UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

Wildlife Management in Conservations Parks – General and Veterinary Intervention

Dissertação de Mestrado Integrado em Medicina Veterinária

Catarina Cabral Oliveira

Orientadores: Doutor Simone Angelucci

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DECLARATION

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UTAD, Novembro 2015

(Catarina Cabral Oliveira)

TO MY PACK,

“Não sou nada.
Nunca serei nada.
Não posso querer ser nada.
À parte isso, tenho em mim todos os sonhos do mundo.”

(Tabacaria, Fernando Pessoa)

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A special thank you to Nature…

ABSTRACT

My thesis of master degree is entitled "Wildlife management in conservation parks – general and veterinary intervention", being this a very comprehensive theme, which I tried to explore, as much as possible, in its plenitude. However, I elected to highlight the management and monitorization of one of the most emblematic and controversy species, the wolf (*Canis lupus*). My internship was performed on Majella National Park, on Abruzzo, Italy.

The creation of protected areas is a benefit to the conservation of nature and of the beings, whom inhabit it. Although not always in a pacific way, the park represent the link/connection between nature and people, through environmental education, promotion of activities (to increase the contact of man with nature), prevention and reduction of conflicts between human and predators among other intervention areas. The ecotourism became a "survival" tool to small populations and inhabitants (humans and wildlife) of the park.

The possibility of accompanying the management, functional and conservation measures of a park, through the contact with a multidisciplinary team, provided me with a very enriching personal and professional experience. As the park involves the entire ecosystem, I had the opportunity to expand my knowledge on fauna, flora, history, culture and people.

This document approaches the methods of animal monitoring, invasive and non invasive, which allows the acquisition of general information on the ecosystem or information regarding only the target specie. These techniques can be followed by the capture of the animals, which influence the method of choice, in the case of the wolf, a leg hold is used, which is constructed by the trap itself, the snare, anchor, alarm system and bait. The immobilization protocol applied to the trapped animal varies in accordance to the experience and preference of the veterinary, always considering the pharmacological actions and drugs interactions and in also, to the evaluation of the general health status of the animal and monitoring of vital parameters (at least heart and breathing rate and body temperature). During the immobilization, it is performed the collection of biological samples, evaluation of lesions, biometric measures and photos. In the end of the

immobilization process it is placed a collar with a radio transmitter or another telemetry device which after the release of the animal allows the attainment of information on their physiology, behaviour and ecosystem.

Nature is target to constant direct and indirect threat. The purpose of the park (although utopian), resides on the balanced and sustainable management of the natural resources, in addition to the promotion of an ecotourism, with the integration of the population. Unfortunately, sometimes the economic “value” overlaps the conservationist value. All effort must be applied on the professional side, but also on the citizens’ part, in order to care for the most precious asset, nature.

KEY WORDS:

Wolf; Immobilization; Chemical immobilization, Capture; Welfare, Ecosystem

RESUMO

A minha dissertação de mestrado está intitulada de "Wildlife management in conservation parks – general and veterinary intervention" (Gestão e conservação da vida selvagem em parques – intervenção geral e veterinária), sendo este um tema bastante abrangente, que tentei explorar na sua plenitude dentro do que foi possível, tendo, no entanto, elegido como área de destaque a gestão e monitorização de uma das mais emblemáticas e controversas espécies, o lobo *(Canis lupus)*. O meu estágio foi efectuado no Parque Nacional de Majella, na região de Abruzzo em Itália.

A criação de áreas protegidas, é uma mais valia na conservação da natureza e dos seres que lá habitam. Embora nem sempre de forma pacífica o parque representa o elo/ligação entre a natureza e as pessoas, através de educação ambiental, promoção actividades (para aumentar o contacto do homem com a natureza), prevenção e redução de conflitos entre homem e predadores, entre outras áreas de intervenção. O ecoturismo passou a ser uma ferramenta de "sobrevivência" para as pequenas populações e habitantes (humanos e selvagens) que habitam no parque.

A possibilidade de acompanhar a gestão, funcionamento e medidas de conservação de um parque, através do contacto com uma equipa multidisciplinar, proporcionou – me uma experiência bastante enriquecedora, a nível profissional e pessoal. Como o parque engloba todo o ecossistema tive a oportunidade de expandir os meus conhecimentos sobre fauna, flora, história, cultura e pessoas.

