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#### Editorial



#### Deer Specialist members welcome to the new quadrienium

With the conclusion of the last quadrienium, the DSG membership reported their activities and expressed a willing to continue as part of our conservation network. Our main goals for the next quadrienium (2013-2016) will be to continue supporting research in: a) the deer "hotspot diversity regions" of Asia and Latino America to obtain scientific knowledge, b) to evaluate the status of each deer species and propose conservation guidelines and, c) to obtain funds to support the small grants to qualified young field biologists.

One of our main tasks for the next period will be to assess all deer species as part of the Global Mammal Assessment which starts this year (2013). We will need the expertise of all our members to accomplish this task. Towards that aim we asked each of you to indicate your areas of expertise so we can efficiently divide the tasks among members.

Our main goals for the next quadrienium will be to continue encouraging our membership to be more active and participative in deer conservation. We decided the best way to increase efficiency and participatory activity is to reorganize the DSG around regions of interest and identify leaders for each sub-group. These 6 regions will be: European Temperate and Arctic, South Asia (India/Nepal), SE Asia, Tropical Asia Island, Asia Temperate, and Americas. We will be approaching members who have been active conservationists in each region to assume a leadership role and to coordinate activities by their sub-group.

We begin this new period with two new Red List Authorities: Dr. Eveline Zanetti (New World Deer Species, eveline\_zanetti@yahoo.com.br) and Dr. Sarah Brook (Old World Deer Species, sarah.brook@wwfgreatermekong.org). Their roles will be critical in the next quadrienium as we strive to complete the reassessments and deal with the issues of species taxonomy. We urge everyone to support their efforts and to welcome their willingness to contribute their time and abilities to this effort. If you have issues with conservation status for a specific species, please contact the RLAs for this committee and start a dialogue.

We want to acknowledge to all who contribute in this edition. In this issue we are including four interesting original articles one short communication and the report including the abstracts of the deer Symposium 2012 in Uruguay.



Finally, we want to thank to all of you for being part of the DSG, we apologize for the delay in the issuing of this newsletter; at times it seemed all the world's events were contriving against its production. Dr. Patricia Black (black.patricia@gmail.com) is the new chair editor in charge of the Newsletter and we are looking forward to be able to efficiently produce subsequent issues. We invite you to submit articles to the next issue, which will be with a submission deadline of September 30.

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## Changing abundance in migratory tundra caribou and wild reindeer and the role for a circumpolar collaborative network

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#### Abstract

Wild reindeer and caribou (*Rangifer*) are among the most widespread members of the deer family. They are found in the polar deserts on high arctic islands, the circumpolar tundra and boreal forest and into the mountain forests and alpine tundra. Those extensive and diverse habitats across national boundaries correspond with a diversity of sub-species and ecotypes that add complexity and uncertainty to status assessment and monitoring. We describe a recent international collaboration to establish a circumpolar network and monitor one of the most widespread forms of *Rangifer* – the migratory tundra caribou and wild reindeer. Range contractions and declining abundances are general themes as well as the importance and pivotal role of indigenous people's knowledge and experience.

Keywords: diversity, migratory caribou, reindeer, Rangifer, circumpolar declines

*Rangifer* is one of the most widespread genera in the deer family occurring in both northern Eurasia and America. *Rangifer* likely evolved in Asia during the Miocene before dispersing across Beringia possibly during the late Pleistocene (Gilbert et al. 2006). *Rangifer* has a single species *Rangifer tarandus* which has diverged into about 13 sub-species and ecotypes with markedly different evolutionary strategies for trading off the risks of predation and parasites relative to foraging. The current taxonomy (Banfield 1961), however, being dependent on skeletal measurements, antler form and pelage is not well supported by recent genetic information (Flagstad &Røed 2003). Caribou of the boreal forests tend to be large-bodied, long-legged forms and dispersed as individuals or small groups. This contrasts with the highly gregarious migratory caribou of the tundra and northern boreal forests. The mountain forms

likewise cover the spectrum from dispersed to migratory gregarious behavior and use habitats from dense mountain forest to alpine tundra. Because of variation in distinct characteristics within groups and recent advances in molecular tools used to define taxonomic units, opinions will continue to differ on the current number of sub-species and ecotypes.

The global IUCN status of the species is Least Concern (Henttonen & Tikhonov 2008). However, within sub-species there are conservation concerns, especially in Canada, which has the highest abundance and diversity. Peary caribou of the High Arctic Islands are nationally recognized as Endangered (COSEWIC 2004); and boreal (woodland sub-species) as Threatened (COSEWIC 2002). Caribou distribution has shrunk with the disappearance from the eastern Maritime Provinces and a distribution receding northward in central Canada (Hummel & Ray 2008). The sub-species which are numerically the most abundant and widely distributed are the migratory tundra ecotype which is termed wild reindeer (Russia) or caribou (North America and Greenland).

On High Arctic islands, there are distinct forms of caribou typically with smaller bodies, notably shorter faces and limbs and lighter coloured-pelage. These caribou of arctic islands are often in small bands as they follow their seasonal migration pathways, which often involve crossing the sea-ice between islands. Novozemel'sk reindeer are Russia's distinct type of arctic island reindeer, *R. tarandus pearsoni*, and are found on Novaya Zemlya archipelago. Svalbard's (Norway) reindeer *R. t. platyrhynchus* are dwarfed and with a marked propensity to fatten up for the long winter. Greenland's distinct form of reindeer, *R. t. egroenlandicus*, were small, shortfaced and with a white winter coat. They ranged along the east coast and became extinct by 1920. Canada's Peary caribou, *R. t. pearyi*, are found on the north and central arctic islands.

The migratory tundra caribou are notable on a global scale for the extent of their migrations (often considered the longest migration for any terrestrial mammal), their sheer numbers and their long-time importance to the culture and subsistence of arctic peoples. Migrations from the winter ranges to the calving grounds often are journeys of several 100 km, up to 1000 km (straight line distance measured from satellite-collared cows). The cows typically show fidelity

to their natal calving grounds where the cows reach densities of 50-100 cows km<sup>-2</sup>. Most calves are born within a few days of each other and are able to keep up with their cows within a day or two. The magnitude and scale of these migrations is not just the sheer numbers but also the persistence over time. For example, the Beverly herd's crossing of the Thelon River to its traditional calving grounds near Beverly Lake was part of the lives of the Dene aboriginal people for 8,000 years, as revealed by an unbroken archaeological record of deep layers of caribou bones and stone tools in the banks of the Thelon River (Gordon 2005).

We suggest that the widespread distribution and abundance of the migratory tundra caribou across Eurasia and North America raises particular conservation concerns. The often high abundance and widespread distribution of caribou and wild reindeer means that they are sometimes considered 'common'. Common species shape ecosystems by their sheer strength of numbers but there is a tendency to under-estimate their conservation concerns (Gaston & Fuller 2008). We need to be able to read danger signals for those common species which include contracting distributions and even the loss of populations—exactly the current pattern for *Rangifer* (Festa-Bianchet et al. 2011).

The current habitat of migratory tundra caribou appears largely intact as agriculture and forestry are limited across their range. However, the cumulative presence of human activities such as roads, recreational tourism, oil, gas development and mining is increasing which is a trend that introduces uncertainty about long term effects. Behavioral responses to such activities as open pit diamond mines on the Canadian tundra are known to produce a 10-15 km surrounding zone of reduced caribou abundance (Boulanger et al. 2012). This contrasts with boreal caribou which have been extirpated from between 40 and 60% of their historic distribution across Canada (Hummel & Ray 2008). In these cases, the loss of caribou followed sweeping habitat changes including shifts in predator-prey relationships following oil and gas development and logging.

Adequate tracking of herd size is dependent on the frequency of monitoring. In Russia (some herds), Alaska and Canada, monitoring of most herds is based on photographic counts on the calving ground or post-calving aggregations with support from demographic indices such as

calf and adult survival and sex ratios. In Canada's Nunavut Territory, the status of the several smaller herds on the northeast mainland and Baffin Island is unknown as the herds are rarely monitored. Gaps in monitoring can lead to uncertainties about whether herds such as Canada's Beverly herd have declined or not (Gunn et al. 2011).

For those herds where adequate monitoring has taken place, we have seen a trend to declining numbers of migratory tundra caribou in recent years (CARMA 12). Currently, circumpolar wild reindeer and caribou have declined by about 33% since peaks in the 1990s and early 2000s (3.8 million compared to 5.6 million) which followed almost universal increases in the 1970s and 1980s. The changes are not unexpected as longer-term and traditional knowledge reveal cyclic abundance. An index to abundance based on dendrochronology uses hoof scars scuffed on tree roots exposed across migratory pathways; it also reveals cyclic abundance related to recurring patterns in climate (Zalatan et al. 2006).

In Canada, herds peaked in size to about 3.4 million caribou during the 1980s and 1990s, and by 2009, they had declined to some 1.1 million caribou. Ten of 12 major herds have declined across Alaska and Canada, some by as much as 70-90% in abundance. The declines were at mostly annual rates of 5-10% with an acceleration of the rate toward the later stages. Subsequently, at least some of those herds have stabilized although at low numbers after measures were taken to reduce mortality through restrictions on hunting. East of Hudson Bay, over the last 20 years, total numbers for the two large herds have decreased with the George herd declining from a peak of about 700,000 in 1993 to about 27,000 in 2012. The other herd, the Leaf River, has declined from 1,193,000 in 2001 to 430,000 in 2011. The largest herd in coastal Alaska, the Western Arctic herd has declined from its peak size in the 1990s, while the Porcupine, Teshepuk and Central Arctic herds have all increased during the 1990s until present (2012).

In west Greenland, one major population had declined to 31,000 by 2010, while the trend for the second major herd appears to have increased to 98,000 in 2010. Iceland's introduced domesticated reindeer became feral and have been increasing since 2000 with over 6,500

animals in fall 2009. Further east in Norway, the mountain reindeer total in 2004 was about 22,000 to 29,000 animals in 23 separate herds (Lund 2004). In Finland, numbers of wild forest reindeer have recently declined to about 1800 (2009). In Russia, three of five major wild reindeer herds in the north are declining; one herd appears stable and one herd increased between 1986 and 2002, which coincided with a decline of domestic reindeer after elimination of state support for reindeer herding. The Taimyr herd is one of the largest migratory herds in the world and declined to number about 650,000 wild reindeer in 2009. In Russia, uncontrolled poaching, range deterioration, increased incidence of disease and parasites are some of the leading factors in the decline (CARMA 1212).

