourced from diet, there can still be significant differences in $\delta^{13}C$ values among tissues (DeNiro and Epstein 1978). Like a large body of previous work (see Schoeninger and DeNiro 1984; Koch 1998; Kelly 2000; Koch et al. 2007), I found collagen to have the highest $\delta^{13}C$ values of the tissues I examined. I anticipated that differences in diet (carnivory vs. omnivory) would result in different $\varepsilon^{13}*$ values between scat and other tissues. My results suggest that this is the case, in that I observe a higher $\varepsilon^{13}*_{keratin-scat}$ value for coyotes than for the gray fox (see Table 3.3) and these are both, in turn, greater than published $\varepsilon^{13}*_{keratin-scat}$ values for mammalian herbivores (Sponheimer et al. 2003b). My field estimated $\varepsilon^{13}*_{scat-diet}$ for coyotes is larger than the experiment derived value of -0.8‰ reported for mammalian herbivores (Sponheimer et al. 2003b), but consistent in the direction

of offset. Sponheimer et al. (2003b) found this negative diet-to-feces carbon isotope fractionation to be counterintuitive; they found a higher concentration of plant acid-detergent fibers (which are enriched in $^{13}C$) in feces than in the herbivore diets, which led them to hypothesize that bulk scat values would also have higher $\delta^{13}C$ values. It may be that the microfloral component of feces is the source of the low $\delta^{13}C$ values, but Sponheimer et al. 2(003b) did not observe an increase in fecal $\delta^{13}C$ following their removal. Lipids are a known source of light carbon (DeNiro and Epstein 1978), but canines tend to utilize lipids quite efficiently and Coffey et al. (1940) found that fecal excretion of fat by healthy dogs varied from just 2 to 4% and was mostly composed of fatty acids. The bulk diet-to-lipid fractionation can be quite large (e.g. -3‰ in gerbils [Tieszen et al. 1983]; -3.3‰ in striped skunks [Hobson and Quirk 2014]), and given that fecal $\delta^{13}C$ values are generally lower than diet, but not as low as pure lipids, a small proportion of fatty acids in the feces may be enough to account for low fecal carbon isotope values. Future work on fecal $\delta^{13}C$ values is necessary to resolve the source of the light carbon. Nevertheless, the application of our derived $\varepsilon^{13}*_{scat-diet}$ for coyotes consistently places coyote scats into the isotopic mixing space created by scat components (Figure 3.4), suggesting that it is appropriate.

Regardless of tissue type, the observed increase in $\delta^{15}N$ values with trophic level has been broadly attributed to the combined effects of (1) the preferential excretion of $^{14}N$ in urea, the main efflux of nitrogen in mammals, resulting in a body pool that is enriched in $^{15}N$ relative to diet and (2) higher dietary protein levels with

increasing trophic level (DeNiro and Epstein 1981; Ambrose 1991). A number of controlled feeding studies have demonstrated that the magnitude of the $^{15}N$ trophic

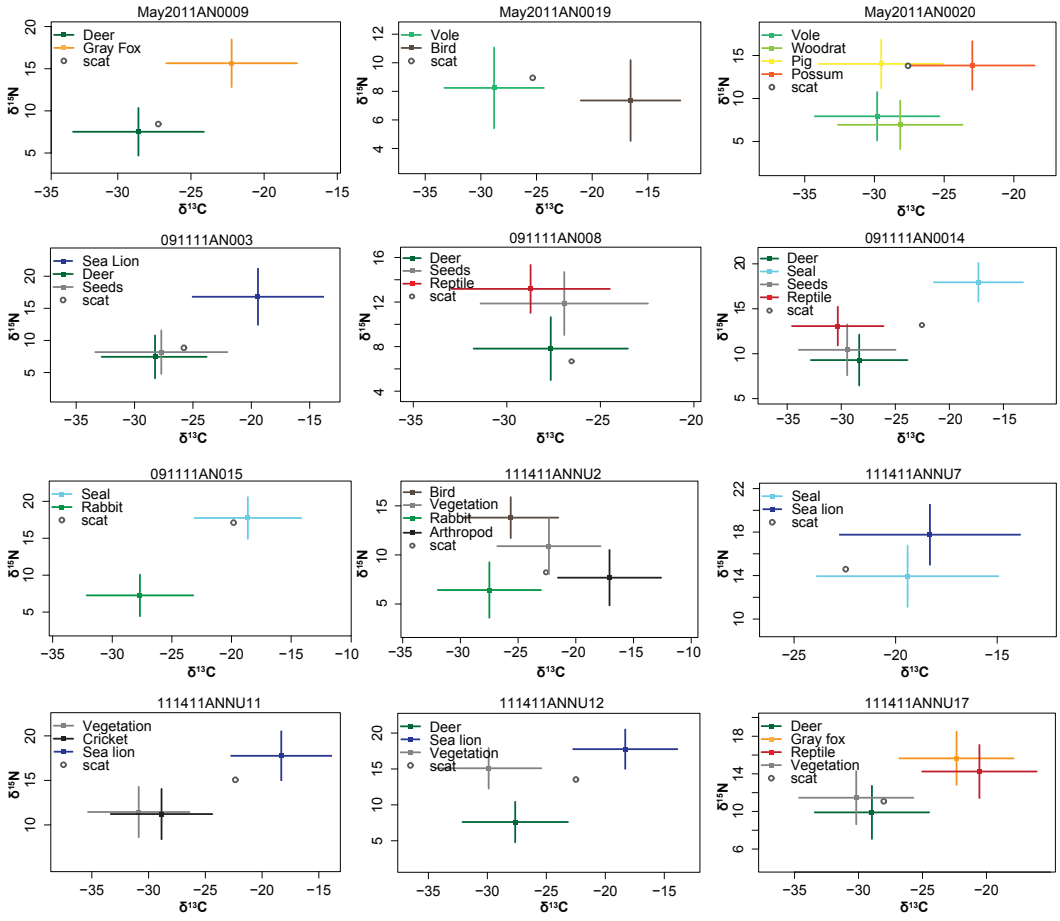


**Figure 3.4.** Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of twelve coyote scats (corrected for trophic discrimination; open circles) from Año Nuevo State Park, CA, plotted in reference to isotope values measured in scat components, also corrected to diet space. Note: SIAR corrects for trophic discrimination by shifting the prey in isotope space, rather than the predators. We use consistent colors for each prey group throughout.

enrichment correlates with dietary protein content (McCutchan et al. 2003; Sponheimer et al. 2003c; Robbins et al. 2005; Robinson et al. 2005), particularly in animals that have high rates of N excretion relative to N assimilation. Protein quality

(Robbins et al. 2010) may also be an important factor. After urea, feces account for the next greatest mammalian nitrogen efflux, yet unlike urea, fecal $\delta^{15}N$ values are consistently enriched in $^{15}N$ relative to diet (Sponheimer et al. 2003a; c; Codron et al. 2005; Codron and Codron 2009), similar to other tissues.

Fecal nitrogen is comprised of undigested nitrogen from food as well as sloughed tissues and microbiota (Sponheimer et al. 2003c). It is difficult to apportion these components, though, previous work suggests that most fecal nitrogen is derived from sloughed endogenous tissues and microbial cells (Van Soest 1994) and fecal $\delta^{15}N$ values elevated above those of diet suggest that the bulk of fecal nitrogen is from the animal itself, rather than undigested food. Schwarm et al. (2009), for example, found that the total endogenous contributions of nitrogen in the feces of herbivores typically account for 60 – 80 % of fecal nitrogen. I observe that coyote scat $\delta^{15}N$ values are consistently higher than those of inferred diet. I also find that $\varepsilon^{15}*_{collagen-scat}$ is greater for coyotes than for gray foxes, suggesting that the proportion of animal-sourced nitrogen in scat scales with dietary nitrogen intake. If this is the case, then I'd expect $\varepsilon^{15}*_{collagen-scat}$ to be greatest in bobcats, which are obligate carnivores, but the one bobcat we examined that had fecal material available has an apparent enrichment factor intermediate between coyotes and gray foxes. Sponheimer et al. (2003c) hypothesized that urinary nitrogen losses will increase rapidly in herbivores that have exceeded their protein requirements, while fecal nitrogen losses should remain somewhat constant. My data suggest a slightly more complex scenario, in which the total amount of the fecal nitrogen efflux still remains

relatively constant, but the proportion that is undigested dietary nitrogen increases with increasing dietary nitrogen content.

**Determining Coyote Diet from Scat**

Klare et al. (2011) reviewed a number of methods to quantify predator diets from scats and found that the less uniform the diet, the larger the disagreements were among different methods. Attention to method is therefore particularly important for omnivores, such as coyotes and foxes. Frequency of occurrence over emphasizes the importance of small food items in our data – small mammals were overwhelmingly identified as the most important – this is likely due to the fact that there are more indigestible parts per unit biomass for small mammals than for larger ones (Floyd et al. 1978; Klare et al. 2011). Frequency of occurrence was the only method, however, to reveal trace items, such as the anthropogenic plastic found in one of the coyote scats. The percent by volume method mitigates small mammal inflation to some degree, but the results are overall quite similar. The mixing model results, on the other hand, do appear to resolve this issue, identifying larger bodied organisms, such as marine mammals and deer, as significantly more important dietary components. Diet estimates based on biomass calculations have also been shown to similarly address this bias, also placing greater emphasis on larger bodied organisms than frequency of occurrence estimates (Klare et al. 2011). This suggests that, although there are fewer indigestible hard parts from these large animals in the scat, a significant proportion of assimilated diet is coming from these animals. Indeed, it

makes sense that a mesocarnivore would avoid the bones of deer to pinniped-sized mammals, with the possible exception of fawns and neonate pups, when plenty of soft tissues are available. The mixing model also de-emphasizes the importance of plant matter to coyote diet. Grass accounted for the majority of the vegetation identified in these scats, and although grass is frequently found in coyote scat, there is no consensus on its role as a food resource; some researchers have suggested that it is ingested incidentally while coyotes are capturing prey (Hawthorne 1972), while others have argued that is may be a necessary source of vitamins (Gier 1968). Our mixing model results suggest that grass is less important to assimilated diet, and therefore lends some support to the idea of incidental consumption or some other non-nutritional explanation.

The strength of the stable isotope approach, then, is that scat isotope values provide a faster, less biased quantitative estimate of assimilated diet than most methods that rely on quantifying purely undigested material. However, potential pitfalls still exist; as with any isotopic study, variation in the system is required to be able to resolve dietary source contributions. Problems will arise if sources are indistinguishable from one another in isotopic space. In the system studied here, a marine resource is one of the major dietary components; marine systems tend to have much higher carbon and nitrogen isotope values than terrestrial systems. Other sources of variation could come from anthropogenic food sources or a more diverse flora containing both $C_3$ and $C_4$ plants. Regardless, questions about resource use may

need to be recast, as traditional organism-based dietary categories may not correspond well with isotopic categories.

Scats capture a very short window of food consumption; the average gut retention time for coyotes is likely on the order of just a few days (Weaver 1993). Stable isotope analyses of scats, then, are particularly useful when working with an organism for which seasonal dietary shifts are important (Hatch et al. 2011; Blumenthal et al. 2012). There are other tissues that turn over rapidly, but their sampling largely requires physical contact with the animal. Scats provide a non-invasive way to gather short-term dietary information. Furthermore, scats can be linked to individuals either through direct observation (as demonstrated by Blumenthal et al. 2012) or potentially through nuclear DNA analyses (Fedriani and Kohn 2001; Prugh et al. 2008), making it possible to non-invasively monitor individual dietary preferences over time.

## CONCLUSION

Data presented on the apparent enrichment in carbon and nitrogen isotopes among collagen, keratin, and muscle in 3 mammalian mesopredators will facilitate comparison among isotopic studies performed on a variety of tissue types. I derived a scat-to-diet discrimination factor for coyotes and validated the use of scat carbon and nitrogen isotopes as proxies for coyote diets. Stable isotopes measured in scat accurately reflect assimilated diet; scat stable isotope values consistently plotted within the mixing space created by the dietary sources found in the scats. Mixing

model estimates of dietary proportions are complementary, though not identical, to estimates derived from traditional scat analysis methods. Given that our mixing model estimates place greater emphasis on larger-bodied prey items, just as previous authors have noted for biomass calculations, these data suggest that scat stable isotopes may provide less biased estimates of diet (given sufficient variation in the ecosystem). Like Blumenthal et al. (2012), I note that scat stable isotope analyses will be most informative in reference to a local isotopic baseline. Furthermore, I suggest that the best approximation of true diet from scat will be derived from a combination of these methods.

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**APPENDIX 3A.** EFFECTS OF RINSING FOLLOWING ACIDIFICATION

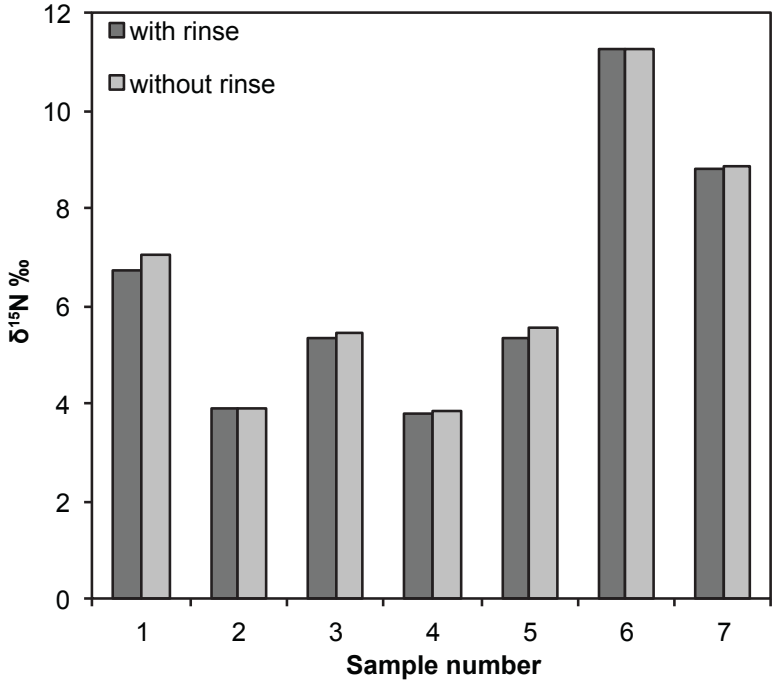


**Figure 3.A1.** $\delta^{15}N$ values measured in split scat samples that were either (1) rinsed after acidification (dark grey) or (2) not rinsed after acidification (light gray). The mean sample difference is -0.1‰, which is indistinguishable from instrumental error (± 0.1‰).

