Metabarcoding of fecal DNA shows dietary diversification in wolves substitutes for ungulates in an island archipelago

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Abstract. Although ungulates are the main prey of wolves (Canis lupus) throughout their range, substantial dietary diversity may allow wolves to persist even when ungulates are declining or rare. Alexander Archipelago wolves (Canis lupus ligoni) inhabit distinct mainland and island biogeographic units, each with a unique assemblage of available prey. We quantified biogeographic variability in wolf diets across the archipelago using DNA metabarcoding of prey in 860 wolf scats collected during 2010–2018 in 12 study sites. We hypothesized that wolves would increase their dietary diversity and niche breadth as the proportion of ungulate species in their diets decreased, but that this could be mediated by the availability of coastal resources. Application of DNA metabarcoding achieved fine taxonomic resolution of prey remains and identified 55 diet items representing species from 42 genera and 29 families, many previously undetected in coastal wolf diets. Overall, ungulates made up the largest proportion of wolf diets but were also most variable between study sites (occurrence per item index [O/I] = 0.130–0.851). On islands, Sitka black-tailed deer (Odocoileus hemionus) were the most consumed ungulate species, whereas moose (Alces alces) and mountain goats (Oreamnos americanus) contributed more to mainland wolf diets. Wolves responded to biogeographical variation in availability of their primary prey by altering their foraging patterns. Wolves increased the number and diversity of species consumed and widened their dietary niche as the proportion of ungulates in their diet declined rather than prey switch to one or few individual diet items. Across all study sites combined, beaver (Castor canadensis; O/I = 0.125), marine mammals (O/I = 0.113), and black bears (Ursus americanus; O/I = 0.067) were important alternate prey. In areas where ungulates had become scarce, sea otters (Enhydra lutris) were particularly important, in one case even becoming the primary diet item suggesting that the ongoing expansion of sea otter populations postreintroduction restores an important food source for these cryptic predators. Here, we show extensive variation in the diet of wolves and elucidate regional consumer–resource interactions across an archipelagic landscape.

Key words: Alexander Archipelago; Canis lupus; dietary niche breadth; Enhydra lutris; foraging ecology; non-invasive sampling; predator–prey ecology; scat.

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INTRODUCTION

Ungulates are the primary prey of wolves throughout their range (Paquet and Carbyn 2003, Peterson and Ciucci 2003, Mech and Peterson 2003), and individual ungulate species constitute between 25% and 74% of wolf diets on average across North America (Newsome et al. 2016). The observed increase in wolf densities with ungulate biomass has led to suggestions that wolf populations are only meaningfully limited by ungulate availability (Messier 1994, Fuller et al. 2003, McRoberts and Mech 2014). Despite their strong association with ungulates, wolves
use a wide variety of alternate prey including beaver (*Castor canadensis*), small mammals, mus-telids, birds, bears, and plants that may supple-ment wolves when ungulates are scarce or less vulnerable (Okarma 1995, Paquet and Carbyn 2003, Peterson and Ciucci 2003, Newsome et al. 2016).

The contribution of alternate prey to wolf diets is spatially heterogenous with some populations exploiting alternate prey at high rates. This may particularly be the case in coastal regions where fish and marine mammals are important alter-nate prey (Darimont et al. 2004, Newsome et al. 2016, Watts and Newsome 2016, Stanek et al. 2017). The archipelagic landscape of Southeast Alaska presents unique variation in prey avail-ability with which to probe wolf dietary plastic-ity. Island and mainland sites across the Alexander Archipelago (Fig. 1) vary in the pres-ence and availability of ungulate prey including Sitka black-tailed deer (*Odocoileus hemionus*), moose (*Alces alces*), and mountain goat (*Oreamnos americanus*). Previously observed alternate prey of wolves in some sites in Southeast Alaska include beaver, salmon (*Oncorhynchus* spp.), black bears (*Ursus americanus*), seals (*Phoca* spp.), and birds and small mammals (Smith et al. 1987, Kohira and Rexstad 1997, Szepanski et al. 1999, Lafferty et al. 2014). However, the bioge-o-graphic variation in wolf diets has not been studied in this region.

Understanding how wolves respond to changes in ungulate abundance is an important consideration for predicting the ability of wolves to persist in areas of variable ungulate occupancy and species composition throughout the Alexander Archipelago. In addition, because the use of alternate prey can subsidize wolf populations, refining our knowledge of alternate prey contribu-tions to wolf diets can shed light on predator–prey dynamics. For example, Szepanski et al. (1999) found that wolves used salmon to a greater extent on the southeast Alaskan main-land where deer abundance was relatively lower than on Prince of Wales (POW) and Kupreanof (KUP) Islands. Furthermore, use of salmon increased during periods when deer abundance was low, providing evidence marine subsidies can uncouple the numerical response of wolves from ungulate abundance. The resulting appar-ent competition between marine resources and ungulates could plausibly depress ungulate pop-u-lations (Adams et al. 2010), which are managed in southeast Alaska for subsistence harvest.

Marine subsidies could also positively affect the conservation status of wolves in Southeast Alaska (*Canis lupus ligoni*), which have repeatedly been considered for listing under the Endan-gered Species Act (USFWS 2016). Although listing was determined not warranted at the time of the decisions, the reviews raised questions about the long-term viability of wolves. The most recent U.S. Fish and Wildlife status review included efforts to update previous wolf viability models (Person and Bowyer 1997, USFWS 2016). A key assumption of these models was that car-rying capacity of Sitka black-tailed deer will lar-gely drive population abundance of wolves, a reasonable premise considering that deer were found to be the key prey item of wolves on POW (Kohira and Rexstad 1997). However, this assumption might not be valid at the regional scale (Southeast Alaska) given evidence of prey switching when ungulate abundance shifts (Laff-erty et al. 2014), reliance on alternate prey in geographical regions where deer abundance is relatively lower (Szepanski et al. 1999), and season-al use of specific prey items (e.g., salmon; Szepanski et al. 1999, Darimont and Reimchen 2002). Therefore, it is necessary to expand the breadth of knowledge about wolf diets in other areas of this heterogeneous environment because geographical and biological differences between islands and coastal mainland systems influence ungulate occurrence and abundance, and these differences could be reflected in wolf diets (Dari-mont et al. 2004).