It is easy to fall into the trap of complacency when a species numbers in the millions or when traditional knowledge tells us that population declines in migratory caribou herds have happened regularly in the past. But the message of conservation is that it is rate of change that determines a species' persistence, not the size of the starting population (Caughley & Gunn 1996). The high rates of decline for some herds are of particular current concern especially when steps to halt them through regulation of hunting were slow to be implemented. While some herds at peak size in the 1990s appeared to be at historic levels, this is not the case for other herds such as the Fortymile herd shared between Canada and Alaska. It has not returned to the historically high numbers of 100s of 1000s in the 1920s, having only reached a peak of 50,000 by 2010 despite years of recovery planning. Although early estimates of abundance are not always known for the Canadian herds of migratory tundra caribou, the southern extent of their winter distribution into the northern boreal forests has contracted since the 1940s and 1950s (Thomas et al. 1998). This suggests that peak herd sizes are below historic peak sizes. The concern about a sliding scale of recoveries is that it becomes an example of the shifting baseline syndrome (Pauly 1995). We tend to treat the ecological conditions at the beginning of our careers as the starting point to measure recovery which then produces ever-shrinking expectations of what constitutes species recovery.

Wild reindeer and caribou are less well-served than other migratory hoofed mammals because they occur in nations not all of which are signatories to the Bonn Convention on Migratory Species (CMS). CMS is an international treaty, under the United Nations Environment Program, which promotes the conservation of wildlife and habitats on a global scale. Progress on monitoring the status of circum-arctic caribou and wild reindeer has been made under the Arctic Council programs. The Arctic Council, through their Conservation of Flora and Fauna (CAFF) program, and CAFF's Circumpolar Biodiversity Monitoring Program (CBMP) initiative, recognizes caribou and wild reindeer as an indicator species, which reflects their importance in arctic ecosystems (Gill & Zockler 2008).

However, the widespread distribution in the circumpolar arctic countries means that assessing global status for wild reindeer and caribou requires navigating the different languages, institutions and cultures from different countries and agencies involved in sharing information about the circumpolar wild reindeer and caribou. A real limitation is that information is often unpublished and widely scattered. However, it is also a necessity in order to benefit from the collective experience in managing caribou and wild reindeer in the face of a changing world with increasing human presence and the onset of global warming, predicted to be rapid in the arctic. Recognizing the need to work together, a circumpolar group of government agencies, comanagement boards, university researchers, and graduate students started the steps toward collaboration by forming a network. Circum Arctic Rangifer Monitoring and Assessment (CARMA) Network held their first formal meeting in 2004. Participants are from all the circumpolar countries with tundra caribou and wild reindeer. With the financial backing of the Canadian International Polar Year Program, CARMA developed standard methods of monitoring herds, and encouraged targeted research and monitoring projects. CARMA has invested heavily in tools such as computer models, technical manuals and databases on climate variables to better integrate our knowledge and support decision-making.

The CARMA network is web-based which has transformed the availability of information that previously was mostly scattered in government files. The web site acts a single place with updated information, maps, manuals, tools, contacts and current status. As part of the Arctic Council's Circumpolar Biodiversity Monitoring Program (CBMP), considerable progress is being made in the sharing and availability of information among agencies and users. Sharing

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knowledge across geographic and political boundaries is where CARMA initially built its strength. However, most recently, CARMA is moving from describing *what* is happening to caribou (symptoms) to understanding *why* it is happening (causes) and *how can* we can use this knowledge to manage into the future.

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## Distribution and loss of potential habitat of the Central American red brocket deer (*Mazama temama*) in the Sierra Madre Oriental, Mexico

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**Abstract:** Habitat degradation and excessive hunting has reduced the distribution and abundance of the Central American red brocket deer *Mazama temama* in Mexico. The objectives of the study were to establish the potential distribution of this species in the mountains of the Sierra Madre Oriental in Mexico to estimate the area currently occupied by the species, and determine the proportion of the potential range within natural protected areas (NPA). We obtained records of the species from data bases, literature and field studies and modeled the potential distribution using MaxEnt. Range reduction and the potential area occupied by NPA were analyzed by using a GIS. We estimated that the range of the species in the Sierra Madre Oriental was approximately 91.818 km<sup>2</sup>. We observed that 49.7% of the original potential range was already lost and estimated that only 7.9 % of this area is currently within a NPA. We discuss some conservation strategies for the species in the Sierra Madre Oriental.

Key words: ecological niche modeling, MaxEnt, natural protected areas.

**Resumen:** La pérdida y degradación de hábitat así como la cacería excesiva han disminuido notablemente la distribución y el tamaño de las poblaciones del venado temazate rojo centroamericano *Mazama temama* en México. Los objetivos de este trabajo fueron determinar la distribución potencial de la especie en la Sierra Madre Oriental, estimando el área en que

habita actualmente y la proporción que actualmente se encuentra en áreas naturales protegidas (ANP). Se obtuvieron registros mediante bases de datos, literatura y en campo. Se modeló su distribución potencial en MaxEnt, la cual se analizó con un SIG. Se obtuvo un área de distribución potencial de aproximadamente 91.818 km<sup>2</sup>. Se estima que la especie ha perdido 49,7% de su hábitat potencial original, y solo el 7,9% está dentro de alguna ANP.

Palabras clave: modelación nicho ecológico, MaxEnt, áreas naturales protegidas.

#### Introduction

The Cervidae family in Mexico is represented by five deer species. Of these, the Central American red brocket deer (Mazama temama Kerr 1792) and Yucatan brown brocket deer (M. pandora Merriam 1901) are the least studied (Mandujano 2004). Deforestation has been the main threat affecting biodiversity over the last 40 years. Consequently, forests are mainly found in inaccessible higher areas while the rest of the landscape has transformed into a matrix of pastures and crops with some isolated vegetation patches (Guevara et al. 2004). Local hunting has become another threat, putting pressure on deer populations. These factors have caused a decline in the distribution of these deer. In Mexico, the Central American red brocket deer is found from the South of Tamaulipas to the Yucatan Peninsula (Hall 1981). This range extends from the coastal plains to mountainous areas; in the latter regions we have less knowledge about the presence and distribution of the Mexican red brocket deer (Fig. 1). The Sierra Madre Oriental runs parallel to the Gulf of Mexico until the Transverse Volcanic Axis, and is comprised of coniferous and oak forests in the highest areas with montane cloud forest and evergreen tropical forest in the lower regions (Rzedowski 1978); both are vegetation types inhabited by the Central American red brocket deer. The specific objectives of this paper are: (1) to determine the potential distribution of this species in the Sierra Madre Oriental, (2) to estimate the area it currently occupies in this region and (3) to determine the proportion of the population currently within a Natural Protected Area (NPA).

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Figure 1. (a) Central American red brocket deer *Mazama temama*in a zoo (photography by Alberto González-Gallina). (b) First photographic recording of the species in the area near Tehuacán-Cuicatlán Biosphere Reserve in Puebla and Oaxaca states (Pérez-Solano *et al.* 2012).



#### Methods

Records regarding the presence of the Central American red brocket deer were obtained from databases of scientific collections, literature and fieldwork (tracks, photo-trapping and interviews with local inhabitants). Geographic distribution modeling was estimated using the Maximum Entropy approach (MaxEnt, Phillips *et al.* 2006), which uses associations among environmental variables and species occurrence records (Peterson 2001). We used 22 environmental information layers: 19 from WorldClim (Hijmans *et al.* 2005) and 3 topographic (elevation, orientation and slope digital model). We worked with these variables in Raster format, at an approximately 1 km<sup>2</sup> pixel resolution. The potential model was reclassified into a presence-absence binary model in ArcView 3.2. Classifying both suitable (tropical, cloud and temperate forests) and non-suitable habitats (human settlements and crop lands) for the species (Bello-Gutiérrez *et al.* 2010), the original presence-absence map was divided following habitat distribution according to the National Forest Inventory (Velázquez *et al.* 2002). Difference in obtained area between the potential distribution of brocket deer and vegetation classification was used to determine habitat loss. In addition, NPAs were overlapped with the model formed by habitats, in order to determine the proportion of protected habitat.

#### Results

Some 89 records of the presence of the Central American red brocket deer were obtained in the studied zone. The model predicts a potential area of 91,818 km<sup>2</sup> (Fig. 2a). However, considering actual suitability of vegetation type, the potential area reduces to 46,141 km<sup>2</sup> (Fig. 2b). The species has therefore lost 49.7% of its original potential habitat to the impact of human activity. It was estimated that only 3,685 km<sup>2</sup> (7.9%) are under the protection of any NPA in the Sierra Madre Oriental (Fig. 3).

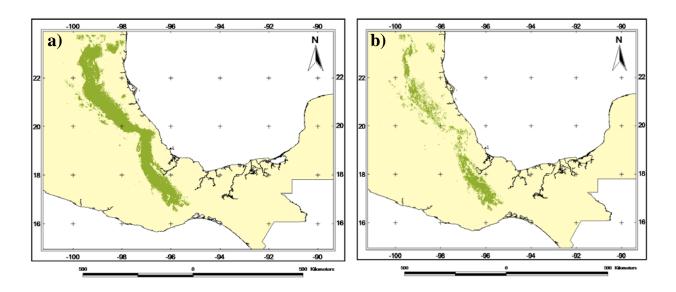


Figure 2. (a) Potential distribution (in green) modeling of Central American red brocket deer in the Sierra Madre Oriental, Mexico. (b) Potential distribution considering type of vegetation and land use.

#### Discussion

Considering the environmental conditions in the Sierra Madre Oriental, the potential habitat for the Central American red brocket deer is over 90,000 km<sup>2</sup>. However, this area has been dramatically reduced by about 50% due to human activity, mainly deforestation, agriculture and settlement. Moreover, the potential area for distribution that is under protection remains minimal (7.9%). The most important part of this potential area for the species is in the southern mountainous region of Oaxaca State, within some cloud and temperate forests of the Tehuacán-Cuicatlán Biosphere Reserve. Most recent studies of the Central American red brocket deer have been performed on plains (e.g., Weber 2005; Reyna-Hurtado & Tanner 2007). Consequently, there is little information regarding mountainous regions. Additional protection and potential use of the Central American red brocket deer could be possible through Wildlife Conservation, Management, and Sustainable Utilization Units (UMA by their Spanish acronym), but this would first require the urgent implementation of studies in this mountain region.