**APPENDIX 3B.**

**Table 3.B1.** Isotope values and digestible [C] and [N] values input into the mixing models for each scat. Food source $\delta^{13}C$ and $\delta^{15}N$ values are corrected to muscle values (see Table 3.1). Digestible [C] and [N] values are derived from sample weight % C and N or sourced from the USDA nutrient database.

| Scat Sample | Food Source | Tissue | $\delta^{13}C$ | SD $\delta^{13}C$ | [C] | SD [C] | $\delta^{15}N$ | SD $\delta^{15}N$ | [N] | SD [N] |
|---|---|---|---|---|---|---|---|---|---|---|
| May2011AN009 | *Odocoileus hemionus* | hair | -26.91 | 1 | 45 | 8 | 5.72 | 1 | 14 | 3 |
| | *Urocyon cinereoargenteus* | hair | -20.42 | 0.5 | 45 | 8 | 13.85 | 1 | 14 | 3 |
| May2011AN0019 | *Microtus californicus* | bone collagen | -26.52 | 0.2 | 45 | 8 | 6.44 | 1 | 14 | 3 |
| | unknown bird | feather | -14.75 | 0.5 | 45 | 8 | 5.56 | 0.3 | 14 | 3 |
| May2011AN0020 | *Microtus californicus* | bone collagen | -27.61 | 1 | 45 | 8 | 6.13 | 1 | 14 | 3 |
| | *Neotoma fuscipes* | hair | -26.22 | 0.2 | 45 | 8 | 5.14 | 1 | 14 | 3 |
| | *Sus scrofa* | hair | -27.18 | 0.5 | 45 | 8 | 12.22 | 1 | 14 | 3 |
| | *Didelphis virginiana* | hair | -21.18 | 1 | 45 | 8 | 12.04 | 1 | 14 | 3 |
| 091111AN003 | *Zalophus californianus* | hair | -17.63 | 0.5 | 58 | 8 | 15.00 | 0.4 | 10 | 3 |
| | *Odocoileus hemionus* | hair | -26.50 | 1 | 45 | 8 | 6.21 | 1 | 14 | 3 |
| | unknown seed | seed | -25.91 | 2 | 48 | 8 | 6.66 | 1 | 2 | 2 |
| 091111AN008 | *Odocoileus hemionus* | hair | -26.55 | 0.5 | 45 | 8 | 6.22 | 1 | 14 | 3 |
| | *Vaccinium ovatum* | seed | -25.83 | 1 | 48 | 8 | 10.27 | 1 | 2 | 2 |
| | unknown reptile | skin | -27.63 | 0.7 | 45 | 8 | 11.58 | 0.4 | 14 | 3 |
| 091111AN0014 | *Odocoileus hemionus* | hair | -27.24 | 1 | 45 | 8 | 7.69 | 1 | 14 | 3 |
| | *Mirounga angustirostris* | hair | -16.21 | 0.5 | 58 | 8 | 16.35 | 0.4 | 10 | 3 |
| | unknown seed | seed | -28.35 | 1 | 48 | 8 | 8.83 | 1 | 2 | 2 |
| | unknown reptile | skin | -29.22 | 0.7 | 45 | 8 | 11.47 | 0.4 | 14 | 3 |
| 091111AN0015 | *Zalophus californianus* | hair | -16.83 | 0.5 | 58 | 8 | 15.95 | 0.4 | 10 | 3 |
| | *Sylvilagus bachmani* | hair | -25.88 | 1 | 45 | 8 | 5.46 | 1 | 14 | 3 |
| 111411ANNU02 | unknown bird | feather | -23.84 | 0.5 | 45 | 8 | 12 | 0.3 | 14 | 3 |
| | *Sylvilagus bachmani* | hair | -25.69 | 1 | 45 | 8 | 4.62 | 1 | 14 | 3 |
| | unknown vegetation (grass) | leaf | -20.53 | 1 | 39 | 8 | 9.09 | 1 | 4 | 2 |
| | unknown arthropod | chitin | -15.28 | 1 | 43 | 8 | 5.88 | 1 | 13 | 3 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 111411ANNU07 | *Mirounga angustirostris* | hair | -17.61 | 0.5 | 58 | 8 | 12.14 | 0.4 | 10 | 3 |
| | *Zalophus californianus* | hair | -16.51 | 0.5 | 58 | 8 | 15.96 | 0.4 | 10 | 3 |
| 111411ANNU11 | unknown vegetation | leaf | -29.07 | 1 | 39 | 8 | 9.65 | 1 | 4 | 3 |
| | *Stenopelmatus* spp. | chitin | -27.06 | 1 | 40 | 8 | 9.42 | 1 | 10 | 3 |
| | *Zalophus californianus* | hair | -16.51 | 0.5 | 58 | 8 | 15.96 | 0.4 | 10 | 3 |
| 111411ANNU12 | *Odocoileus hemionus* | hair | -26.37 | 1 | 45 | 8 | 5.81 | 1 | 14 | 3 |
| | *Zalophus californianus* | hair | -16.51 | 0.5 | 58 | 8 | 15.96 | 0.4 | 10 | 3 |
| | unknown vegetation (seaweed) | leaf | -28.09 | 1 | 38 | 8 | 13.3 | 1 | 4 | 3 |
| 111411ANNU17 | *Odocoileus hemionus* | hair | -26.92 | 1 | 45 | 8 | 8.1 | 1 | 14 | 3 |
| | *Urocyon cinereoargenteus* | hair | -20.54 | 0.5 | 45 | 8 | 13.85 | 1 | 14 | 3 |
| | unknown reptile | skin | -18.75 | 0.7 | 50 | 8 | 12.55 | 0.5 | 10 | 3 |
| | unknown vegetation (grass) | leaf | -28.38 | 1 | 38 | 8 | 9.66 | 1 | 4 | 3 |

# Chapter 4

## Tracing a subsidy through time: Marine resource use by modern California coyotes (*Canis latrans*) is a new behavior

**Keywords:** *Canis latrans*, interspecific competition, paleoecology, resource subsidy, stable isotopes

**ABSTRACT**

Considerable interest exists in how resource subsidies impact predator populations and whether they have cascading effects on other predators and prey. Coyotes (*Canis latrans*) have been known to consume marine foods, but both the degree to which they rely on marine subsidies and the length of time the subsidy has occurred are unknown. I investigated the importance of marine foods to modern coastal coyotes in the central coast region of California and compared their present-day diet to that of Holocene coyotes via stable isotope analyses. I measured $\delta^{13}C$ and $\delta^{15}N$ values in modern coyote scats sourced from three coastal sites and in coyote bone collagen from six Holocene archaeological sites spanning in age from ~3000 – 750 BP. I found evidence for marine resource use by modern coastal California coyotes at only one site, Año Nuevo, which hosts a mainland northern elephant seal (*Mirounga angustirostris*) breeding colony. Seals and sea lions account for about 20% of Año

Nuevo coyote diets throughout the year and this subsidy is having a positive impact on coyote population size. My sub-fossil isotope data suggest that Holocene coyotes did not consume marine-derived foods, even at sites adjacent to a past mainland northern fur seal (*Callorhinus ursinus*) rookery. Marine resource use by contemporary California coyotes is a new behavior relative to their recent ancestors. This change in behavior is likely enabled by reduced competition with humans, other, now-absent consumers (e.g., grizzly bears, *Ursus arctos californicus*), or both.

## INTRODUCTION

Marine and terrestrial environments are linked through cross-habitat transfers of energy and nutrients, the flux of which can subsidize a diverse array of consumers and have significant consequences for local communities and food webs (Polis and Hurd 1996; Polis et al. 1997; Jefferies 2000). The magnitude and direction of a subsidies' impact, however, can be quite variable, since subsidies can vary spatially and temporally, can enter recipient habitats at any trophic level, and can have major impacts on food web dynamics (Polis et al. 1997, 2004). For example, the input of allochthonous prey often allows predators to increase in local abundance (Henschel et al. 2001), and top-down effects can follow when an enlarged population of subsidized predators depresses local resources (Flaherty 1969; Senft et al. 1987; Rose and Polis 1998; Rand and Louda 2006; Gompper and Vanak 2008). If predators are supported by an imported resource on which they have no negative impact, their success may become decoupled from local productivity, making overexploitation of resident prey

(even to extinction) possible (Polis et al. 1997). On the other hand, should the subsidy become the predators' preferred resource, resident prey may be released from predation pressure (Nakano et al. 1999; Sabo and Power 2002). Similarly, if an intra-guild predator prefers a subsidized resource, otherwise contested niche space may become available to a competitor. For example, Gomez et al. (2010) observed that consumption of marine prey by native otter (*Lontra provocax*) and fox (*Pseudalopex vison*) likely allowed for their coexistence with two exotic carnivores (mink, *Neovison vison*, and grey fox, *Pseudalopex griseus*) in coastal Argentina. Generalist carnivores such as coyotes (*Canis latrans*) are likely important drivers of top-down dynamics (Schmitz et al. 2000; Borer et al. 2005; Jiang and Morin 2005) and are documented intra-guild predators (e.g. Cypher and Spencer 1998).

Coyotes have a rapidly expanding North and Central American range (Hidalgo-Mihart et al. 2004; Chubbs and Phillips 2005; Fener et al. 2005; Mendez-Carvajal and Moreno 2014), have been shown to benefit from marine subsidies in desert environments (Rose and Polis 1998) and can also have cascading impacts on other predators and prey (Crooks and Soule 1999). Observational evidence (Marine Mammal Center 2014) has suggested that coyotes on the central coast of California consume marine resources, but the importance of marine resources in their diets is unknown. It's possible that coyotes in coastal areas have an advantage over their interior counterparts and that the delivery of a marine subsidy to coastal coyotes has contributed to their success. If the subsidy is new or has a recent onset, it may be facilitating coyote expansion along coastal routes, as has been demonstrated for red

foxes (*Vulpes vulpes* L.) expanding into the arctic (Killengreen et al. 2011). In light of these possibilities, I seek to quantify the current marine subsidy to coyotes on the central coast and evaluate its spatial and temporal coverage. My research goals were: (1) to characterize the extent and importance of a marine subsidy to modern coyotes on the central California coast, and (2) to determine whether this marine subsidy is recent or has roots deeper in the Holocene.

The central California coast is an ideal region to investigate questions about the magnitude and continuity of a marine subsidy to coyotes. The marine environment is highly productive, offering numerous opportunities for the delivery of subsidies to adjacent terrestrial communities. Today, Año Nuevo State Park supports a breeding colony of northern elephant seals (*Mirounga angustirostris*), which was established in the 1960s (Le Boeuf and Panken 1977). Mainland rookery sites such as this are likely to provide terrestrial predators and scavengers with easy access to live and dead seal pups, both of which are possible coyote food sources (Steiger et al. 1989; Way and Horton 2004). Año Nuevo Island is also a favored haul out for sea lions and consequently dead sea lions wash up on the mainland beach with regularity (Burton et al. 2002; P. Morris, personal communication). In addition, people have occupied the coast for thousands of years, allowing for the accumulation of subfossil assemblages in archaeological middens. A preponderance of evidence now points to the existence of a mainland northern fur seal (*Callorhinus ursinus*) rookery active ~2000 BP coincident with human occupation at Moss Landing, CA (Burton et al. 2001; Newsome et al. 2007; Gifford-Gonzalez and Sunseri 2009; Gifford-Gonzalez

2011), allowing for a past-present comparison between sites with very similar resource availabilities.

I present stable isotope data from both modern coyote scat from 3 coastal sites and Holocene coyote bone collagen from 6 coastal archaeological sites spanning periods of occupation from ~3000 – 750 BP. The $\delta^{13}C$ and $\delta^{15}N$ values of animal tissues and scat reflect the isotopic composition of an animal's diet, offset by a characteristic trophic increase in both $\delta^{13}C$ and $\delta^{15}N$, though the increase in $\delta^{15}N$ is more pronounced (Schoeninger and DeNiro 1984; Kelly 2000; Koch et al. 2007). Marine ecosystems are isotopically distinct from terrestrial ecosystems in part because of baseline differences in the isotopic composition of primary producers; marine primary producers are enriched in $^{15}N$ and $^{13}C$ relative to terrestrial plants. Furthermore, marine food chains are generally longer than terrestrial food chains, leading to greater trophic enrichments in the heavier isotopes. Apex predators in marine systems have $\delta^{15}N$ values in the range of +16-19‰, while terrestrial apex predator $\delta^{15}N$ values range between +7-12‰ (Newsome et al. 2010). Coastal California is dominated by $C_3$ plants (Suits et al. 2005) and therefore its coastal food webs are characterized by relatively low $\delta^{13}C$ values ranging from -22 to -28‰, while marine sourced materials tend to have slightly higher values (-16 to -20‰, Craig 1953; Newsome et al. 2010). Consumers relying on a mixture of marine and terrestrial resources will have $\delta^{13}C$ and $\delta^{15}N$ values that fall somewhere in between these end members. To estimate the proportional contribution of marine foods to both modern and Holocene coyote diets I use a Bayesian multiple source stable isotope

mixing model (SIAR) (Parnell et al. 2010). In the modern, I am able to corroborate the mixing model results with analysis of scat contents.

## MATERIALS AND METHODS

### Scat Sample Collection and Processing

I collected mesopredator scat samples quarterly in spring 2011 through summer 2013 along 3 coast-to-inland transects in the central coast region of California at 3 sites: Año Nuevo State Park, Younger Lagoon Reserve/Moore Creek Preserve, and Andrew Molera State Park (Figure 4.1, Appendix 4A). To ensure that

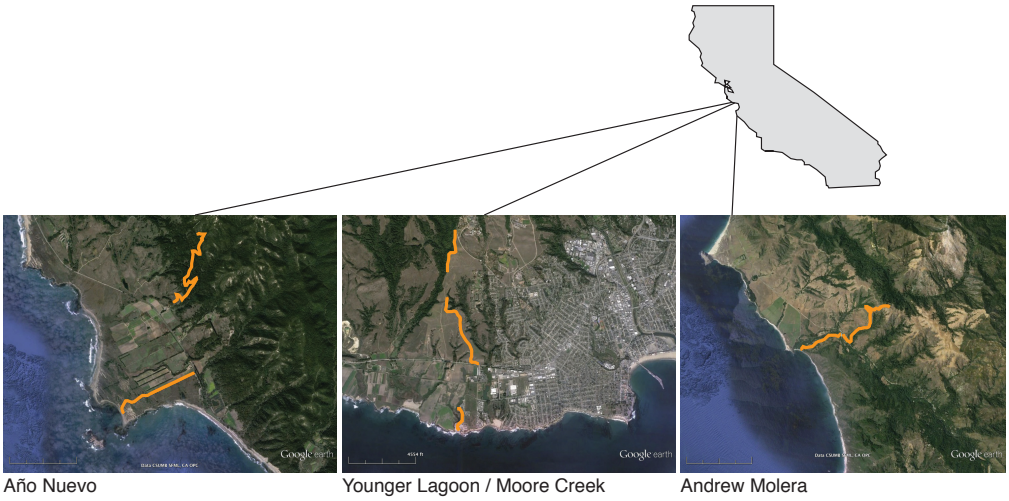


**Figure 4.1.** Map illustrating the locations and extent of the three modern transects. On the ground distances at each site are shown in orange. Images are sourced from Google Earth.

collected scats were fresh and deposited within a known time period, I cleared transects of all scats about one week prior to collection. At the time of collection, I recorded scat locations with a GPS, placed scats in individually marked Ziploc bags, and recorded the date/time, morphological measurements, and a description of the

scat morphology and contents on a data sheet attached to the bag. I walked the length of each transect twice during both clearing and collection to better ensure that no scats were missed. Scats were stored frozen (-4˚C) until processing. I took DNA samples by swabbing the exterior of the scats with a Q-tip, and then freeze dried and oven dried (60˚C for 48 hours) the scats to kill any potentially harmful parasites. I then subsampled scats for isotopic analysis, washed the remaining material in nylon stockings in an automatic clothes washer, and once dry, I placed the scat contents on a gridded sorting tray to estimate the percent by volume contributions of mammal, bird, reptile, invertebrate and plant components to the nearest 5% (McDonald and Fuller 2005). While the fur was spread out, I sampled guard hairs from the center of each grid cell until I examined 40-50 hairs and identified them to the finest taxonomic level possible through comparison with a local mammal guard hair reference collection housed at UCSC and with published keys (Mayer 1952; Tumlison 1983; Debelica and Thies 2009). I determined the optimal number of hairs to analyze by performing rarefaction on a set of well-sampled scats.

I measured the $\delta^{13}C$ and $\delta^{15}N$ values in the fine-grained scat matrix and compared these values with isotope values from coyote food sources. To prepare scat samples for isotopic analysis, I extracted the matrix by gently breaking apart oven-dried scats over a fine mesh sieve. The matrix passes through the sieve while other scat components, such as fur, feathers or bone are captured above. I then cleaned the powdery matrix by placing it in filter paper cones and rinsing it first with MilliQ water, then with 0.1N HCl to remove $CaCO_3$, and again with MilliQ. After the scat

samples are fully dry and homogenized, I weighed approximately 5 mg of scat matrix into 5 x 9-mm tin boats for isotopic analysis.

To enable comparison among scat and diet component isotopic values, I cleaned and analyzed a subset of identified scat components, hair samples from live-trapped small mammals, and berry and insect samples collected along the transects. I rinsed hair and feather samples with MilliQ water, immersed them in petroleum ether and sonicated for 15 min, rinsed them again and dried them at 60°C overnight. I decalcified bone fragments in 0.5 N HCl for ~72 hours or until bubbles stopped being produced. This was followed with an NaOH treatment to remove humic acids (0.1N NaOH for 24 hours) and then by lipid extraction: samples were immersed in petroleum ether and sonicated 2-3 times for 15 min with MilliQ rinses in between (Dobush et al. 1985). I then rinsed the collagen samples five times in MilliQ water, froze them and freeze dried them overnight. Arthropod and berry samples were repeatedly rinsed and sonicated in MilliQ water (4x for 15 min), dried (60°C oven overnight) and then crushed with an agate mortar and pestle. I weighed ~0.7mg of hair, collagen and arthropod samples into 5 x 9-mm tin boats. I divided berry samples into aliquots of ~0.4 mg for C isotopes and ~3 mg for N isotopes, and sealed them in 5 x 9-mm tin boats.

