Ultrasound speed in red deer antlers: a non–invasive correlate of density and a potential index of relative quality

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Abstract
Ultrasound speed in red deer antlers: a non–invasive correlate of density and a potential index of relative quality. Deer antlers can be used as an index of individual performance both in ecological and productive contexts. Their quality is often measured only by their biometrical features, such as size, asymmetry or weight. Mechanic characteristics cannot normally be measured without destroying the antler and hence losing the commercial value of the trophies. Here, we studied ultrasonic velocities, density, and tensile strength across various sections of cast antlers of Iberian red deer (*Cervus elaphus hispanicus*). We found that the speed value depended on the section of the antler and the propagation direction. For antler sections, velocities were lowest for mid–beam and highest for brow tine. Results were similar for density and indirect tensile strength, probably related to differences in functionality among antler sections. Density explained most of the variability of ultrasound–speed. The time elapsed from antler shed affected density more than ultrasound speed. The indirect tensile strength showed a non–linear, decelerating relationship with ultrasound speed. We discuss the applications of ultrasound speed as a non–invasive tool to measure density and physical properties of antlers and antler sections, and their potential use as an index of quality.

Key words: *Cervus elaphus hispanicu*s, Deer antlers, Deer population management, Hunting trophies, Iberian red deer, Ultrasound

Resumen
Velocidad de ultrasonidos en las cuernas del ciervo ibérico: una medición no invasiva de la densidad y un posible índice de la calidad relativa. Las cuernas de ciervo pueden usarse como un índice de calidad individual, tanto en contextos ecológicos como productivos. Por lo general, la calidad de las cuernas se mide solo por sus características biométricas, como la longitud, la asimetría o el peso, mientras que las características mecánicas normalmente no pueden medirse sin destruir la cuerna y, por lo tanto, sin perder el valor comercial de los trofeos. En este trabajo medimos la velocidad de transmisión de ultrasonidos en distintas secciones de cuernas de desmogue de ciervo ibérico (*Cervus elaphus hispanicus*), junto con la densidad y la resistencia a la tracción. Encontramos que el valor de la velocidad depende de la sección de la cuerna y de la dirección de propagación. En relación con las secciones de las cuernas, las velocidades más bajas se obtuvieron en el tronco medio y las más altas, en las luchaderas. Los resultados fueron similares con respecto a la densidad y la resistencia a la tracción indirecta, probablemente debido a la diferencia de funcionalidad de las distintas secciones de cuerna. La densidad fue la principal causa de la variabilidad en la velocidad de los ultrasonidos. El tiempo transcurrido entre el desmogue y la medición afectó a la densidad más que a la velocidad de los ultrasonidos. La resistencia a la tracción indirecta mostró una relación curvilínea de desaceleración con la velocidad de los ultrasonidos. Analizamos las aplicaciones de la velocidad de los ultrasonidos como herramienta no invasiva para medir la densidad y las características físicas de las cuernas y de sus secciones, así como su posible utilidad como índice de la calidad.

Palabras clave: *Cervus elaphus hispanicu*s, Cuernas de ciervo, Gestión de la población de ciervos, Trofeos de caza, Ciervo ibérico, Ultrasonidos

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Introduction

Deer antlers are deciduous bony structures that males shed and re–grow each year (Gross, 1983). They are used as weapons in conspecific fights for female access (Clutton–Brock, 1982) and probably also as visual signals of dominance (Lincoln, 1972; Geist, 1966). This sexual character is particularly costly to grow and maintain (Andersson, 1994; Gaspar–López et al., 2008; Foley et al., 2012) and its development relates to tooth wear and potential lifespan (Carranza et al., 2004, 2008; Pérez–Barbería et al., 2015). Antler growth demands a great amount of calcium and phosphorus within a short period of time (Chapman, 1975), and therefore usually involves demineralization of the skeleton since the animal's daily intake cannot supply these minerals at the required rate (Chapman, 1975; Meister, 1956). Due to their high cost of growth, antlers may be used as an honest signal of individual quality (Weladji et al., 2005; Pérez–González et al., 2010), as an indicator of genes related to immune response (Ditchkoff et al., 2001), and as an example of sexual selection, particularly relating to competition between males (Lincoln, 1972; Gesist, 1966; Clutton–Brock et al., 1982). In addition, females may use these traits as reliable signals when choosing mates (Wong and Candolin, 2005). Deer antlers may encode information of the quality of the male based on their size and asymmetry (Mateos et al., 2008; Parsons, 1992), their body condition and body size (Kruuk et al., 2002), or the density of antler bone (Landete–Castillejos et al., 2007).

In the last decades, the impact of human activities on the environment has increased. Management of red deer populations in Europe, and particularly in Spain, has intensified and better procedures are needed to assess their consequences. The study of antler characteristics may provide important information not only on the bearer but also concerning environmental variations (Weladji et al., 2005; Carranza and Vargas, 2007; Foley et al., 2012). Climate change, for instance, may affect the size of antlers (Torres–Porras et al., 2009) and likely other antler features. Until recently, length, asymmetry and weight or density have been studied external measurements (Swaddle, 2003; Mateos et al., 2008). To study mechanical properties, destructive techniques (Landete–Castillejos et al., 2007) have been studied but they present limitations. Antlers are trophies in hunting management, valuable for managers and hunters, and cannot be destructively analyzed without losing their commercial value. A new technique is thus needed to determine the physical/mechanical properties without decreasing their value. Ultrasound velocity measures could be an interesting tool for this purpose.

Ultrasound is a travelling mechanical vibration whose speed depends on the mechanical properties of the medium (Njeh et al., 1997). It is an alternative to conventional absorptiometry to obtain information on structure and density (Gluer et al., 1993; Tavakoli and Evan, 1991). The speed of ultrasonic waves has been extensively used to measure both the elastic properties of bone and its proportion of compact and porous tissue (e.g. Bonfield and Tully, 1982; Hartman et al., 2004; Drozdzowska and Pluskiewicz, 2005).