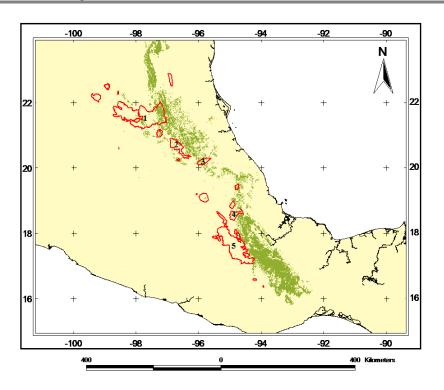


Figure 3. Natural Protected Areas (circled in red) in the potential distribution area of the Central American red brocket in the Sierra Madre Oriental, Mexico. Sierra Gorda Biosphere Reserve in Guanajuato and Querétaro (1), Barranca de Meztitlan Biosphere Reserve in Hidalgo (2), Cuenca Hidrográfica del Río Necaxa Natural Resources Protected Area in Hidalgo and Puebla (3), Parque Nacional Cañón del Río Blanco in Veracruz and Puebla (4) and Tehuacán-Cuicatlán Biosphere Reserve in Puebla and Oaxaca (5).

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#### Restoration of Bukhara deer(Cervus elaphus bactrianus Lydd.) in Central Asia in 2000-2011

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#### Abstract

The goal of this study was to monitor the recovery of the populations of Bukhara deer (BD) within its historical distribution area in Central Asia. Restoration activities were based onscientific knowledge gained from a 30 year study of its ecology, behavior and communication. The research methods included: mapping acoustic communication - recording and acoustic analyses of the vocalizations; visual recording of animals in the field; long-term visual observations of individually recognized animals; recording and mapping of traces. In1989 there were around 900 BD in all groups, with potential for population growth up to 4000 – 5000 animals. The Bukhara deer restoration activities (WWF project) included technical support for the nature reserves still inhabited by the species, anti-poaching activities, reintroduction in suitable sites at the limits of the historical distribution, ecological education, local communities involvement, etc. All restoration activities were accompanied by species monitoring. As a result of this scientifically based approach to species restoration the total Bukhara deer number increased from 350 in 1999 to 1900 in 2011; the efficiency and cost effectiveness of the suggested methods were proven by the results obtained.

Key words: Bukhara deer, behavior, monitoring, ecological adaptations, conservation.

#### Resumen

El objetivo del estudio fue monitorear la restauración de las poblaciones de ciervos de Bukhara en su área histórica en Asia Central. Las actividades de restauración se basaron en los antecedentes científicos del estudio de 30 años de su ecología, comportamiento y comunicación. Los métodos de investigación incluyeron: mapeo de comunicación acústica; grabación y análisis acústico de las vocalizaciones; registro visual de los animales en las rutas; observaciones visuales de largo plazo de los animales individualmente identificados; registro y asignación de huellas. En 1989 hubo unos 900 BD en total en todos los grupos, con potencial de crecimiento de la población hasta 4000-5000 animales. Las actividades de restauración de ciervos de Bukhara(proyecto WWF) incluyen apoyo técnico a las reservas naturales todavía habitadas por la especie, las actividades anti caza furtiva, reintroducción en lugares adecuados en los límites de la zona histórica, la participación de las comunidades locales, educación ecológica, etc. Todas las actividades de restauración son acompañadas por la vigilancia de la especies. Como resultado del enfoque basado en el conocimiento científico de la especie en la restauración de los ciervos Bukhara, el número total aumentó de 350 en 1999 a 1900 en 2011; los resultados demostraron la eficacia de los métodos sugeridos.

**Palabras clave:** ciervos de Bukhara, comportamiento, seguimiento, adaptacionesecológicas, conservación.

#### Introduction

According to various taxonomic systems, the Bukhara deer (*C. e. bactrianus*) differs both from the groups of Eastern and Western red deer subspecies (Geptner et al. 1961). It is a relatively small deer, the skull length is only about 390 mm and the shoulder height is 120 cm (Geist, 1998). Its 5-pronged antlers, (velvet coated during the period of growth, similarly to other red deer), which are considered to be primitive relative to related subspecies within *Cervuselaphus*, weigh only between 3.4 and 5.5 kg (measured from cast antlers) (Geist, 1998).

*C. e. bactrianus* inhabits riparian forests of Central Asia and it is considered to be a part of a major systematic group described as relatively primitive and seriously endangered (Nowak & Ronald, 1999). This group appears to have given rise to both the red deer to the west and the wapiti to the north and east (Nikolskii, 1984; Nowak, 1999; Pereladova, 2004). According to the latest genetic studies, it is closest to the group of Indian and Chinese deerand even to the sika deer (*Cervusnippon*). In the publication ofLudt et al. (2004) it is stated that there is "a very high probability that there exist two different species of red deer with three subspecies in Asia and America (Eastern Red Deer) and four subspecies in Eurasia (Western Red Deer) and an additional one or two primordial subspecies in Central Asia (Tarim group:*C.e. yarkandensis*,

*C.e. bactrianus*). The origin of the genus *Cervus*seems to be in Central Asia near today's Hindu Kush".

Various characteristics of the Bukhara deer also differ from those of the other red deer subspecies. Its dietary preferences are one of those. Although the main habitats of the Bukhara deer are riparian forests, its diet includes grasses, sedges and shrubs, and tree shoots to a minor extent. Its diet is regularly very limited most of the year (e.g. when inhabiting reeds and brush). As opposed to other red deer subspecies, which use branches preferably of young deciduous trees of up to 15 mm thick, Bukhara deer eat only branches of willow, poplar, some other riparian forest species and saxaul in deserts up to 5 mm (Sablina, 1970). Moreover, other subspecies of red deer as well as sika deer brought to Tajikistan (experiments on deer velvet farms in 1970-1980), were obliged to use the typical Bukhara deer diet –and it caused various diseases: problems with bones, blindness. Special analysis has shown that all diseases were caused by a lack of a number of vitamins (mainly ruined by Asian insolation) and minerals. Bukhara deer in the same system of pens, using the same diet didn't suffer any of those diseases (Prisiazjnuk, oral communication). And an opposite example: a group of Bukhara deer were kept for a short time in a specialized breeding centre - Reserve de la Haute-Touché, France, and were fed with the same forage as other deer species. In a week the dominant stag died from abdominal distension, the rest were sick, but recovered after the diet was changed for dry poor hay (expertise and recommendations of the author in 1995).

Another specific physiological characteristic of Bukhara deer unusual in other red deer subspecies – is the order of various phenological processes (the rutting season in well - developed populations under natural conditionsprecedes the molting period (growth of winter fur) in this subspecies and vice versa for other red deer subspecies).

In order to identify specific characteristics of the subspecies, the behavior and communication of the Bukhara deer were compared to similar characteristics of 5 other deer subspecies of the former Soviet Union (research of the author in 1978-1990, and other species subspecies of deer (data from other authors).Comparison of specific characteristics of acoustic communication, territorial and social behavior allowed us to identify which are specific to the Bukhara deer, and which are dependent on the population characteristics. The subspecies-specific characteristics are the following: the acoustic characteristics of the signals (a low frequency component with format structure and a high frequency component with a harmonic structure – both independently modulated in one and the same signal); the typical size of groups/ harems; pure leks(in the version of Clutton-Brock et al. 1992, 1993) e.g. – not registered for any other red deer subspecies. The variability of different characteristics allowed us to suggest methods of behavioral monitoring, which permit characterizing the population condition without direct impact on the animals. The set of morphological, ecological, recent genetic and behavioral characteristics of Bukhara deer suggest, that it differs greatly from other red deer subspecies (Pereladova, 1993a, 1993b, 1998, 2009.) – and it is critically important to save this unique form of deer.



**Figure 1**.Bukhara deer male, female and fawn in Zarafshan, Uzbekistan. Courtesy Natalya MARMAZINSKAJA/WWFRussia

#### **Conservation status**

Historically, Bukhara deer inhabited all river valleys of Amudaria and Syrdaria, as well as Murgab and Tedjen to the west (Turkmenistan), Zarafshan, various minor rivers – and Ily to the

east (Fig.1, map 1989; Geptner et al. 1961, Bannikov, 1978). It was already seriously threatened in the 1960s, first of all because of habitat destruction (only 10% of natural riparian forests remained in the Amudaria river valley; the rest had been replaced by fields) and direct elimination (poaching). Deer disappeared from Tedjen, Murgab, lower Amudaria, Zarafshan, and the entire Syrdaria valley. In the 1960-1970s a number of special reserves were established for protection of the Bukhara deer, and special activities were undertaken in order to restore the deer to its natural habitats (Zarafshan, Dashtidjum –Tajikistan -1961, the lower reaches of Amudaria - Badai-Tugai, Uzbekistan -1976, Ily river-Karatchingil,Kazakhstan-1981) and to establish additional populations in suitable habitats in mountain valleys (Romit, Karatau, Sarykhosor, etc. – Tajikistan – 1961-1978). By 1989 there were about 900 deer totally in 13 populations/groups, with potential for population growth up to 4000 – 5000 animals (Flint et al. 1990, Pereladova 1993a, 1993b).

After the breakup of the former Soviet Union, only 350 Bukhara deer remained in all populations throughout the area, as a result of poaching. Moreover, the major groups which survived in the Amudaria river valley were under a common threat of various disasters (floods, diseases, etc.), so it became critically important to restore populations in other parts of the historical distribution area to ensure the species' survival. Depending on geographical location, deer were found to move either several hundreds of meters or 2-3 kilometers while searching for food during a twenty-four period (Bannikov 1978). Because Cervus e. bactrianus has very narrow habitat preferences, there is little opportunity for individuals to disperse to other areas when their habitat is threatened (Geist 1998). Bannikov (1978) indicated that it was unusual for Bactrian deer to migrate, especially in good habitat conditions. However seasonal migrations of Bukhara deer were recorded in the neighboring Haloxylondeserts, returning the following summer. In addition, non-periodical migrations of the deer were observed during major spring and summer river floods and fires such as those caused by reed burnings (Bannikov 1978). In addition, in cases of high population density and high numbers of young stags, their migrations were initiated by adult stags (driving younger ones away from the lek in mid-September, Pereladova, 1998). That is the major way in which the area inhabited by the deer is initially expanded. Therefore, while Bukhara deer populations demonstrate a tendency to remain within defined areas, they are able to migrate outside these localized regions in search of more food or better habitat, or in response to social pressures. Overall, 30 to 50% of all populations have been shown to migrate every year and to cross national borders. This was the reason why the CMS / Bonn Convention Bukhara Deer Memorandum of Understanding and Action Plan was developed (on the basis of the Bukhara deer restoration programme, Flint et al., 1990) and signed by all Central Asian countries in 2002.

The goal of the study was to monitor the results of the Bukhara deer (*Cervus elaphus bactrianus Lydd*.) population's recovery in its historical distribution area in Central Asia and to verify the efficacy of suggested measures of restoration/predictions of population dynamics development based on the data of comparative behavioral monitoring.

#### Materials and methods

The behavioral study and population monitoring covered the period from 1972 until 2011, including 1999 - 2011, the period of practical activities on the restoration of the species in its historical area (excluding Afghanistan), and regular census of all populations in the project area.