**Sub-Fossil and Historical Sample Collection and Processing**

The Monterey Bay area has a significant archaeological record that contains evidence of human hunting, collecting, and resource processing occurring as early as

10,000 BP (Moratto 1984). Ecological subsistence models suggest that peoples resided along the area's coastlines where terrestrial and marine resources were both available (Hylkema 1991) and there have indeed been a number of sites discovered and excavated along the coast (Jones 1991). To evaluate marine resource use by Holocene coyotes, I selected sites that span a range of occupational periods, contain coyote bones, and have already been the subject of extensive research (Figure 4.2, Table 4.1, Appendix 4B). I acquired archaeological and historical bone and fur samples from the UC Santa Cruz Monterey Bay Archaeology Archives, Moss Landing Marine Lab, and the California Academy of Sciences. Archaeological bone samples were primarily identified to species by D. Gifford-Gonzalez through comparison with reference materials. I avoided bones that appeared burned or charred because heating can change stable isotope ratios (DeNiro et al. 1985). I prepared collagen and hair samples for isotopic analysis in the same manner as I did for modern samples.

**Table 4.1.** List of archaeological sites from which our samples were sourced and their ages of occupation. Ages are calibrated and were compiled by Gifford-Gonzalez 2011.

| Site | $^{14}$C YBP 2σ | Reference/Notes |
|---|---|---|
| CA-SCR-35 | 2870 - 2970 | Newsome et al. 2007 |
| CA-MNT-234 | 2470 - 2438 | Newsome et al. 2007 |
| CA-MNT-229 | 900 - 2700 | Dietz et al. 1988; Jones 2002 |
| CA-SMA-18 | 1070 - 1480 | Hylkema et al. 2006; Newsome et al. 2007 |
| CA-SMA-113 | 880 - 940 | Beta Analytic #238125, 238126, 238127; Gifford-Gonzalez 2011 |
| CA-SMA-115 | 575 - 835 | Hylkema 1991 |

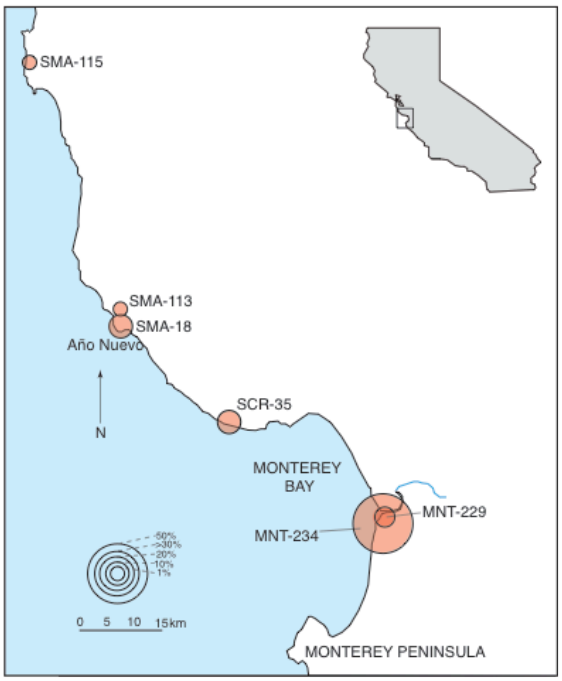


**Figure 4.2.** Map of the central California coast illustrating the locations of the six Holocene archaeological sites used in this study. Also illustrated are the proportions of *Callorhinus ursinus* bone found in the sites. Modified from Gifford-Gonzalez (2011).

**Stable Isotope Analysis**

All samples were subjected to Dumas combustion using a Carlo Erba 1108 elemental analyzer and then $\delta^{13}C$ and $\delta^{15}N$ values were measured on a ThermoFinnigan Delta Plus XP continuous flow isotope ratio mass spectrometer at the UC Santa Cruz Stable Isotope Laboratory. I report our results using δ notation, in which

$$\delta^{H}X = ((R_{sample}/R_{standard}) - 1) \times 1{,}000 \qquad (4.1)$$

where R is the ratio of the heavy isotope to light isotope for element X (e.g. Sulzman et al. 2007). Carbon isotope values are reported relative to Vienna Pee Dee Belemnite (a marine carbonate) and nitrogen isotope values are reported relative to air, and the resulting value is expressed in parts per thousand (i.e., per mil, ‰). Sample isotopic values are corrected for size, drift and source stretching effects. I calculated the

average analytical precision for the scat data as the *SD* of the $\delta^{13}C$ and $\delta^{15}N$ of 41 replicates of an internationally calibrated in-house standard (PUGel); precision was 0.2‰ for carbon and 0.1‰ for nitrogen. I similarly calculated the average analytical precision for the muscle, collagen, keratin, and arthropod samples as the *SD* of the $\delta^{13}C$ and $\delta^{15}N$ of 20 replicates of a different internationally calibrated in-house standard (Acetanilide); precision was also 0.2‰ for carbon and 0.1‰ for nitrogen.

**Data Analysis**

I performed all statistical analyses in R (R Development Core Team, 2013). I used Hotelling's $T^2$-test, the multivariate analogue to the univariate t-test, to evaluate whether coyotes from Año Nuevo and Younger Lagoon have statistically different multivariate stable isotopic means. I used Stable Isotope Analysis in R (SIAR) (Parnell et al. 2010), a Bayesian stable isotope mixing model, to estimate the proportional contributions of various scat components to coyote diets. SIAR is capable of accounting for error in estimates of trophic enrichment factors as well as for variations in the elemental concentrations of C and N in the food sources, which could otherwise bias model output (Phillips and Koch 2002). Scat components, which largely represent the indigestible portion of animal diet, are proxies for assimilated diet. To convert stable isotope values measured in these indigestible components, such as hair or bone, to assimilated coyote diet space (i.e. the muscle tissue that is digested and assimilated) I applied published organism and tissue specific correction factors (see Table 3.1). Using these transformed values, I ran the mixing models for

the species-verified scat samples collectively by site. I also corrected scat isotope values for trophic discrimination (Table 4.2) by adding 1.8‰ ± 1‰ for $\delta^{13}C$ values and subtracting 1.8‰ ± 2‰ for $\delta^{15}N$ values (Reid, *Chapter 3*). I similarly corrected fossil coyote specimens for trophic fractionation (Table 4.2) by combining collagen-to-diet corrections derived for wolves (Fox-Dobbs et al. 2007) and coyotes (Schwarcz 1991), subtracting 1.3‰ ± 0.6‰ for $\delta^{13}C$ values and 2.8‰ ± 0.9‰ for $\delta^{15}N$ values. I used the Schwarcz (1991) $\delta^{15}N$ correction in place of that calculated for wolves by Fox-Dobbs et al. (2007) because a correction of 4.6‰ ± 0.7‰ made little sense with our data, placing the majority of the coyotes below terrestrial herbivores and completely outside of the mixing space. Trophic discrimination values have also been experimentally derived for red foxes (Roth and Hobson 2000), but collagen isotope values were not included in that study.

**Table 4.2.** Organism and tissue-specific corrections applied to modern and Holocene stable isotope values.

| **Trophic Discrimination Corrections** | | | | |
|---|---|---|---|---|
| Organism | Tissue | $\delta^{13}C_{organism\ -diet}$ | $\delta^{15}N_{organism\ -diet}$ | Citation |
| *Canis latrans* | scat | +1.8‰ ± 1‰ | -1.8‰ ± 2‰ | Reid, Chapter 3 |
| *Canis latrans* | bone collagen | -1.3‰ ± 0.6‰ | -2.8‰ ± 0.9‰ | C correction - Fox-Dobbs et al. 2007; N correction - Schwarcz 1991 |
| **Holocene Diet Space Corrections** | | | | |
| Organism | Tissue | $\delta^{13}C_{collagen\text{-}muscle}$ | $\delta^{15}N_{collagen\text{-}muscle}$ | Citation |
| *Canis latrans* | bone collagen | -4‰ ± 1‰ | no change | Newsome et al. 2004 |
| Terrestrial mammals | bone collagen | -4‰ ± 1‰ | no change | Newsome et al. 2004 |
| Pinnipeds | bone collagen | -5.6‰ ± 1‰ | no change | Newsome et al. 2004 |

To compare isotopic niche widths across different sites and different time periods, I used SIBER (Stable Isotope Bayesian Ellipses in R) metrics (Jackson et al. 2011). Unlike the quantitative metrics proposed by Layman et al. (2007), these metrics are unbiased with respect to sample size and are able to take into account uncertainty in the sampled data. The Bayesian technique is qualitatively similar to bootstrapping; it returns a posterior probability distribution representing estimates of a standard ellipse area that takes into account uncertainty in the sampled data and the sampling process. Following Jackson et al. (2011) I calculated the sample size standard ellipse area for each subgroup ($SEA_c$) and assessed whether they were significantly different by comparing their Bayesian 95% credible limits; ellipse areas are significantly different when the limits do not overlap. In order to directly compare past and present samples I also corrected modern and historical samples for the Suess effect (the isotopic depletion of surface carbon reservoirs due to the burning of fossil fuels) (Quay et al. 1992; Sonnerup et al. 1999). My Suess correction was derived by fitting a spline function to the combined atmospheric $\delta^{13}C$ records from Rubino et al. (2013) and Indermühle et al. (1999) and predicting the $\delta^{13}C$ value of the atmosphere at the time each sample was collected. I then standardized the $\delta^{13}C$ values to the time period of interest. For comparison to archaeological data, I corrected the historical samples to 750 BP, which required the addition of between 0.28‰ for the samples from the late 1800s to 1.5‰ for the sample from 1991.

## RESULTS

### Modern Coyotes

Between May 2011 and August 2013, I collected a total of 687 mammalian mesopredator scats. The overall scat deposition rates were on average highest at Andrew Molera, followed by Año Nuevo and finally by Younger Lagoon (Table 4.3). Because of considerable difficulty in making morphological distinctions between scats produced by coyotes, bobcats, and gray foxes (Reid, *Chapter 2*), I first compared dietary breadth among sites at the mesopredator-, rather than species-, level. At this resolution, mesopredators at Año Nuevo have a significantly greater isotopic dietary breadth ($SEA_c$ = 14.4 ‰$^2$) than those at either Younger Lagoon or Andrew Molera ($SEA_c$ = 10.8 ‰$^2$ and 6.2 ‰$^2$, respectively; $p = 0.0194$ and $p < 0.0001$; Figure 4.3). Año Nuevo is also the only site where I observe evidence for marine resource use by any mesopredators.

To take the dietary analysis to the predator species-level, I submitted a subset of 135 scats from Año Nuevo and Younger Lagoon to Wildlife Genetics International for mitochondrial (mt) DNA-verification (Appendix 4C: Table 4.C1). I did not verify any of the scats collected at Andrew Molera to species with mtDNA. Of the 115 scats analyzed from Año Nuevo, 20 were identified as coyote. I identified an additional 10 scats as coyote based on the presence of 1 – 5 coyote guard hairs in the scats, which are a product of self-cleaning; this is a method of scat identification that has proved successful previously for pumas (Miotto et al. 2007). I have not carefully dissected every scat in our collection and I anticipate that there are more coyote scats than just

**Table 4.3.** Scat depositions rates at each site in scats/km/day. I initially collected scat about 10 days after clearing, but shortened this
time period to 7 days with some variation depending on scheduling.

| Site | Transect length (km) | May 2011 | Sept 2011 | Nov 2011 | Jan 2012 | Apr 2012 | June 2012 | Oct 2012 | Jan 2013 | May 2013 | August 2013 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Año Nuevo | 6 | 0.68 | 0.63 | 0.50 | 0.45 | 0.50 | 0.88 | 0.90 | 0.48 | 0.38 | 1.14 | 0.65 |
| Younger Lagoon/Moore Creek | 4 | - | 0.33 | 0.45 | 0.78 | 0.86 | 0.57 | 0.88 | 0.41 | 0.46 | 0.43 | 0.57 |
| Andrew Molera | 4.5 | - | 0.71 | 0.98 | 0.78 | 1.21 | 0.73 | - | - | - | - | 0.88 |

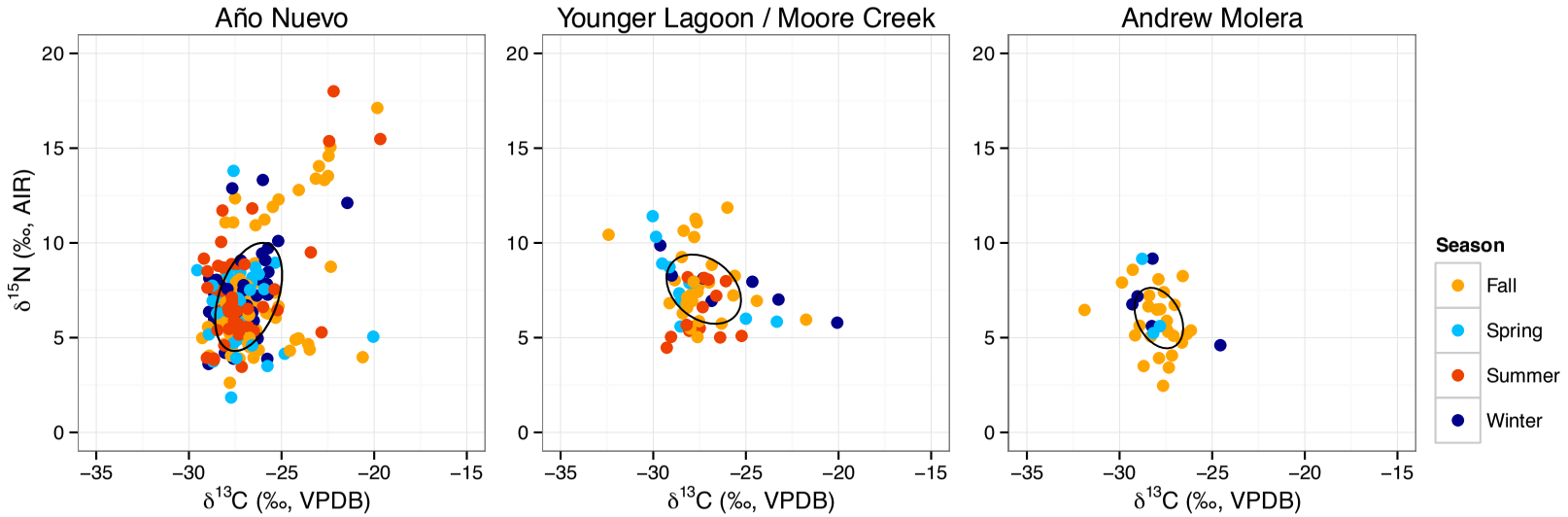


**Figure 4.3.** Stable isotope values of mesopredator scats collected seasonally from three sites ($n$ = 203 at Año Nuevo, $n$ = 61 for Younger Lagoon/ Moore Creek, and $n$ = 35 at Andrew Molera). Also plotted as black ovals are the sample standard ellipses (Jackson et al. 2011) for the mesopredators at each site.

these 30 in my current sample. The Año Nuevo coyote scats have a mean $\delta^{13}C$ value of -24.76 ± 2.6‰ and mean $\delta^{15}N$ value of 10.54 ± 4.0‰ and the multivariate means are significantly different from those for the 10 mtDNA- and 4 hair-verified Younger Lagoon coyote scats ($\delta^{13}C$ = -27.01 ± 2.6‰ and mean $\delta^{15}N$ value of 7.64 ± 1.2‰; $F_{2,41} = 4.96$, $p = 0.01$; see also Appendix 4A: Table 4.A1). Of the 30 verified coyote scats collected at Año Nuevo, 46% contain isotopic and/or physical evidence of marine resource consumption (Figure 4.4). Marine material found in the scats is largely northern elephant seal and California sea lion (*Zalophus californianus*) hair and sometimes kelp or sea bird feathers; I found no evidence for fish or shellfish consumption (Reid, *Chapter 3*). I found marine material in scats during all seasons. Indeed, there is little seasonal dietary variation; the difference in the isotopic breadth of coyote diet between the winter wet and summer dry seasons is not statistically significant ($SEA_c$ = 33.4 and 28.1 $‰^2$, $p = 0.57$; Figure 4.5).

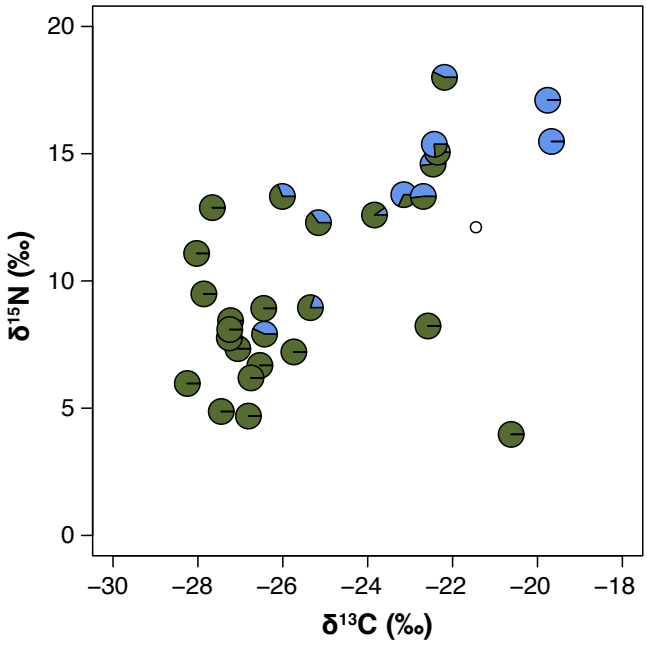


**Figure 4.4.** Stable isotope values of verified coyote scats collected at Año Nuevo between May 2011 and August 2013. Overlain over the isotope values are pie charts depicting the proportion of marine (blue) vs. terrestrial (green) food remains identified in the dissected scats. One sample contained no identifiable remains and is shown as an open circle.