Some of the advantages of ultrasound diagnostic techniques are that they are straightforward, non–invasive, and non–destructive (Bowling and Frank, 1981; Laugier, 2012). For these reasons, they have been extensively used for bone diagnosis (Lakes et al., 1986; Lasaygues and Pithioux, 2002; Laugier, 2006; Tonni et al., 2012) but to our knowledge, ultrasound has been used to describe the characteristics of antler tissues in only one study (Lees, 1982).

Here we studied the relationships between the speed of ultrasound through antler bone and the physical/mechanical properties of antlers. Our objective was to investigate the relationship between ultrasound speed and antler density and mechanical properties, as well as the possibility to use ultrasound as a non–invasive procedure to assess the quality of red deer antlers. This work led to the design of equipment for measuring ultrasound speed in antlers. It has been registered as a utility model (Del Río et al., 2012).

Methods

Study area

Antlers were obtained from free–ranging red deer from four estates in Extremadura, a region in south–western Spain. The study area was divided into two zones, with two states located in the Sierra de San Pedro and the other two in the Monfragüe National Park. The vegetation cover in these estates is open–managed forest 'dehesa' (main tree species *Quercus rotundifolia* and *Q. suber*) and Mediterranean scrub (genus *Cistus, Erica, Arbutus, Mirtus, Pistacia, Phyllyrea*).

Procedure

Antlers were collected in the field after being shed at the end of March and beginning of April over three consecutive years. We used 29 antlers from different individual males. Because we only had access to the shed antlers from the field, not to the males, we could not register the age of the stag (a rough estimation from antler size and shape ranged 2–4 years; see e.g. Clutton–Brock et al., 1982; Gross, 1983). Most antlers were measured within one year after they were cast. For some, however, between one and four years elapsed between casting and measurement. This allowed us to see the effect of time from casting by including in models a dichotomous variable 'recently shed' with level 1 = less than or equal to 1 year elapsed between casting and measurements, and level 0 = more than 1 year between casting and measurements. To study properties at different antler sections, antlers were cut in 4–7 (depending on the size and morphology of the antler) fragments (~15 cm length, 3–4 cm diameter) at the positions shown in fig. 1. Thus, we obtained a total of 150 antler specimens or fragments belonging to 29 antlers from different individual stags. However, a variable number of antler fragments were used for measurements, and for statistical models we used only

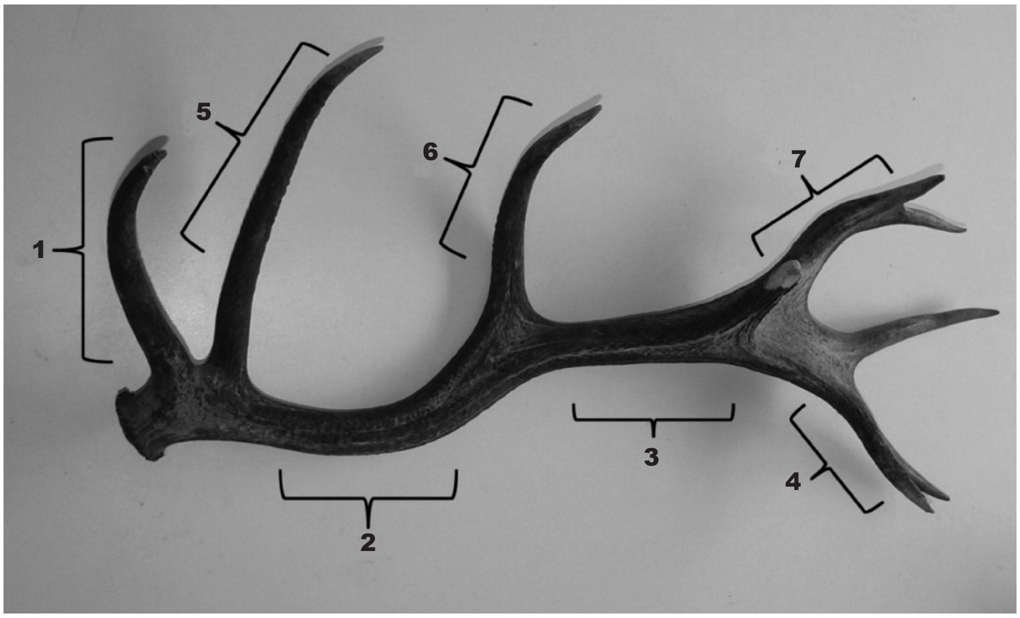

Fig. 1. Position of antlers sections: 1, brow tine; 2, low beam, or section between bez tine and trez tine; 3, mid beam, or section between trez tine and crown; 4, palm beam; 5, bez tine; 6, trez tine; 7, crown tine.

Fig. 1. Posición de cada sección de las cuernas: 1, luchadera; 2, zona media baja o sección entre las luchaderas y la punta central; 3, zona media alta o zona entre la punta central y la corona; 4, palma; 5, contraluchadera; 6, punta central; 7, punta de corona.

those four sections (numbers 1 to 4 in fig. 1) present in all the antlers as repeated measures (see below). We established a measurement protocol to allow all the following tests to be performed on each fragment. First we determined the thickness of the antler bone tissue by means of various measurements on both surfaces of the cut with an electronic caliper. The time of flight of ultrasonic wave was then measured and the ultrasound velocity was determined. We determined the density, porosity, and strength.

Ultrasound measurements

In order to apply this method for quality assessment of antlers in different situations, it is essential to use the through transmission (T–T) technique with a portable, compact, robust, and easy to use device (Steinkamp BP–V with transducers of 50 kHz nominal frequency). The low frequency of transducers (50 kHz), together with the high voltage pulse applied to the emitting transducer (600 Vpp), ensures that the wave will be transmitted through the material despite the high attenuation in the porous material in the interior of antlers. We used plasticine as coupling material to ensure the correct transmission of the wave between the transducers and the rough antler surface, checking the received waveform on an oscilloscope, subtracting in each measure the propagation time of the wave in the coupling medium. This attenuation, below 0.1 MHz, particularly in porous material such as bone or antlers, is relatively insensitive to frequency and above 1 MHz signal–to–noise becomes a significantly limiting factor (Langton and Njeh, 2008). It also is important to consider the length (~ 15 cm) and thickness (~ 3 cm) of the samples to choose the transducers. For these two reasons, porosity and sizes of samples, we selected transducers of 50 kHz to detect the signal into the received transducer.

