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Endangered species, archaeology, and stable isotopes: huemul (*Hippocamelus bisulcus*) isotopic ecology in central-western Patagonia (South America)

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ABSTRACT

The management and conservation of animal species should be based upon a long-term analysis that considers its geographical distribution and feeding patterns. The study of carbon and nitrogen stable isotopes on skeletal remains provides a quantitative approach to the paleodietary reconstructions and constitutes a potent tool for comparing behavioral aspects of the fauna. In this paper we present the first set of isotopic values for modern and archaeological samples of Huemul (*Hippocamelus bisulcus*) from the forests of Central-western Patagonia, Chile. Contrary to initial expectations, our analysis indicates that there is no evidence of an important incidence of the canopy effect on the δ^{13}Ccollagen values, which we suggest is due to an ecological selection that drives the huemul to focus its predation on open sectors within the forest. On the other hand, a wide range of δ^{15}Ncollagen values for huemul was verified. This could reflect the N impoverishment of the forest soils and provide an interesting ecological indicator. The long-term information offered by the archaeological record provides the necessary context for decision-making conducive towards the preservation of *Hippocamelus bisulcus* in Patagonia.

1. Introduction

The management and ecological and genetic conservation of animal species should be based upon a long-term analysis that considers its geographical distribution and foraging patterns. Within this context, archaeological analysis of faunal assemblages provides one of the principal sources of information with temporal depth (Burton et al., 2001; Lyman and Cannon, 2004; Wolverton, 2008). This paleoecological perspective is particularly necessary in the case of several South American ecosystems, which have suffered important transformations since the beginning of Western colonization. Therefore, it could be that neoecological observations do not correspond to prehistoric distributions and ecological interactions. The study of carbon (^{13}C) and nitrogen (^{15}N) isotopic compositions on skeletal remains provides a quantitative approach to paleodietary reconstructions (Ambrose, 1993; Koch, 2007) and constitutes a strong tool for comparing behavioral aspects of the fauna, thus contributing to an understanding of past ecosystems. In this paper we present the first set of isotopic values for modern and archaeological samples of Huemul (*Hippocamelus bisulcus*) from central-western Patagonia, Chile (Fig. 1).

The Huemul is a medium sized cervid with short legs and a well built body that inhabits the Chilean and Argentinean Andes between the 40° and the 49° S, with the presence of a relict population towards the 36-37° S (Vila et al., 2006, Fig. 2), which reflects a more widespread ancient distribution. It is in a clear process of population decrease, genetic impoverishment and drastic retraction within its area of distribution, reasons for which it has been classified as a species “in danger of extinction” by the International Union for the Conservation of Nature (IUCN) and considered in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and in the Red Books of Chile and Argentina. For comparative reasons, we also present local preliminary information for the camelid guanaco (*Lama guanicoe*), the largest terrestrial wild mammal in southern South America that preferably inhabits open areas with grasslands (González et al., 2006); and for pudu (*Pudú pudú*), the smallest cervid in the world that inhabits the Andean evergreen forests (Iriarte, 2008). Since the guanaco is mainly an inhabitant of the open steppe plains and the pudu is an exclusive inhabitant of the deep evergreen forests, altogether with the huemul they provide an initial framework for herbivore species with different dietary and geographical patterns widely studied at an ecological level (Section 1.2).

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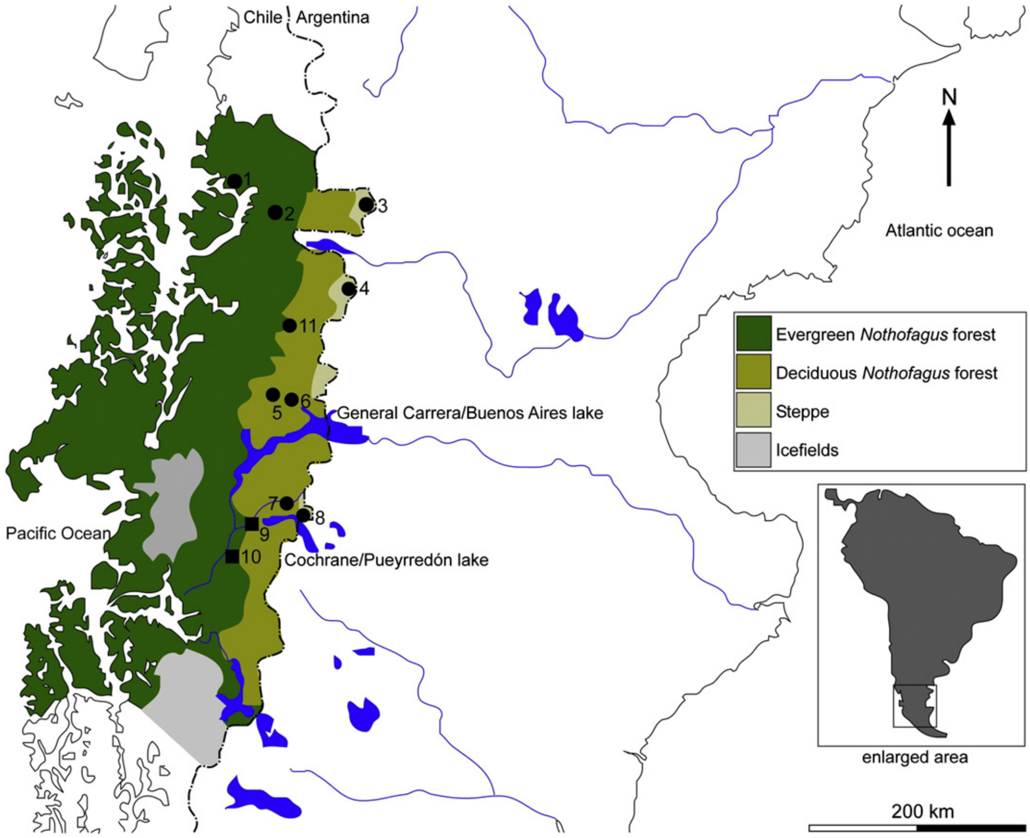

Fig. 1. Map of Aisén within Central-western Patagonia, current vegetation distribution (SERPLAC XI Region, 2005) and sites discussed in the text. References: 1. Seno Gala 1, 2. El Toro rockshelter, 3. El Chueco 1 rockshelter, 4. Baño Nuevo 1 cave, 5. Fontana rockshelter, 6. Las Guanacas (two samples), 7. Gianella rockshelter, 8. Entrada Baker rockshelter, 9. Cerro Tamango, 10. Los Ñadis, 11. Lomo de Dragón Bajo rockshelter. Circles: archaeological sites, squares: recent samples.