Neste documento são abordados os métodos de monitorização animal, invasivos e não invasivos, que permitem a obtenção de informação geral sobre o ecossistema ou informação somente referente à espécie alvo. Estas técnicas podem ser seguidas pela captura de animais cujo método vai depender da espécie animal, no caso do lobo é usado uma armadilha de perna, que é construída pela armadilha em si, laço, âncora, sistema de alarme e isco. O protocolo de imobilização química a aplicar ao animal armadilhado varia de acordo com a preferência e experiência do médico veterinário, tendo sempre em conta as acções farmacológicas e interacções dos fármacos, acompanhado de uma avaliação do estado geral e monitorização de alguns parâmetros vitais (pelo menos da frequência

cardíaca, respiratória e temperatura). Durante a imobilização são efectuadas colheita de amostras biológicas, avaliação de lesões, medidas biométricas e feitas fotografias. No fim do processo de imobilização é colocado um colar com um rádio emissor ou outro meio de telemetria que após a libertação do animal permita obter informações sobre a sua fisiologia, comportamento e ecossistema onde ele vive.

A natureza é alvo de constante ameaça, tanto directa como indirectamente. O papel dos parques (embora utópico), reside na gestão equilibrada e sustentável dos recursos naturais, conjuntamente com a promoção de um ecoturismo, com a integração da população. Infelizmente por vezes o "valor" financeiro sobrepõe – se ao valor conservacionista. Sendo que todos os esforços devem ser implementados tanto por parte dos profissionais da área, mas também por parte do cidadão comum, de zelar pelo bem mais precioso que temos, a natureza.

PALAVRAS CHAVE:

Lobo; Monitorização; Imobilização química, Captura; Bem – estar; Ecossistema

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LIST OF ABBREVIATION

-	Negative
%	Percentage
+	Positive
µ	Morphine
µ g	Micrograms
AIHTS	Agreement on International Humane Trapping Standards
Alb	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
Amy	Amylase
ANOVA	Analysis of Variance
AST	Aspartate Transaminase
AV blocks	Atrioventricular blocks
Bas	Basophils
bpm	Beat per Minute
BSE	Bovine Spongiform Encephalopathy
BUN	Blood Urea Nitrogen
c	Colorimetric
Ca^{2+}	Calcium
Chol	Cholesterol
cm	Centimeters
CM	Capture Myopathy
CNS	Central Nervous System
CO_2	Carbon dioxin
CR	Council Regulation
Creat	Creatinine
D	Dopamine
DABP	Diastolic Arterial Blood Pressure
DNA	Deoxyribonucleic acid
e	Enzymatic
EC	European Commission
ECG	Electrocardiogram
EDTA	Ethylene diaminetetraacetic acid
EEC	European Economic Community
Eos	Eosinophils
F	Female
fl	Femtoliters
FM	Frequency Modulation
g	Grams
GABA	Gamma aminobutyric acid
GGT	Gamma glutamyltransferase

Glob	Globulin
Glu	Glucose
GMRS	General Mobile Radio Service
GPRS	General Packet Radio System
GPS	Global Position System
GSM	Cellular Network Standard
h	Hours
HCT	Hematocrit
HGB	Hemoglobin
HR	Heart Rate
i.e.	*id est* (That is)
IM	Intramuscular
ISPRA	Institute for Environmental Protection and Research
IU	International Unit
IV	Intravenous
IZSAM	Istituto Zooprofilattico Sperimentale d' Abruzzo e Molise
k	Kinetic
$\mathbf{K^+}$	Potassium
Kg	Kilograms
Km	Kilometers
$\mathbf{Km^2}$	Kilometers Squared
l	Litters
$\mathbf{LD_{50}}$	Median Lethal Dose
LUC	Large Unstained Cells
Lymp	Lymphocytes
m	Meters
M	Male
MABP	Mean Arterial Blood Pressure
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
mEq	Milliequivalent
mg	Milligram
min	Minutes
ml	Milliliters
mm	Millimeters
mmHg	Millimeters of Mercury
Mmol	Milimol
MMS	Multimedia Messaging Service
MNP	Majella National Park
Mon	Monocytes
MPV	Mean Platelet Volume
mV	Millivolts
n	Sample size
$\mathbf{Na^+}$	Sodium