When using transducers 50 kHz and with measurements made on samples sized an order of magnitude of the wavelength of the ultrasonic signal, overlapping of waves might occur. However, using an oscilloscope we verified that the start of the first cycle allowed the device to determine (with accuracy of 0.1 µs) the time taken for the ultrasonic signal to reach the receiver from the transmitter.

The value of the flight time of the signal between the transmitter and the receiver was obtained through measurements made in each fragment, over the central part where the thickness was previously measured with an electronic caliper. For each test, the measurements were repeatedthree times, withdrawing and returning the transducers to the fragment every time. Therefore, we obtained a total of nine measurements obtained in three different tests. From these we obtained the corresponding mean and associated error. The standard errors obtained for each measurement ranged between 5–10 %. However, to further ensure the reproducibility of the measurements obtained following the method described above we carried out a specific analysis with three different sets of measures with 50 kHz transducers. In all cases we obtained ratios between the speeds of each series next to the unit (0.98–1.10). These results allow us to ensure the reproducibility of measurements within the range that provides the associated error.

To obtain the maximum information on the speed of propagation of ultrasound in the antler samples, for each fragment we made measurements in the three principal axes: the longitudinal axis (V_L, in the direction of the antler beam) and the two transverse axes of the oval transversal shape of the antler (the mayor axis: V_{T1}, and the minor one: V_{T2}), taken at the center of the fragment.

Non–ultrasound measurements

For each sample we measured the density, porosity, and strength. For density and porosity we applied an adaptation of the European standard test used for calculating these properties in natural stone (EN1936, 2006). The bulk density (γ) is determined by the equation:

$$\gamma = [W_d / (W_{sat} - W_{sum})] \cdot \gamma_{H2O} \qquad \text{(Eq. 1)}$$

where, W_d is the dry weight (after removing ambient moisture using an oven at 40º for 24–48h until weight stabilized), W_{sat} is the weight saturated with methanol, W_{sum} is the weight of the saturated sample submerged in the hydrostatic balance and γ_{H2O} is the water density.

We defined the bulk porosity ($\rho_{\%}$) as the ratio between the volume of accessible pores and the total sample volume, with the former parameter being determined by the weight gain of the sample saturated with methanol divided by the density of methanol. The formula used is thus:

$$\rho_{\%} = \frac{[(W_{sat} - W_d) / \gamma_{met}]}{[(W_{sat} - W_{sum}) / \gamma_{H2O}]} \times 100 \qquad \text{(Eq. 2)}$$

The proportion of cancellous and compact areas ($A_{ca/co}$) was determined by measurements made in both areas, for a selected group of specimens from all sections of each antler. These measurements were made in four different directions of the outer and the inner diameters of the two sections of each sample, obtaining the average value and the area assuming circular sections for the samples. Although there is a transition zone between compact and cancellous areas, the most contrasting line of separation occurred between cancellous and the rest (see fig. 2), and hence for practical purposes we decided to include the transition zone in the compact measure.

To determine the tensile strength we elaborated an indirect tensile strength (ITS) test (the Brazilian test) as the method to use for the basic mechanical characterization of the material. The strength trials had to be designed bearing in mind the need to relate this mechanical property with the speed of ultrasound. We therefore needed to know the strength of the entire fragment of antler (both the compact and the porous parts) because the speed is measured through these two concentric layers.

Initially, we attempted to use the bending or flexural test (EN12372, 2006) as most representative of the stresses to which antlers are subjected in conspecific combat. This test was found to be unusable because it was impossible to obtain fragments with sufficiently flat and smooth support surfaces for forces to be applied to them without causing movement of the fragment. A further problem was the plasticity of the material, which in some cases the test equipment could not be used without fragment breaking, a limitation acknowledged in other studies (Landete–Castillejos et al., 2007).

We then tried compression (EN1926, 2006) and point load stress tests, but both presented major difficulties in implementation, in the former because the fragment had a curved geometry, and in the latter because the steel cones that are used to press on a point in the material dig into the antler bone without breaking the sample. This variable is an adaptation of testing procedures used to determine the quality of materials such as rocks, concrete and bituminous mixtures (Balbo, 2013; Sivakugan et al., 2014).

The indirect tensile strength test (ITS) or Brazilian test is used to determine the tensile properties of the samples. This test allows a relatively wide range of values for specimen geometry, defined in terms of length to diameter ratio, and loading rates, defined as either time to failure or stress rate It is hence a useful procedure when specimens are approximately cylindrical, as is the case for antler sections. The indirect tensile strength of cylindrical specimens was calculated by subjecting them to a compressive force applied in a narrow band along their entire length. The result of the force resulted from the orthogonal traction making the sample break under tension. The trials were kept as close as possible to the requirements of the published norms (EN12390–6, 2009), transmitting the stress to the antler sample through metal plates about twice the diameter of the antler fragment. Thus, in our case, the stress was applied diametrically across the circular cross section, in the direction of the axis, corresponding to that of the measurement of the V_{T2} speed of ultrasound (fig. 2).

The indirect tensile strength (ITS) was determined by the equation:

$$ITS = (2P_{max}) / (\pi e \phi) \qquad \text{(Eq. 3)}$$

where P_{max} is the maximum load applied and *e* and ϕ are respectively the height and the base diameter of the cylindrical sample.

Statistical analysis

Statistical analyses were performed with the computer software packages SPSS. Relationships between variables were explored using Spearman correlation coefficients, since not all correlations were lineal.

Descriptive statistics were carried out for all the fragments of the antler but the models were conducted with only the main four antler fragments or

Fig. 2. Experimental setup for indirect tensile strength test (left). Result of its application to a cylindrical fragment from the antler of one of the deer (right).