The paleoecological contribution of stable isotopes in the context of species conservation has been widely demonstrated for terrestrial herbivores (Ambrose and De Niro, 1986; Tieszen, 1994; Koch et al., 1995), and cervids in particular (Bocherens, 2000; Ben-David et al., 2001; Stevens et al., 2006); terrestrial omnivores and carnivores (Bocherens et al., 1995; Szpak et al., 2009); pinnipeds (Etnier, 2004; Newsome et al., 2007); and birds (Chamberlain et al., 2005; Lambertucci et al., 2009), amongst others. Specifically, the Patagonian region presents abundant isotopic data for guanaco (Barberena et al., 2009, 2010; Tessone, 2010; Tessone and Belardi, 2010), which is integrated and discussed here.

1.1. Study area

The study area corresponds to the Aisén Region (Chile) located at the western margin of Patagonia between ~43° and 48° S. It is characterized by an abrupt biogeographic gradient controlled by the moisture-laden westerly winds and the barrier effect of a massif mountain range. Four geomorphologic units can be observed from west to east: the Channels and the Pacific archipelagos, the Andes mountain range which includes volcanoes and ice fields, the sub-Andean mountain range that has a lower relief, and extensive sedimentary plains with geoforms of glacial and fluvio-glacial origin including large lakes. According to the rainfall gradient, formations of evergreen forests dominate the west, making way for deciduous forests and, towards the east, open terrain with steppe vegetation (Fig. 1). Available paleoenvironmental information suggests that this phytogeographical distribution did not substantially vary since the Early Holocene, except for minor fluctuations in the location of the forest/steppe margin (Bennett et al., 2000; Markgraf et al., 2007; Reyes et al., 2009). This is relevant for the spatial analysis that takes place in the discussion.

1.2. Ecology of *Hippocamelus bisulcus*

The simultaneous development of conservation efforts (Povilitis, 1977) and a better access to periglacial sites and to the Patagonian forests has allowed for significant advances in the knowledge on the huemul, including studies concerning their dietary habits (Colomés, 1978; Galende et al., 2005; Vila et al., 2009, 2010), social organization (Povilitis, 1983), history (Díaz, 1990) and genetics (Corti et al., 2009), in addition to an extensive bibliography concerning conservation policies (Díaz and Smith-Flueck, 2000; Serret, 2000; Corti, 2008).

Huemul populations occur from close to sea level up to 3.000 masl, usually at the forest edge at either upper or lower timberline and in association with forests of southern beech (*Nothofagus* spp.). This species has been recorded at a variety of habitats including valley floors and mountain slopes, ranging from open grasslands to closed forested settings (Povilitis, 1977; Díaz and Smith-Flueck, 2000; Vila et al., 2010). As a browser, huemul relies primarily on buds, twigs and other parts of woody plants. It requires concentrated nutrients, precluding the heavy use of forage of low digestibility, such as dried grasses. However, huemul seasonally consume newly growing grasses (Vila et al., 2010:95; see also Colomés, 1978). Annual home range sizes have been estimated to be ca. 350–650 ha with daily travel distances rarely exceeding 5 km (Gill et al., 2008). Huemul and guanaco have ruminant digestive systems, allowing for a preliminary comparison of their isotopic values in dietary terms.

Fig. 2. *Hippocamelus bisulcus* during winter season (left female, right male; location: Cochrane area; photograph by René Millacura).

1.3. Stable isotopes and palaeoecological research

Paleodietary studies by means of carbon and nitrogen stable isotopes are based on the experimental observation of a correlation between the isotopic compositions of an organism and the values of the foods that comprise its diet (De Niro and Epstein, 1978, 1981; Ambrose and Norr, 1993; Koch, 2007). The isotopic compositions of the different fractions that compose a bone -or any other organic tissue- offer an averaged record of the dietary intake of an organism, and its temporal extent varies according to the type of tissue sampled. In the case of bones, the estimate is around the last five to ten years of life of the organism, depending on the turnover rate of the different elements (Ambrose, 1993). The isotopic values in this paper have been obtained from the organic fraction of the bone, usually named collagen or gelatin (Ambrose, 1990; see section 2).

The δ^{13}Ccollagen in herbivores is largely determined by the isotopic composition of the plant species regularly consumed. Two main photosynthetic pathways exist that predominate respectively under different climatic and ecological conditions. Plant species that have the C_4 photosynthetic pathway (Hatch–Slack cycle) present a global average of $-12.5‰$ and are adapted to arid and warm conditions. On the other hand, plant species with the C_3 photosynthetic pathway (Calvin cycle) have δ^{13}C average values around $-26.5‰$ (Ehleringer and Cerling, 2001). These are less well adapted to arid and warm conditions than C_4 plants (Tieszen, 1991). Because of the humid climate conditions that prevail in the study area, one cannot expect a high representation of C_4 species.