Comparative behavioral monitoring included: mapping\*\*\* of acoustic communication (all active periods of the day in all seasons of activity); visual identification and recording of animals in the field (including size of the groups, sex-age ratio); visual long-term observations of individually recognized animals (behavior, phenology, etc.); recording and mapping of traces (footprints, beds, droppings, marks on trees, etc.). Comparative analysis of behavior and acoustic communication was conducted in populations which varied in (a) geographical location, (b) population number/density, (c) phase of the population development (growth, stabilization, decline, etc.), (d) type of habitat (riparian forests, reed brushes, mountain river valleys); (e) particular ecological conditions of the year; (f) population origin (natural, reintroduced, introduced). The investigations were carried out in the following study areas: Tajikistan (3 sites), Uzbekistan (3) Kazakhstan (1), Turkmenistan (1) – in total more than 1000 km from the north to the south, and more than 1500 km from the west to the east. Research activities were repeated in different populations in different phases of population development;

for example, surveys of the model natural population in the south of Tajikistan – 1972, 1973 (350 deer in population), 1978, 1984 (200 deer), 1996, 2004 (about 100 deer).

\*\*\* Mapping of acoustic communication is a special method (*first publication - Nikolskii*, Novikova, Naumov, 1975; Space-time characteristics of biological communication in the field – on the example of Bukhara deer rutting-calls //. J. Ecology, N1, 100-102. – in Russ.)

The method is the following: throughout the whole rutting period, during the time of daily rutting activities (usually from 17-18 until 10-11 o'clock next day – 16-18 hours a day), each rutting call is marked on a schematic map of the region of the investigation. The observer is placed on a special observation point (e.g., a tower in a flat area), and the map is changed each hour. Later on, the data are processed, allowing the analysis of the space-time dynamics of rutting activities).

Activities on Bukhara deer restoration (WWF project) included technical support for the nature reserves still inhabited by the species, special anti-poaching activities, reintroduction in suitable sites at the limits of the historical distribution area, ecological education/local community involvement, etc. All restoration activities were accompanied by species monitoring which was conducted by national teams at the project sites, using a number of methods (autumn census during the rutting activity; visual spring and autumn censuses using transects; regular recording of deer traces, etc.). Periods of observation / census were repeated at each site every year.

Reintroduction was initiated at the limits of the historical area outside the Amudaria river basin – in the most suitable habitats: (1)Zarafshan (Uzbekistan), where the first deer were set free in 2005, (2) Turkestan (Kazakhstan, Syrdaria) and (3) Altyn-Emel (Kazakhstan, Ili). Reintroduction activities were based on the following principles: (1) breeding in pens / releasing second and later generations; (2) sex-age composition of the groups for releases, (3) ensuring slow and optimal area expansion, (4) arriving at critical population density / sex-age ratio in each local group. Regular observations at all sites (deer in the pens and released groups) allowed us to adjust the process to the conditions of the group development.

Another important component of our practical activities is the habitat of one of the last populations of Turanian tiger (the last record was in 1958) and Bukhara deer. This is the

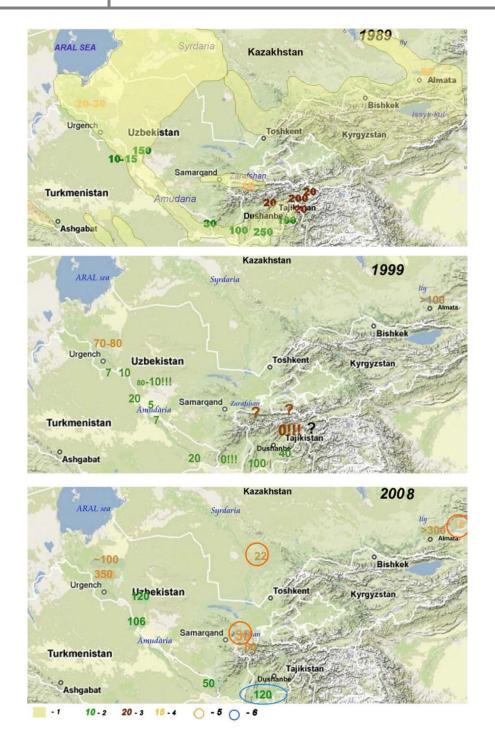
Bukhara deer habitat restoration in Tigrovajabalka ("Tiger's valley"). It is one of the first strict nature reserves (zapovedniks) of the former Soviet Union and the oldest zapovednik in Tajikistan; it was established in 1938 in order to protect a unique massive area of riparian forests in the confluence of the Vakhsh and Panj rivers. Unfortunately, all ecosystems of the reserve suffer from a lack of water, caused by construction of dams and hydroelectric stations, and systems of dams and canals for agricultural irrigation. This leads to an absence of the floods needed for the natural development of riparian forests, and to the overgrowth of natural streams such that the lakes do not receive necessary water. Starting in 2003 with small-scale support from WWF Netherlands, Minnesota Zoo and the Disney Fund, and continuing in 2007, with greater funding from MFA/WWF Norway, WWF implemented special measures for the ecosystem's restoration, ensuring a natural regime of water supply. Dredging of the overgrown canals permitted the restoration of normal water levels to the lakes and the normalization of the level of underground waters. This resulted very quickly in the restoration of various components of the ecosystems and the consequent growth of the Bukhara deer population.

#### Results

Since 1999, WWF has maintained a project supporting the restoration of the Bukhara deer. By 2009 the free ranging groups in the reintroduction sites had exceeded 30 animals in Zarafshan, 21 in Turkistan and 10 inAltyn-Emel, with 15-20 deer still kept in pens at each of the sites for reintroduction and future releases.

#### Results of 10 years of restoration activities and data on populations monitoring

As a result of this scientifically-based approach, Bukhara deer numbers increased from **350** in 1999 to **1900** in 2011, including: Uzbekistan - in 4 sites - from 180-190 to 1200; Kazakhstan – 2 sites - from 80 to 400, Turkmenistan – 8 sites – from 50 to 150, Tajikistan – 2 sites – from 30-50 to 290. A successful reintroduction process is continuing at 3 sites.



**Figure 2.** Map of Bukhara deer distribution and population numbers in Central Asiain 1989, 1999 and 2008. 1-historical area, 2-natural populations – number of deer, **3-**introduced populations (mountain

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valleys)- number of deer, **4**- reintroduced populations – number of deer, **5**-new reintroduction sites (WWF projects and 6-habitat's restoration site

	Year (data from	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	September-													
	October)													
U	Badai-Tuagai NR	~100	120	140	150	160	220	250	265	316	346	374	517	666
z												37%	2% juv	
в												juv	+30 in	
													pens	
	Kyzylkumskii NR	76	40	75	80	95	120	80	110	120	120	130	~130	~130
	Other territories	~50	~50	~50	~50	~60	~90	~90	~90	~100	~100	~100	~140-	160 + 80
													180	
	Zarafshan -	9	10	14	18	20	23	26	32	33	39	46	~30-32?	40 (+140
	reintroduction												(+22 in	transbou
													pens)	ndary
														with
														TJ)**
	Subtotal	~190	180	240	330	385	450	480	490	560	600	700	~900	1200
к	Karatchingil	80	100	150	~200	~250	~250	280	300	>300	>300	320-	350	>350
Α												350		
z	Turkestan (S-D)	0	0	4	6	8	10	12	15	19	22	34	18 (+22	24 (+30 in
													in pens)	pens)
	Subtotal	80	100	150	~200	~250	250	280	300	280	350	370	390	>400
т	Middle reaches of	30	35	35	45	50	60	70	80	94	106	100	60-70	50-70
U	Amudaria													
R	(7 sites)	*20	**25	*25	45	250	250				250			
к	Djazguzer (A-D	~20	~25	~35	45	~50	~50	~50	~50	~50	~50	~50	~50	~50
	upper reaches ) <b>Subtotal</b>	50	60	70	90	100	110	120	130	140	150	150	120	120
т		50	00	70	30	80	>50	>50	>50	>80	>140	>150	>150	>150
A	Tigrovajabalka Zarafshan (upper					25	~50	35	35	40	60	60-65	60-65	×150 140
Ĵ	reaches)					25		55	55	40	00	00-05	00-05	transbou
1	reaches													ndary
														with Uz**
	Subtotal	?	?	?	60	~100	?	~100	~100	120	200	210	210	~290
BD	) in the Region	~350	400	500	650	800	850	>900	1000	1100	1300	1430	1620	1900
	TOTAL													
			1	1										

#### Table 1. Population dynamics of Bukhara deer

\*\* - when calculating "Total number of Bukhara deer" – last line - this figure was once taken into consideration, as those 140 animals are

migrating from Uzbekistan to Tajikistan and back, and in fact the same animals are included in national reports of both countries.

#### Discussion

Continuous and stable population growth at all project sitesis the best proof of the efficacy of the suggested measures of restoration. The project was comparatively small-scale (€0 000 a year over all four countries for all activities, including infrastructure preparation/breeding pens for reintroduction, special measures of territorial protection, establishment of additional Protected Areas, and human dimension activities (Pereladova, 2011). That is why we suggest using this experience in other cases of rare deer restoration (similar approaches can be adapted to other rare species). In addition to traditional measures of species protection, which includes anti-poaching activities and ecological education/prevention of poaching, the following key measures should be recommended:

- ensuring safe local and seasonal migrations of animals (including cases of migrations in extreme conditions, like floods): establishment of a system of ecological corridors with special regimes of protection;
- special biotechnical measures (e.g. forage fields, seasonal feeding during some years) which allows the population to arrive at sustainable numbers and to achieve a sex –age ratio, which supports the highest reproductive success;
- regulation of the total number of animals and sex ratio of the populations (artificial withdrawal of surplus males from local populations lacking natural ways of migrations / area expansion) in order to avoid local overpopulation and depletion of ecosystem resources;
- reintroduction into suitable habitats and ensuring connectivity (necessary protection of ecological corridors for local migrations) / possibilities of gene exchange between minor populations.

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March, 2013

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#### On the purity of Mesopotamian fallow deer

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#### Abstract

Mesopotamian fallow deer originating from the world herd that was established at the Opel Zoo in 1957 have been suspected of being hybridized with European fallow deer. I review existing evidence relevant to this issue. Findings indicate that there is no evidence of hybridization in animals originating from the Opel Zoo. I conclude that the Semeshkandeh captive herd in Iran and the Israeli reintroduced population are pure bred Mesopotamian fallow deer.

#### Resumen

Los ciervos *Dama mesopotamia* que provienen originalmente del Zoológico de Opel han sido cuestionado posibles epidsodios hibridación con ciervos *Dama dama* de Europa. En este trabajo revisamos la evidencia existente. Los datos indican que no hay evidencia de hibridización animales en el Zoológico de Opel. Concluyo que los animales cautivos en Semeshkandeh en Iran y la población re-introducida en Israel corresponden al stock puro de *Dama Mesopotamia*.