Fig. 2. Dispositivo experimental para la medición de la resistencia a la tracción indirecta (izquierda). Resultado de aplicar esta técnica a una de las secciones de cuerna con forma cilíndrica (derecha).

sections (brow tine, low beam, mid beam and palm beam; see fig. 1).

However, as the four fragment measurements in the same antler are not independent cases, to obtain predictive models on the real relationship between the V_{T2} and the antler characteristics, we conducted a General Linear Mixed Model (GLMM) with V_{T2} as the dependent variable and antler fragments as repeated measures. The chosen type of the covariance structure for the repeated effect was *Toeplitz: Heterogeneous,* which assumes heterogeneous variances and heterogeneous correlation between sections. Estate identity was introduced as a random factor.

Explanatory variables were the bulk density, the indirect tensile strength (ITS) and the categorical variable 'recently shed' that controlled for the time elapsed since antler shedding until the ultrasound measurement was carried out, in two levels: recent, i.e. less than one year since casting (level = 1), and not recent, i.e. between 1 and 4 years since casting (level = 0). We used this variable in two levels instead of all the years after exploring its relationships with V_{T2}, bulk density and ITS, as well as the sample size for each year and the adequacy of models. Tensile strength (ITS) was fitted as an orthogonal quadratic relationship, as suggested in the explorative stage of analyses. The percentage of porosity was so highly correlated with density that it cannot be considered a different measure. Similarly, we avoided introducing the cancellous–to–compact area ratio in the analysis because it was also highly correlated with density and it was measured in fewer cases (r_s=–0.84, see table 2; quadratic regression: $R^2 = 0.652$, $F_{2,41} = 38.46$, $P < 0.001$), which greatly reduced the sample size and produced a serious collinearity problem (mainly increasing the standard error for the estimate, so that any effect of the problematic variable on V_{T2} cannot be detected).

In the model, we first introduced the main effects and their meaningful double interactions. We then removed the non–significant interactions step–by–step using the p–values. The coefficients of the model were estimated using REML (restricted maximum likelihood), and the degrees of freedom were calculated following Satterthwaite's approximation.

Normality and homoscedasticity were verified by using bivariate plots. The dataset was checked for outliers using plots of fitted values against residuals and one odd record was removed.

Results

Descriptive analysis of the variables

Table 1 shows descriptive information on the ultrasonic velocities under each of the three axes measured in the fragments of antlers and the rest of antler measurements included in this study. The mean value of the velocity in the longitudinal axis (V_L) appeared higher than the velocities in the transversal axes (V_{T1}, V_{T2}), both transversal velocities being very similar to each other. This anisotropy is due to various factors such as the structure of concentric cylindrical layers of the antler bone, as occurs in other materials with similar geometry and structure (tree trunks, bones, etc.), and other structural characteristics such as orientation of mechanically efficient microstructures (collagen and mineral particles).

The physical and mechanical variables measured ITS, bulk density (γ), porosity (ρ) and cancellous vs compact area ratio ($A_{ca/co}$) showed considerable variability, indicating the large differences between antler fragments, mainly in their relative composition of cancellous and compact tissue and other related properties: indirect tensile strength, density, bulk porosity, and cancellous–to–compact area ratio.

Correlations between variables

The ultrasound velocities were highly correlated, obtaining the following values of Pearson's correlation coefficient: r(V_L,V_{T1}) = 0.709, r(V_L,V_{T2}) = 0.825 and r(V_{T2},V_{T1}) = 0.905. For subsequent analyses we chose the V_{T2} measurement because: (1) using V_L implies sectioning the antler, which is not possible in a non–destructive procedure to measure deer antler quality; (2) V_{T1} provides a smaller plain surface of measure which may make transducer coupling with the sample difficult.

Table 2 shows the Spearman correlations between ultrasound and non–ultrasound variables. The ultrasound speed (V_{T2}) was positively correlated with indirect tensile strength (ITS) and bulk density (γ) and negatively with porosity and cancellous to compact area ratio ($A_{ca/co}$). Similarly, the physical variables were correlated with each other. Porosity was so highly correlated with bulk density (r_s= –0.94, table 2) that the two could be considered the same variable, so we only included bulk density in the regression analyses below.

Differences between antler sections

Table 3 and Figure 3 show the differences between antler sections in ultrasound speed and other physical characteristics.

Ultrasound speed, V_{T2} (m/s) was higher in the brow tine and palm beam, whereas the mid beam presented lower values (fig. 3A). Post–hoc multiple comparisons (using Sidak correction after applying a repeated measured model) showed significant differences between brow tine and low beam (233 ± 55, df = 28, P = 0.001) and between brow tine and mid beam (356 ± 65, df = 34, P < 0.001).

Bulk density (g/cm^3) followed the same pattern: with the brow tine being the denser antler section, decreasing towards the distal sections and increasing again just in the palm beam (fig. 3B). There were significant differences between brow tine and the other sections (with low beam: 0.23 ± 0.03, df = 42, P < 0.001; with

Table 1. Summary description of the statistical data obtained for ultrasonic velocities and other physical and mechanical variables studied in the antlers.

Tabla 1. Descripción resumida de los datos estadísticos obtenidos para las velocidades de los ultrasonidos y otras variables físicas y mecánicas estudiadas en las cuernas.

	N	Min	Max	Mean	SD
V_L (m/s)	25	3,076	3,877	3,482	224
V_{T1} (m/s)	25	1,781	2,899	2,394	296
V_{T2} (m/s)	108	1,700	3,121	2,389	278
ITS (MPa)	120	0.86	19.23	5.53	3.83
γ (g/cm^3)	130	0.79	1.64	1.19	0.21
ρ (%)	130	13.73	57.10	32.73	9.86
$A_{Ca/Co}$	79	0.06	2.52	0.75	0.56

Table 2. Spearman's correlation coefficient (r_s) between variables studied in antler fragments: N, sample size in each correlation. (All correlations are significant at 0.01 level, 2–tailed).