The feasibility of large-scale regional diet anal-ysis is facilitated by advances in molecular meth-ods due to increased accessibility and efficiency of next-generation sequencing technologies. Environmental DNA methods allow the extraction and identification of individual organisms from complex mixtures of DNA, such as in scats or stomach contents (Taberlet et al. 2012). A stan-dardized DNA region is amplified and sequenced from the pooled material; then, sequences are compared to a reference library for automated identification of multiple species. DNA metabarcoding of scat contents is an increasingly used method in terrestrial wildlife studies that provides finer taxonomic resolution.
Fig. 1. Locations of wolf scat samples from study sites (BER, Berners Bay; DOU, Douglas Island; GST, Gustavus; HEC, Heceta Island; JNU, Juneau, KUIU, Kuiu Island; KUP, Kupreanof Island; MIT, Mitkof Island; PI, Pleasant Island; POW, Prince of Wales Island; REV, Revillagigedo Island; SNOW, Snow Pass Islands) in Southeast Alaska, USA, 2010–2018.
than traditionally used methods such as morphological identification (Kartzinel et al. 2015, Alberdi et al. 2019). Using non-invasive samples such as scats can shed light on diets of elusive and cryptic species such as terrestrial carnivores (Smith et al. 2018), although low DNA quality may hamper accurate identification of taxonomic units. However, a recent assessment found DNA metabarcoding to reveal higher dietary diversity than mechanical sorting of prey remains with fewer misassigned species even with degraded wolf scats (Massey et al. 2019).

Here, we conduct a biogeographic analysis of wolf diets at twelve study sites across the Alexander Archipelago using DNA metabarcoding. We identified and quantified geographic variability in wolf use of prey species across systems with distinct ungulate species composition and abundance. As the use of ungulates declines, wolves could respond either by prey switching to one or few alternate prey or generally expanding their niche breadth as the most energetically profitable prey becomes less available (Stephens and Krebs 1986). We hypothesized that wolves would increase their dietary diversity and niche breadth as the proportion of ungulate species in their diets decreased, but that this could be mediated by the availability of coastal resources as indexed by the ratio of land area to shoreline in each study area.

**Materials and Methods**

**Study area**

We studied wolf diets in southeast Alaska, a geographically complex region within the Alexander Archipelago (Fig. 1). This area is composed of over 2,000 named islands ranging from one to 6700 km² in size and bordered to the east by the heavily glaciated Coast Mountains (with elevations ranging from sea level to 4600 m) on the mainland and covering 29,000 km of coastline (MacDonald and Cook 2007). The ecosystem is dominated by temperate rainforests, with marine estuaries, wetlands, and alpine tundra interspersed throughout, and is characterized by mild temperatures (mean annual temperature −5° to 8.5°C) and abundant precipitation (200–600 cm annually). The Alexander Archipelago is naturally fragmented by steep terrain and expanses of ocean which contribute to variation in species distribution and assemblages between island and mainland areas of this region.

Wolves occur across Southeast Alaska except for Admiralty, Baranof, and Chichagof islands. We collected wolf scat samples originating from 12 biologically and geographically distinct study sites (Fig. 1, Table 1), including separate islands and mainland units with varying ungulate prey distributions (e.g., Sitka black-tailed deer, moose, and mountain goats; Table 1). Sitka black-tailed deer occur at moderate densities on the central and southern island study sites (Douglas, DOU; Kuiu, KUIU; Kupreanof, KUP; Mitkof, MIT; Revillagigedo, REV; Snow Pass, SNOW) and at high densities on the islands of the outer coast (Prince of Wales Island, POW; Heceta, HEC). Coastal mainland systems are rugged, glaciated, and receive more snow contributing to low Sitka black-tailed deer abundance in Berners Bay (BER), Gustavus (GST), and Juneau (JUN; Schoen and Kirchhoff 2016). Sitka black-tailed deer on Pleasant Island (PI) are scarce (McCoy 2017). Deglaciation and vegetation succession patterns have allowed moose to colonize specific mainland drainages (Klein 1965) including GST where moose occur at high densities (White et al. 2014) and less isolated island systems (KUIU, KUP, MIT) where moose density is low to moderate (Lowell 2018). Moose were introduced to BER and occur at moderate densities (White et al. 2012). Mountain goats occur throughout the mainland mountains and were introduced to REV in 1983 (Paul 2009).

**Sample collection**

We collected scats during 2012–2018 along wolf travel routes, near den sites, on secondary roads during planned scat collection surveys and opportunistically coinciding with other wolf monitoring field work. We estimated the age (fresh [<one week], medium-aged [>one week−three months], old [>three months]) of scat based on appearance and time since last site visit (Ciucci et al. 1996) and exposure time considering that scats decompose rapidly in rainforest environments (Darimont et al. 2004) and exposure time considering that scats decompose rapidly in rainforest environments (Darimont et al. 2004). Collected scats were stored in Ziploc bags, labeled with location, date, and perceived age of the scat prior to analysis, and frozen (−20°C). Frozen scats were shipped to Oregon State University for sample preparation and analysis. To minimize...
autocorrelation of scat diet data, we both randomly subsampled scats collected on the same day at the same location when the number of scat samples was \( n > 5 \) and sampled the same locations at intervals \( \geq 2 \) months.