The SIAR dietary mixing model for Año Nuevo coyotes predicts that pinnipeds constitute a modal proportional contribution of 22%, with deer and the various *Peromyscus* spp responsible for the next highest proportions (Figure 4.6; Appendix 4D: Table 4.D1). This relatively high reliance on marine resources is corroborated by the scat dissections, which similarly indicate that pinnipeds make up about 20% of coyote diets at Año Nuevo. I decided not to include marine fish or shellfish as mixing model inputs because I found no evidence for their consumption by any modern coyotes. After marine mammals, the most frequently identified species in the coyote scats was black-tailed deer (*Odocoileus hemionus*) (Reid, *Chapter 3*). These results from Año Nuevo differ somewhat from the SIAR mixing model predictions for Younger Lagoon coyotes, which identify deer as the most important dietary component (modal contribution of 19.7%) followed closely by *Peromyscus californicus*, rabbits and wood rats (modal proportions of 17.1%, 14.4%, and 14.3%, respectively).

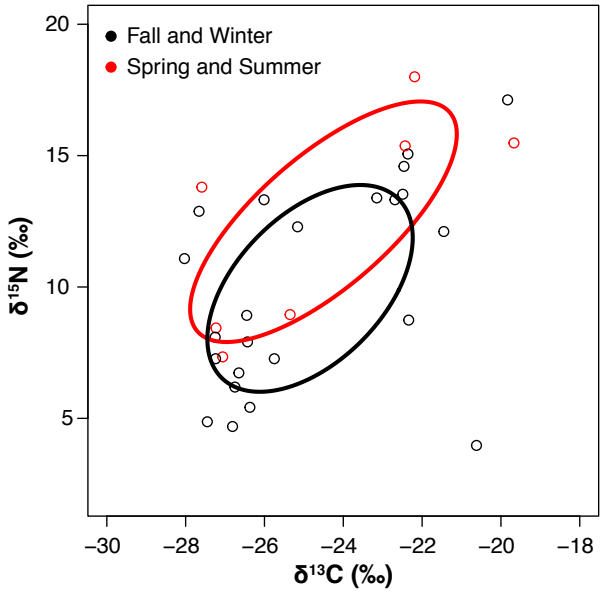


**Figure 4.5.** Comparison of fall and winter wet season coyote scat stable isotope values (black) to spring and summer dry season values (red) isotope space. The sample standard ellipses for the wet and dry seasons are plotted in black and red, respectively; they do not have significantly different areas.

**Table 4.4**. C and N isotope values of modern coyote dietary sources at Año Nuevo State Park and Younger Lagoon/Moore Creek Preserve, CA. Values have been corrected to coyote diet space (see Table 3.1).

| Food Source | Tissue | $\delta^{13}C$ | SD $\delta^{13}C$ | [C] | $\delta^{15}N$ | SD $\delta^{15}N$ | [N] | n | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Berries | fruit | -25.3 | 1.5 | 48 | -2.6 | 2.5 | 2 | 10 | Reid et al. 2013 |
| Deer | hair | -27.3 | 0.9 | 45 | 3.3 | 1.1 | 14 | 10 | this study |
| Pinnipeds | collagen | -19.2 | 0.9 | 58 | 18.4 | 1.0 | 10 | 57 | Burton and Koch, 1999 |
| Vole | hair and collagen | -29.1 | 0.7 | 45 | 4.9 | 1.0 | 14 | 4 | this study |
| Woodrat | hair | -28.8 | 0.5 | 45 | 5.6 | 0.7 | 14 | 2 | this study |
| *P. boylii* and *P. maniculatus* | hair | -25.3 | 0.5 | 45 | 4.3 | 1.5 | 14 | 45 | this study |
| *Peromyscus californicus* | hair | -23.5 | 0.7 | 45 | 9.1 | 2.0 | 14 | 30 | this study |
| Rabbits | collagen | -28.0 | 0.5 | 45 | 6.0 | 0.8 | 14 | 5 | this study |
| Jerusalem cricket | chitin | -26.6 | 0.5 | 43 | 1.3 | 0.5 | 10 | 3 | this study |

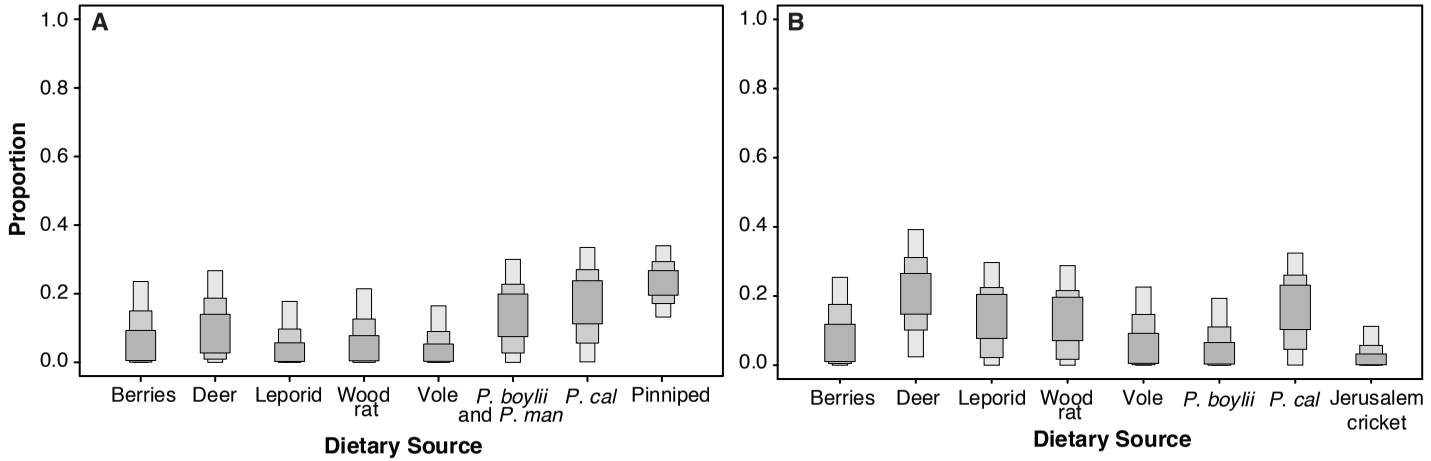




**Figure 4.6.** SIAR mixing model predictions of dietary source contributions to coyote diets at (A) Año Nuevo State Park and (B) Younger Lagoon/Moore Creek. Boxplots show the relative proportional contributions of each prey type with 50% (darkest grey), 75% and 95% (lightest grey) credibility intervals. Prior to running the model, I corrected coyote scat values for trophic and tissue specific discrimination (+1.8 ‰ C and -1.8 ‰ N; Reid, *Chapter 3*).

In order to evaluate coyote dietary selectivity for at-risk nocturnal small mammal species (e.g. *Neotoma fuscipes annectens* – a California species of special concern), I took a live-dead approach (Terry 2010a; b), comparing the modern live community with the scat-bound "dead" community (Appendix 4E). Agreement between the modern live surveys and the coyote scat small mammal death assemblage is relatively high (Tables 4.5 and 4.6); live trap and scat data are nearly indistinguishable in richness, but they are less similar in evenness. The Jaccard similarity index also reveals high agreement between the species lists of modern live surveys and coyote scats (0.68). However, the two assemblages start to diverge when rank-order and proportional abundances are considered. The Spearman rho correlation between the two pooled assemblages is positive (0.54) but not statistically significant ($p = 0.3$) and the Bray-Curtis similarity with sample-size-standardized data is just 0.51. These differences in rank-order and proportional abundances are driven by the fact that *Peromyscus* spp. are by far the most common live-trapped nocturnal small mammals (Appendix 4E: Table 4.E1), while *Microtus californicus* is the most common nocturnal small mammal species found in the coyote scats (Strauss's $L = 0.51$ for *M. californicus*). Coyotes consume *Neotoma fuscipes* in proportion to their abundance (Strauss's L = -0.005 for *N. fuscipes*), suggesting that coyotes do not negatively impact this at risk species.

**Table 4.5.** Rarefied richness (S ± 95% C.I.) and evenness (Probability of Interspecific Encounter [PIE] ± 95% C.I.) for all data pooled by type and survey. See Appendix 4E for additional information on these measures.

| | Trapping Surveys | Scat Surveys |
|---|---|---|
| S at $n = 5$ | 2 ± 0.24 | 1.90 ± 0.19 |
| PIE | 0.39 ± 0.07 | 0.57 ± 0.13 |

**Table 4.6.** Mean agreement (± 95% C.I.) between modern trapping surveys and scat samples as measured by Jaccard similarity, Spearman rho, and Bray-Curtis similarity.

| | | Trapping surveys | | | | | |
|---|---|---|---|---|---|---|---|
| | | Jaccard similarity | | Spearman rho | | Bray-Curtis similarity | |
| | | All surveys | Pooled | All surveys | Pooled | All surveys | Pooled |
| Scat surveys | All surveys | 0.74 ± 0.03 | 0.74 ± 0.09 | 0.42 ± 0.05<br>8.3% | 0.41 ± 0.08<br>0% | 0.62 ± 0.04 | 0.62 ± 0.11 |
| | Pooled | 0.70 ± 0.03 | 0.68 | 0.58 ± 0.08<br>0% | 0.54<br>$p = 0.3$ | 0.54 ± 0.03 | 0.51 |

**Holocene and Historical Coyotes**

I analyzed 23 Holocene coyote bones from 6 archaeological sites spanning occupation times from ~3000 to 750 BP (See Table 4.1). This is the maximum possible sample of unburnt *C. latrans* specimens from all the site collections, which are 100% analyzed. Carnivore remains are quite uncommon in regional archaeological sites, in which the focal prey are ruminants, lagomorphs and larger rodents; this, plus the relatively lower numbers of carnivores relative to these herbivores in animal communities, result in lower rates of carnivore occurrence. I also analyzed 16 historical coyote bone and hair specimens collected between 1893 and 1992 (Appendix 4B: Table 4.B1). There is little evidence for marine resource use by

coastal coyotes across these time periods; coyote collagen $\delta^{13}C$ and $\delta^{15}N$ values fall squarely in the range expected for an exclusively terrestrial diet at all six archaeological sites and continue to do so into historical times (Figure 4.7, Appendix 4B: Table 4.B2). Additionally, the Bayesian stable isotope mixing models consistently predict that marine foods are highly unlikely to have contributed to past coyote diets at all but two sites where relatively high $\delta^{15}N$ values (without corresponding high $\delta^{13}C$ values) are driving the result (Figure 4.8; see also Appendix 4D, Table 4.D2 and Figure 4.D1). The only coyote samples to exhibit a strong marine influence are the modern Año Nuevo coyote scats. A direct comparison between CA-MNT-234, which was adjacent to a mainland seal rookery at the time of occupation, and Año Nuevo today indicates that coastal coyote dietary breadth has expanded into the present (SEAc = 28.7 $‰^2$ in the present and 3.6 $‰^2$ at MNT-234, $p = 0.2158$). Two historical California grizzly bear (*Ursus arctos californicus*) samples exhibit isotope values indicative of marine resource consumption (CAS 24360 and 27342, see Table B2), both of which are from the San Francisco Bay area and of unknown age. The remaining three historical grizzly samples have isotopic signatures suggestive of purely terrestrial diets.

**DISCUSSION**

At Año Nuevo, modern coastal coyotes take full advantage of marine resources. The obvious difference between Año Nuevo and the two other sites I considered is the presence of the northern elephant seal rookery. Still, coyote scats

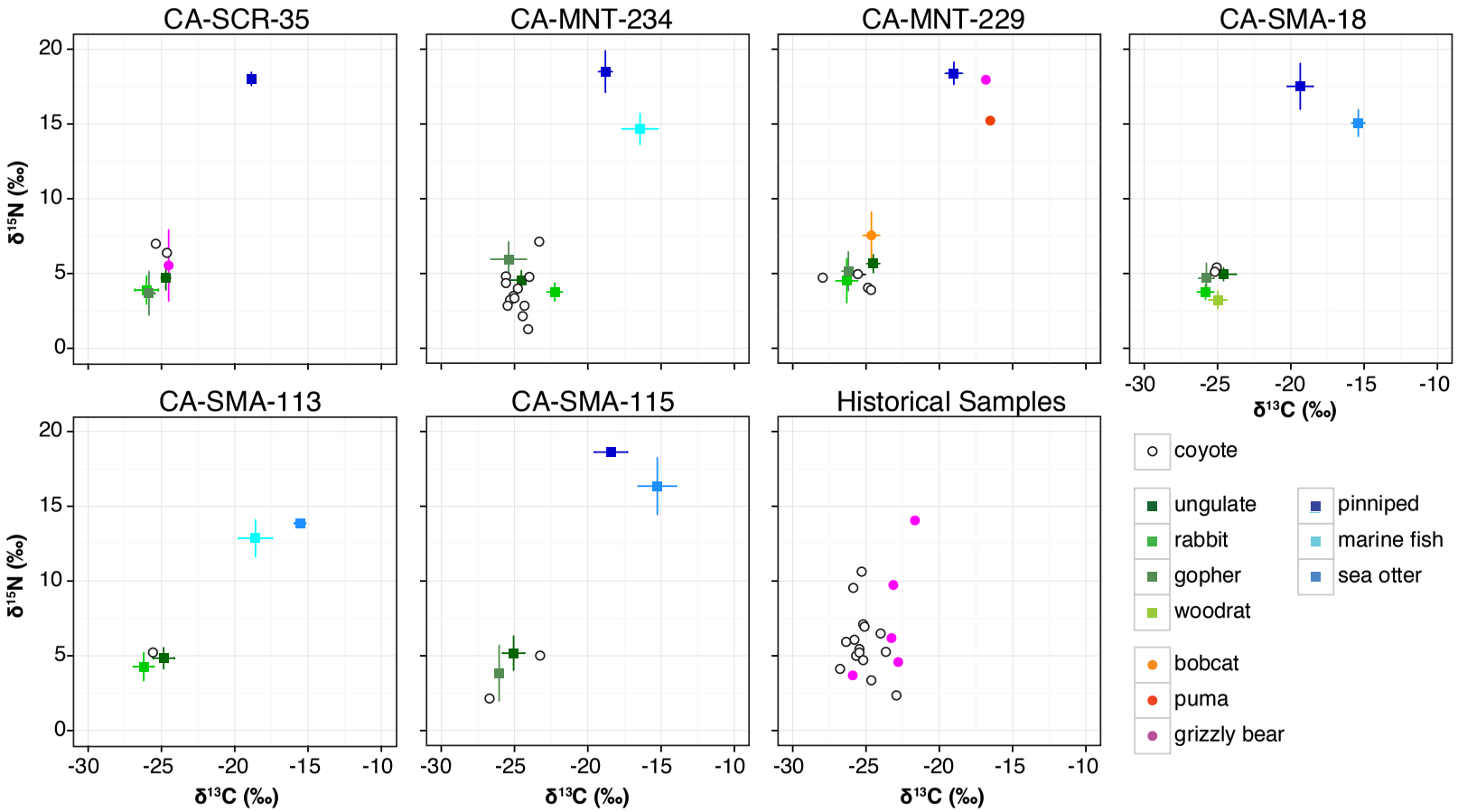


**Figure 4.7.** C and N isotope values measured in bone collagen from coastal CA archaeological and historical materials. Sites are ordered from oldest (CA-SCR-35) to youngest (Historical Samples). Coyote stable isotope values are corrected for trophic discrimination and terrestrial and marine mammal collagen values are corrected to muscle (Table 4.2). Historical carbon isotope values are corrected for the Suess effect. I did not correct the other carnivores (bobcat, puma, and grizzly) for trophic discrimination.