Tabla 2. Coeficiente de correlación de Spearman (r_s) entre las variables estudiadas en los fragmentos de cuernas: N, tamaño de la muestra en cada correlación (todas las correlaciones son significativas P < 0.01, 2 colas.

		V_{T2} (m/s)	γ (g/cm^3)	ρ (%)	$A_{Ca/Co}$	ITS (MPa)
V_{T2} (m/s)	r_s	1.00	0.68	–0.62	–0.51	0.58
	N	108	105	105	46	95
γ (g/cm^3)	r_s	0.68	1.00	–0.94	–0.84	0.70
	N	105	130	130	71	117
ρ (%)	r_s	–0.62	–0.94	1.00	0.78	–0.58
	N	105	130	130	71	117
$A_{Ca/Co}$	r_s	–0.51	–0.84	0.78	1.00	–0.83
	N	46	71	71	79	71
ITS (MPa)	r_s	0.58	0.70	–0.58	–0.83	1.00
	N	95	117	117	71	120

Table 3. Values (Mean ± SD) and simple size (N) obtained in each section of the antlers for the speed of ultrasound and the other physical–mechanical variables.

Tabla 3. Valores (media ± DE) y tamaño de muestra (N) obtenidos en cada una de las secciones de las cuernas para la velocidad de los ultrasonidos y del resto de variables físicas y mecánicas.

	V_{T2} (m/s)	γ (g/cm^3)	$A_{Ca/Co}$	ITS (MPa)
Brow tine	2,520 ± 260 (23)	1.33 ± 0.16 (25)	0.58 ± 0.49 (12)	7.69 ± 4.03 (23)
Low beam	2,330 ± 230 (25)	1.11 ± 0.16 (26)	1.06 ± 0.45 (12)	3.04 ± 1.64 (24)
Mid beam	2,210 ± 190 (25)	0.98 ± 0.11 (26)	1.45 ± 0.62 (12)	2.64 ± 1.56 (24)
Palm beam	2,360 ± 260 (18)	1.18 ± 0.15 (20)	0.56 ± 0.37 (11)	5.65 ± 3.05 (19)

mid beam: 0.36 ± 0.03, df = 42, $P < 0.001$; with palm beam: 0.18 ± 0.04, df = 48; $P = 0.01$); and between low and mid beam (0.13 ± 0.3; df = 40, $P < 0.001$).

In accordance with the decrease in density, the proportion of cancellous to compact tissue area increased toward distant sections, decreasing just in the palm beam (fig. 3C). The differences between brow tine and mid beam (–0.87 ± 0.23, df = 19, $P = 0.007$) and between palm beam and the two central sections (low beam: –0.50 ± 0.17, df = 22, $P = 0.04$; mid beam: 0.88 ± 0.20; df =16, $P = 0.003$) were significant.

Similarly, brow tine and palm beam were the sections with highest ITS (MPa), whereas the central sections such as low beam and mid beam were more fragile (fig. 3D). The differences between brow tine and low beam (5.05 ± 0.76, df = 21, $P < –0.001$) and between brow tine and mid beam (5.37 ± 0.85, df = 26; $P < 0.001$) were significant, and so were the differences between palm beam and the central sections of the antler (low beam: 2.52 ± 0.70, df = 23, $P = 0.008$; mid beam: 2.84 ± 0.62, df = 19, $P = 0.001$).

Relationship between ultrasound speed and antler mechanical properties

We used mixed model analysis with the different fragments of each antler as repeated measures to study the relationship between mechanical properties of antler fragments and ultrasound speed (table 4). Density had the highest effect to explain ultrasound speed (V_{T2}), but tensile strength (ITS) and its quadratic term were also significantly related to V_{T2}. Time elapsed since antler shedding (recently shed) showed a significant interaction with bulk density to explain V_{T2}, so that for a given density, antlers shed more than one year ago showed higher ultrasound speed (table 4; fig. 4).

Indirect tensile strength (ITS) showed a non–linear, decelerating relationship with ultrasound speed (V_{T2}) (table 4). As tensile strength of antler samples increased, the ultrasound speed did not increase with the same slope (fig. 5).

We did not find a significant variation between estates (i.e. areas or deer populations) in the relationships between antler features and ultrasound speed (table 4).

Discussion

Our results showed that the speed of ultrasound in antlers correlated significantly with the physical properties studied. We found the highest effect for bulk density, meaning that V_{T2} may be a good indicator of density. Therefore, the differences in ultrasound speed can inform about conditions during antler development, such as mineralization (Currey, 1987, 1990), likely related to diet (Landete–Castillejos et al., 2012; Gómez et al., 2013), and leading to variations in density. Measuring ultrasound speed may be more feasible in practice than measuring density, particularly because of its non–destructive procedure. Our results also indicate however, that ultrasound speed may be a less sensitive than density due to variations caused by drying up or any degradation of organic matter remaining in the antler, when they are collected from the field and stored in indoor conditions. For recently shed antlers, high weight (and hence the estimated density) may be due to water (Currey et al., 2009) or any non–mineral tissue content within the bone matrix that might degrade with time. As ultrasound speed is lower in water or soft tissue than in solid material (e.g. in wood, Oliveira et al., 2005) it may be a better indicator of quality (i. e. mineralization) than density. Further experimental studies should focus on the variations of the relationships between ultrasound speed, mineral content and other variables throughout the desiccating/degradation process of individual antlers.