According with these patterns, forested ecosystems are exclusively composed of C_3 species. Under certain conditions, forested plants can be characterized by even lower δ^{13}C values than coeval plants in open environments. This effect is caused by photosynthetic recycling of CO_2 produced via soil respiration that is depleted in ^{13}C, and also by low light levels. This depleted signature can be transmitted throughout the successive steps that conform a food chain (Ambrose and De Niro, 1986; van der Merwe and Medina, 1991; Heaton, 1999; Krigbaum, 2003; Drucker et al., 2008). This situation has been discussed for different cervid species around the world (Stevens et al., 2006; Drucker et al., 2008), and is relevant in the case of the huemul, described as a habitant of the forest. Nevertheless, discrepancies do exist regarding its preference for closed forest habitats or forest borders and meadow patches (Díaz and Smith-Flueck, 2000; Vila et al., 2006). δ^{13}C data for samples with prehistoric chronology could indicate the importance of foraging in 'closed' forest environments (evergreen and deciduous forests) versus environments with more open characteristics (forest steppe ecotone and steppe). This would provide an important marker of the dietary ecology of this species, as has been previously discussed for the guanacos (Barberena et al., 2009).

Bone collagen δ^{15}N values are utilized as a trophic indicator because there is a consistent enrichment of 3–4‰ that occurs at each trophic level, which has been evaluated in terrestrial and marine food chains (Ambrose, 1991; Bocherens et al., 1995; Bocherens and Drucker, 2003; Hedges and Reynard, 2007; Szpak et al., 2009). In different contexts in the world, particularly low values of δ^{15}N have been observed in soils and plant communities of forested environments (Garten, 1993), which are transferred to successive trophic levels. As suggested by Craine et al. (2009:990, highlight added), "if the general regional relationships between N availability and foliar δ^{15}N extend to the global scale, then the global foliar δ^{15}N patterns suggest that: *warm and dry sites have high N availability relative to cold and wet sites*; plants with high N concentrations occupy sites with high N supply". The verification of this effect on the collagen of European cervids is particularly interesting (Rodière et al., 1996; Bocherens, 2000).

In summary, from a methodological perspective, one can expect the existence of an isotopic signal of δ^{13}C and δ^{15}N proper of the more closed environments inhabited by huemul and pudu, particularly when compared with guanacos, which tend to inhabit more open environments.

2. Materials and methods

The samples analyzed in this paper principally come from archaeological sites and in two cases correspond to samples of recently recorded dead animals in the study area. In the case of archaeological samples, taxonomical identification was performed by the observation of diagnostic anatomical features of selected bones in reference skeletons. The samples were selected from archaeological contexts with known ages. The chronological data was determined by the sample's stratigraphic location and, in two cases, by direct radiocarbon dating of the bone specimen. Radiocarbon ages are expressed in calendar years before the present year (cal yr BP) and were calibrated using the OxCal 4.1 program (Bronk Ramsey, 2009) utilizing the ShCal 04 curve. The sites and samples are evenly distributed throughout the region. The majority of the sampled sites correspond to stratified deposits in rockshelters, where the bone samples were dry and in an excellent state of preservation.

Diverse processes have a bearing on the formation of an archaeological faunal assemblage, principally human decisions concerning the transportation of anatomical parts (Lupo, 2006). This could disguise the geographical distribution of a particular species in the past. Nevertheless, there are contextual criteria (i.e., taphonomic and zooarchaeological) that allow assessing the fidelity of the context of recovery of fossil faunal remains to the ecological context where an animal lived (Behrensmeyer et al., 2000; Grayson, 2005; Lyman, 2011). On the other hand, it is also necessary to consider that the surrounding vegetation of the sample sites could have been different in the past. For this reason it is necessary to calibrate the results with the available paleoenvironmental information.

With the exception of one case, the samples were processed by the Center for Applied Isotope Studies, University of Georgia (USA). The crushed bone was treated with diluted 1 N acetic acid to remove surface absorbed and secondary carbonates. Periodic evacuation insured that evolved carbon dioxide was removed from the interior of the sample fragments. The chemically cleaned sample was then reacted under vacuum with 100% H3PO4 to dissolve the bone mineral and release carbon dioxide from bioapatite for carbon isotope ratio analysis. The residue was filtered, rinsed with deionized water and under slightly acid condition ($pH = 3$) heated at 80 °C for 6 h to dissolve collagen and leave humic substances in the precipitate. The collagen solution was then filtered to isolate pure collages and dried out. The dried collagen was combusted at 575 °C in evacuated/sealed Pyrex ampoule in the present CuO. The carbon dioxide and nitrogen were cryogenically separated. The isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were measured separately, using a stable isotope ratio mass spectrometer. The ratios are expressed in parts per thousand (‰) using the δ notation, and are measured with respect to the limestone Vienna Pee Dee Belemnite (VPDB), in the case of δ^{13}Ccollagen, and to atmospheric air nitrogen (AIR), in the case of δ^{15}Ncollagen. Error is of 0.1‰ for $\delta^{13}C$ and less than 0.2‰ for $\delta^{15}N$. Standard procedures for the evaluation of collagen post-mortem alteration (De Niro, 1985; Ambrose, 1990) were applied.

One of the δ^{13}Ccollagen pudu values, from Seno Gala 1 archaeological site, is the product of an AMS radiocarbon dating. Abundant information has been presented suggesting that these values can be confidently used for stable isotopic research (van Klinken et al., 2000). All the individuals that were sampled are adults; thirteen samples correspond to different bones and three to teeth dentine. A + 1.5‰ correction was applied to the two modern samples in order to account for the so-called 'industrial effect' producing modern plants to have lower $\delta^{13}C$ values than ancient plants due to the burning of ^{13}C-depleted fossil fuels (Marino and McElroy, 1991).

The degree of isotopic discrimination between the samples belonging to different species and subareas are tested by means of the multivariate Kruskal–Wallis test, in the case of δ^{13}Ccollagen, and by the bivariate Mann–Whitney test for δ^{15}Ncollagen (involving only two variables). The Kruskal–Wallis test is a non-parametric Analysis of Variance (ANOVA), comparing the medians of several univariate groups. It can also be regarded as a multiple-group extension of the Mann–Whitney test. It does not assume normal distribution, but does assume equal-shaped distribution for all groups. The null hypothesis is: the samples are taken from populations with equal medians. A post-hoc Mann–Whitney pairwise test is also given for all pairs of groups with a Bonferroni conservative correction for multiple testing (Zar, 1996).