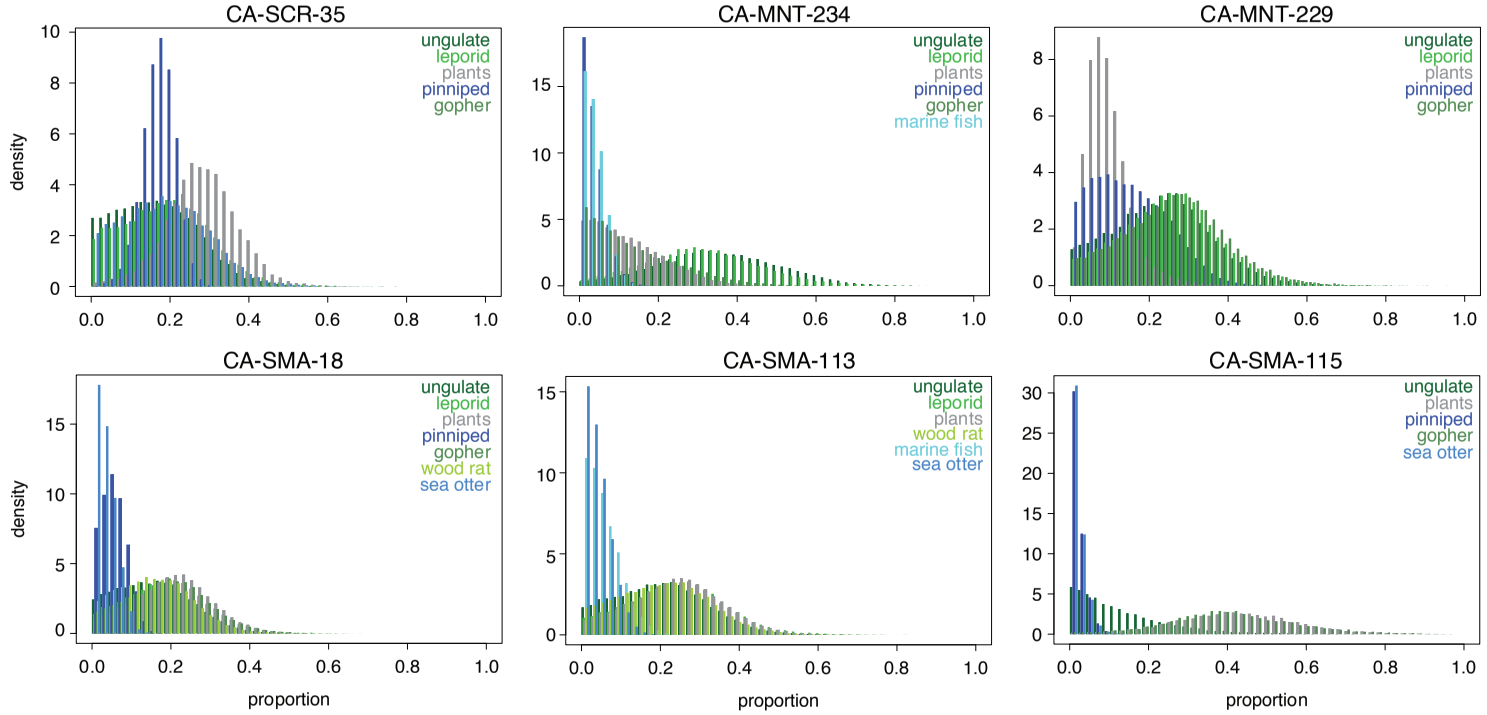


**Figure 4.8.** SIAR mixing model results for the coyote bones from the six archaeological sites. Possible dietary sources are color-coded to match Figure 4.7. There is little evidence for marine resource use by coyotes in the past and the sites at which the models do suggest possible marine resource use by coyotes (CA-SCR-35, CA-MNT-229 and CA-SMA-18), the coyote isotope values can be better explained in other ways (see Discussion).

from Año Nuevo contain sea lion hair just as frequently as they contain elephant seal hair, suggesting that marine resources are gained primarily through scavenging stranded marine mammal carcasses. Elsewhere around Monterey Bay, marine mammal strandings are not uncommon, but are a less frequent occurrence; California sea lions and harbor seals (*Phoca vitulina*) are the most frequently encountered stranded marine mammals with peak monthly mean deposition rates around Monterey Bay of 0.27 and 0.36 mammals per kilometer, respectively (Nevins et al. 2011). It may be that coyotes at the other sites would also readily consume marine foods if given the opportunity, but the opportunities at any one locality are generally few and far between. At Año Nuevo, the marine resource subsidy is not only significant, but also essentially constant – peaks in harbor seal strandings in Monterey Bay coincide with their breeding season in early spring (Nevins et al. 2011), while California sea lion stranding rates tend to be highest in the summer/early fall (Greig et al. 2005), and elephant seals begin pupping in the winter (Le Boeuf and Panken 1977). Additionally, Año Nuevo is a particularly well-protected area; human traffic is forbidden on the beaches, with exceptions made for researchers and park personnel, who do not disturb carcasses beyond taking samples from them. As such, coyotes at Año Nuevo are perhaps more shielded from human disturbance than on other greater Monterey Bay beaches, where humans are often present recreationally from morning to night and carcasses are collected by research groups for necropsy (NOAA Fisheries Service 2011).

Scats provide a relatively short snapshot of coyote diet (gut retention times for wolves are on the order of a few days – Weaver 1993), so it is possible that I happened to collect scat immediately following a stranding event and therefore marine foods are over-represented in our sample. Two lines of evidence refute this idea, however: (1) I find remains of marine foods in coyote scats during all seasons and it is unlikely that all of our scat sampling surveys happened to occur right after a stranding event, and (2) I find that C and N isotope values measured in coyote bone collagen from two modern road kill coyotes collected near Año Nuevo also point to marine resource consumption ($\delta^{13}C$ = -22.2 and -22.0, $\delta^{15}N$ = 13.9 and 13.4). The timescale of isotopic turnover in bone collagen is on the order of years (Tieszen et al. 1983; Hobson and Clark 1992) instead of days, so these collagen isotope values suggest that individuals in that area consistently rely on marine foods throughout their lifetimes.

Marine resources were not important components of Holocene coyote diets, which appear to be much more closely aligned with our observations for modern coyotes at Younger Lagoon/Moore Creek than those at Año Nuevo. Although the mixing models for CA-SMA-18, CA-SCR-35 and CA-MNT-229 suggest possible marine resource use by coyotes, these results are more likely an artifact of an inaccurate mixing space. An assumption behind all mixing models, including SIAR, is that the dietary sources input into the model represent the entirety of diet, which I know is not true for the Holocene models. I am lacking isotopic information for insects, which are a component of modern coyote diets at Younger Lagoon, and for

mice, which I found to be important for modern coyotes at both Año Nuevo and Younger Lagoon. At CA-SCR-35, the two sampled coyotes both have nitrogen isotope values that are elevated in comparison to the considered terrestrial prey. Nevertheless, these values are not accompanied by higher carbon isotope values (as would be expected with the inclusion of marine resources), and are therefore better explained by a missing higher trophic level terrestrial food source, such as the California mouse (*Peromyscus californicus*), which consumes enough insects to have $\delta^{15}N$ values that place it a trophic level higher than its sympatric congeners (Reid et al. 2013). At CA-MNT-229, the mixing model predictions for coyotes make little sense; all three coyotes have $\delta^{15}N$ values equal to or less than the terrestrial prey considered. Given the correlation structure of the possible dietary sources (Appendix D: Figure D2), it is clear that the model is trading off the inclusion of pinnipeds at the expense of other terrestrial prey. The model fit is generally poor (modal residual error term of 1.7 for C and 0.2 for N) and it seems most likely that a lower trophic level terrestrial food source is missing in the space where fruit or Jerusalem crickets (*Stenopelmatus*) sit in the modern mixing space.

To evaluate behavioral changes over time, I need to compare past and present sites at which I know there has been a constant marine subsidy in place. CA-MNT-234 contains significant marine mammal remains; 50% of non-rodent mammal bones in the primary midden at CA-MNT-234 are from northern fur seals with the number of identifiable specimens equaling 2334 (Gifford-Gonzalez 2011). Given the proportion of bone elements from breeding aged females and young-of-the-year as

well as isotopic evidence suggesting that the young were not yet weaned, a mainland rookery on or very near Moss Landing Hill was likely present at the time of human occupation (Burton et al. 2001). This suggests that marine resource availability to coyotes at this site should be comparable to that of Año Nuevo today. Nonetheless, it appears that coyotes were not taking advantage of this resource (Figure 4.7); 1 out of the 12 coyote specimens exhibits slightly elevated $\delta^{13}C$ and $\delta^{15}N$ values in comparison to the rest, but even these values are likely better explained by individual consumption of a slightly higher trophic level terrestrial prey, such as the California mouse. Our results imply that, relative to the Holocene, the consumption of marine food by modern coastal coyotes is a new behavior.

What could have caused such a shift in behavior? One possible explanation is that relaxed interspecific competition with grizzly bears, humans, or a combination of the two, allowed modern coyotes to broaden their niche to include marine resources. Two methods have been widely used to assess the role interspecific competition may play in producing dietary niche shifts (Korpimäki 1987). The first contrasts niches in situations where potential competitors are absent with niches where they are present (Huey et al. 1974; Schoener 1975; Diamond 1978; Schmitt and Coyer 1983) and the second compares prey use during periods of food abundance with periods of food shortage (Schoener 1982). Here I can contrast coyotes from CA-MNT-234 and -229, when both grizzlies and humans are present, with coyotes from present day Año Nuevo, when grizzlies are absent and humans are still present (but exhibiting

significantly different behavior). I can't assess interspecific competition by way of the second method, because I do not have past prey abundance information.

Brown bears in Alaska are known to limit marine resource use by wolves when the two species co-occur (Darimont et al. 2009). Historical evidence suggests that California grizzlies were abundant along the coast and that they consumed marine foods (Storer and Tevis 1996). During a few week's visit to Monterey in the early 1600s, Sebastian Vizcaíno observed bears heading down to the beach at night to feed on a whale carcass (Storer and Tevis 1996). It's possible, then, that the extirpation of the California grizzly bear afforded coyotes the opportunity to change their diets and move into the grizzlies' former niche. As expected if interspecific competition were to affect resource utilization, I see that dietary overlap is reduced in the presence of grizzlies; I observe that coyotes at CA-MNT-234 and -229 do not consume marine foods, but the one grizzly specimen I was able to sample from those sites consumed marine foods almost exclusively (Figure 4.7).

Changes in human behavior over the last several thousand years may also have contributed to modern coyote dietary expansion. Past peoples in the central coast region of California were without question relying heavily on marine resources, as evidenced by their midden contents and isotopic values (Newsome et al. 2004; Bartelink 2009; Beasley et al. 2013), and could have been closely protecting those resources from potential competitors, such as coyotes. Although I do not have isotope data from humans from the sites I analyzed, Bartelink (2009) observed that San Francisco Bay area human bone collagen $\delta^{13}C$ and $\delta^{15}N$ values from the Early Period

(ca. 4950 to 2150 BP) are reflective of heavy consumption of high trophic level marine prey, such as pinnipeds and marine fish. Newsome et. al (2004) also reported that marine resources comprised a significant proportion of early and middle Holocene human diets at Harkins Slough (SCR-60/130), a site located very near to Moss Landing. Again, reduced dietary overlap in the presence of past humans conforms to expectations for interspecific competition.

In contrast to Holocene peoples, modern humans are more recently making an effort to reduce exploitation of coastal resources; the Monterey Bay National Marine Sanctuary was established in 1992, 20 years after the enactment of the Marine Mammal Protection Act (MMPA). In their 40-year review of the MMPA, Roman et al. (2013) found that, although population trends are unknown for most stocks, pinniped stocks with known trends have largely been increasing since the MMPA was enacted. Indeed, all pinnipeds along the California coast (with the exception of Northern fur seal and Stellar sea lion) have been increasing (Costa et al. 2006; Le Boeuf et al. 2011; Rick et al. 2011). Furthermore, a significant proportion of coastal land in California is protected in parks, perhaps leaving coastal habitats open to coyotes and other animals.

Interestingly, both past human and past grizzly bear isotope data suggest that high trophic level marine resources may have declined in abundance into the Late Holocene. Results from analyses of human remains from Harkins Slough (SCR-60/130) demonstrate a drop in dependence on marine resources; Newsome et al. (2004) estimated that marine mammals, marine fish and shellfish comprised 70-84%

of early Holocene (~7000 BP) human diets, but that number drops to 48-58% in the middle Holocene. Similarly, both Bartelink (2009) and Beasley et al. (2013) observed that San Francisco Bay area human bone collagen $\delta^{13}C$ and $\delta^{15}N$ values are higher in the the Early Period (ca. 4950 to 2150 BP) than in the Middle (ca. 2150 to 1050 BP) and Late Periods (ca. 1050 to 200 BP). Although I have very few grizzly specimens, the specimen from CA-MNT-234 also has significantly higher $\delta^{13}C$ and $\delta^{15}N$ values than the known-age historical sample from 1909 (Figure 4.7). Northern fur seals also drop in abundance in Monterey Bay area archaeological sites in the Middle period, disappearing from the record completely by AD 750, and may have been overexploited for nutritional needs as well as for the exchange of their furs (Gifford-Gonzalez and Sunseri 2009). Together, these data suggest that marine resources were declining across this time period. It may be that it has taken until very recently for marine mammals to recover from this earlier period of exploitation and that recovery was a requirement for them to become an important food source for any modern coyotes. Analysis of additional grizzly (and human) specimens may shed more light on the past relationships between grizzlies, people, and coyotes, but, given the difficulty in obtaining and analyzing such specimens, it may be difficult to further resolve the reason for the shift in the coastal coyote dietary niche into the present.

Regardless of the reason for modern coyote dietary niche expansion, the marine subsidy to coastal California coyotes clearly has a recent onset. How then, is this new subsidy affecting coyotes and the greater coastal ecosystem? While the determination of true coyote abundances is not possible without capture-recapture

data, two lines of indirect evidence suggest that the coyote density near the coast at Año Nuevo is elevated relative to that further inland. First, I recorded a higher level of coyote activity on a coastal Año Nuevo trail camera than on either a camera on the inland portion of the Año Nuevo transect or at Younger Lagoon (Appendix 4F: Table 4.F1). Second, mtDNA-verified coyote scats collected at Año Nuevo consistently occurred in greater abundance on the coastal side of the transect (Appendix 4F: Figure 4.F1). Given that the coyotes at Año Nuevo continue to consume terrestrial foods in significant proportions in addition to marine resources, theory predicts that this increased consumer density should depress terrestrial resources (Flaherty 1969; Senft et al. 1987; Rose and Polis 1998; Rand and Louda 2006; Gompper and Vanak 2008). My small-mammal trapping data suggest that coastal small mammal populations are slightly reduced relative to inland populations. Over the course of 6 trapping sessions, I recorded 162 individuals in the coastal willows and coastal coyote brush plots versus 206 from the inland coyote brush and inland forest plots (standardized by trap effort; 540 trap nights per plot). If I normalize for habitat type and compare just the coastal and inland coyote brush plots, I see the same trend, with only 46 individuals captured on the coastal side and 100 individuals captured on the inland side. As Rose and Polis (1998) point out, however, the relative importance of bottom-up and top-down effects are difficult to decipher in a system where a subsidy could be received at multiple trophic levels.

I can assess the potential for coyote impacts on particular small mammal prey species by comparing the live community to the organisms found in the coyote scats:

a mismatch between these two records should reflect coyote dietary preferences. I saw that voles are more common in the coyote scat assemblage than they are in the live community. Prior research indicates that California vole populations are generally limited by both predation (Pearson 1966) and food availability (Lidicker 1973) while their reproductive success is determined by microhabitat differences in herbaceous vegetation (Ostfeld et al. 1985). Voles are found in a range of habitats, including grasslands, shrublands, marshes and even oak savanna (Cudworth and Kropowski 2010). Although I did not expect to capture voles in the inland forest plot, I expected them to be present in the remainder of the micro-plots; yet, I only caught voles in the two coastal micro-plots. Given that coyotes are likely less abundant on the inland side of the transect, the lack of voles in the inland coyote brush plot is more likely driven by food availability and potentially microhabitat differences rather than by predation pressure (at least from coyotes). While the two coyote brush plots are similar, the inland plot does have a higher coyote brush density than the coastal plot. Vole behavior could also be biasing the live community data; if voles are relatively more trap shy than other nocturnal small mammals they would be underrepresented in the live community. However, in their meta-analysis of live-trapping data Hammond and Anthony (2006) found that *Microtus californicus* is a trap prone rather than trap shy species, suggesting that voles are preferentially consumed by coyotes and they are therefore at the greatest risk for population depression.