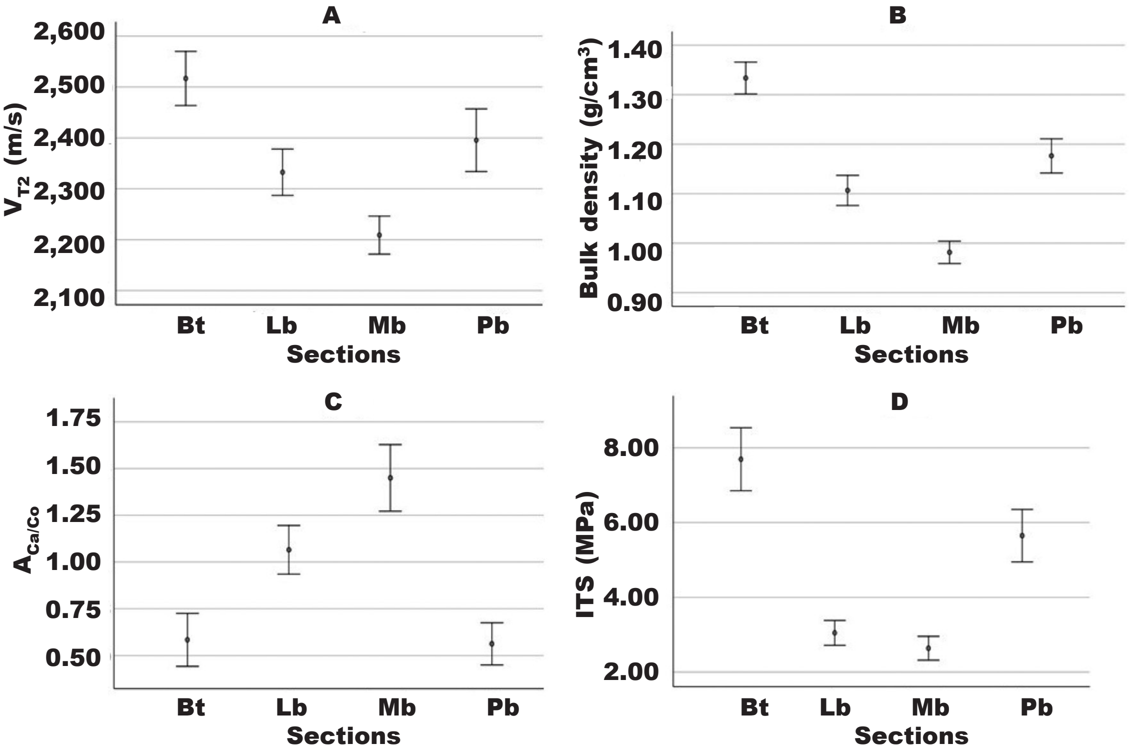

Fig.3. Differences in the speed of ultrasound V_{T2}, density, tensile strength and cancellous to compact area ratio, between sections at different parts of the antler: Bt, brow tine; Lb, low beam; Mb, mid beam; and Pb, palm beam. (The figure shows mean ± SD for each section).

Fig. 3. Diferencias en la velocidad de los ultrasonidos V_{T2}, la densidad, la resistencia a la tracción y la proporción entre el área esponjosa y el área compacta, entre diferentes secciones de la cuerna: Bt, la luchadera; Lb, la zona media baja; Mb, la zona media alta; Pb, la palma. (La figura muestra la media ± DE de cada sección).

We expected that ultrasound speed would be related to the resistance of antlers, in our case to tensile strength. Our results showed that this relationship is not linear and little predictive for values of tensile strength (fig. 5). We do not have a clear explanation for this nonlinear behavior. It may be related to the mechanical behavior of cylindrical structures such as the antler samples, for which the increase in section diameter may cause the total density to decrease (and therefore V_{T2} to decrease as well), keeping the value of ITS stable, because less strong material (cortical) is needed to maintain the same mechanical properties of the whole structure (Gere and Goodno, 2011). It is also worth considering that the equation for indirect tensile strength was designed to correct for the size of the specimen, assuming homogeneous features in the material. In the case of biological structures, the composition and density are not likely to be independent of the size of the specimen, so it may be that the ITS equation developed for materials does not capture these peculiarities. But tensile strength may also be related to the composition of antler bone, rather than simply density or mineralization. The mineral composition of antler bone may affect its mechanical properties without changing density and hence ultrasound speed. For instance, Landete–Castillejos et al. (2010) reported that some minerals, notably manganese (Mn), highly influenced the mechanical properties of antler bone (see also review in Picavet and Balligand, 2016). Therefore, ultrasound speed may not be a useful indicator of antler bone composition besides its effects on variations in density.

Values reported in the literature for physical and mechanical properties of deer antlers are variable and depend on the species (Chen et al., 2009). For the flexural strength of antlers, Landete–Castillejos et al. (2007) reported values between 81.9 and 103.7 MPa for cortical antler bone of free ranging and captive raised populations Iberian red deer, respectively. For hog deer (*Axis porcinus*) the reported value was 246 MPa (Kitchener in Chen et al., 2009). Chen et al. (2009) measured bending strength in cortical bone antlers of North American elk *(Cervus canadensis*) and found values of 197.3 ± 24.0 MPa, and 66.7 ± 10.7 MPa, for longitudinal and transversal axis, respectively. These authors also reported tensile strength va-

Table 4. Results of the general linear mixed model (GLMM) with bulk density (γ in g/cm^3) and indirect tensile strength (ITS in MPa) as the explanatory variables for ultrasound speed (V_{T2} in m/s). Time elapsed from antler cast to measurement was introduced as the categorical variable 'Recently shed' (reference level = 1, see Statistical analysis in Methods).

Tabla 4. Resultados del modelo lineal general mixto (MLGM) con la densidad aparente (γ en g/cm^3) y la fuerza de tracción indirecta (ITS, en MPa) como variables explicativas de la velocidad de los ultrasonidos en las cuernas (V_{T2} en m/s). El tiempo transcurrido entre el desmogue de la cuerna y la medición se introdujo como variable categórica, "Recently shed" (nivel de referencia = 1, véase el subapartado "Statistical analysis" en "Methods").