**Molecular scat analysis**

We identified prey species using metabarcoding of amplified target DNA sequences in wolf scats following methods in Massey et al. (2019) and Eriksson et al. (2020). Briefly, we pooled three subsamples taken from across the interior of the scat, with a sample amount ranging from 200 to 500 mg. We used a slightly modified protocol from the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Modifications include 500 µL Buffer ATL, 50 µL Proteinase K, and 1.0 mm Zirconia/Silica beads (BioSpec Products, Bartlesville, Oklahoma, USA). Samples were vortexed for 10 min at maximum speed before incubating at 56°C for 4–6 h. Each extraction batch (between 13 and 23 scats per batch) included a blank control to identify possible cross contamination.

Vertebrates make up most wolf prey species; thus, we used PCR (AmpliTaq Gold DNA polymerase, Life Technologies, Carlsbad, California, USA) to amplify a ~100 bp region of the mitochondrial 12S region to target the vertebrate genome. We used the forward primer as in Riaz et al. (2011) but modified the first base pair of the reverse primer to allow broader binding of vertebrate targets (Massey et al. 2019). We tagged each of 384 forward and reverse primers with unique matching 8 bp indices to identify individual scat samples and eliminate samples without matching indices to reduce error originating from tag jumping (Schnell et al. 2015). Each sample was amplified in triplicate with each replicate receiving a unique index. We included three no-template controls per 96-well plate. PCR was performed in 20 µL reactions consisting of 10 µL Kapa HiFi HotStart High Fidelity ReadyMix (Kapa Biosystems), 5.6 µL of each primer (0.25 µmol/L final concentration), 2.4 µL of water, and 2 µL DNA extracts (including extraction blanks and PCR no-template controls). Cycling conditions were 95°C initial denaturation for 3 min, followed by 35 cycles of 98°C for 20 s, 58°C for 15 s, 72°C for 30 s, and a final extension at 72°C for 1 min. We quantified DNA concentration of the samples using a fluorescence microplate reader with the AccuBlue dsDNA Quantitation Kit (Biotium, Hayward, California, USA) to amplify a ~100 bp region of the mitochondrial 12S region to target the vertebrate genome. We used the forward primer as in Riaz et al. (2011) but modified the first base pair of the reverse primer to allow broader binding of vertebrate targets (Massey et al. 2019). 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Following normalization, 3 μL from each sample per 96-well plate was pooled into a 0.65-mL Eppendorf tube. We used NEBNext Ultra II Library Prep Kit (New England BioLabs, Ipswich, Massachusetts, USA) to adapt the pools of 384 PCR products into Illumina sequencing libraries each with a unique 6 bp library index following the manufacturer’s instructions. This allowed us to reuse the unique 384 indexes in multiple sequence libraries to achieve a higher level of multiplexing. Library pools were purified using Solid Phase Reversible Immobilization (SPRI) paramagnetic beads (Aline Biosciences, Woburn, Massachusetts, USA), quantified using a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, California, USA), and normalized before sent for 150 bp paired-end sequencing on an Illumina HiSeq 3000 at the Center for Genome Research and Biocomputing, Oregon State University. Library size distribution was checked prior to sequencing using a High-Sensitivity D5000 DNA ScreenTape assay on an Agilent Tapestation 4200 (Agilent Technologies, Santa Clara, California, USA).

Raw sequence reads were analyzed using a bioinformatics pipeline designed to trim and demultiplex the sequence reads to identify each PCR replicate (whether corresponding to a scat or blank) based on its corresponding 8 bp index. Sequences from each sample were clustered by 100% similarity and taxonomically assigned using BLAST against 125 vertebrate sequences in GenBank and from a custom 125 database of vertebrate tissue from the Pacific Northwest maintained by the Levi Lab to fill gaps in GenBank. Because wolf diets are low diversity and contain larger-bodied prey that are well-represented in GenBank, the custom database was only helpful for improved taxonomic assignment for infrequently detected murid rodents and birds. We assigned taxa with 100% match and high query cover (>99%) to species-level after ensuring that no other taxa in GenBank also had 100% match. Taxa that could not be assigned to species were manually BLASTed again and assigned to genus or family based on percent match of related taxa.

Filtering and quality control measures were carried out on taxonomically assigned sequences as described in Massey et al. (2019). In brief, we first removed *Canis* spp. and contaminants based on read counts in no-template controls and extraction blanks. Specifically, in rare cases in which an extraction blank contained a low-level contaminant (low read count in 2 or 3 reps), that species was removed from scats extracted with that blank unless high read count clearly supported inclusion. This happened very infrequently, and no contamination was observed in PCR no-template controls. We then excluded prey items occurring in fewer than 2 of 3 PCR replicates.

Sequence filtering for metabarcoding requires balancing errors of commission (falsely including a species that was not present) and errors of omission (falsely removing a species that was present). To avoid errors of commission, we eliminated sequences that made up less than 0.5% of the total read count for a sample. However, to avoid errors of omission we then manually examined removed taxa and retained rare smaller-bodied prey items that might be erroneously excluded based on low read count (e.g., a songbird present in 2 or 3 PCR replicates of a scat that primarily contained deer). If a rare species occurred in low counts (~400 given average sequencing depth) in 3 reps and was not observed in blanks, we felt confident retaining that species even if its reads represented a small proportion of reads in the scat.

Finally, we compared taxonomic assignments with known fauna of southeast Alaska (MacDonald and Cook 2007) to replace non-regional species identified with BLAST with closely related regional taxa. This was necessary for some birds and murid rodents that assigned to related congeners or confamilials sometimes with 100% match but did not occur for any large-bodied prey typically consumed by wolves.