The possibility also exists that other mesopredators, such as bobcats and gray foxes, are supported in greater numbers at Año Nuevo because coyotes have in part

shifted out of otherwise contested niche space (Gomez et al. 2010). The bobcat population at Año Nuevo is thriving; nearly half of the mtDNA-verified scats collected there were attributed to bobcats (Appendix 4C: Table 4.C1) and they were recorded by both the coastal and inland camera traps with higher relative abundance indices than at Younger Lagoon (Appendix 4F: Table 4.F1). Their spatial distribution along the Año Nuevo transect is also more complete than that of the coyote (Appendix 4F: Figure 4.F1). A more detailed assessment of dietary and spatial niche partitioning by coyotes and bobcats at these coastal sites will be necessary to delineate whether or not the situation at Año Nuevo is unique.

## CONCLUSION

I have shown that marine subsidies to coyotes in coastal California have a very recent onset and that coyotes are likely positively impacted by a marine subsidy where it occurs. Today, marine resources comprise about 20% of coyote diets during all seasons at Año Nuevo, where there is an active northern elephant seal rookery and an essentially constant delivery of marine resources to land through pinniped stranding inputs. Coyotes do not consume marine resources in significant enough proportions for us to detect at other modern coastal sites where marine resources are more scarce. In the past, coyotes did not consume marine foods, even at localities adjacent to mainland seal rookeries, such as Elkhorn Slough, CA (CA-MNT-234). Both past peoples and California grizzly bears, however, relied heavily on marine resources and could have prevented coyotes from gaining access to a subsidy from

the sea. Onset of heavy marine resource use by California coyotes appears to have been delayed until marine mammal populations began to recover following the MMPA and designation of marine sanctuaries.

Evidence from elsewhere in North and Central America is mounting that coyotes in other coastal areas also benefit from the sea. Coyotes are turtle egg predators in both Costa Rica (Eckrich and Owens 1995) and Florida (Atencio 1994, Lewis 1996), seabirds and shorebirds make up ~50% of coyote diets in parts of Baja California (Alvarez-Castaneda and Gonzalez-Quintero 2005), and Rose and Polis (1998) found that marine foods comprised 48% of coyote diets in another part of Baja California. At the very edge of their range in Panama, where coyotes first arrived in 1995, coyotes are observed more frequently in coastal areas than in the interior (Mendez-Carvajal and Moreno 2014). The same is true at the other end of their expanding range in Labrador; three out of the five coyote sightings reported by Chubbs and Phillips (2005) were in coastal areas. The narrative around coyote expansion has primarily invoked a combination of apex predator extirpation and deforestation as the key drivers (Ripple et al. 2013). Our results add another piece to this narrative, suggesting that release from competition in coastal areas can confer the benefit of access to a resource subsidy, making coastal routes particularly lucrative for range expansion. I suspect that marine resources are important for coyotes along their expanding edge, though future work on coyotes in Panama and Labrador is required in order to test this hypothesis.

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**APPENDIX 4A.** MODERN SITES and SCAT RESULTS

I selected three sites on the central California coast at which to quantify marine versus terrestrial resource use by modern coyotes, each with access to a slightly different suite of marine resources during different seasons. All three transect sites are bisected by coastal Highway One, but, at these locations, the highway is only a two-lane road without significant nighttime traffic. All three transects also follow both an elevation and habitat gradient: elevations increase with distance from the coast as does forest cover.

Año Nuevo State Park and Reserve is located north of Monterey Bay, ~20 miles from Santa Cruz. Año Nuevo is a haul out for California sea lions (*Zalophus californianus*) and home to a breeding colony of northern elephant seals founded in the 1960's (Le Boeuf and Panken 1977). The breeding season starts in December when females begin to arrive and give birth. After pups are weaned (~30 days) females mate and return to the sea. Most adults leave by mid-March, though individuals of all ages return later in the year to molt. The scat transect at Año Nuevo follows a gravel road on the coastal side that is restricted to park personnel and researchers needing access to the beach. There is private property directly across Highway 1 from the coastal portion of the park, so the inland portion of the transect requires a short jog up the Highway and then continues up Chalk's Road, another restricted access gravel road that ultimately connects with Big Basin State Park.

Andrew Molera State Park is located on the Big Sur coast ~25 miles south of Monterey, CA. The park is divided by the Big Sur River, which supports an annual

steelhead run. Adult steelhead in small coastal streams tend to migrate upstream from the ocean after several prolonged storms; the migration seldom begins earlier than December and may extend into May if late spring storms develop (Shapavolov and Taft, 1954). On the coastal side, the Andrew Molera transect follows the Beach Trail, a wide, well-used hiking trail extending from the main parking lot out to the beach. The inland portion follows the East Molera Trail, the first portion of which follows an old road bed that ultimately narrows to a foot path.

Finally, Younger Lagoon Reserve and Moore Creek Preserve are respectively part of the University of California Natural Reserve System and green space belonging to the City of Santa Cruz. Younger Lagoon Reserve is a Y-shaped lagoon on the south side of Highway 1, providing protected habitat for 100 species of resident and migratory birds. Moore Creek Preserve on the north side of Highway 1 has high quality coastal prairie and riparian forest habitat. The scat transect here follows a narrow footpath through Younger Lagoon, breaks for private property, then continues up a restricted access dirt road through Moore Creek Preserve. Parts of Moore Creek are grazed during the winter.

**Table 4.A1.** Stable carbon and nitrogen isotope results for mtDNA- and hair- verified coyote scats from Anu Nuevo and Younger Lagoon/Moore Creek.

| ID | Site | Species | Month | Season | $\delta^{13}C$ | $\delta^{15}N$ | C:N | Notes |
|---|---|---|---|---|---|---|---|---|
| AN0015 | ANNU | coyote | May | Spring | -27.1 | 7.3 | 6.4 | |
| AN0019 | ANNU | coyote | May | Spring | -25.4 | 9.0 | 6.9 | |
| AN0020 | ANNU | coyote | May | Spring | -27.6 | 13.8 | 7.8 | |
| AN009 | ANNU | coyote | May | Spring | -27.2 | 8.4 | 7.1 | |
| 091111AN003 | ANNU | coyote | September | Fall | -26.4 | 7.9 | 7.3 | |
| 091111AN006 | ANNU | coyote | September | Fall | -27.2 | 7.3 | 8.4 | hair-verified |
| 091111AN008 | ANNU | coyote | September | Fall | -26.6 | 6.7 | 8.6 | DNA failed, but hair-verified |
| 091111AN0012 | ANNU | coyote | September | Fall | -26.4 | 5.4 | 5.4 | hair-verified |
| 091111AN0014 | ANNU | coyote | September | Fall | -23.1 | 13.4 | 7.6 | DNA failed, but hair-verified |
| 091111AN0015 | ANNU | coyote | September | Fall | -19.8 | 17.1 | 7.3 | |
| 102212ANNU19 | ANNU | coyote | October | Fall | -27.3 | 8.1 | 15.4 | |
| 102212ANNU20 | ANNU | coyote | October | Fall | -26.8 | 4.7 | 6.7 | DNA failed, but hair-verified |
| 102212ANNU24 | ANNU | coyote | October | Fall | -26.7 | 6.2 | 6.4 | |
| 102212ANNU25 | ANNU | coyote | October | Fall | -20.6 | 4.0 | 4.9 | |
| 111411ANNU02 | ANNU | coyote | November | Fall | -22.3 | 8.7 | 6.5 | |
| 111411ANNU03 | ANNU | coyote | November | Fall | -22.7 | 13.3 | 10.4 | |
| 111411ANNU04 | ANNU | coyote | November | Fall | -26.5 | 8.9 | 7.7 | |
| 111411ANNU07 | ANNU | coyote | November | Fall | -22.5 | 14.6 | 9.6 | |
| 111411ANNU11 | ANNU | coyote | November | Fall | -22.4 | 15.1 | 7.0 | |
| 111411ANNU12 | ANNU | coyote | November | Fall | -22.5 | 13.5 | 6.6 | |
| 111411ANNU16 | ANNU | coyote | November | Fall | -25.2 | 12.3 | 7.4 | |
| 111411ANNU17 | ANNU | coyote | November | Fall | -28.0 | 11.1 | 9.7 | |
| 111411ANNU18 | ANNU | coyote | November | Fall | -27.4 | 4.9 | 6.6 | |
| 012712ANNU01 | ANNU | coyote | January | Winter | -21.4 | 12.1 | 6.4 | hair-verified |
| 012712ANNU11 | ANNU | coyote | January | Winter | -25.8 | 7.3 | 21.1 | DNAmixed, but hair-verified |
| 062412ANNU32 | ANNU | coyote | June | Summer | -22.2 | 18.0 | 6.0 | |
| 062412ANNU34 | ANNU | coyote | June | Summer | -19.7 | 15.5 | 7.8 | hair-verified |

**Table 4.A1.** Continued.

| ID | Site | Species | Month | Season | $\delta^{13}C$ | $\delta^{15}N$ | C:N | Notes |
|---|---|---|---|---|---|---|---|---|
| 062412ANNU37 | ANNU | coyote | June | Summer | -22.4 | 15.4 | 6.7 | |
| 020313ANNU03 | ANNU | coyote | February | Winter | -27.7 | 12.9 | 7.7 | |
| 020313ANNU09 | ANNU | coyote | February | Winter | -26.0 | 13.3 | 7.9 | |
| 091211MC1 | YLMC | coyote | September | Fall | -27.5 | 7.7 | 7.0 | hair-verified |
| 091211MC4 | YLMC | coyote | September | Fall | -27.7 | 7.9 | 7.0 | hair-verified |
| 091211MC5 | YLMC | coyote | September | Fall | -28.8 | 8.0 | 9.4 | hair-verified |
| 091211MC6 | YLMC | coyote | September | Fall | -27.9 | 6.9 | 7.9 | hair-verified |
| 111511YLMC08 | YLMC | coyote | November | Fall | -26.8 | 8.8 | 6.8 | |
| 111511YLMC14 | YLMC | coyote | November | Fall | -25.7 | 7.2 | 10.1 | |
| 012612YLMC09 | YLMC | coyote | January | Winter | -26.8 | 6.9 | 8.5 | |
| 012612YLMC11 | YLMC | coyote | January | Winter | -29.6 | 9.9 | 11.3 | |
| 012612YLMC17 | YLMC | coyote | January | Winter | -20.1 | 5.8 | 11.5 | |
| 012612YLMC19 | YLMC | coyote | January | Winter | -23.2 | 7.0 | 10.6 | |
| 012612YLMC22 | YLMC | coyote | January | Winter | -29.0 | 8.3 | 9.0 | |
| 041412YLMC09 | YLMC | coyote | April | Spring | -27.8 | 7.9 | 7.3 | |
| 041412YLMC15 | YLMC | coyote | April | Spring | -29.5 | 8.9 | 7.5 | |
| 062612YLMC14 | YLMC | coyote | June | Summer | -27.5 | 5.5 | 13.4 | |

**APPENDIX 4B.** HOLOCENE SITES AND HISTORICAL SAMPLES

**CA-SCR-35 – Red, White and Blue Beach Site**

CA-SCR-35 is located about 4.8 km south of Davenport, CA in the northernmost reaches of Monterey Bay. Dating to 2870 – 2970 BP, this is the oldest of the sites we worked with. Under the direction of Diane Gifford-Gonzalez, the bulk of the site was analyzed as a practicum in archaeological laboratory techniques by students at UCSC. Northern fur seals are present and account for about 11% of the number of identifiable specimens (NISP = 19). Carnivores are relatively scarce in this assemblage, but I was able to analyze 2 coyote bones and one grizzly bear mandible.

**CA-MNT- 234 – Moss Landing Hill Site**

CA-MNT-234 is located on a stabilized sand dune very near the junction of Elkhorn Slough and the Monterey Submarine Canyon, at the center of the Monterey Bay shoreline. The primary midden deposit is more than 3 m deep and covers an area of ~16,500 $m^2$ (Gifford-Gonzalez and Sunseri 2009). Direct Accelerator Mass Spectrometry (AMS) radiocarbon dating on bones from the site combined with the re-analysis of previous radiocarbon dates on single shells suggest that the primary midden deposit represents just a few hundred years between 2300-2700 BP (Newsome et al. 2007). According to Gifford-Gonzalez (2011), 50% of non-rodent mammal bones in the primary midden are Northern fur seal (NISP = 2334). I analyzed 12 coyote specimens from this site.

**CA-MNT-229 – Vierra Site, Elkhorn Slough**

CA-MNT-229 is an extensive shell midden (230 cm maximum thickness) on the south bank of Elkhorn Slough near its present outlet to the sea at the southern side of the Hwy. 1 bridge, about 1.6 km northeast of MNT-234. It contains faunal and artifactual constituents typical of Central California coast estuarine shell middens, including an abundance of mussel, clam, and oyster shell fragments as well as vertebrate remains (Jones and Jones 1992). Radiocarbon dates from shells, charcoal, and collagen suggest primary site occupation dates between 900-2700 BP, as well as an older component that dates from 6000-8200 BP (Jones and Jones 1992, 2002). The Elkhorn Slough site also contains northern fur seal remains, but significantly fewer than at CA-MNT-234 (NISP = 114) (Gifford-Gonzalez 2011). We were able to analyze a number of different predator specimens from this site, including 4 from coyotes, 6 bobcat, one mountain lion and one grizzly bear.

**CA-SMA-18 – Point Año Nuevo**

CA-SMA-18 is located at Point Año Nuevo and dates to 1070-1480 BP (Newsome et al. 2007). The site was on a stabilized sand dune and required a rapid-recovery salvage excavation because elephant seal traffic was causing site erosion. Ten percent of the identifiable bones at SMA-18 are from northern fur seals (NISP = 111), including those of adult males, females, juveniles, and young-of-the-year (Gifford-Gonzalez 2011). The site represents a relatively short period of occupation, likely less than 200 years (Boone 2012). Just two coyote specimens from this site

were suitable for analysis.

**CA-SMA-113 – Quiroste Valley**

CA-SMA-113 is a more recently occupied site located in Quiroste Valley along the western edge of the Santa Cruz Mountains within Año Nuevo State Park. Northern fur seals make up only ~3% of its identifiable elements (NISP = 11). Their appearance in the SMA-113 fauna at all is of interest, however, because SMA-113 derives from a later period, 880-940 BP, which in part spans the Medieval Climatic Anomaly, a period of transient warm climate, particularly in North America (Gifford-Gonzalez 2011). Of the two coyote specimens we analyzed from this site, only one had sufficiently well preserved collagen.

**CA-SMA-115 – Montara State Beach Site**

CA-SMA-115 is located on the coastal terrace at Montara State Beach on property under the jurisdiction of the California Department of Parks and Recreation. It is both the youngest and northernmost site we considered. Excavation of the site ensued in 1983 after severe El Niño storms washed away some of the terrace and threatened to wash away the site entirely. The site dates to 575 – 835 BP based on a single calibrated radiocarbon date from a *Mytilus* shell (Hylkema 1991). In 2009, the site was re-excavated in a rapid recovery effort to restabilize the erosion front. At 57.29% of mammalian NISP, sea otters dominate the faunal assemblage (NISP = 55).

I sampled four coyote specimens from the second excavation (Gifford-Gonzalez 2010).

**Historical Samples**

I obtained historical coyote and grizzly bear bone and fur samples from the California Academy of Sciences. I chose specimens from coastal counties that roughly span the period of grizzly bear extirpation in California. The last hunted California grizzly was shot in 1922 in Tulare County, CA and the last recorded California grizzly bear sighting was in 1924 in the southern Sierras (Storer and Tevis 1996). Of the five grizzly bear specimens I obtained from the Cal Academy, only one has a known age (CAS 129, collected in 1909); the other specimens were all collected post grizzly extirpation.