Fixed Factors	Estimate	S.E.	df	*t*	Sig.
Intercept	1,061.379	175.046	41.464	6.063	0.000
Recently shed	381.886	215.732	49.706	1.770	0.083
Bulk_density	1,137.478	176.301	31.783	6.452	0.000
ITS	54.988	22.476	53.988	2.446	0.018
Square_ITS	–3.803	1.572	50.125	–2.419	0.019
[Recently shed] * Bulk_density	–453.115	193.162	44.104	–2.346	0.024

Random Factors	Estimate	S. E.	Wald Z	Sig.
Variance between estimates	1,922.185	3,795.980	0.506	0.613

lues (direct) of 115.4 ± 16.6 MPa (longitudinal) and 20.3 ± 6.0 MPa (transversal) for antlers of the same species, and values from Currey (1990) of 158 MPa from red deer antlers. We did not find any studies using the ITS–Brazilian test for deer antlers. For small pieces (2 x 4 x 30 mm) of archaeological bones, this technique yielded values ranging between 60 MPa and 4 MPa for bone bulk densities between 2.0 g/cm^3 and 0.8 g/cm^3, respectively (Turner–Walker, 1995).

Values reported here for tensile strength of antlers, ranging from 0.86 to 19.23 MPa and with an average value of (5.5±3.8) MPa (table 1), are lower than those reported in the literature. Because we used a different technique to determine strength (indirect tensile) our results are little comparable with those of other authors who used flexural or direct tensile strength. Moreover, the fragments of specimens used in our case have different geometry and dimensions to those used by these authors (fig. 2). In fact, we performed the tests on antler specimens that included the complete structure with a compact outer layer and a highly porous inner layer, with cylindrical geometry of approximately 0.15 m length and 0.03–0.04 m in diameter at the base, while other studies only tested the compact part on different geometries and smaller fragments: Chen et al. (2009) on rectangles (25–30 x 3 x 2 mm) and Landete–Castillejos et al. (2007) on antler bars (50 x 2; 5 x 4 mm) both extracted samples only from the external compact tissue, thus leading to possible differences between axes and excluding any differences in cancellous *vs* compact composition of antlers.

Chen et al. (2009) reported a complete set of values for the static elastic modulus (destructive) obtained by different authors, ranging from 2.2 GPa for roe deer (*Capreolus capreolus*) (Currey, 1987) to 17.1 GPa for chital deer (*Axis axis*) (Rajaram and Ramanathan 1982). For Iberian red deer, mean values reported for the static elastic modulus are (Landete–Castillejos et al., 2007) 5.27 ± 0.33 GPa (free ranging population) and 6.87 ± 0.28 GPa (captive raised).

For a medium with infinite dimensions the wavelength of ultrasound can be established when its velocity (v) depends on the properties of the medium through which it is propagating and its mode of propagation, according to the following formula (Czichos et al., 2006):

$$v = (E/\gamma)^{1/2} \qquad \text{(Eq. 4)}$$

where E is the dynamic Young's modulus and γ is the density of the medium. Generally, this formula is used to estimate the value of Young's modulus of different materials using an indirect and non–destructive technique, (Del Río et al., 2007). Therefore, the values that we obtain through this equation are only indicative of the possible mechanical behavior of antlers respect to this elastic modulus. The average value of the young modules calculated with the Eq. 4 in our study for dynamic elastic modulus for Iberian red deer was: 5.9 ± 2.8 GPa, consistent with those obtained by the aforementioned authors.

The differences in the speed of ultrasound according to the direction of measurement and the

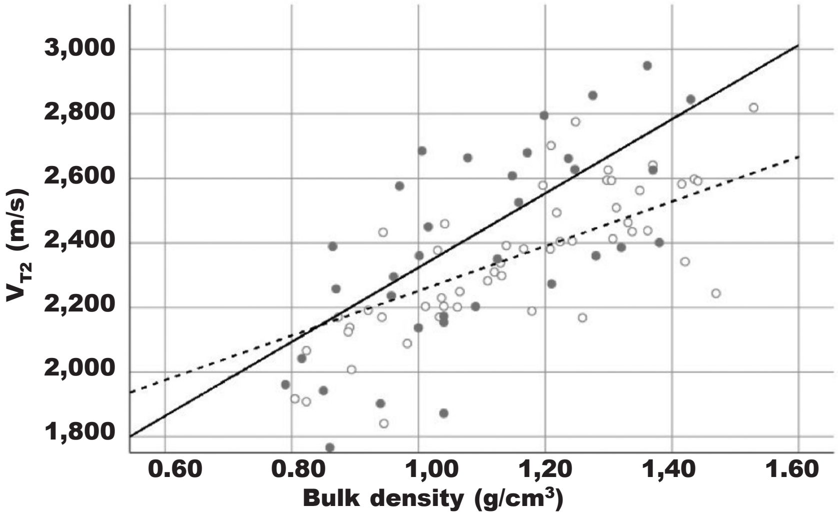

Fig. 4. Relationship between antler density and ultrasound speed (V_{T2}) for two groups of antlers according to time elapsed from shed to measurement: recently shed, open circles and discontinuous line; older antlers, black dots and continuous line. The figure shows raw data and prediction lines from the mixed model in table 4, with other variables fixed at their mean values.

Fig. 4. Relación entre la densidad de la cuerna y la velocidad de transmisión de los ultrasonidos (V_{T2}) en dos grupos de cuernas según el tiempo transcurrido entre el desmogue y la medición: cuernas recientes, puntos blancos y línea discontinua; cuernas más antiguas, puntos negros y línea continua. En el gráfico se muestran los datos sin tratar y las líneas de predicción del modelo mixto de la tabla 4 con las demás variables fijadas en sus valores medios.

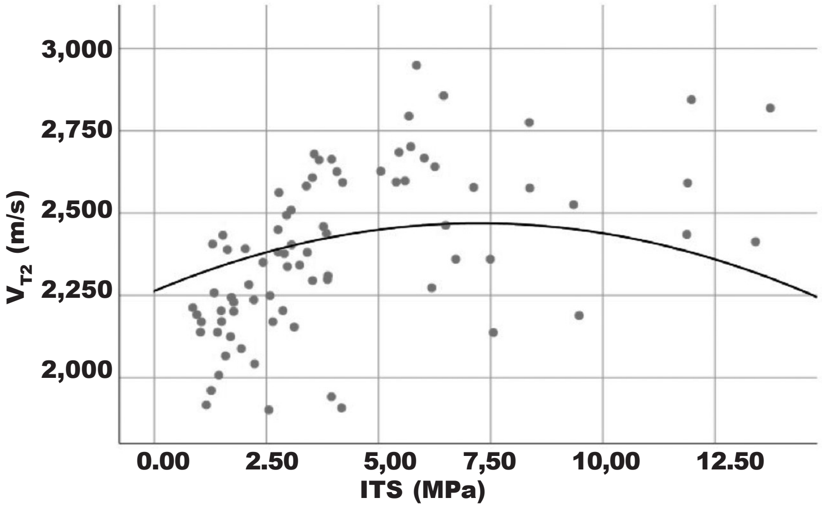

Fig. 5. Relationship between the indirect tensile strength (ITS in MPa) and the speed of ultrasound (V_{T2}). The figure shows raw data and the prediction line from the mixed model in table 4 with other variables fixed at their mean values.