#### Introduction

The Mesopotamian fallow deer (*Dama mesopotamica*) is one of the rarest deer in the world. In the mid 20<sup>th</sup> the century (ca. 1945) the species (then still considered a subspecies) was categorized as globally extinct. In 1957 a remnant population, estimated then at one-two dozen animals, was discovered in Iran in the Karcheh area (Jantschke 1991). In 1957 and 1958 a male and female (respectively) were trapped from this population and sent to the Opel Zoo in Frankfurt (Germany), forming the basis of today's world captive herd. In 1960, a first fawn (a

female) was born in captivity, but the male died before it could sire a male. It took four more years before another male was obtained from Iran and in the meantime the two females were kept with European fallow deer (*Dama dama*) and produced several hybrids. After the arrival in 1962 of a new male from Iran, the hybrids were reportedly either transferred to the Munich zoo or euthanized (Grenz 1978). Then in 1973 the founder female and the first born female fawn were returned to Iran, as part of an original agreement, together with 5 other individuals (2M,3F). These animals eventually ended up in a breeding facility in Semeshkandeh where their offspring (to the best of my knowledge) still exist. At Semeshkandeh another captive herd originating from the wild in Iran was held as well, but the animals from the different sources were kept separately. For reasons that are yet unclear, the Semeshkhandeh herd originating from the Opel zoo was labeled as suspected hybrids (Jantschke 1991) in an Iranian governmental report (Chapman and Chapman 1980). To date no copy of this report has ever been made available.

In 1976 Israel received 2 pairs of Mesopotamian fallow deer directly from the Opel zoo, of which one female perished shortly after arrival. At the end of 1978 during the height of Iranian revolution, Israel obtained 4 more females from the Sameshkandeh facility. This translocation was based on a previous agreement Israel had with the Shah's regime. While the Israeli crew that transported the deer report they obtained the animals from the enclosure holding the deer from the Iranian source (M. VanGrevenbroek, *Pers. Comm.*), Iranian sources claim the animals were taken from the Opel zoo lineage, and that the reintroduced herd in Israel is, therefore, a hybrid herd.

#### Evidence regarding the purity of the Sameshkandeh herd

What evidence exists regarding the purity of Opel zoo lineage at Sameshkandeh? Of the 7 animals returned to Iran by the Opel Zoo, 4 have full parental records in the studbook dating back to the Iranian source animals (these include the original female and her first daughter). Three of the 7 (1M,2F) have one parent listed in the studbook as unknown. This is not an uncommon occurrence in studbooks. Nevertheless the possibility that one or more of these



Figure 1: Stags of European fallow deer (left) and Mesopotamian fallow deer at Hai-Bar Israel (right). Note the differences in the antlers and tail.

individuals was a hybrid can not be ruled out. That said, all existing evidence suggests that the Sameshkandeh herd originating from the Opel zoo is pure:

- 1. Already back in 1991, Jantschke reports on an official document by the Opel Zoo veterinarian, Grenz (1978) that states that when the transfer from the Opel Zoo to Iran took place, there were no more hybrids left at the Opel Zoo.
- 2. Also, in Jantschke's 1991 report, he testifies to the fact that both T. Haltenorth and H. Diller, who had seen the Mesopotamian fallow deer in the wild, stated that in 1975 there were no morphological signs of hybridization in the Opel Zoo herd. It is important to note here that there are two prominent morphological differences between the European fallow deer and the Mesopotamian fallow deer that are clearly visible (Plate 1): (a) The European fallow deer have pronounced palmate antlers while there is no such

appearance in their Mesopotamian counterparts. (b) The tail is very different as well, with the European fallow deer tail being long and wide (somewhat like a white-tail deer, *Odocoileus virginianus*) having a pronounced black stripe in the middle with white borders, while the Mesopotamian tail is narrow and short (like a mule deer, *Odocoileus hemionus*) with only a hint of a center black stripe.

- Two genetic studies carried out in the 1980s by J. Pemberton and H.G. Schiel (both cited in Jantschke 1991) found no evidence of hybridization in Mesopotamian fallow deer bred in European zoos.
- 4. Of the more than 300 Mesopotamian fallow deer bred at the Hai-Bar facility in Israel, none have ever shown European fallow deer traits (antlers or tails).
- 5. A preliminary study carried out on 8 animals from the Hai-Bar herd to try and select a genetically diverse group for reintroduction (Hillel 1992) indicated that the average band sharing was 91%. The probes and enzymes used produced 158 bands and an overall genetic similarity of 95%. This extremely low genetic variance suggested that further genetic screening for the purpose of enhancing genetic diversity of the reintroduced animals was useless, but also suggested that there was no past hybridization.
- 6. In the mid 1980's P. Dratch (personal communication) analyzed 6 Mesopotamian fallow deer blood samples he received from Hai-Bar Carmel while in New Zealand. Similar to J. Pemberton's results he found the same protein that is distinct to Mesopotamian fallow deer and not found in the European fallow deer in all six samples.
- 7. Finally, in a recent study (Fernández-García 2012) 29 European fallow deer antler material samples were genetically compared with 9 samples of Persian fallow deer of European sources, 3 blood samples from the Israeli herd, and 17 antler samples obtained from the wild in Iran prior to 1960. Results showed no signs of hybridization and the Israeli samples were the closest to the Iranian wild stock (supporting Van Grevenbroek's testimony).

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#### Conclusions

There appears to be no hard evidence of hybridization in the current captive herds of Mesopotamian fallow deer in Europe or Israel. Although the possibility of hybridization can not be disproved unless all animals of Opel Zoo descent are genetically analyzed, the data available suggest that the probability of past hybridization in the European, Semehkandeh, or Israeli captive herds of Mesopotamian fallow deer is extremely small, if not negligible. All these captive populations should be considered pure *Dama mesopotamica*.

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### Modeling Habitat for the Conservation of Huemul in southern Chile and Argentina

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In part of its central-south distribution range, huemul deer (*Hippocamelus bisulcus*) are found in the Valdivian Rainforest Ecoregion, a world biodiversity hotspot. In this large area, most of the huemul records are from the Argentine side, where the National Park Administration (APN) has recorded direct and indirect huemul signs. However, there is little information about huemul distribution on Chile's side of this ecoregion, although the species is likely to occupy similar habitats.

We are using MAXENT, a machine learning algorithm for maximum entropy parameter estimation, to build a species distribution model (SDM) for huemul. The main goal is to predict areas within the region studied that satisfy habitat requirements for this endangered deer and thus its potential distribution range. We used previously recorded presence data from two Argentinean National Parks, Los Alerces and Lago Puelo, two surrounding provincial protected areas, Río Azul-Lago Escondido and Lago Epuyén, and all additional presence records outside of the protected areas system. We added new data for Los Alerces and Lago Puelo NPs and Futaleufú National Reserve, with the collaboration of APN in Argentina and National Forestry Service (CONAF) in Chile.

Our aim is to build a SDM using presence records from protected areas where huemul are known to occur and project the model predictions northwards to regions where no huemul presence records exist. The absence of records in these areas is because of the lack of surveys searching for huemul on the Chilean side, and that only a few have been done in Argentina. The predictions of the model will enhance the identification of areas with suitable habitat, which will be used to guide prospective field campaigns. The predictions may also help to identify existing and potential biological corridors between protected areas and new priority areas where huemul dispersion is possible and could be fostered.

Nowadays, national protected area systems in Argentina and Chile are under pressure because new large touristic and energy development projects are being assessed for these areas. The use of an accurate spatial representation of possible huemul distribution will improve information available to decision makers to evaluate the conservation risks of implementing such projects in those areas.



#### Advances in the scientific research for the Neotropical deer

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Last December during the *II Congreso Uruguayo de Zoología* we organized the Symposium "*Advances in the scientific research for the Neotropical deer*". The symposium was attended by representative researchers, students from Uruguay and the region. In the following section we included the abstracts of the oral and poster presentations. We acknowledge the Scientific Conference Committee for hosting the symposium and authorized the abstract publication in the DSG Newsletter.

#### Seminal characteristics of Pampas deer (Ozotoceros bezoarticus)

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The aim of this study was to characterize pampas deer semen during the breeding season (January to April). Semen was obtained in 43 extractions by electroejaculation in males maintained under general anesthesia. The ejaculate volume ranged between 20 µl and 1655 µl (415.9 ± 52.6 µl; mean ± SEM), and the pH between 7.0 and 8.5, varying between transparent to white or yellow color; the consistency varied between aqueous milky to creamy. The quality of semen was  $3.3 \pm 0.2$  (scale of 0 to 5); the percentage of motile spermatozoa was  $65.2 \pm 3.7\%$ , and of progressive motile spermatozoa was  $56.4 \pm 4.1\%$ . The total amount of spermatozoa in the ejaculate was  $286.2 \pm 60.1$  (x10<sup>6</sup>), with a percentage of live spermatozoa of  $80.0 \pm 5.8\%$ ;  $65.3 \pm 3.2\%$  of spermatozoa presented morphological abnormalities. The percentage of spermatozoa with abnormal head, mid-piece and tail was  $17.4 \pm 3.1\%$ ,  $35.0 \pm 3.2\%$  and  $47.6 \pm$ 

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2.2%, respectively. The percentage of spermatozoa with damaged acrosomes was 22.7  $\pm$  2.4%, and with lost acrosomes, 4.8  $\pm$  0.8%. Finally, the spermatozoa head had a length of 7.7  $\pm$  0.5  $\mu$ m, a width of 4.4  $\pm$  0.3  $\mu$ m, an area of 28.3  $\pm$  2.6  $\mu$ m<sup>2</sup>, and a perimeter of 22.0  $\pm$  1.4  $\mu$ m. This is the first report characterizing pampas deer semen and spermatozoa parameters.

#### Preservation of pampas deer semen (Ozotoceros bezoarticus)

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Under the project "Reproductive biology of pampas deer (*Ozotoceros bezoarticus*)" semen collections were performed from March 2008 to February 2012. All samples were frozen, generating a semen bank. Six different diluents were tested to determine the best extender for pampas deer semen preservation. We used one homemade extender: Fructose-Tris-Glycine (FTG), with 10 or 20% off egg yolk added. Commercial extenders used were Red extender (10% and 20% egg yolk), Andromed (which does not include egg yolk), and Triladyl (20% egg yolk). There were no differences in the preservation of frozen samples with 10% or 20% egg yolk. Results were better with FTG than Red extender when both had 10% egg yolk. DNA integrity was better with Red extender with 20% egg yolk and FTG with 10% egg yolk, compared with Red with 10% egg yolk. Samples diluted with Andromed and Triladyl are being compared now. Our aim now is to continue obtaining semen, freeze it with the most suitable extender, and therefore increase the semen bank and potentially use it for artificial insemination.