This short 12S region is highly conserved taxonomically such that congeners, such as wolves and coyotes, cannot always be distinguished. To distinguish wolves from coyotes, we amplified a fragment of the mtDNA control region using a single dye-labeled forward primer paired with a reverse primer and analyzed the fragment size on an AB3730 capillary DNA sequencer (Applied Biosystems, Foster City, California, USA), as suggested by De Barba et al. (2014). We modified the forward primer from Wasser et al. (1997), LTPROBB13(mod) –CAACCATCAGCACC CAAG, labeled with FAM on the 5’ end, and
the reverse primers from Murphy et al. (2000), H16145mod –GCAARCCATTAATGCAG. Our primer modifications allowed binding to Canis spp. without mismatches to produce fragment sizes of 147 bp for wolves and 142 bp for coyotes.

**Data analyses**

We estimated diet diversity and specialization for wolves in each study site using Shannon’s diversity index ($H'$; Shannon 1948) and Levins’ (1968) measure of niche breadth ($B$), respectively. Niche breadth values close to zero indicate a narrow diet niche with a high degree of specialization, whereas higher values indicate greater dietary generalization. We also measured species richness ($S$) as the total number of wolf diet items identified per study site. We calculated individual-based rarefaction curves using EstimateS 9.1.0 (Colwell 2013) to determine if samples reached a species diversity asymptote ($H'$) indicating completeness of samples for comparison of wolf diet diversity between areas despite differences in sample size.

To compare wolf diet composition between regions, we grouped consumed species or taxonomic units into diet item categories (Appendix S1: Table S1). We calculated indices of the relative frequency of occurrence as (1) the occurrence per feces ($O/F$) index (the number of occurrences of a diet item divided by the total number of scat samples) and (2) the occurrence per item ($O/I$) index (the number of occurrences of a diet item divided by the total number of diet items). We calculated $O/F$ and $O/I$ indices for each study site; we included $O/F$ for comparison to previous wolf diet studies, but restricted statistical tests to $O/I$ indices to avoid overcounting prey items that co-occur in scats containing multiple species (Kohira and Rexstad 1997). We also quantified wolf diet composition using relative read abundance (RRA) calculated as the proportion of prey DNA sequence reads in a scat sample divided by the total number of prey DNA sequences in that sample (Massey et al. 2019). The RRA proportions were averaged across all scat samples for each diet item in each study site. Massey et al. (2019) demonstrated strong evidence for a positive correlation between RRA and the estimated volume of diet item per scat estimated from mechanical sorting.

We evaluated the significance of variation in diet item consumption between study sites and seasons using a permutation-based multivariate analysis of variance (PERMANOVA, Anderson 2001). We generated separate Bray-Curtis dissimilarity matrices using the $O/I$ index and RRA data and ran analyses with 99,999 permutations and pairwise comparisons using Bonferroni’s correction. For tests of seasonal differences of diet item groups, we used the estimated age of the scat and the date of collection to group scat samples into seasons (summer, May–September; winter, October–April). If we could not determine which season the scat was grouped to, samples were used in analyses testing the significance of differences in diet item consumption between regions only (all data pooled by study site). Because PERMANOVA results indicated that wolf diet composition did not vary significantly by season ($O/I$ $F = 0.806, P = 0.578$; RRA $F = 898, P = 0.489$) and variation in the ungulate composition of wolf diets did not vary by season ($O/I$ $F = 0.695, P = 0.579$; RRA $F = 0.534, P = 0.744$), all subsequent analyses combine data from scats pooled at the level of study site but not season.

To assess which diet item groups were primarily responsible for observed differences in wolf diets between study sites, we used similarity percentage analysis (SIMPER; Clarke 1993) based on Bray-Curtis dissimilarity matrices of the $O/I$ index and RRA data. We visualized differences in diet composition between study sites by conducting a correspondence analysis (CA), which calculates eigenvalues and eigenvectors of a chi-squared distance matrix of diet items across study sites. Correspondence analysis identifies major axes of variation in wolf prey consumption and the distribution of diet item groups between study sites in ordination space. We performed SIMPER and PERMANOVA analyses using PAST v 3.25 software (Hammer et al. 2001). Because ungulates are the primary prey of coastal wolves (Kohira and Rexstad 1997, Szepanski et al. 1999, Darimont et al. 2004, Lafferty et al. 2014), we conducted separate analyses to test for differences in consumption of each ungulate species (Sitka black-tailed deer, moose, and mountain goats) by study site and season using PERMANOVA.
When ungulates are less available, wolves may prey switch or increase their niche breadth. We used a Gamma generalized linear model to assess how indices of dietary diversity (\(H'\) and \(S\)) and specialization (\(B\)) are affected by the proportion of ungulates (species combined) in the diets of wolves at each study site as measured by the O/I index. Because shoreline availability could influence the accessibility of coastal resources (Darimont et al. 2004, Collins et al. 2019), we additionally tested whether the ratio of shoreline length to land area in each study area influenced these metrics of dietary diversity and specialization.

To calculate the ratio of area to shoreline, a polygon map of the shoreline of Alaska was clipped to a buffer constructed around all scat locations in a geographic area. We used GPS collar data from 16 Southeast Alaskan wolves collected during 2012–2019 to determine the median displacement during a 48-hour period (11.54 km, range = 8.67–18.68 km), as scats represent wolf diets for the proceeding 24- to 60-hour interval (Peterson and Cuicci 2003). Within this 11.55-km buffer, the land/shoreline ratio was calculated as the planar area of land divided by the length of shoreline (excluding any offshore islands). Buffering, clipping, area, and length calculations were performed in R (R Core Team 2019) with the rgeos and sp packages.

**RESULTS**

Amplification success rate of the scat samples was 89.5%. After screening protocols (removing [1] scats that did not amplify, [2] scats that were determined to originate from species other than wolf, [3] scats from geographic areas with inadequate sample sizes), 860 of the 1121 scats collected were included in subsequent analyses. After filtering artifacts that typically slightly mismatched wolves or the dominant prey items, we retained ~95% of reads for downstream analysis.