3. Results

Table 1 shows the δ^{13}Ccollagen and δ^{15}Ncollagen isotopic data obtained for the three species in consideration along with the chronological information and data concerning the current ecological context. Table 2 presents the descriptive statistics of the data, along with sample groups of guanacos from forest and steppe environments in neighboring regions (Barberena et al., 2009; Tessone and Belardi, 2010), which are integrated in the previously mentioned analysis. We will begin addressing the data regarding huemul ($N = 12$) in greater detail.

At the δ^{13}Ccollagen level, once the two modern samples from Cerro Tamango and Lago Cochrane have been corrected for the industrial effect, one can observe a relatively defined range of variation of ca. 3‰ and a standard deviation of 0.84‰. The average for the twelve huemul samples is 20.16‰. On the other hand, the δ^{15}Ncollagen values present a considerably wider variation range of 6‰ with an average of 2.7‰ and a standard deviation of 2.04‰.[1] This is significant considering that the average enrichment associated with each trophic level is of ca. 3‰ (cf., Drucker et al., 2003), and that we are dealing with a single herbivore species here.

A low correlation between δ^{13}Ccollagen and δ^{15}Ncollagen values ($r = 0.22$, $p = 0.48$, Fig. 3) is present, although the presence of six samples with low δ^{13}Ccollagen and δ^{15}Ncollagen values should be noted.

3.1. Temporal trends

In order to evaluate the existence of temporal trends, we have organized the samples in five chronological intervals in calendar years BP: modern samples (last 200 years), 200–600 cal yr BP, 600–2100 cal yr BP, 2300–3300 cal yr BP and, lastly, two sample that correspond to the Early Holocene (8400–7700 cal yr BP). The results indicate a slightly negative and not significant correlation between chronology and δ^{15}Ncollagen ($r = -0.27$, $p = 0.37$, $N = 12$). In the case of δ^{13}Ccollagen, a more negative relation is verified, although also not significant ($r = -0.63$, $p = 0.02$, $N = 12$) (Fig. 4).

In synthesis, temporal changes of magnitude in the isotopic information for huemul were not registered, although an increase in the size of the sample could modify this situation. In particular, δ^{13}Ccollagen values show a stronger negative relation that requires further testing and may have paleoclimatic implications in terms of extent and density of forest cover. Nevertheless, it must be noted that broader studies of southern Patagonian guanacos did not detect temporal variations during the Holocene (Barberena et al., 2009; Borrero et al., 2009; Tessone, 2010; Tessone and Belardi, 2010).

[1] Recently, isotopic information has been presented for an artifact elaborated from huemul horn recuperated at the Atlantic coast, away from the known huemul habitat (Cruz et al., 2010). The sample dates back to 1150 ± 70 ^{14}C yr BP and presents a δ^{15}Ncollagen value of 0.34‰ and a δ^{13}Ccollagen value of −21.02‰, placing it within the range for the Aisén huemules. The low δ^{15}Ncollagen value coincides with the lowest cases registered by us and constitutes an anomalous case within the information for herbivores that inhabit the steppe (Tessone and Belardi, 2010). On the other hand, Tessone et al. (2011) are analyzing huemul samples from northern and southern Patagonia.

Table 1
Isotopic results for Patagonian herbivores.

Locality	Position	Species	Sample	Lab Code	δ^{13}Ccollagen	δ^{15}Ncollagen	Chronology (cal yr BP)	Modern vegetation	Context reference
Cerro Tamango	47°11′S 72°34′W	*H. bisulcus*	femur	UGAMS 05218	−22,1 (-20.6)[a]	5.4	Modern	Forest steppe transition	–
Los Ñadis	47°30′S 72°59′W	*H. bisulcus*	femur	UGAMS 6676	−21,1 (-19.5)[a]	1.3	Modern	Evergreen forest	–
Gianella	47°04′S 72°16′W	*H. bisulcus*	mandible	UGAMS 6675	−19.9	5.3	2900–2750	Steppe	–
Entrada Baker	47°10′S 71°52′W	*H. bisulcus*	tarsal	UGAMS 6674	−19.8	5.3	450-recent	Steppe	Mena and Jackson, 1991
Las Guanacas	46°15′S 71°59′W	*H. bisulcus*	metapodial	UGAMS 6680	−18.8	4.4	> 500	Forest steppe transition	Mena, 1983
Las Guanacas	46°15′S 71°59′W	*H. bisulcus*	metapodial	UGAMS 6679	−18.8	2.6	650–300	Forest steppe transition	Mena, 1983
Fontana	46°12′S 72°13′W	*H. bisulcus*	mandible	UGAMS 6677	−20.4	1.5	2100–650	Deciduous forest	Mena et al., 2004
Fontana	46°12′S 72°13′W	*H. bisulcus*	tooth	UGAMS 6678	−20.5	0.9	2100–650	Deciduous forest	Mena et al., 2004
Baño Nuevo 1	45°17′S 71°32′W	*H. bisulcus*	radius	UGAMS 6673	−20.6	2.2	8400–8200	Steppe	Mena and Stafford, 2006
El Toro	44°42′S 72°13′W	*H. bisulcus*	tooth	UGAMS 6671	−20.8	0.7	2750–2350	Evergreen forest	Méndez et al., 2006
El Toro	44°42′S 72°13′W	*H. bisulcus*	long bone	UGAMS 6672	−20.6	−0.6	2760–2360	Evergreen forest	Méndez et al., 2006
Lomo de Dragón Bajo	45°40′S 72°04′W	*H. bisulcus*	scapulae	UGAMS 8191	−21.7	3.5	7870–7770	Forest steppe transition[c]	–
El Toro	44°42′S 72°13′W	*Pudú pudú*	long bone	UGAMS 6670	−21.6	1.8	2760–2360	Evergreen forest	Méndez et al., 2006
Seno Gala 1	44°02′S 73°08′W	*Pudú pudú*	tooth	BETA 230515	−23.6	–	1300–1200[b]	Evergreen forest	Reyes et al., 2007a
El Chueco 1	44°31′S 71°07′W	*L. guanicoe*	Tibia	UGAMS 06106	−20.2	6.1	3300–3100[b]	Steppe	Reyes et al., 2007b
El Chueco 1	44°31′S 71°07′W	*L. guanicoe*	humerus	UGAMS 06107	−20.1	4.5	250-recent[b]	Steppe	Reyes et al., 2007b

[a] values corrected for 'Industrial effect'.
[b] direct ^{14}C AMS ages
[c] paleoclimatic data suggest placing this mid-Holocene sample in the closed forest set.