#### First description of pampas deer (*Ozotoceros bezoarticus*) sperm ultrastructure: a qualitative-quantitative study

Poster

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Our aim was to provide a detailed, qualitative and quantitative description of the sperm ultrastructure of the pampas deer (Ozotoceros bezoarticus, an endangered South American deer species). Five adult males from the semicaptive population at Estación de Cría de Fauna Autóctona, Pan de Azúcar, Uruguay (33°47' S, 54°00' W) were anesthetized with blowpipe and dart (mixture of xylazine, ketamine and atropine) and sampled by electroejaculation. One sample/animal was diluted (PBS buffer), centrifuged (120G), fixed (3% glutaraldehyde, 4°C), postfixed in osmium tetroxide, dehydrated in acetone and embedded in plastic resin. 100 ultrathin sections were stained (uranyl acetate plus lead citrate) and examined with a transmission electron microscope (up to 500 sperm/sample were evaluated, and classified either as intact or damaged). Sperm abnormalities were classified as either head, mid-piece or principal piece defects. We calculated the percentage of normal sperm and of spermatozoa with each defect. The sperm ultrastructure coincided, generally, with that of ruminants, but some minor particularities were noted: the acrosome was flattened, with a thinner and more dense equatorial segment, as compared to the apical and principal acrosomial segments; the epinuclear zone was electron-lucent, with paracrystaline structure; the nucleus was also flattened, with oval or falciform sections and very condensed chromatin; small nuclear vacuoles were very abundant, suggesting frequent defects in chromatin condensation; the mid-piece had a very

small, irregular annulus and several dense bodies juxtaposed to the fibrous sheath. Sperm defects were frequent (53.2% of sperm studied). The most frequent defects were disruptions and folds of the plasma membrane, acrosomal dilation, vesiculization of the external acrosomial membrane, multiple tails enveloped by one plasma membrane, and frequent cytoplasmic droplets. In summary, we have described for the first time the ultrastructure of the pampas deer sperm. Its general organization was similar to other ruminants' sperm, although several minor particularities were noted, as well as a high percentage of sperm defects.

#### Different types of communication in solitary brocket deer (Mazama sp.)

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Small solitary cervids that live principally in tropical forests need to communicate with each other in order to maintain their territories and to find each other for reproduction: males and females; females and fawns. Many of these species maintain localized defection sites (latrines), which are used to delimit territories, establish presence and indicate reproductive state, especially female estrus. Male brocket deer (*Mazama gouazoubira*) are territorial and establish latrines; captive males can distinguish their own dung in a latrine from introduced dung from an unknown individual and they react differently to dung from males or females. They respond to male dung especially by counter-marking, urinating and defecating close to the introduced dung. Counter-marking is important in territory defense in many mammals. Females also distinguish between their own dung and that of unknown individuals, but their counter-marking reactions were not statistically significant; they also do not maintain exclusive territories. Acoustic communication is important in close range interactions between males and females and fawns. Males of 3 species of *Mazama (M. americana, M. gouazoubira* y *M. nemoorivaga*) were found to produce courtship bleats with females. These bleats differ

significantly among species in fundamental frequencies (F0) and duration, but the F0s are not correlated with body size. In contrast, formants (emphasized frequencies due to vocal tract resonances) do reflect body size; females thus can distinguish male species and body size based on different acoustic parameters. Females and fawns also produce the same type of bleat (with different acoustic parameters), which are important in their finding each other for lactation. Fawns remain hidden in their first few weeks of life and females seek them out periodically to feed them. In conclusion, chemical communication is more important for communication over greater distances and longer periods of time, while acoustic communication is important in close range communication.

### Male and female relationships in the pampas deer population of Los Ajos: observations that generate new questions for understanding the biology of the species Ozotoceros bezoarticus

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The Los Ajos pampas deer population is an endemic subspecies from Rocha-Uruguay with about 450 individuals. The conservation of this biological heritage requires an understanding of biological processes associated with the adaptation of individuals to the environment they occupy. Our objective was to characterize demography associated with the sex ratio and sexual segregation. We conducted 44 surveys in seven years, and we described 2149 groups. The sex ratio was 1.64 Q: 1  $\mathcal{J}$ , significantly different from the 1:1 found in captive births. Tracking adults with radio collars showed strong site fidelity. The comparison between populations detected a correlation between density and sex ratio, where environments with better pastures have higher density and fewer males per female. This pattern would be expected in a polygyny system where the females are conditioned by the carrying capacity of the environment, while the males defend their territories. This population is gregarious with a preference for mixed groups. Segregation of the sexes is observed annually at a time coinciding exactly with the birth of fawns and the growth of antlers. These results open new questions. The analysis of the sex

ratio at birth in the wild will be interesting as well as the dispersion behavior of females and juvenile males, in order to determine the origin of the sex ratio differences observed in adults. Male hormonal studies would help us understand the development of dominance status of different males and its impact on access to females. Sexual segregation patterns support the hypothesis of "predation risk" in females. It would be important to characterize the types of environments and their potential as a source of high-powered pasture forage or shelter capacity for females with calves. Our results give new insights into the response of this species to environmental changes.

#### Review of parasites reported for native cervids (Mammalia, Cervidae) from Uruguay. I. Endoparasites

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Three species of native cervids are known from Uruguay: the Marsh Deer, *Blastoceros dichotomus*, a species "probably extinct" since the middle of the past century; the Brown Brocket Deer, *Mazama gouazoubira*, that inhabits the entire country except the Southwest and the lower basin of Santa Lucía river; and the Pampas Deer, *Ozotoceros bezoarticus*, with only two surviving populations in the counties of Salto and Rocha, corresponding to two subspecies, *O. b. arerunguaensis* and *O. uruguayensis*, respectively. The aim of this communication is to provide a summary of the endoparasites reported up to the present for native cervids from Uruguay. No reports of parasites from Marsh Deer are known for the country. The following endoparasitic taxa have been reported from the other two cervid species (abbreviations: *Mg: M. gouazoubira; Ob: O. bezoarticus; Oba: O. b. arerunguaensis; Obu: O. b. uruguayensis*; WL: wild life; Cap: captivity; Copr: coprological diagnosis; Nec: necropsy; Hist: histopathology). Protozoa, Apicomplexa, Eimeriidae: *Eimeria* sp. (*Oba/Obu/*WL/Copr); *Mg/*Cap/Copr); Sarcocystidae: *Sarcocystis* sp. (*Mg/*WL/Hist); Platyhelminthes, Digenea, Fasciolidae: *Fasciola hepatica (Oba/Obu/*WL/Copr); Paramphistomatidae: *Paramphistomum* sp. (*Obu/*WL/Copr);



Taeniidae: Cysticercus tenuicollis *Ob/*Cap/Nec; Cestoda. (*Ob*/?/Nec; Mg/WL/Nec; Mg/Cap/Nec); Anoplocephalidae: Moniezia sp. (Oba/WL/Copr; Ob/Cap/Copr); M. benedeni (Mg/WL/Nec); M. expansa (Obu/WL/Copr); Nematoda, Adenophorea, Trichuridae: Trichuris sp. (Obu/WL/Copr); Capillaria sp. (Oba/Obu/WL/Copr); Secernentea, Strongyloididae: (*Obu/*WL/Copr; *Ob/*Cap/Copr; *Mg*/WL/Copr); *Strongyloides* sp. Trichostrongylidae: (Oba/WL/Copr/Nec; Obu/WL/Copr); H. contortus Haemonchus sp. (Mg/WL/Nec);Trichostrongylus spp. (Oba/WL/Copr/Nec; Obu/WL/Copr); T. axei (Mg/WL/Nec); T. colubriformis (Mg/WL/Nec); Ostertagia spp. (Oba/WL/Copr/Nec; Obu/WL/Copr); O. ostertagi (Mg/WL/Nec); Cooperia punctata (Mg/WL/Nec); Strongylidae: Oesophagostomum spp. (Oba/Obu/WL/Copr; Mg/WL/Nec); Protostrongylidae: Muellerius sp. (Obu/WL/Copr). Whereas probably the two taxa of Protozoa are specific to these cervids, all the helminthes (with the exception of the lung nematode Muellerius sp.) are shared with domestic ruminants and they, very probably, colonized the native cervids of Uruguay. This is the first report of the nematodes Strongyloides sp. and H. contortus parasitizing M. gouazoubira in Uruguay.

# Seasonal pattern of marking and agonistic behavior in male pampas deer (Ozotoceros bezoarticus)

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Reproductive seasonality in wild ruminants is usually influenced by different factors (photoperiod, food availability, climate and socio-sexual stimuli). In *O. bezoarticus*, the antler cycle is associated with reproductive patterns because it depends directly on the androgen concentration. The goal of this study was to determine whether there was a seasonal pattern to marking and agonistic behaviors in male *O. bezoarticus*. These behaviors were recorded in two groups of 6 males (adults and juveniles) housed in pens of 0.5 ha in the "Estación de Cría de Fauna Autóctona Pan de Azúcar", Maldonado, Uruguay. Focal observations were conducted weekly for 1 h AM and 1 h PM (May 2008 and April 2009, total = 204 h). Pampas deer males

showed seasonality in both behaviors. The general pattern indicates that these behaviors are synchronized with the antler and female breeding cycles, increasing their frequency 2-3 months before the breeding season onset. The frequency of marking was minimal in winter-spring, coinciding with the peak of births, antler cast, and antlers in velvet. Agonistic interactions were grouped into three types according to the temporal pattern. 1) "Low Aggressiveness": The units in this group did not change throughout the year, so they are probably used to maintain hierarchies, thus requiring little energy. 2) "Medium Aggressiveness": The maximum use of these behaviors was in winter and early spring, before the onset of the breeding season, when animals did not have antlers or had them in velvet. 3) "High Aggressiveness": Behaviors that require the use of the antlers, were risky, and involved a great deal of investment. Overall, marking and aggressive behaviors are synchronized with the antler cycle and the breeding seasonal cycle of females.