Overall the scat samples contained 55 diet items representing species from 42 genera and 29 families (Appendix S1: Table S1) and had on average 47,215 diet item DNA sequences per sample (SE = 2353). The number of diet items per scat ranged from one to eight (mean = 1.40, SD = 0.741; Table 1). Individual-based rarefactions curves for dietary diversity (\(H'\)) reached an asymptote between 30 and 40 samples indicating sufficient sampling effort for all study sites except HEC (\(n = 17\)) and SNOW (\(n = 26\)); thus, it is possible that wolf diet diversity is underestimated in these two study sites (Appendix S1: Fig. S1).

Wolf diet \(H'\) was highest, and \(B\) was widest in GST and KUI, whereas KUP and REV wolves had the lowest \(H'\) and most narrow \(B\) (Table 1). Wolf diet species richness ranged from five to 26 diet items between study sites (mean = 12, SD = 6.4; Table 1). The CAs based on the O/I index and RRA data revealed similar patterns separating wolves that consumed marine mammals and other fish diet items (PI and GST) from wolves more strongly associated with ungulates (KUP, MIT, POW, REV; Appendix S1: Fig. S2).

Across all study sites (poled data), the most frequently occurring diet item group was ungulates (O/I = 0.546), followed by beaver (O/I = 0.125), marine mammals (O/I = 0.113), and bears (O/I = 0.067; Fig. 2). Similarly, the proportion of sequence reads for each prey item across all study sites indicated ungulates made up the largest proportion of wolf diets (RRA = 0.642), followed by marine mammals (RRA = 0.114) and beaver (RRA = 0.092). Ungulates were both the most frequently occurring diet item group and most variable in frequency of occurrence. The proportion of ungulates in wolf diets ranged broadly between study sites from low (PI O/I = 0.130; RRA = 0.132) to high (KUP O/I = 0.851; RRA = 0.904; Table 2). Beaver and bears occurred in the diets of wolves in nine and 10 out of 12 study sites, respectively, and contributed substantially to wolf diets (O/Ibeaver = 0.007–0.112; RRAbeaver = 0.002–0.121; O/Ibeaver = 0.049–0.212; RRAbeaver = 0.019–0.187; Table 2). Marine mammals were consumed at seven study sites (O/I = 0.002–0.645, RRA = 0.001–0.721). Sea otters (Enhydra lutris) were consumed at the highest frequencies (O/I = 0.105–0.522) and proportions (RRA = 0.131–0.633), whereas harbor seals (Phoca vitulina) and Steller sea lions (Eumetopias jubatus) had lower contributions (Fig. 3e,f). Birds were consumed broadly (in 11 out of 12 study sites) but at relatively low frequencies (O/I ≤ 0.006) and proportions (RRA ≤ 0.031); however, on some small islands bird consumption was high (PI O/I = 0.116; RRA = 0.103; SNOW O/I = 0.176; RRA = 0.102; Table 2). Bald eagles
(Gavia pacifica), sandhill cranes (Grus canadensis), and other Corvid and passerine species rarely consumed (Fig. 3c,d). Five mustelid species were also consumed at eight study sites: wolverine (Gulo gulo), river otter (Lontra canadensis) and three species of grouse (Neovision canadensis). kangaroo rat, and mink (Neovision canadensis) and ermine (Mustela erminea) at KUIU and SNOW.

Wolf diet composition varied by study site (PERMANOVA O/I F = 1.401, P = 0.097). Variation in the proportion of ungulates (SIMPER O/I = 28.9%; RRA F = 7.19%) and marine mammals (SIMPER O/I = 21.26%; RRA F = 85.47%) in wolf diets accounted for most of the dissimilarity between study sites (Appendix S1: Table S2). Ungulate species composition of wolf diets varied substantially by study site (PERMANOVA O/I F = 2.735, P = 0.006; RRA F = 2.879, P = 0.002). On islands where Sitka black-tailed deer were the only ungulate available (DOU, HEC, POW, SNOW), they were the dominant species in wolves’ diets (O/I = 0.529–0.632, RRA = 0.648–0.777; Fig. 3a, b). In study sites where multiple ungulate species were available, Sitka black-tailed deer were also most frequently consumed by wolves in KUP, MIT, and REV (O/I = 0.655–0.766; RRA = 0.744–0.873; Fig. 3a, b). Moose occurred in greater proportions in wolf diets than deer in KUIU (O/I = 0.327, O/I = 0.143; RRA = 0.320, RRA = 0.173) and GST (O/I = 0.284, O/I = 0.037; RRA = 0.343, RRA = 0.042), and mountain goats were the most frequently occurring ungulate in BER (O/I = 0.448; RRA = 0.495) and JNU (O/I = 0.362; RRA = 0.381; Fig. 3). On PI, Sitka black-tailed deer (O/I = 0.123; RRA = 0.132) and moose (O/I = 0.007; RRA < 0.001) were consumed less than marine mammals (O/I = 0.645; RRA = 0.721; Table 2, Fig. 3).

Shannon diversity (H’) and niche breadth (B) declined with increased ungulate occurrence per item in a bivariate relationship (H’ β = −1.10; P = 0.001; B β = −1.54; P = 0.01), and the effect increased when controlling for area to shoreline ratio (H’ β = −1.36; P = 0.0004; B β = −2.06; P = 0.003). There was no effect of area to shoreline ratio alone on H’ (β = −0.01; P = 0.77), or B (β = 0.005; P = 0.95), but dietary diversity and niche breadth increased marginally with area to shoreline ratio when controlling for ungulate consumption (H’ β = 0.05; P = 0.07; B β = 0.097; P = 0.08). Species richness of prey declined marginally with increased ungulate occurrence per item in a bivariate relationship (β = −1.45; P = 0.06), but the effect did not increase when controlling for area to shoreline ratio (β = −1.58; P = 0.08), which was neither associated with species richness alone (β = −0.07; P = 0.40) or when controlling for ungulate consumption (β = 0.03; P = 0.72). Thus, S, H’, and B all declined substantially with increased ungulate consumption, and wolf dietary diversity and niche breadth increased in less shoreline influenced areas (higher area to shoreline ratio). However, the effect of the area to shoreline ratio was influenced by Pleasant Island, which was a high leverage point with minimal ungulate consumption, a low area to shoreline ratio, and both low dietary diversity and niche breadth due to extensive specialization on marine mammals.