3.2. *Intra-specific variations in huemul*

We organized the samples in function of the ecological characteristics of the current environment from which they come from (section 1.1). It must be mentioned that most of the area considered presents a very low, and in many cases negligible, recent human impact (with the exception of the Lomo de Dragón Bajo sample, see below). Samples of huemul from evergreen and deciduous forests are included in the "closed" forest category ($N = 7$); on the other hand, samples located near the forest steppe ecotone and at the steppe are considered as coming from "open" settings ($N = 5$). This division does not strictly correspond to two discrete ecological categories, but it provides a useful scheme for an initial exploration of isotopic variation. This heuristic approach needs to be independently evaluated, since environmental changes can modify the spatial configuration of these ecosystems. Paleoecological data (i.e., pollen variations) suggest relative stability since 8200–7500 cal yr BP where, despite minor fluctuations, high relative values of effective moisture allowed the sustainability of the forests (Markgraf et al., 2007; Reyes et al., 2009).

Table 2
Descriptive statistics for δ^{13}Ccollagen and ä^{15}Ncollagen data on Patagonian herbivores.

	Huemul	Pudú	Steppe guanaco	Forest guanaco
δ^{13}Ccollagen				
N	12	2	60	11
Minimum	−21.7	−23.6	−22.3	−24.9
Maximum	−18.8	−21.6	−16.7	−19
Mean	−20.16	−22.6	−19.78	−22.16
Standard deviation	0.84	1.41	0.95	1.86
Median	−20.45	−22.6	−19.73	−22
δ^{15}Ncollagen				
N	12	1	36	0
Minimum	−0.6	1.8	2.26	–
Maximum	5.4	1.8	9.7	–
Mean	2.7	1.8	6.37	–
Standard deviation	2.04	0	1.73	–
Median	2.4	1.8	6.43	–

The sample from Lomo de Dragón Bajo site is currently located near Coyhaique City, an area presenting an important degree of recent anthropic impact (Fig. 1). Intense fires that occurred during the early XXth century changed the forest coverage significantly. Accordingly, paleoclimatic data indicate that a dense forest predominated at this area before ca. 7000 cal yr BP (Markgraf et al., 2007). On this basis, this sample is included in the closed forest set in the following analyses.

A comparison of these two subsamples of huemul by means of Mann–Whitney tests shows that there are not statistically significant differences between the δ^{13}Ccollagen and δ^{15}Ncollagen medians, with values of $p = 0.07$ for ^{13}C and $p = 0.103$ for ^{15}N. There is a tendency to slightly depleted δ^{13}Ccollagen values in the samples from closed environments, although the isotopic ranges are largely overlapping (Table 3, Fig. 3). The differences in huemul δ^{15}Ncollagen values are of greater magnitude. With the exception of one of the samples from Cerro Tamango, which constitutes an outlier, the samples from closed environments present δ^{15}Ncollagen values of less than 2‰, while the samples from open environments are all above this value. As mentioned, the Mann–Whitney test does not

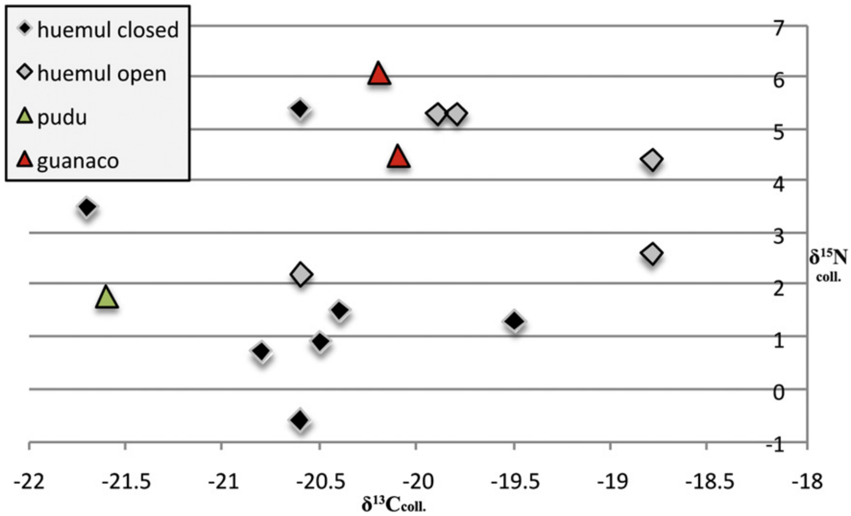

Fig. 3. δ^{13}Ccollagen and ä^{15}Ncollagen values for herbivore samples from Aisén.

show statistically significant differences between the medians, perhaps due to small sample sizes, although there are important differences in average values (1.81‰ for closed environments vs. ± 3.96‰ for open environments, Table 3).

3.3. Inter-specific variation

In Table 4 we present the statistical analysis of differences between the medians for the Patagonian herbivore species with a global $p = <0.001$, which lends strength to the following pair-wise comparisons. In Fig. 5 we present box-plots for δ^{13}Ccollagen and δ^{15}Ncollagen values for Patagonian herbivore species (univariate box-plots were selected because many of the samples do not have information for C and N).