#### Strategies of behavioral dominance in pampas deer (ozotoceros bezoarticus)

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Pampas deer live in groups with hierarchical structure in which strategies of dominance vary according to social status and age. These strategies may be physical or non-physical, and may be more or less energetically demanding. We performed studies with 6 semi-captive breeding groups, each composed of 1 adult male and 4-7 adult females. We recorded all agonistic behaviours (while animals were fed with ration). Males were dominant over females, and used non-energetically costly behaviours more frequently than females. Moreover, males displayed more agonistic behaviours towards high-ranking than towards low-ranking females. They used non-energetically demanding behaviours toward high-ranking and energetically demanding behaviours toward high-ranking and energetically demanding behaviours toward high-ranking females could be more displacements of females by mere presence. In this sense, high-ranking females could be more exposed to aggression than low-ranking females while feeding, as they probably had more

access to food, increasing the probability of conflict. Another possible explanation is that –as occurs in other deer species- individuals that are closer in social rank, as occurs in males and high-ranking females, interacted aggressively more frequently. We also performed a study in two semi-captive breeding groups of young and adult males, and we observed that young males used physical agonistic behaviours more frequently than the group of adult males. Probably this is explained by the shorter period since the establishment of hierarchies. In summary, social status and age affected the agonistic behaviours displayed to establish and to maintain the social hierarchy in pampas deer groups.

# Extractions of semen by Electroejaculation (EE) and physiological changes in anesthetized adult and subadult pampas deer (*Ozotoceros bezoarticus*) males

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The "Pampas deer" (Ozotoceros bezoarticus) is the most endangered cervid species native to Uruguay. The knowledge and application of effective reproductive technologies could contribute to the survival of this species. The aim of this study was to determine the response to different parameters during general anesthesia (GA) and electroejaculation (EE) in two groups: adult and young male test specimens, subjected to a repetitive procedure (AG and EE) applied seasonally (fall, winter, spring, summer, and fall again). The study was conducted by the ECFA (Estación de Cría de Fauna Autóctona -Cerro Pan de Azúcar-Maldonado, Uruguay -33°48'32<sup>--</sup>S, 55°56'52<sup>--</sup>W) using 11 animals, with 53 captures and samples taken by EE in five consecutive seasons. The animals composed two groups of specimens, 6 adults and 5 subadults, in which the following parameters were measured: Heart rate (HR), Pulse rate (PR) and Respiratory rate (RR), Partial oxygen saturation (SpO2) and Rectal Temperature (RT); before (BEE) during (DEE) and after the EE (AEE). Also blood samples BEE and AEE were taken to determine the concentrations of creatine kinase (CK), aspartate aminotransferase (AST), serum alkaline phosphatase (FAS) and cortisol. During the EE the HR and PR increased in both groups, with higher values in the adults.. The concentration of CK, FAS, and AST values increased DEE in both groups; the values of CK were greater in the adults although the FAS

and AST were greater in the group of subadults. Furthermore it was found that the repetition of the GA and EE did not affect the values of the different parameters studied; only effects possibly due to the season when the study was done were observed. It can be concluded that the EE under GA can be used in the same individual repeatedly without any observed significant effects on its health.

#### Vocalizations during electroejaculation in anesthetized adult and subadult male Pampas deer (*Ozotoceros bezoarticus*).

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There are several studies describing the stress response to an electroejaculation (EE) in ruminants, which is probably due to the pain caused by the electric shock applied. Several authors have found that EE causes vocalizations which are probably induced by pain. The aims of this study were to determine whether EE under general anesthesia (GA) in the Pampas deer (Ozotoceros bezoarticus) produces vocalizations, and to characterize and analyze these vocalizations to determine whether they are affected by the category and level of voltage used. The study was conducted in the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (ECFA) (Maldonado, Uruguay). 10 EE were conducted and recorded in adults (5-7 years) and 11 EE in subadults (2 years old). Vocalization emissions were recorded with a directional microphone connected to a recording system in mp4 format. We analyzed the total number of vocalizations emitted, the total time spent vocalizing, the duration of vocalizations, the fundamental frequency (F0) parameters (Hz), F0 start (Fstart), F0 final (Fend), F0 maximum (Fmax) and F0 minimum (Fmin). Both groups (adult and young deer) produced vocalizations during the EE, but in young specimens each vocalization lasted longer, and the total time spent vocalizing also was longer than in the adults. For each case studied the F0 start, F0 maximum, F0 final and F0 minimum were higher in young animals. In conclusion, the Pampas deer vocalizations emitted during EE under GA and the total time of vocalization were longer and the frequencies were higher in subadult deer than in adults.

#### Ecoethology of mule deer in the Chihuahuan Desert, Mexico.

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Mule deer (Odocoileus hemionus) were studied in the Mapimí Biosphere Reserve (MBR), Durango, Mexico, in the Chihuahuan Desert, because of their importance as an herbivore and hunting resource. They share this habitat with cattle under extensive management. During 1996-1997, using the counting of faecal groups in 8 transects of 800 m as an indirect method, the estimated density was considered very low (between 2 and 3 deer / km<sup>2</sup>). In 2006-2007 the same sites were sampled, estimating a density between 4 and 6, although during the 2010 -2011count, the average was 3 deer  $/ \text{ km}^2$ . In order to determine the distribution and distances traveled by mule deer, six transects of 5 km long and 20 m wide were used. We found that deer seem to concentrate their activities at the bottom of the hill San Ignacio at distances no greater than 1.5 km. During 2010 and 2011, in order to find out whether there is spatial competition between deer and cattle, faecal groups were obtained in the same transects for both species. The average was 86.58 deer faecal groups / ha and 32.21 cattle faecal groups/ ha. Measures of habitat variables such as visibility, slope, elevation and vegetation were also taken. Correlations of habitat variables with density ratios and principal component analysis (PCA) were used to identify the most important: the volume of vegetation and visibility for one component, the density and the slope for the second component. Differences in habitat use between deer and cattle related to the slope, altitude and vegetative cover were found.

### Preliminary analysis of the seed dispersal role of the pampas deer

### (Ozotocerosbezoarticus, L. 1758)

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The Pampas deer was the most characteristic and abundant ungulate of the Uruguayan grasslands, with a fundamental role in its landscape diversity. Previous observations on its feeding ecology indicate that the species is a mixer feeder with preference for grasses, fruits and flowers. The objective of this research was the description and analysis of the seeds found in pampas deer faeces from two Uruguayan populations. The study was conducted at private ranches of the "ÁreaPrioritaria Arerunguá", Salto, and the "Sierra de Los Ajos" area in Rocha. We made transects in each enclosure and collected only fresh faeces. The sampling covers more than two years, with a total of 130 samples. At the laboratory the seeds without fractures were isolated, and considered as potentially viable seeds. Then, we photographed, generating the first digital image bank for grasslands seeds in Uruguay. We identified taxonomically the potentially viable seeds using catalogues and the Botanic Laboratory seed collection. We also classified the seed species according to their productive type, which determines their value and efficiency as cattle fodder, using Rosengurtt criteria. Identified species include "fine grasses" and "minor shrubs". Primary classification confirms feeding observations of pampas deer feeding ecology, since it includes a broad number of species of different families, with Poaceae, Cyperaceae and Caryophyllaceae standing out. The species we identified could be indicators of the pampas deer's potential role as a high quality forage seed disperser.

#### **Conservation Genetics Units in Neotropical deer using molecular markers**

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Molecular markers are useful in confirming the existence of several cryptic Neotropical deer species that occur in sympatry. The main goal was to analyze the phylogenetic relationship and phylogeographic patterns of Neotropical deer using molecular markers. We analyze the intraspecific variability in a biogeographic context using markers useful for characterizing species with DNA from materials collected noninvasively from materials such as carcasses and feces. We used Cytocrome b, D loop, Citocrome Oxidase I and nuclear markers associated with sexual chromosomes such as Amelogenine. The interspecific analysis using Cytocrome b was useful to infer the phylogenetic relationships within Neotropical deer. Applying the divergence time among the species and the evolutionary rates, we detected the existence of two clades that diverged 5 MYA ago. We designed a set of primers to amplify a fragment of 156 bp of Citocrome Oxidase I, useful for taxonomic identification from low quality DNA. We determined ESU (Evolutionarily Significant Units) using D loop in pampas deer Ozotocerosbezoarticus, and in the marsh deer Blastocerusdichotomus. We are using a fragment of the Amelogenine gene for sexing samples collected non-invasively and useful for analyzing introgression among species. Finally, the combined use of these molecular markers will be adequate to determine the presence, abundance and genetic diversity of Neotropical deer.

Project funded by CSIC.

### Population dynamics of endoparasites in wild Pampas deer (Ozotoceros bezoarticus), sheep (Ovis aries) and cattle (Bos taurus) in the region of Arerunguá, Salto - Uruguay.

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are useful for analyzing ecological dynamics and their role as possible reservoirs. Since 1992 we have been monitoring endoparasitic fauna in the Pampas deer (*Ozotoceros bezoarticus*) in Arerunguá, Salto and the Sierra de los Ajos, Rocha, populations in Uruguay. The genera of the endoparasites identified by the morphology of eggs, larvae and/or adults were *Trichuris, Capillaria, Strongyloides, Fasciola, Paramphistomum, Moniezia, Haemonchus, Ostertagia, Trichostrongylus, Oesophagostomum* and *Coccidias ooquiste*. The aim of this study was to compare seasonal changes in parasitic loads of pampas deer, sheep and cattle that occupy the same paddocks. We are correlating the annual seasonal composition of the parasitic load with climatic variables and livestock stocking densities. Sampling is being conducted in three paddocks inhabited by pampas deer, cattle and sheep. The coproparasite analysis was performed in adult cattle and calves, sheep and deer that live together in the same paddock. We found *Paramphistomum* eggs in the cattle and pampas deer. We also detected *Moniezia* eggs in calves. We found that the sheep parasite loads had highly significant values for the number of gastrointestinal nematode eggs eliminated and these could be a source of infection for the deer and the ecosystem.

# First Pampas deer (*Ozotoceros bezoarticus*) ancient DNA amplification: its relevance to Conservation Biology and Archaeology

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The Pampas deer was one of the most heavily consumed animals in Uruguayan prehistory, used for food and as a source of raw material. In the recent past the pampas deer was very abundant in Uruguay based on the archaeological record, the ethno-historical chronicles and the genetic diversity that the species maintains. We estimated that the species was consumed for more than 3500 years. Currently there are no more than 1500 individuals of Pampas deer in Uruguay. We are reporting the first DNA isolation records detailing the methodology used and the results obtained in the extraction, amplification and sequencing of mitDNA from an archaeological sample of Pampas deer bone dated at 1700 years old from a prehistoric mound in the area of San Miguel (Rocha). This result and its implications are discussed and evaluated from the point of view of archeology, as well as from conservation biology. This is the first record of pampas deer ancient DNA isolation's providing an interesting source of new knowledge about Pampas deer natural history and its interaction with past human prehistoric populations.



Abundance, distribution and habitat use of Central American red brocket deer (*Mazama temama*) in the municipality of San Bartolo Tutotepec, Hidalgo, Mexico.