**Discussion**

Across the Alexander Archipelago at twelve study sites, we found that wolves increased the number and diversity of species consumed and increased their dietary niche breadth as the proportion of ungulates in their diet declined. Thus, rather than prey switch to one or few individual prey items, wolves broadly increased their niche breadth as ungulates became less available. Ungulates were the most prevalent diet item, echoing previous coastal wolf diet research (Fox and Streveler 1986, Kohira and Rexstad 1997, Darimont et al. 2004, Lafferty et al. 2014). However, our results revealed substantial variation in the proportion of ungulates in wolf diets across study sites, geographic differences in ungulate species consumption among island and mainland sites, and relegation of ungulates to secondary prey at one site. Sitka black-tailed deer were the most widely consumed ungulate species, and on most islands, wolves demonstrated a high degree of deer specialization (Figs. 3, 4). Wolves on these islands had the
The largest proportion of ungulates in their diets and correspondingly narrow niche breadth and dietary diversity. Conversely, in areas where the relative proportion of ungulates was low, wolves had a wide niche breadth and high dietary diversity indicating increased use of alternate prey.

The proportion of ungulates in wolf diets followed regional patterns of prey distribution and abundance in Southeast Alaska. Sitka black-tailed deer are the most common and widespread large mammal, with the highest densities on the central and southern islands (Schoen and Kirchhoff 2016) where they made

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**Fig. 2.** Composition of wolf diets based on occurrence per item (O/I) index of diet items identified in wolf scats, Southeast Alaska, USA, 2010–2018. Diet items occurring at <0.05 frequency in any study site were excluded. Size of pie chart is proportional to sample size. Study site abbreviations are listed in Table 1 and the Fig. 1 legend.
Table 2. Contribution of diet item groups to wolf diet composition between study sites in Southeast Alaska, USA, 2010–2018.

| Index by study site | Diet item groups |
|---------------------|------------------|
|                     | AM   | BR†  | BE  | BI  | DO  | HR  | MM  | MA  | MI  | MU  | FI  | PO  | SA  | SQ  | UN  |
| BER                 | O/F  | 0.125| 0.042|     | 0.125| 0.083|     | 0.042| 0.792|     |     |     |     |     |     |
|                     | O/I  | 0.103| 0.035|     | 0.103| 0.069|     | 0.035| 0.655|     |     |     |     |     |     |
|                     | RRA  | 0.101| 0.007|     | 0.114| 0.033|     | 0.038| 0.706|     |     |     |     |     |     |
| DOU                 | O/F  | 0.033| 0.033| 0.033| 0.067| 0.100| 0.033| 0.033| 0.133| 0.867|     |     |     |     |     |
|                     | O/I  | 0.025| 0.025| 0.025| 0.050| 0.075| 0.025| 0.025| 0.100| 0.650|     |     |     |     |     |
|                     | RRA  | 0.002| 0.001| 0.033| 0.031| 0.097| 0.002| 0.006| 0.051| 0.777|     |     |     |     |     |
| GST                 | O/F  | 0.158| 0.070| 0.070| 0.070| 0.316| 0.018| 0.071| 0.053| 0.035| 0.140| 0.018| 0.457|     |     |
|                     | O/I  | 0.111| 0.049| 0.049| 0.049| 0.222| 0.012| 0.049| 0.037| 0.025| 0.099| 0.012| 0.321|     |     |
|                     | RRA  | 0.074| 0.070| 0.031| 0.018| 0.251| 0.015| 0.028| 0.039| 0.006| 0.081| 0.002| 0.385|     |     |
| HEC                 | O/F  | 0.188| 0.063|     |     | 0.188|     |     |     | 0.750|     |     |     |     |     |
|                     | O/I  | 0.158| 0.053|     |     | 0.158|     |     |     | 0.632|     |     |     |     |     |
|                     | RRA  | 0.188| 0.004|     |     | 0.150|     |     |     | 0.658|     |     |     |     |     |
| JNU                 | O/F  | 0.029| 0.118| 0.059|     | 0.235|     |     |     | 0.029| 0.911|     |     |     |     |
|                     | O/I  | 0.021| 0.085| 0.043|     | 0.170|     |     |     | 0.021| 0.660|     |     |     |     |
|                     | RRA  | 0.011| 0.051| 0.015|     | 0.182|     |     |     | 0.029| 0.712|     |     |     |     |
| KUIU                | O/F  | 0.013| 0.158| 0.171| 0.026| 0.184|     | 0.092|     | 0.026| 0.618|     |     |     |     |
|                     | O/I  | 0.010| 0.122| 0.133| 0.020| 0.143|     | 0.071|     | 0.020| 0.480|     |     |     |     |
|                     | RRA  | 0.013| 0.121| 0.140| 0.026| 0.131|     | 0.053|     | 0.023| 0.492|     |     |     |     |
| KUP                 | O/F  | 0.012| 0.094| 0.047|     |     | 0.012|     |     |     | 0.941|     |     |     |     |
|                     | O/I  | 0.011| 0.085| 0.043|     |     | 0.011|     |     |     | 0.851|     |     |     |     |
|                     | RRA  | 0.012| 0.061| 0.020|     |     | 0.004|     |     |     | 0.904|     |     |     |     |
| MIT                 | O/F  | 0.021| 0.125|     | 0.042|     |     |     |     | 0.042| 0.917|     |     |     |     |
|                     | O/I  | 0.018| 0.109|     | 0.036|     |     |     |     | 0.036| 0.800|     |     |     |     |
|                     | RRA  | 0.014| 0.125|     | 0.021|     |     |     |     | 0.028| 0.813|     |     |     |     |
| PI                  | O/F  | 0.011| 0.169|     | 0.937|     | 0.011| 0.011| 0.126| 0.190|     |     |     |     |     |
|                     | O/I  | 0.007| 0.116|     | 0.645|     | 0.007| 0.007| 0.087| 0.130|     |     |     |     |     |
|                     | RRA  | 0.000| 0.103|     | 0.721|     | 0.011| 0.011| 0.022| 0.132|     |     |     |     |     |
| POW                 | O/F  | 0.003| 0.163| 0.351| 0.062| 0.017| 0.003| 0.003| 0.024| 0.042| 0.076| 0.910|     |     |
|                     | O/I  | 0.002| 0.099| 0.212| 0.038| 0.010| 0.002| 0.002| 0.015| 0.025| 0.046| 0.549|     |     |
|                     | RRA  | 0.000| 0.036| 0.156| 0.004| 0.007| 0.000| 0.003| 0.001| 0.017| 0.022| 0.753|     |     |
| REV                 | O/F  | 0.033| 0.100| 0.067|     | 0.017| 0.050| 0.017|     |     |     | 0.917|     |     |     |
|                     | O/I  | 0.028| 0.083| 0.056|     | 0.014| 0.042| 0.014|     |     |     | 0.764|     |     |     |
|                     | RRA  | 0.031| 0.048| 0.009|     | 0.001| 0.009| 0.012|     |     |     | 0.890|     |     |     |
| SNOW                | O/F  | 0.039| 0.231|     | 0.115|     | 0.193| 0.039|     | 0.750|     |     |     |     |     |
|                     | O/I  | 0.029| 0.176|     | 0.088|     | 0.147| 0.029|     | 0.529|     |     |     |     |     |
|                     | RRA  | 0.019| 0.102|     | 0.062|     | 0.130| 0.038|     | 0.648|     |     |     |     |     |