A first interesting observation is that Huemul cannot be differentiated from steppe guanaco on the basis of δ^{13}Ccollagen values, indicating the absence of a 'canopy' depleted signature. It must be mentioned that the conservative Bonferroni correction applied to the test results in Table 4 impedes discrimination of huemul and forest guanaco δ^{13}Ccollagen medians, since these samples are statistically different before applying this correction (shown in parentheses in Table 3). This may be of exploratory value pointing to predatory differences between huemules and guanacos living within the forest or near its margins (as already suggested).

The δ^{15}Ncollagen median for huemul is statistically different to the median for steppe guanacos (the only species available for comparison; Table 4). We suggest this difference is related to differences in N availability between steppe and forest settings (cf., Craine et al., 2009). Data for pudu is very limited and shows relatively depleted δ^{13}Ccollagen and δ^{15}Ncollagen values; this is consistent with expectations derived from ecological literature (Iriarte, 2008).

4. Discussion: isotopic ecology of Patagonian herbivores

Even though the results presented here are based on a small number of data, it is noteworthy that this is the first systematic record of isotopic information for huemul from Chilean Patagonia. Considering the huemul's low population density and its behavior, it is a species that is difficult to sample nowadays. This information is valuable in the identification of preliminary patterns and for generating hypotheses. Additionally, the high frequency of archaeological findings in western Patagonia suggests that this resource constituted a recurrent game for hunter-gatherer groups inhabiting the forests (Mena, 1983; De Nigris, 2004; Mena et al., 2004; Fernández, 2010; Fuentes and Mena, 2010). For this reason, the isotopic information presented is crucial for the understanding of human dietary variability in western Patagonia.

4.1. δ^{13}Ccollagen: canopy effect on temperate forests

We have suggested that huemul cannot be differentiated from steppe guanaco on the basis of δ^{13}Ccollagen values. This indicates the absence of a 'canopy' depleted signature. Two alternative hypotheses can be suggested:

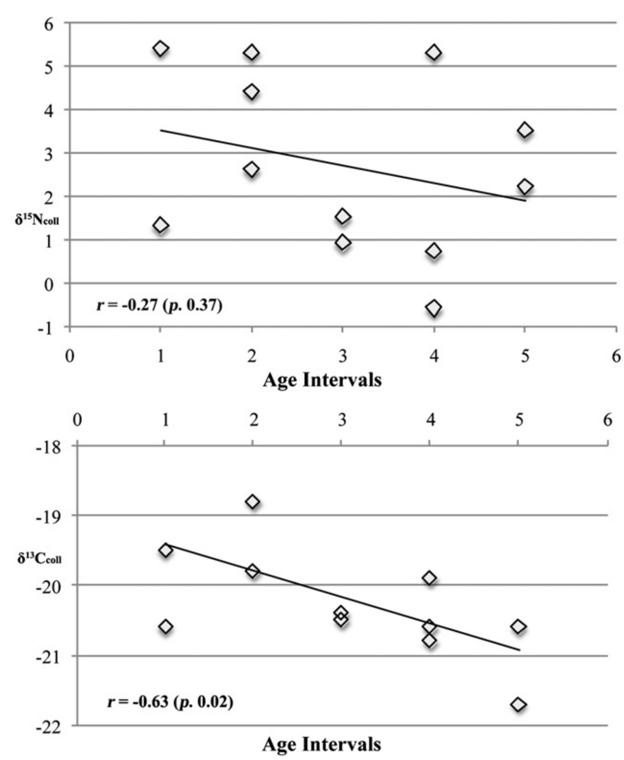

Fig. 4. Relation between δ^{13}Ccollagen and δ^{15}Ncollagen values with chronology for huemul samples.

Table 3
Descriptive statistics for huemul samples from closed and open areas. Mean values are highlighted in bold.

	Closed forest huemul	Open forest huemul
δ^{13}Ccollagen		
N	7	5
Minimum	−21.7	−20.6
Maximum	−19.5	−18.8
Mean	**−20.58**	**−19.58**
Standard deviation	0.64	0.77
Median	−20.6	−19.8
δ^{15}Ncollagen		
N	7	5
Minimum	−0.6	2.2
Maximum	5.4	5.3
Mean	**1.81**	**3.96**
Standard deviation	1.99	1.47
Median	1.3	4.4

(a) The cold and high-latitude forests of Patagonia do not produce a significant canopy effect.
(b) The foraging behavior of the huemul does not regularly include plants affected by the canopy effect.

This reproduces to some extent recent debate regarding cervid species from the northern hemisphere (Stevens et al., 2006; Drucker et al., 2008, 2010). Information available for guanacos is relevant for this issue (Barberena et al., 2009, 2010; Tessone, 2010) since, based on relatively large sample sizes, steppe guanacos can be statistically differentiated from forest guanacos, which would display an incidence of the canopy effect (Table 4, Fig. 5). This finding indicates that the canopy effect does not have a significant incidence in huemul, whereas it appears to have an effect on guanacos inhabiting the forests or forest borders, therefore lending support to hypothesis (b).

This allows us to suggest that, even though the huemul effectively inhabited forested spaces, they did not mainly consume closed-canopy plants. This is consistent with proposals for red deer (*Cervus elaphus*) in Europe: "… there is a difference between living in a forest and feeding in a forest, thus occupancy within a forest habitat does not necessarily result in low faunal δ^{13}C" (Stevens et al., 2006:16). Studies of this European species mark a preference for open spaces within the forest ecosystem (Latham et al., 1999).

In the case of the huemul, this could also indicate the importance of open areas within the forest. It is important to note that all current dietary studies (Colomés, 1978; Galende et al., 2005; Vila et al., 2009, 2010) describe the huemul as an understory browser, with a tendency to consume bushes like chilca (*Fuchsia magellanica*)

Table 4
Kruskal–Wallis and Mann–Whitney test results of differences between δ^{13}Ccollagen and δ^{15}Ncollagen medians (in parenthesis, *p* value before applying the Bonferroni correction). Significant values are highlighted in bold.