Fig. 5. Relación entre la resistencia a la tracción indirecta (en MPa) y la velocidad de los ultrasonidos (V_{T2}). En el gráfico se muestran los datos sin tratar y la línea de predicción del modelo mixto de la tabla 4 con las demás variables fijadas en sus valores medios.

correlation between these values have been studied in cancellous bone (Hans et al., 1999). As with our results, Hans et al found a positive correlation between density and the speed of ultrasound, and differences according to the direction of measurement that they attributed to the trabecular structure of bone tissue. Similar results were reported for bovine cortical bone (Lasaygues and Pithioux, 2002), with greater longitudinal speeds (in the direction of the collagen fibers) than radial speeds, and both were significantly correlated.

Regarding the correlations between the physical properties of bone, several studies have shown that the density of bone is the most important property determining its flexibility (Keller, 1994; Carter and Hayes, 1977). But density alone does not fully explain the variability in flexibility (Rice et al., 1988). Instead, structural information is also needed to improve estimates of bone flexure and fracture risk (Ulrich et al., 1999).

Not surprisingly, we found that the ration of cancellous to compact bone was strongly negatively related to density and, to a lesser extent, to the indirect tensile strength and velocity of ultrasound (see table 2). The density values we obtained varied widely (between 0.79 and 1.64 g/cm^3) due to the different values of the cancellous to compact area ratio of the fragments analyzed, this ratio varying between 2.2 and 0.06. The average value of the bulk density we obtained (1.19 ± 0.21 g/cm^3) for fragments containing both porous and compact parts is consistent with the value provided by Chen et al. (2009) for full antler (1.3 ± 0.10 g/cm^3), and for cortical (1.72 ± 0.04 g/cm^3) and porous parts (0.50 ± 0.05 g/cm^3).

The mineral content is considered an important factor to determine density, and consequently the tensile strength, of bone tissue (Currey, 1987, 1990). The mineral content of antlers is incorporated via two routes during antler formation: from the diet and from re–absorption of minerals from the skeleton. Changes in diet affect the formation of the antler (Landete–Castillejos et al., 2012), so that the habitat where deer live could be an important factor in explaining variability in ultrasound speed. Differences in ultrasound speed for different sections within an antler can indicate differences in mechanical properties that could be related to functionality. The differences in V_{T2} and other physical properties between the distinct parts of the antler, with a range of mean values from 2,209 ± 37 m/s for the mid beam to 2,565 ± 68 m/s for the brow tine reflect differences in their resistance to breakage. Antler formation is differentially influenced by selection so that some parts are more susceptible than others to harsh conditions during development, affecting asymmetry for instance (Mateos et al., 2008). According to Gómez et al. (2016), in their study with PIXE and PIGE techniques they observed that mineralization decreases upwards from the base of the antler, being higher in low beam than in mid beam. This different mineralization would explain in part the porosities (and the ITS) of the different sections of the antlers, that is the main cause of the differences obtained between the speeds of propagation by ultrasound.

Our results indicate that antler resistance to breakage is not uniform throughout antler morphology, probably related to the costs for male deer of antler breakage at different sections. Antler base, main beam and proximal tines are stronger than more distal parts of the antler. Moreover, our results reveal a weak zone of the antler at mid beam section that might serve to facilitate breakage at this point rather than in more proximal parts of the antler. Antlers are subjected to directional selection because of their role in male–male competition (Kruuk et al., 2002). Antler tines and beam break in fights during the rutting season (Johnson et al., 2005; Karns and Ditchkoff, 2012) and male mating success is affected by antler breakage (Johnson et al., 2007). In white–tailed deer (*Odocoileous virginianus*) antler tines were found to break more easily than beam (Karns and Ditchkoff, 2012), probably because they are more exposed to impact, but it may also be that a broken beam has more serious consequences for the bearer than broken tines. Further studies on the differences in fighting behaviour between deer species may also contribute to our understanding of differences between antler parts.

As our results show, antler structure and density explain much of the variability of the speed of ultrasound, and they in turn relate to the tensile strength. This relationship, however, is not linear as we have shown. We are aware that we did not measure the actual resistance of antler sections in the way they are used by stags in real fighting. However, the differences in ultrasound speed for different sections within an antler can indicate differences in mechanical properties that could be related to its functionality.

Thus, as in the diagnosis of osteoporosis, the use of ultrasound in antlers may be an interesting non–invasive tool to study their mechanical properties as well as the functionality and susceptibility to stress conditions of different antler parts. On the other hand, since antler properties are related to the conditions of animals during antlerogenesis, ultrasound speed appears to be a useful non–invasive tool with a potentially wide range of applications in the management of deer populations.

In conclusion, ultrasound speed is an interesting tool to estimate the density of antler bone. It is less influenced by moisture or soft tissue remains than total antler density, and hence may be used (along with other measurements) as an indicator of quality. The main advantages of this method, registered as a utility model (Del Río et al., 2012), are that it is non–destructive and rapid, it does not require any complicated training, and it can be used in outdoor conditions with portable equipment. Consequently, compared to currently used methods to estimate antler mechanical properties or density on the basis of antler weight, we suggest that this procedure can be implemented as a rapid, straightforward, and reliable method to assess antler quality for research, population management, and trophy measurement.

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