Notes: Proportions are displayed as the occurrence per feces (O/F) index, the occurrence per item (O/I) index, and the relative read abundance (RRA) estimates. Study site abbreviations are listed in Table 1 and the Fig. 1 legend. Diet item groups are as follows: AM, amphibian; BR, bear; BE, beaver; BI, bird; DO, domestic; HR, hare; MM, marine mammal; MI, microtine; MU, mustelid; FI, other fish; PO, porcupine; SA, salmon; SQ, squirrel; and UN, ungulate.

† All bears detected in wolf scat were black bears (Ursus americanus) except for 1 brown bear (Ursus arctos) detected in a scat sample from Mitkof Island.
up the largest proportion of wolf diets (Figs. 2, 3a,b). On islands where Sitka black-tailed deer were sympatric with moose or mountain goats, deer were generally consumed at a higher rate, as has been demonstrated in other systems (Fritts and Mech 1981, Benson et al. 2017). Conversely, moose and mountain goats were the most consumed ungulate species on mainland study sites. Mountain goats made up a larger portion of wolf diets than other ungulates in
There were some notable exceptions to ungulate-dominated wolf diets, and in these areas, wolves generally had wide dietary niches and high dietary diversity. Wolves in Gustavus had the widest niche breadth, highest dietary diversity, and the second largest number of species consumed of all study sites. Despite high moose densities in the Gustavus study site (White et al. 2014), moose represented only 28% of the wolf diet items, with marine mammals (22%), black bear (11%), and salmon (10%) making substantial contributions. Of the marine mammals, sea otters (*Enhydra lutris*) were the most consumed species (O/I = 0.210; RRA = 0.251) and harbor seals (*Phoca vitulina*) in small proportions (O/I = 0.012; RRA < 0.001; Fig. 3e,f). The low prevalence of moose in Gustavus wolf diets could have resulted from a strategy by wolves to reduce their risk of injury, as moose can be dangerous prey because their defensive behavior when attacked (Mech et al. 2015). Salmon are seasonally abundant, and predating or scavenging marine mammals may be possible year-round given the ample accessible shoreline in Gustavus. In addition, killing and handling times of these species is likely shorter than a large terrestrial ungulate such as a moose, making them an efficiently obtained and beneficial alternate prey (Darimont and Reimchen 2002, Paquet and Carbyn 2003).

Another exception to ungulate-dominated wolf diets was found on Pleasant Island, where ungulates made up only 13% of the wolf diet items, and wolf diet species richness and diversity were correspondingly high. Despite the scarcity of Sitka black-tailed deer and moose, wolves continued to occupy this small island (49 km²) during our study period. Marine mammals were the most prevalent diet category in wolf diets on Pleasant Island, with sea otters dominant (O/I = 0.522; RRA = 0.633), and Stellar sea lions (*Eumetopias jubatus*; O/I = 0.058; RRA = 0.044) and harbor seals (O/I = 0.065; RRA = 0.045) in small proportions (Fig. 3e,f). Sea otter populations have reached high levels of abundance since reintroduced individuals became established in the adjacent Glacier Bay National Park in the late 1980s (Esslinger et al. 2015, Williams et al. 2018) and are now substantial or primary diet items by wolves in Gustavus and on the mainland study sites of Berners Bay and Juneau (Figs. 2, 3a,b).
Pleasant Island, respectively. The unexpected high levels of sea otter consumption represent the recovery of a lost species interaction for these archipelagic wolves, but it is still unknown whether wolves are scavenging or predating sea otters. Although Pleasant Island wolves had a relatively wide dietary niche breadth, it was not as wide as would be predicted given that wolves have the lowest the ungulate consumption of all study sites (Tables 1 and 2). The degree of specialization on marine mammals could provide explanation for this pattern.