δ^{13}Ccollagen (Kruskal–Wallis, Mann–Whitney post-hoc)	Steppe guanaco (*N* = 60)	Pudu	Forest guanaco
Huemul (*N* = 12)	$p = 1$	$p = 0.2293$	$p = 0.02$ ($p = <0.005$)
Pudu (*N* = 2)	$p = 0.1186$	–	$p = 1$
Forest guanaco (*N* = 11)	**$p = <0.001$**	–	–
δ^{15}Ncollagen (Mann–Whitney)			
Huemul (*N* = 12)	**$p = <0.001$**	–	–

or maitén (*Maytenus boaria*). Given the restricted nature of the home range of these herbivores (Gill et al., 2008; Corti et al., 2009), and the important distance that separates them from the steppe in many of the cases that have been considered, the existence of a highly selective and biased foraging behavior towards plants from *open areas within the forest* arises as an adequate explanation for the δ^{13}Ccollagen values. The characteristics of the Aisén forest, emplaced on mountain ranges, produces important altitudinal differences in vegetation cover due to the existence of an important gradient in temperature, allowing access to open spaces within the forest and making the former explanation likely.

In synthesis, a comparison of the δ^{13}Ccollagen values for the herbivore species analyzed indicates that, globally, the huemul does not exhibit an incidence of the canopy effect. Contrarily, the two samples of pudu present relatively low δ^{13}Ccollagen values with respect to the huemul and guanacos from open environments, being within the range of values for the guanacos from forested environments (Fig. 5). This is consistent with the influence of the canopy effect in this species, as it also is with the available ecological information (Iriarte, 2008). This could mark a greater foraging selectivity on behalf of the huemules, which leads them to select plants from open spaces within the forest in their diet.

Based on historical accounts, some authors have suggested that the huemul was an inhabitant of the eastern steppe plains during pre-Hispanic times (Díaz, 1990; Díaz et al., 2007). Our results, indicating the regular consumption of plants from open areas within the forest, may be seen as consistent with this suggestion. Nevertheless, the abundant zooarchaeological record from the steppes of southern Patagonia fails to support this hypothesis, since there are practically no huemul bones (v.g., Borrero, 1990, 2001:28; Miotti, 1998; Mengoni Goñalons, 1999; Bourlot, 2009). The few cases recorded correspond basically to isolated horn specimens that can be better explained as long-distance transported artifacts (cf., Cruz et al., 2010), rather than consumption of locally available resources.

4.2. δ^{15}Ncollagen: isotopic signature of forested ecosystems

The huemul δ^{15}Ncollagen values present a wide range of variation with an average of 2.6‰ and a tendency towards very low values. As mentioned in the introduction, studies in soil isotopic ecology and plant communities in cold-forested environments have registered an important depletion of $\delta^{15}N$ values due to the low availability of N in these ecosystems (Garten, 1993; Craine et al., 2009). This explains the low $\delta^{15}N$ values recorded in animal communities that inhabit closed settings, including the case of certain cervid species (Rodière et al., 1996; Bocherens, 2000; van Klinken et al., 2000). According to this, we suggest that the low δ^{15}Ncollagen values presented for huemul are produced by the low availability of N in the forested environments of Aisén. In this sense, these values provide ecological and geographical information of the association with certain forested ecosystems.

The high moisture that predominates in Aisén could offer an alternative explanation for the depleted δ^{15}Ncollagen values if we take into consideration that, in certain contexts, a negative correlation with humidity has been registered (Gröcke et al., 1997; Schwarcz et al., 1999; Pate and Anson, 2008). Nevertheless, we consider this explanation as inadequate in contexts where mean annual precipitations exceed 400 mm, as in this case (Sealy et al., 1987; Drucker et al., 2003; Stevens et al., 2006; see also Cormie and Schwarcz, 1996).

The δ^{15}Ncollagen values presented for huemul and pudu can be compared with an important body of data published by Tessone and Belardi (2010; see also Tessone et al., 2009; Tessone, 2010) for guanacos from the steppe, a short distance away from Aisén. The huemul values and the sole pudu sample analyzed overlap. On the other hand, this range virtually does not overlap with guanaco