**Table 4.B1.** Historical coyote and grizzly bear specimens acquired from the California Academy of Sciences for isotopic analysis.

| Sample ID | Species | Material Sampled | County | Locality | Year Collected |
|---|---|---|---|---|---|
| CAS 20549 | Canis latrans | nasal turbinates | Lake County | Lakeport | 1893 |
| CAS 6431 | Canis latrans | skull fragment | San Mateo County | unspecified | 1894 |
| CAS 263 | Canis latrans | hair | San Mateo County | Portola | 1908 |
| CAS 76 | Canis latrans | nasal turbinates | Kern County | Buttonwillow | 1909 |
| CAS 92 | Canis latrans | hair | Santa Barbara County | Cuyama Range | 1909 |
| CAS 120 | Canis latrans | hair | Santa Barbara County | Cuyama Range | 1909 |
| CAS 165 | Canis latrans | hair | Mendocino County | Sherwood | 1910 |
| CAS 928 | Canis latrans | hair | Santa Cruz County | Summit of Santa Cruz Mountains | 1913 |
| CAS 955 | Canis latrans | hair | Contra Costa County | Lafayette | 1914 |
| CAS 1328 | Canis latrans | hair | Humboldt County | Weitchpec | 1916 |
| CAS 1329 | Canis latrans | hair | Humboldt County | Weitchpec | 1916 |
| CAS 1128 | Canis latrans | hair | Alameda County | Warm Springs | 1916 |
| CAS 8115 | Canis latrans | fragment | Monterey County | Military reservation near Monterey | 1939 |
| CAS 12633 | Canis latrans | nasal turbinates | Santa Cruz County | Quail Hollow Rd., 2 miles east Ben Lomond | 1961 |
| CAS 21220 | Canis latrans | skull fragment | Marin County | 11.3 miles east of Highway 1 | 1978 |
| CAS 23923 | Canis latrans | fragment | Solano County | Grizzly Id. State Wildlife Area | 1991 |
| CAS 129 | Ursus arctos | nasal turbinates | Santa Barbara County | Cuyama Plain | 1909 |
| CAS 5567 | Ursus arctos | nasal turbinates | Monterey County | Marina P.O. | unknown |
| CAS 9377 | Ursus arctos | skull fragment | Santa Clara County | Indian Mound, San Antonio Road, 2-1/2 miles SE Mayfield | 1948 |
| CAS 24360 | Ursus arctos | mandible | San Francisco County | Under house at #36 Broderick St. | 1999 |
| CAS 27342 | Ursus arctos | scapula | San Francisco County | San Francisco, Golden Gate Park, near Steinhart Aquarium. | 1973 |

**Table 4.B2.** Stable carbon and nitrogen isotope results for archaeological and historical coyote and grizzly bear specimens. These values are not corrected for trophic fractionation or for the Suess effect.

| Site | Sample ID | Species | $\delta^{13}C$ | $\delta^{15}N$ |
|---|---|---|---|---|
| SCR-35 | 100213 | *Canis latrans* | -19.3 | 9.1 |
| SCR-35 | 101882 | *Canis latrans* | -20.1 | 9.8 |
| SCR-35 | 101892 | *Ursus arctos* | -20.5 | 5.5 |
| MNT-234 | 3406 | *Canis latrans* | -20.2 | 5.7 |
| MNT-234 | 1697 | *Canis latrans* | -20.0 | 6.1 |
| MNT-234 | 1817 | *Canis latrans* | -19.8 | 6.2 |
| MNT-234 | 1761 | *Canis latrans* | -20.3 | 7.5 |
| MNT-234 | 1701 | *Canis latrans* | -18.8 | 4.0 |
| MNT-234 | 3411 | *Canis latrans* | -20.3 | 7.2 |
| MNT-234 | 1891 | *Canis latrans* | -18.0 | 9.9 |
| MNT-234 | 1200 | *Canis latrans* | -19.0 | 5.6 |
| MNT-234 | 1699 | *Canis latrans* | -18.7 | 7.5 |
| MNT-234 | 3409 | *Canis latrans* | -19.1 | 4.9 |
| MNT-234 | 1836 | *Canis latrans* | -19.5 | 6.7 |
| MNT-234 | 1703 | *Canis latrans* | -19.8 | 6.2 |
| MNT-229 | 836 | *Canis latrans* | -19.4 | 6.7 |
| MNT-229 | 856 | *Canis latrans* | -19.3 | 6.7 |
| MNT-229 | 1272 | *Canis latrans* | -20.2 | 7.7 |
| MNT-229 | 1222 | *Canis latrans* | -22.7 | 7.5 |
| MNT-229 | 1242 | *Ursus arctos* | -12.8 | 18.0 |
| SMA-18 | E030-024 | *Canis latrans* | -19.8 | 8.2 |
| SMA-18 | E144-009 | *Canis latrans* | -19.9 | 7.9 |
| SMA-113 | 1259 | *Canis latrans* | -20.3 | 7.9 |
| SMA-115 | 142 | *Canis latrans* | -21.4 | 4.9 |
| SMA-115 | 109 | *Canis latrans* | -18.0 | 7.9 |
| Lake Co. | CAS 20549 | *Canis latrans* | -20.0 | 9.8 |
| Monterey Co. | CAS 6431 | *Canis latrans* | -20.2 | 9.9 |
| San Mateo Co. | CAS 263 | *Canis latrans* | -22.4 | 8.4 |
| Kern Co. | CAS 76 | *Canis latrans* | -18.8 | 12.9 |
| Santa Barbara Co. | CAS 92 | *Canis latrans* | -19.8 | 7.7 |
| Santa Barbara Co. | CAS 120 | *Canis latrans* | -20.0 | 9.0 |
| Mendocino Co. | CAS 165 | *Canis latrans* | -20.7 | 5.9 |
| Santa Cruz Co. | CAS 928 | *Canis latrans* | -21.2 | 7.2 |
| Contra Costa Co. | CAS 955 | *Canis latrans* | -21.6 | 7.7 |
| Humboldt Co. | CAS 1328 | *Canis latrans* | -21.9 | 8.5 |
| Humboldt Co. | CAS 1329 | *Canis latrans* | -21.9 | 8.5 |
| Alameda Co. | CAS 1128 | *Canis latrans* | -21.5 | 7.9 |
| Monterey Co. | CAS 8115 | *Canis latrans* | -22.0 | 6.9 |
| Santa Cruz Co. | CAS 12633 | *Canis latrans* | -19.5 | 6.9 |
| Marin Co. | CAS 21220 | *Canis latrans* | -21.5 | 7.8 |
| Solano Co. | CAS 23923 | *Canis latrans* | -21.8 | 12.2 |
| Santa Barbara Co. | CAS 129 | *Ursus arctos* | -18.2 | 4.6 |

| | | | | |
|---|---|---|---|---|
| Monterey Co. | CAS 5567 | *Ursus arctos* | -18.7 | 6.2 |
| Santa Clara Co. | CAS 9377 | *Ursus arctos* | -21.4 | 3.7 |
| San Francisco Co. | CAS 24360 | *Ursus arctos* | -17.0 | 14.0 |
| San Francisco Co. | CAS 27342 | *Ursus arctos* | -18.5 | 9.6 |

**APPENDIX 4C.** DNA SAMPLING

I conducted fecal genotyping in collaboration with Wildlife Genetics International (WGI). As per their recommendations, prior to being dried, I swabbed the scats with Q-tips, which were then stored dried in unwaxed coin envelopes. DNA is extracted by clipping a small (~3 mm x 3 mm) piece of each swab and processing the clippings as tissue samples using QIAGEN DNeasy Blood and Tissue Kits. The species test is a sequence-based analysis of the mitochondrial 16S rRNA gene (Johnson and O'Brien 1997). The specific primers and analytic conditions that Wildlife Genetics uses are not published, but their results could be reproduced using published methodology. Two variants of this analysis are employed using either primers that amplify across all mammals or primers designed to amplify Carnivora sequence in preference to other mammals. The results are compared to reference data from over 125 species of mammals.

**Table 4.C1.** WGI results by site and species.

| Species | Año Nuevo | Younger Lagoon | Total |
|---|---|---|---|
| bobcat | 55 | 9 | 64 |
| coyote | 20 | 10 | 30 |
| gray fox | 28 | - | 28 |
| spotted skunk | 1 | - | 1 |
| mixed | 2 | - | 2 |
| failed | 9 | 1 | 10 |
| Total | 115 | 20 | 135 |

**APPENDIX 4D.** SIAR MIXING MODELS

## Modern Samples

**Table 4.D1.** SIAR mixing model predictions for coyote diets derived from 30 verified Año Nuevo coyote scats and 14 verified Younger Lagoon/Moore Creek coyote scats.

| **Año Nuevo** | | | | |
|---|---|---|---|---|
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Berries | 0.000 | 0.236 | **0.023** | 0.103 |
| Deer | 0.000 | 0.268 | **0.109** | 0.127 |
| Pinnipeds | 0.122 | 0.326 | **0.220** | 0.223 |
| Voles | 0.000 | 0.168 | **0.014** | 0.064 |
| Woodrats | 0.000 | 0.214 | **0.018** | 0.089 |
| *P. boylii* and *P. maniculatus* | 0.000 | 0.291 | **0.136** | 0.148 |
| *P. californicus* | 0.003 | 0.334 | **0.178** | 0.177 |
| Rabbits | 0.000 | 0.180 | **0.014** | 0.070 |
| SD1 | 1.89 | 3.42 | **2.53** | 2.62 |
| SD2 | 2.86 | 5.16 | **3.80** | 3.95 |
| **Younger Lagoon/Moore Creek** | | | | |
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Berries | 0.000 | 0.254 | **0.023** | 0.116 |
| Deer | 0.024 | 0.392 | **0.197** | 0.213 |
| Voles | 0.000 | 0.168 | **0.014** | 0.064 |
| Rabbits | 0.000 | 0.297 | **0.144** | 0.147 |
| Woodrats | 0.000 | 0.288 | **0.143** | 0.143 |
| *P. boylii* | 0.000 | 0.193 | **0.015** | 0.076 |
| *P. californicus* | 0.000 | 0.324 | **0.171** | 0.168 |
| Jerusalem cricket | 0.000 | 0.112 | **0.009** | 0.040 |
| SD1 | 1.71 | 4.03 | **2.53** | 2.78 |
| SD2 | 0.00 | 1.72 | **0.77** | 0.85 |

**Holocene Samples**

**Table 4.D2.** SIAR mixing model predictions for coyote diets derived from Holocene coyote bone collagen samples. Because of small sample sizes, we ran the models individually for each coyote bone from sites SCR-35, SMA-113, and SMA-115. For those sites, we simply averaged the results together for an overall coyote diet prediction.

| **CA-SCR-35** | | | | |
|---|---|---|---|---|
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Ungulate | 0.002 | 0.380 | **0.211** | 0.197 |
| Leporid | 0.002 | 0.380 | **0.221** | 0.200 |
| Plants | 0.060 | 0.397 | **0.238** | 0.233 |
| Pinniped | 0.093 | 0.252 | **0.174** | 0.173 |
| Gopher | 0.001 | 0.383 | **0.214** | 0.197 |
| **CA-MNT-234** | | | | |
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Ungulate | 0.039 | 0.638 | **0.319** | 0.345 |
| Leporid | 0.028 | 0.599 | **0.294** | 0.323 |
| Plants | 0.000 | 0.287 | **0.026** | 0.121 |
| Pinniped | 0.000 | 0.088 | **0.009** | 0.035 |
| Marine Fish | 0.000 | 0.089 | **0.010** | 0.038 |
| Gopher | 0.000 | 0.352 | **0.025** | 0.139 |
| SD1 | 0.00 | 1.04 | **0.49** | 0.54 |
| SD2 | 1.03 | 3.68 | **1.91** | 2.25 |
| **CA-MNT-229** | | | | |
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Ungulate | 0.000 | 0.449 | **0.256** | 0.237 |
| Leporid | 0.007 | 0.479 | **0.267** | 0.258 |
| Plants | 0.005 | 0.205 | **0.067** | 0.097 |
| Pinniped | 0.000 | 0.314 | **0.078** | 0.150 |
| Gopher | 0.007 | 0.480 | **0.258** | 0.258 |
| SD1 | 0.50 | 6.27 | **1.62** | 2.75 |
| SD2 | 0.00 | 2.74 | **0.17** | 0.90 |

**Table 4.D2. Continued.**

| CA-SMA-18 | | | | |
|---|---|---|---|---|
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Ungulate | 0.000 | 0.320 | **0.140** | 0.164 |
| Leporid | 0.001 | 0.351 | **0.152** | 0.185 |
| Plants | 0.008 | 0.354 | **0.198** | 0.189 |
| Pinniped | 0.000 | 0.153 | **0.025** | 0.066 |
| Gopher | 0.000 | 0.338 | **0.237** | 0.177 |
| Wood rat | 0.001 | 0.324 | **0.202** | 0.167 |
| Sea otter | 0.000 | 0.141 | **0.010** | 0.052 |
| SD1 | 0.00 | 20.23 | **0.23** | 7.48 |
| SD2 | 0.00 | 12.81 | **0.26** | 3.57 |
| **CA-SMA-113** | | | | |
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Ungulate | 0.000 | 0.405 | **0.238** | 0.207 |
| Leporid | 0.004 | 0.456 | **0.252** | 0.242 |
| Plants | 0.009 | 0.454 | **0.264** | 0.248 |
| Marine Fish | 0.000 | 0.138 | **0.014** | 0.056 |
| Wood rat | 0.000 | 0.398 | **0.200** | 0.206 |
| Sea otter | 0.000 | 0.100 | **0.010** | 0.041 |
| **CA-SMA-115** | | | | |
| Source | Low 95% hdr | High 95% hdr | mode | mean |
| Ungulate | 0.000 | 0.466 | **0.158** | 0.204 |
| Plants | 0.068 | 0.513 | **0.249** | 0.285 |
| Pinniped | 0.000 | 0.151 | **0.013** | 0.061 |
| Gopher | 0.078 | 0.651 | **0.353** | 0.370 |
| Sea otter | 0.001 | 0.158 | **0.085** | 0.079 |

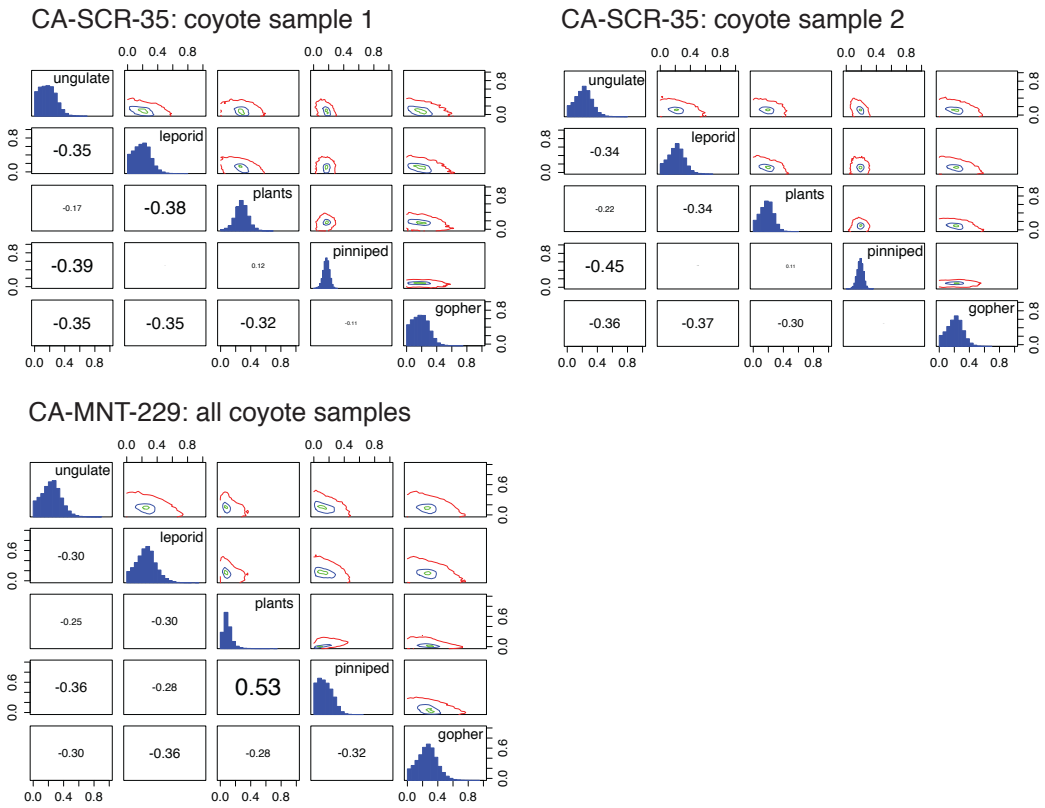


**Figure 4.D1.** Matrix plots for the two archaeological sites for which the mixing models are suggesting that coyotes were consuming marine foods. Since there are only two samples from CA-SCR-35, we ran the model separately for each sample. The posterior correlation coefficients are displayed in the lower left hand portion of each set of plots; they are scaled by strength. For both coyotes from CA-SCR-35, there is a strong negative correlation between pinnipeds and ungulates. This is because, after pinnipeds, ungulates have the highest $\delta^{15}N$ and $\delta^{13}C$ values of the possible dietary sources, so solutions that include ungulates do not need to rely as heavily on pinnipeds and vice-a-versa. Because the two coyote samples have $\delta^{15}N$ values that are higher than any of the possible terrestrial sources, the model requires some amount of pinniped to resolve these values. Given that the higher $\delta^{15}N$ values are not accompanied by any increase in $\delta^{13}C$, it seems most likely that we are missing a higher trophic level terrestrial food source (e.g. *Peromyscus californicus*), which would negate any need for the model to rely on pinnipeds to explain the coyotes. The situation at CA-MNT-229 is similar; pinnipeds are negatively correlated with all of the terrestrial meat sources. Here, the model is fairly certain that pinnipeds aren't part of the diet (the histogram is strongly skewed), but what those negative correlations tell us is that solutions that rely less heavily on any of the terrestrial meat sources then require the inclusion of a bit more pinniped.