Wolves on Kuiu Island were also an exception to the pattern of high ungulate consumption (Fig. 2, Table 2). Unlike on other islands where Sitka black-tailed deer and moose were sympatric, wolves favored moose over deer (Fig. 3a, b). Sitka black-tailed deer made up only 14.3% of Kuiu wolf diets (compared to the average of 65% on other islands), whereas black bear, beaver, and marine mammals each contributed at similar or equal quantities at this site (Table 2), making these species important alternate prey. Sitka black-tailed deer occur at low abundance on Kuiu Island, which is attributed to wolf predation and extensive alteration of their winter habitat from logging (Schoen and Kirchhoff 2016). In contrast, Kuiu has one of the highest densities of black bears in North America (1.5 per km²; Peacock 2004). Wolves consumed black bears more on Kuiu Island than any other study site (O/I = 0.122, RRA = 0.121; Table 2). Marine mammal consumption was also relatively high (O/I = 0.143, RRA = 0.131) and consisted entirely of sea otters.

Access to marine environments can provide a wider variety of forage species including marine mammals, fish, and marine invertebrates available in the intertidal zone. Increased consumption of these species could be advantageous for wolves because of reduced handling times and risk, allowing wolves to forage individually (Collins et al. 2019). Canids with greater access to marine resources can have wider dietary breadth and more dietary trophic diversity (Rose and Polis 1998, Szepanski et al. 1999). Salmon are well-recognized as an important seasonal food source for wolves in coastal regions (Szepanski et al. 1999, Darimont and Reimchen 2002, Stanek et al. 2017), and use of marine mammals has also been documented (Klein 1995, Darimont et al. 2004, Lewis and Lafferty 2014, Watts and Newsome 2016) but is less understood. Although wolves have been observed scavenging large marine mammals and hunting smaller species (Watts et al. 2010, Lewis and Lafferty 2014, Collins et al. 2019), a greater understanding of the relative contribution of marine mammals to coastal wolves’ diets is still emerging. Here, we show extensive wolf consumption of marine mammals, particularly sea otters. The ongoing recolonization of sea otters is thus likely to restore an important diet item for wolves in this archipelagic landscape.

In addition to extensive use of marine resources, terrestrial and avian alternate prey species played a substantial role and varied biogeographically. Beaver was the second most frequently occurring diet item after Sitka black-tailed deer on Prince of Wales and Revillagigedo Islands, corroborating previous work (Smith et al. 1987, Kohira and Rexstad 1997). Beaver are a prevalent part of wolf diets in North America and Europe and provide seasonal subsidies during summer, are an important food source for pups (Newsome et al. 2016, Sidorovich et al. 2017, Gable et al. 2018, Myslajek et al. 2019), and access to beaver may promote higher rates of pup survival (Benson et al. 2013). The role of beaver as alternate prey may contribute to increased incidental wolf predation of ungulates due to shared seasonal habitats (Latham et al. 2013), illustrating how diet subsidies may have proximate consequences to ungulate populations. Black bears were also important alternate prey on both mainland and island sites. Black bear have been previously identified in wolf scats on Prince of Wales (Kohira and Rexstad 1997), Kuiu (ADF&G, unpublished data), and British Columbia (Darimont et al. 2004), and wolves have been observed preying on black bears in British Columbia, Manitoba, and Minnesota (Rogers and Mech 1981, Horejsi et al. 1984, Paquet and Carbyn 1986). This relationship is sometimes reversed as wolves may be wounded or killed during conflicts with black bears (Kohira and Rexstad 1997). Thus, black bears represent a food source as well as a source of competition for food. In northern mainland study sites and adjacent Douglas Island, marmots were an important food item (representing between 10% and 17% of all diet items) where wolves commonly use...
alpine habitats and may encounter marmots while hunting mountain goats (Fox and Streveler 1986).

Diets of coastal wolves inhabiting Southeast Alaska and British Columbia have been previously quantified using mechanical sorting of prey remains in scats, but diet items were often pooled into categories (Fox and Streveler 1986, Smith et al. 1987, Kohira and Rexstad 1997, Darimont et al. 2004, Lafferty et al. 2014). Using DNA metabarcoding, we could identify each diet item to the genus or species level and therefore described a greater diversity of foraging habits. For accurate taxonomic identification, we relied on comprehensive reference libraries (GenBank) and a custom database of 12S sequences from vertebrate species in the Pacific Northwest to fill gaps in GenBank. This database was not necessary for the bulk of wolf diet but was helpful for identifying some birds and murid rodents. We have assembled this database over time in an attempt to identify species that imperfectly match sequences in GenBank. Additionally, our use of the modified 12S mtDNA primer resulted in a broad binding of prey targets without base-pair mismatches for the taxa amplified here. However, our focus on vertebrate species does obscure some dietary diversity as wolves are known to consume intertidal and terrestrial invertebrates (Okarma 1995, Meriggi et al. 1996, Darimont et al. 2004).

By using metabarcoding for high-throughput wolf diet analysis, this research elucidates regional consumer-resource interactions at a broad geographical scale. With the exception of one study site where wolves have specialized on marine mammals, we found that wolves increased their overall dietary diversity instead of switching to one or few alternate prey and this trend increased as the proportion of ungulates in their diets decreased. This pattern could either be explained by low ungulate abundance (i.e., Pleasant Island or Kuiu) or low ungulate availability due to prey refugia, variation in ungulate vulnerability, or wolf exposure to risk while hunting. It may be more profitable in these cases for wolves to acquire smaller terrestrial mammals or marine resources if they are abundant and seasonally and spatially predictable, requiring less searching and handling effort. The extensive variation of prey species identified in this research corroborates the recognized dietary flexibility of wolves and indicates that wolves are an adaptable predator of terrestrial, marine, and avian species. In a rapidly changing and heterogenous landscape, a diverse diet may be a successful strategy to increase resilience to fluctuations in prey abundance over time and space.

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