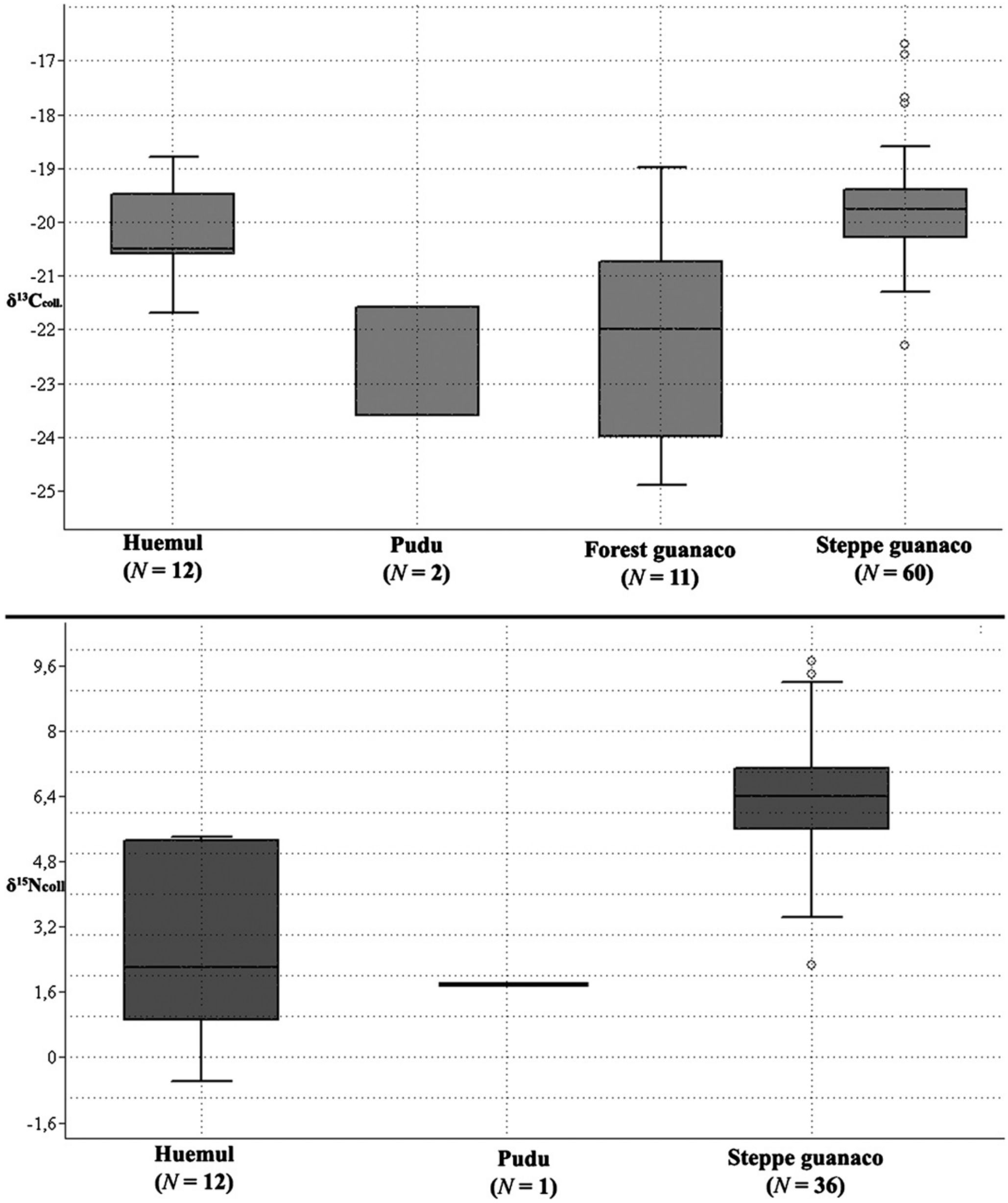

Fig. 5. Box-plots for δ^{13}Ccollagen and ä^{15}Ncollagen values for Patagonian herbivore species.

δ^{15}Ncollagen values, which are considerably enriched (Fig. 5). This inter-specific comparison reinforces the low N availability in the soils of cold-forested environments as the cause of the low δ^{15}Ncollagen values in huemul. In this way, δ^{15}Ncollagen values provide an isotopic marker of ecological association with cold-forest ecosystems, complementing δ^{13}Ccollagen, which has been proposed as a geographical marker for guanacos that inhabited the forest (Barberena et al., 2009).

5. Conclusions and implications for human paleodietary studies

We have presented a set of isotopic data for huemul from the southernmost Chilean forests. Contrary to the initial expectations, our analysis indicates that there is no evidence of a significant incidence of the canopy effect, which could be due to an ecological selection that drives the huemul to focus its predation on open sectors within the forest ecosystems. As mentioned beforehand, this is consistent with reconstructions developed for other cervid species. On the other hand, a wide range of δ^{15}Ncollagen values for huemul was verified, although with a minimum superimposition with respect to guanaco's range (Tessone and Belardi, 2010; Tessone et al., 2011). We suggest this reflects the low N availability in cold-forest soils and provides an important ecological indicator (cf., Rodière et al., 1996; Drucker et al., 2003). Therefore, δ^{13}Ccollagen values for huemul do not show an unambiguous canopy effect whereas low δ^{15}Ncollagen values provide an evidence of foraging in N-depleted forest settings. At face value, these findings can be seen as incompatible. Nevertheless, C and N biochemical cycles are conditioned by different regulating factors (Ambrose, 1991; van der Merwe and Medina, 1991; Cormie and Schwarcz et al., 1996; Ehleringer and Cerling, 2001; Drucker et al., 2008, 2010; Craine et al., 2009; Ugan and Coltrain, 2011), not completely understood yet, and may not vary in conjunction.

The zooarchaeological record from the western Patagonian forests indicates that human groups inhabiting the area regularly hunted and consumed huemul (Mena, 1983; Belardi and Gómez Otero, 1998; De Nigris, 2004; Mena et al., 2004; Méndez et al., 2006; Fernández, 2010). Therefore, the data assemblage presented here is relevant for the development of paleodietary studies on human remains. The information presented indicates that, in the context of terrestrial food chains, the consumption of huemul could be distinguished through δ^{15}Ncollagen compositions, generating low values in the human samples. Huemul δ^{13}Ccollagen values are too similar to those of the guanaco (the main terrestrial resource in the steppe) to allow differentiating the consumption of one species versus the other.

In the case of the western coastal environments of Aisén (Fig. 1), where guanaco was not available, marine resources constituted the principal alternative source of protein. Considering that marine mammals and birds are highly enriched in ^{13}Ccollagen and ^{15}Ncollagen in comparison to terrestrial animals (Barberena, 2002; Zangrando et al., 2004; Borrero et al., 2009), the isotopic discrimination of the consumption of huemul could be feasible, depending on the magnitude of its consumption by humans in the past.

Abundant existing ecological and genetic information indicates an important geographic and demographic retraction experienced by the huemules in recent times (Díaz and Smith-Flueck, 2000; Vila et al., 2006; Corti et al., 2009). The preliminary isotopic information here presented is relevant in the context of management decision of this species that is currently in danger of extinction. Corti et al. (2009) recommend the necessity to generate connectivity and increase the gene flow between different huemul subpopulations in Aisén, thereby emphasizing the inclusion of adequate geographical corridors for the species. Our isotopic data indicates, in concordance with the available ecological information, that during the Late Holocene, the huemules exercised a marked predatory selection towards open environments within the forest ecosystems. We consider that the long-term information offered by the archaeological record provides the necessary context for decision-making conducive towards the preservation of *Hippocamelus bisulcus* in Patagonia.

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