**APPENDIX E.** SMALL-MAMMAL TRAPPING AND LIVE-DEAD COMPARISON

**Modern small-mammal trapping surveys**

I conducted small mammal trapping surveys seasonally in 2012-2013 in four habitats along our scat collection transect at Año Nuevo (Figure 4.E1). In order of increasing distance from the point, these habitats included (1) coastal willows – a dune environment with willow clumps and ephemerally wet areas; (2) coastal coyote brush – a grassy and brambly field pockmarked with large clumps of coyote brush and poison oak; (3) inland coyote brush – similar to its coastal counterpart with a slightly higher density of large coyote brush clumps; and (4) inland forest – a mixed evergreen forest with an open understory.

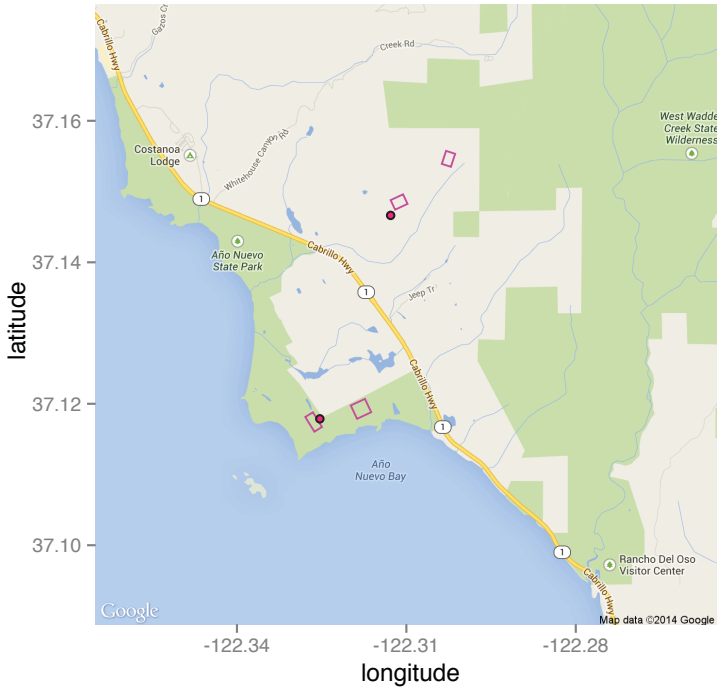


**Figure 4.E1.** Small mammal trapping plots at Año Nuevo State Park. Plots are outlined in pink. From left to right, the plots include (a) coastal willows, (b) coastal coyote brush, (c) inland coyote brush and (d) inland forest. Pink dots mark the location of my trail cameras.

My trapping surveys targeted the nocturnal small mammal community, as coyotes largely hunt at night (Fox 1975) and because our initial scat content data suggested that nocturnal small mammals such as wood rats, mice and voles were important to coyote diets. Trapping surveys lasted three nights apiece, with traps set in the early evening and checked in the early morning (2160 trap nights in total). I followed a targeted transect approach (Wilson et al. 1996), setting 30 Sherman live traps spaced ~10-15 m apart in each microhabitat. Traps were set in the same areas (though not necessarily in exactly the same positions) in each of the survey periods. I baited the traps with oats alone, as I was concerned that repeated peanut butter consumption could bias small mammal hair stable isotope values. Captured animals were tagged (self-piercing ear tags model 1005-1; National Band and Tag Company, Newport, Kentucky) and I sampled their fur for isotopic analysis. I occasionally caught one diurnal species (Merriam's chipmunk, *Tamias merriami*), which I excluded from further analysis because it's not a member of the nocturnal small-mammal community. A number of possible biasing factors make trapping an imperfect means of assessing the local small-mammal community (e.g. Jaksic et al. 1999; Torre et al. 2004). For example, differential trapability can be caused by trap or bait type, the spatial arrangement of traps, or by species specific differences in relative trap shyness (Pizzimenti 1979; Drickamer and Mikesic 1993; Wilson et al. 1996). Still, most small mammal census data are collected via trapping (Wilson et al. 1996) and, by taking this approach, my data become comparable with many other studies. I conducted trapping in compliance with the most recent American Society of

Mammalogists' guidelines (Sikes and Gannon 2011) and with the approval of both the University of California Santa Cruz Animal Care and Use Community and State of California (Department of Fish and Game scientific collecting permit 11995 to R. Reid).

**Coyote scat small-mammal archive**

To quantify the scat-bound "dead" community, I identified small-mammal craniomandibular elements and guard hairs to genus (and when possible to species) by comparison with reference materials. I focused on the DNA-verified coyote scats. I calculated the minimum number of individuals (MNI) of each species in a sample by incorporating information on side (left vs. right) and age (juvenile vs. adult). Because my modern survey data is restricted to the nocturnal small mammal community, I also restricted the coyote scat community data to the nocturnal small mammal community for the purposes of this comparison. I pooled data by scat collection periods (Table 4.E2).

**Live-Dead Comparison**

In order to evaluate coyote dietary preferences, I chose to take a live-dead approach (Terry 2010a; b), comparing the modern live community with the scat-bound "dead" community; differences can then be attributed to predator selectivity. I compared community richness (number of nocturnal small-mammal species), evenness (uniformity of the distribution of taxonomic abundances), taxonomic composition, and species abundances (rank and proportional). Following Terry

(2010a; b), I used rarefaction to compare live and dead species richness values, Probability of Interspecific Encounter (PIE; Hurlbert 1971) to summarize community evenness, the Jaccard similarity index to assess agreement between live and dead species lists, nonparameteric Spearman rank correlation tests to assess live-dead agreement in rank abundance of species, and finally the Bray-Curtis similarity index to assess live-dead agreement when proportional abundances of species are also considered. I performed all of these calculations in R using the vegan package (Oksanen et al. 2013). I compared live survey periods with scats collected during the same periods. Some scats were collected prior to initiating the live survey and these I paired with live surveys from similar seasons but different years for the sake of comparison.

I also calculated Strauss's linear index, an index of prey selectivity in which $L = r_i - p_i$, where $r_i$ is the relative occurrence of prey item $i$ in a scat sample and $p_i$ is the relative abundance of prey item $i$ in the live community, both expressed as proportions (Strauss 1979). This index is linear across all values between +1 and -1 and is less affected by small sample sizes than Ivlev's elevtivity Index (Strauss 1979).

**Table 4.E1**. Modern live data from repeat trapping surveys at Año Nuevo State Park. Results are shown for each of the four microplots and summed across all sampling localities in each trapping session.

| Species | April 2012 | June 2012 | October 2012 | February 2013 | May 2013 | August 2013 |
|---|---|---|---|---|---|---|
| **Año Nuevo Inland Forest** | | | | | | |
| *Neotoma fuscipes* | - | 2 | 1 | 1 | 2 | 4 |
| *Peromyscus boylii* | 8 | 4 | 7 | 2 | 4 | 2 |
| *Peromyscus californicus* | 13 | 7 | 10 | 7 | 13 | 12 |
| *Sorex trowbridgii* | 1 | 2 | 4 | - | - | - |
| **Año Nuevo Inland Coyote Brush** | | | | | | |
| *Neotoma fuscipes* | 2 | 3 | 5 | - | 3 | 6 |
| *Peromyscus boylii* | 1 | - | 1 | - | - | - |
| *Peromyscus californicus* | 10 | 6 | 3 | 8 | 9 | 4 |
| *Peromyscus maniculatus* | 2 | 1 | 3 | 5 | 4 | 4 |
| *Rattus norvegicus* | - | 1 | - | - | - | - |
| *Reithrodontomys megalotis* | 5 | 2 | 4 | 2 | - | 2 |
| *Sorex trowbridgii* | - | - | 1 | - | - | - |
| *Tamias merriami* | 2 | - | - | - | - | 1 |
| **Año Nuevo Coastal Coyote Brush** | | | | | | |
| *Microtus californicus* | - | 9 | 1 | - | - | - |
| *Peromyscus maniculatus* | 8 | 3 | - | 2 | - | 10 |
| *Reithrodontomys megalotis* | 5 | 3 | 3 | 2 | - | - |
| **Año Nuevo Coastal Willows** | | | | | | |
| *Microtus californicus* | 1 | 1 | 2 | 1 | 1 | - |
| *Peromyscus californicus* | 6 | 5 | 4 | 4 | - | - |
| *Peromyscus maniculatus* | 7 | 7 | 23 | 16 | 13 | 15 |
| *Reithrodontomys megalotis* | 3 | 5 | - | - | - | 2 |
| **Totals Across All Plots** | | | | | | |
| *Microtus californicus* | 1 | 10 | 3 | 1 | 1 | 0 |
| *Neotoma fuscipes* | 2 | 5 | 6 | 1 | 5 | 10 |
| *Peromyscus boylii* | 9 | 4 | 8 | 2 | 4 | 2 |
| *Peromyscus californicus* | 29 | 18 | 17 | 19 | 22 | 16 |
| *Peromyscus maniculatus* | 17 | 11 | 26 | 23 | 17 | 29 |
| *Rattus norvegicus* | - | 1 | - | - | - | - |
| *Reithrodontomys megalotis* | 13 | 10 | 7 | 4 | - | 4 |
| *Sorex trowbridgii* | 1 | 2 | 5 | - | - | - |
| *Tamias merriami* | 2 | - | - | - | - | 1 |

**Table 4.E2.** Scat-bound "dead" data for the nocturnal small mammal community at Año Nuevo State Park. Results are shown for DNA-verified coyote scats from each scat collection period.

| Species | May 2011 | September 2011 | November 2011 | April 2012 | June 2012 | October 2012 | Total |
|---|---|---|---|---|---|---|---|
| *Microtus californicus* | 6 | 1 | 3 | 0 | 5 | 0 | 15 |
| *Neotoma fuscipes* | 0 | 0 | 0 | 1 | 0 | 1 | 2 |
| *Peromyscus* spp | 1 | 1 | 3 | 2 | 1 | 0 | 7 |
| *Rattus norvegicus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Reithrodontomys megalotis* | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| *Sorex trowbridgii* | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 7 | 2 | 8 | 3 | 6 | 1 | 27 |

**APPENDIX 4F.** COYOTE ABUNDANCE MEASURES

Two lines of evidence suggest that the coyote population is relatively greater where animals are receiving a marine subsidy. First, in the fall of 2012 through summer of 2013, I regularly placed two camera traps along the Año Nuevo transect, one on the coastal portion and one on the inland portion (See Figure 4.E1). Coyote activity was only recorded by the more coastal of these two cameras, where the average relative abundance index is 0.0077 (Table 4.F1). A camera trap set up on the coastal portion of the Younger Lagoon transect during some of the same sampling periods had an average relative abundance index for coyotes of just 0.0021. Second, mtDNA-verified coyote scats collected at Año Nuevo consistently occurred in greater abundance on the coastal side of the transect (Figure 4.F1).

**Table 4.F1.** Photographic count statistics for mammalian predators caught by the Año Nuevo and Younger Lagoon camera traps. Cameras were placed in the same locations for each sampling period. I attached them low to the ground on trees adjacent to the transects and set them to take three photos in quick succession each time they were triggered. Values are reported as the relative abundance index: Number of events / [Number of trap nights) x 100]. One event is equal to a 3-photo set.

| Predator species | November 2012 | February 2013 | May 2013 | August 2013 | September 2013 | October 2013 | Mean |
|---|---|---|---|---|---|---|---|
| **Año Nuevo Inland Camera** | | | | | | | |
| coyote | 0 | 0 | 0 | 0 | na | na | 0 |
| bobcat | 0.00444 | 0.00438 | 0.01100 | 0.00067 | na | na | 0.0051 |
| gray fox | 0 | 0 | 0 | 0 | na | na | 0 |
| mountain lion | 0 | 0 | 0.00400 | 0 | na | na | 0.0010 |
| **Año Nuevo Coastal Camera** | | | | | | | |
| coyote | 0.00889 | 0.00714 | 0.00867 | 0.00600 | na | na | 0.0077 |
| bobcat | 0 | 0.00333 | 0.00400 | 0.00200 | na | na | 0.0023 |
| gray fox | 0 | 0 | 0 | 0 | na | na | 0 |
| mountain lion | 0 | 0 | 0 | 0 | na | na | 0 |
| **Younger Lagoon Coastal Camera** | | | | | | | |
| coyote | na | na | 0 | 0 | 0.00200 | 0.00630 | 0.0021 |
| bobcat | na | na | 0.00250 | 0.00033 | 0.00033 | 0.00037 | 0.0009 |
| gray fox | na | na | 0 | 0 | 0 | 0 | 0 |
| mountain lion | na | na | 0 | 0 | 0 | 0 | 0 |

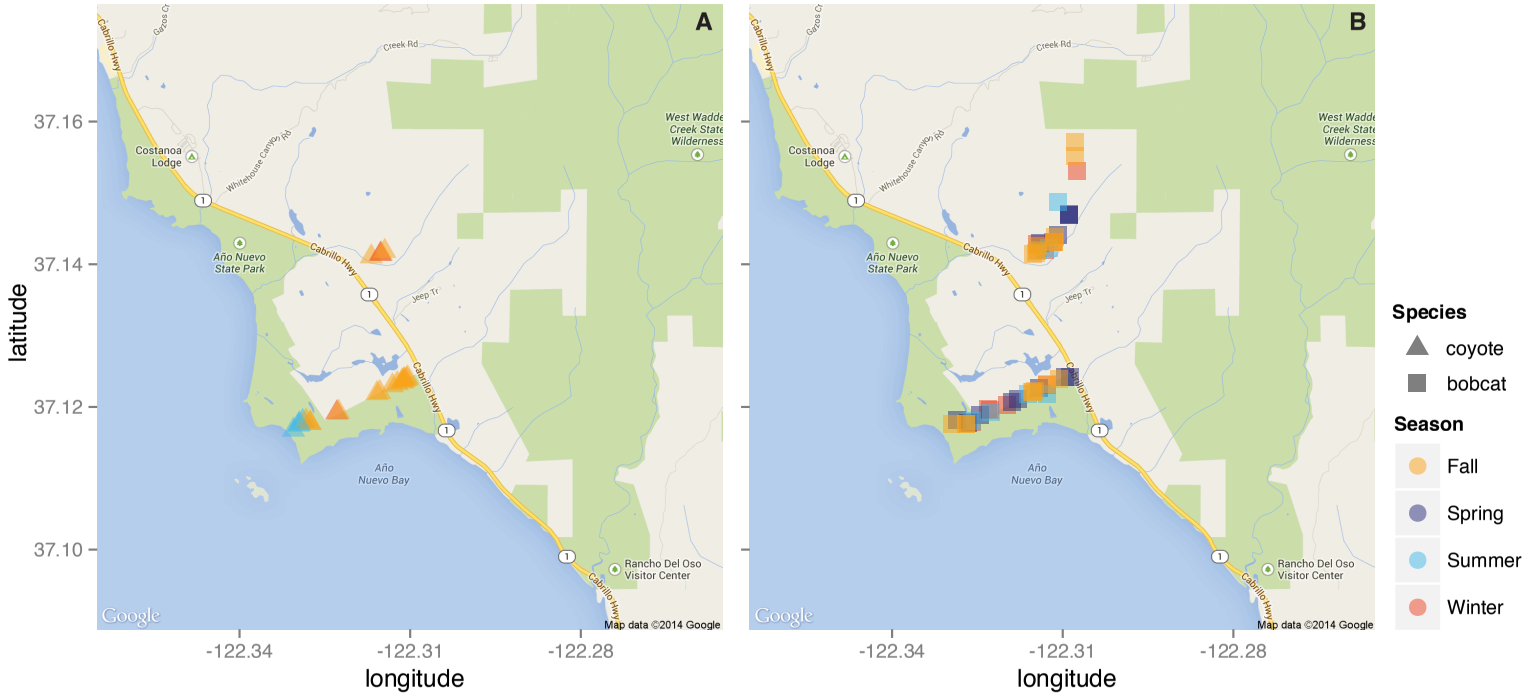


**Figure 4.F1.** Spatial distribution of DNA-verified coyote (A) and bobcat (B) scats along the length of our Año Nuevo transect. Samples are color-coded by the season in which they were collected.