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The Central American red brocket deer (Mazama temama) is one of the lesser known species of deer in Mexico; only 12% of the national studies have examined aspects of its biology and it could be considered as a vulnerable species. The study area was located in the cloud forest (1757.8 ha) of San Bartolo Tutotepec, state of Hidalgo, Mexico. For abundance, distribution and habitat use we established 14 transects 500 m long and 1m wide. We calculated the relative abundance index as number of tracks per km<sup>2</sup>. Eleven points were established within each transect spaced every 25 m to measure the following variables: forest structure, low trees and shrubs, cover protection, canopy cover, available plant species consumed. We performed a principal component analysis and a general linear model of simple regression to determine the relationship between each of the variables and the relative abundance of brocket deer using the program Statistica v. 7.0. The Mann-Whitney test was applied to assess whether there were statistically significant differences between the abundances and the variables in conserved and disturbed sites. There were a total of 352 brocket deer tracks with a sampling effort of 21 linear km. The average relative abundance ratio was 0.034 tracks/km, which is considered low. Variables with significant differences between the sampling points and the presence of deer with respect to those with deer absence were: density, canopy cover, cover protection for young, and adults, linear density and richness of shrubs and herbaceous plants. There was low deer abundance in disturbed sites (less than 10% of the tracks found); this indicates that the brocket deer prefer sites with greater vegetation cover and avoid deforested areas.



#### Anatomy of the stomach of Cervus elaphus barbarus

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The barbary stag (*Cervus elaphus barbarus*), or atlas deer, is a subspecies of red deer that is the only member of the deer family native to Africa, in the countries of Algeria, Tunisia and Morocco. *Cervus elaphus* is considered an intermediate feeder ruminant, with a tendency to include more browse than grass in its natural diet. To the best of our knowledge there is no available information on the macroscopic anatomy of the stomach of *Cervus elaphus barbarus*. In this work we describe the macroscopic anatomy of the stomach of one adult male *Cervus elaphus barbarus* from Tunisia, North Africa. The stomach of this barbary stag was composed of the four classic compartments of ruminants. The weight of the entire stomach was 10.5 Kg. The ruminal papillae were distributed unevenly in the rumen. The maximum height of the cristae reticuli was 0.2 cm. The *Cellulae reticuli* were mostly undivided. The *Curvatura omasi* measured 15.0 cm and the omasum had about 12 *Plicae spirales abomasi*. We concluded that the stomach morphology of the barbary stag had the characteristics of other intermediate feeder ruminants.

#### Anatomy of the gastrointestinal tract in the axis deer (Axis axis)

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The macroscopic anatomy of the stomach and intestines of adult axis deer (Axis axis), a cervid species considered an intermediate/mixed feeder, was observed and recorded. Eight adult wild axis deer from Parque Anchorena (Colonia, Uruguay) of both sexes were used and studied by simple dissection. The ruminal papillae were distributed in the entire area of the inner surface of the rumen, and were larger and more abundant within the atrium. The ruminal pillars had no papillae. There was an accessory ruminal pillar located between the right longitudinal and right coronary ventral pillars connected to the caudal pillar. No dorsal coronary pillars were found and the ventral coronary pillars were connected. The reticulum was the third compartment in size. The Cellulae reticuli were not divided and rarely contained secondary crests. There were no Papillae unguiculiformes. The omasum was the smallest gastric compartment. The abomasum had about 12 spiral Plicae, and a small pyloric Torus was present. The intraruminal papillation was similar to that in species that are characterized by a higher proportion of grass in their natural diet. The finding of the small reticular crests is typical for browser ruminants and was coincident with data reported for other deer. The comparative ratio of the small intestine to the large intestine was 1.69, which appears below that of the 'browser range'. We concluded that the gastrointestinal system of axis deer reflected morphological characteristics typical of both types of ruminants: browser and intermediate feeders.

March, 2013

#### Macroscopic anatomy of the pampas deer (Ozotoceros bezoarticus, Linnaeus

#### 1758) female genital system

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The objective was to describe the macroscopic anatomy of the genital organs of the female pampas deer (Ozotoceros bezoarticus). Organs from animals that had died at a captive breeding station were dissected. Twenty females, 17 adults and 10 subadults, prepuberal and approximately one year old, were studied by gross dissection. The ovaries of adult females without corpora lutea weighed  $0.22 \pm 0.02$  g (right) and  $0.20 \pm 0.01$  g (left), respectively, and represented  $0.03 \pm 0.01\%$  of the body weight. All corpora lutea (n=6) were found in the right ovary, but in cases where there was no corpus luteum present, the right ovary tended to be heavier than the left (P=0.1). The absence of an ovarian bursa, intercornual ligament, and suburethral diverticle are peculiar to this deer species. Only 6 convex caruncles were found in the uterine horns; this situaction differs greatly from domestic ruminants, which present more than 100. Four regular folds that were observed in the cervix of the uterus should not be an obstacle to the passage of a transcervical catheter, allowing intrauterine artificial insemination. The close relationship between the ovarian artery and vein stood out and could be associated with the passage of the uterine luteolytic factor from the vein into the ovarian artery, avoiding its systemic passage, as happens in domestic ruminants. While the plan of organization was similar, there are some differences with the descriptions of other ruminants. Our results may be especially useful as they provide information that should be considered in the development of female reproductive biotechnologies.

March, 2013

#### Macroscopic anatomy of the pampas deer (Ozotoceros bezoarticus, Linnaeus

#### 1758) male genital system

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In order to describe the macroscopic anatomy of the genital organs of the male pampas deer (Ozotoceros bezoarticus), organs from ten animals that had died at a captive breeding station were dissected. The unpigmented scrotum was located in the inguinal region near the body, and was covered by the thighs. In the investments of the testicles the dartos tunic was greatly developed. The cremaster muscle was located dorsocaudal to the testicle, and was caudally divided into three bundles of fibers. The right testicle was significantly heavier than the left, and there was a positive relationship between body weight and weight of both testicles. The tail of the epididymis, ventrally located, had a caudal portion attached to the tail extremity of the testicles by the ligament of the testicles, and a free elongated portion located caudally. The deferent duct was located caudal and medial to the corresponding testicles. The accessory genital glands were the ampullary glands, vesicular glands, and a small pars disseminata of the prostate. The penis was fibroelastic, without sigmoid flexure, with a thick albuginea. The retractor penis muscle was very long, and ended in the distal part of the penis near the rudimentary glans. The general disposition of the male genital organs of the pampas deer were similar to those of other ruminants, with some differences, such as the size and location of the testicles, the absence of the sigmoid flexure of the penis, and the small number of accessory genital glands.

# Pampas deer (*Ozotoceros bezoarticus*) *in vitro* tissue propagation under various conditions and a cell bank creation for the species

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The Pampas deer (Ozotoceros bezoarticus) is an endangered species, so the cryopreservation of biological materials is an essential strategy for storing its genetic information. The objective of this study was to propagate and preserve cells from pampas deer tissues obtained at the Estación de Cria de Fauna Autóctona del Cerro Pan de Azúcar (ECFA), Maldonado, Uruguay, in various culture conditions. Skin biopsies were taken under general anesthesia. Until the samples were processed, they were maintained in a conservation medium at 4°C. Culture growth was compared according to: 1) processing time (begining 6 or 24 h after the tissue extraction); 2) use or not of antibiotics (penicillin-estreptomycin or gentamicin); 3) culture medium used (E-MEM or RPMI); 4) time of tissue culture (fresh implants or two week-culture implants). All tissues were cultivated at 37°C in a controlled atmosphere (5% CO<sub>2</sub>). Fibroblast monolayers were propagated in every culture condition described above. Cellular growth was observed in 90.0% (18/20) and 91.6% (22/24) of the tissues cultured 6 and 24 h after sampling, and in 90.9% (10/11) and 100% (9/9) of the tissues cultured with penicillin-estreptomycin or gentamicin respectively. The culture medium used did not have an impact on the cellular growth results, as 80.0% (8/10) and 88.9% (8/9) of E-MEM and RPMI cultured samples grew. Finally, cellular growth was observed in 90.0% (18/20) and 100% (12/12) of the fresh tissues and the re-explanted tissues respectively. None of the variables analyzed affected the results, so cellular culture had a high rate of success in all conditions reported. Cells were propagated and preserved in liquid nitrogen, creating a cell bank for the species.



#### Neotropical deer Identification using COI by RT-PCR

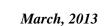
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The Neotropical Cervidae is a part of the family which has a wide variety of taxa (17) and several examples of cryptic species within the genus Mazama. The goal of our research was to design a suitable marker for taxonomic identification by Real Time PCR amplification (RT-PCR) using samples of low quality. We amplified and sequenced the complete COI gene of 52 pampas deer (Ozotocerosbezoarticus), individuals from 3 brown brocket deer (Mazamagouazoubira) and 1 marsh deer (Blastocerusdichotomus). We designed a specificshorter primer to amplify a 156 bp fragment in the COI gene, which is a highly conserved region at the intraspecific level, but variable among species. The effectiveness of the marker was testedby PCR final and Real time PCR. In both cases we obtained highly efficient amplifications (95-98%) for tissue and fecal DNA (42). The advantage of Real time-PCR is, that using High Resolution Melting (HRM), we obtained the melting curves characteristic for each species. The results were confirmed by sequencing the amplified products and the subsequent "Blast Search" on the species available in GenBank, as well as through the comparative analysis with sequences of our data.



# Effect of female presence on reproductive biology of pampas deer males (*Ozotoceros bezoarticus*)

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The aim of the present work was to determine if pampas deer males in permanent contact with females possess greater fecal testosterone concentrations and better seminal characteristics, both in fresh and after dilution, than males isolated from females. We used 11 adult males from Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (Maldonado, Uruguay), from which 5 were allocated with females, and 6 were isolated from them. Fecal testosterone concentration was determined by radioimmunoassay in samples collected weekly from October to May. Semen was collected by electroejaculation before and at the end of the breeding season (November and April respectively). Semen was evaluated in fresh and after the addition of extenders (November: Fructose-Tris-Glycine + 20 % egg-yolk; April: Andromed). Contact with females produced a higher testosterone concentration (P=0.04). In November, fresh semen from males with females had greater quality (P=0.03), a greater percentage of progressive motile spermatozoa (P=0.02), and tended to have a greater percentage of motile spermatozoa (P=0.10). After dilution, semen from males allocated with females had greater quality (P=0.04), percentage of motile spermatozoa (P=0.04), of progressive motile spermatozoa, and the percentage of spermatozoa with an intact acrosome was also greater (P=0.03). In April, fresh semen from both groups of males did not differ. After dilution, semen from males with females had a greater percentage of spermatozoa with a damaged acrosome (P=0.04). We concluded that direct contact with females stimulated pampas deer males to produce more testosterone, and increased seminal quality before the breeding season.

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