ND	No Determined
Neut	Neutrophils
NIBP	Noninvasive Blood Pressure
NMDA	N – Methyl – D – aspartate
No	Number
NOP	Nociceptinorphanin
O_2	Oxygen
^oC	Degree Celsius
P^+	Phosphorus
PA	Protected areas
PCV	Packed Cell Volume
Pg	Picograms
PLT	Platelets
R	*Rectus*(Right)
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
Reg	Regulation
Rpm	Respiratory per Minute
RR	Respiratory Rate
RSA	Respiratory Sinus Arrhythmia
S	*Sinister* (Left)
SA node	Sinoatrial node
SABP	Systolic Arterial Blood Pressure
SC	Subcutaneous
sec	Seconds
SMS	Short Message Service
SPO_2	Hemoglobin Saturation
TB	Total Bilirubin
T^o	Temperature
TP	Total Proteins
Triglyc	Triglycerides
TTDs	Tranquilizers Trap Devices
UC	Uric Acid
UHF	Ultra High Frequency
VHF	Very High Frequency
WBC	White Blood Cell
YNP	Yellowstone National Park
µ m	Micromolar
δ	Delta
κ	Kappa

1. BIBLIOGRAPHIC REVISION

1.1. NATIONAL PARKS

The interest in wildlife and environment is relatively recent. The firsts protected areas (PA) for the preservation of nature were created only in the XIX (nineteenth) century. The Yellowstone National Park (YNP) was established in 1872, as the first world PA. This park is situated in three states, Wyoming, Montana and Idaho . The economic aspect was the basis for the creation of the YNP, due to the interest of the Northern Pacific Railroad Company in monetizing their recent railway line through the passage near the park. Unfortunately, the real problem associated to the “past concern about nature” and its management was the Man. The requirements of the “Park” were to become a PA without human presence (which decolonized and tortured indigenous tribes whose territories covered the park area) and where only "good animals" could live in the park (herbivores and grizzly bears (*Ursus arctos ssp.*), the animals most appreciated by tourists). This became one of the biggest documented harvest against carnivores, mainly wolves which contributed to their extinction in the park for a period almost of 60 years and caused an imbalance in the ecosystem, which due to the absence of predators originated an over population of herbivorous, and ravaged all vegetation (Barber-Meyer, Mech, and White 2008; Sellars 2009; Smith, Peterson, and Houston 2014; How Wolves Change Rivers 2014; «Yellowstone, Unnatural Histories - BBC Four» 2015).

The real concern with nature and wildlife as a whole in parks (with biotic and abiotic factors) began only in the 1920/30s century in some PA of North America, with the interview of George Wright, Joseph Dixon and Ben Thompson, who made the first field fauna survey and were the precursors of some ideas now considered the base to conservation biology (Shafer 2001). Some years later (in 1949) Aldo Leopold, had great influence in the struggle for the defense of wolf, in a time in which other biologists were against the presence of this animal (Mech and Boitani 2007).

After an evolution in perception, the rules and management of the parks, nowadays, include concerns about legal, social, political, economic and cultural conditions which are some of the aspects required in a park frame, but also the ecosystem and sustainability (Andrade and Rhodes

2012). A notorious and ascendant concern on the environment and wildlife ecosystems remodeled the structure of parks, also encompassing the local populations, using the ecotourism as a form of conservation and also a way to minimize the conflicts between humans and wildlife (Coria and Calfucura 2012).

My internship was conducted in Majella National Park (MNP). MNP was founded in 1991, its distribution covers the province of Pescara, L'Aquila and Chieti, in the region of Abruzzo. Not just the fauna and flora are one of the richness of the Park, but also old traditions and archeological findings make MNP a place very popular to tourists. One of the Park's objectives is the involvement of local population in order to take advantage of nature offers. Unfortunately this cooperation and coexistence is not always peaceful, regarding the predators that are part of the "enchantment" and residents park's ecosystem («Majella National Park | Parco Nazionale della Majella» 2015; International Wolf Congress 2013).

The management of wildlife, including relation between predators and human being has always been a major problem in the parks. One of the more problematic predators, who made the area of the MNP his home, is the wolf.

1.2. THE WOLF

The gray wolf (*Canis lupus*) in North America is the most emblematic member of the "wolf family", there are almost thirty subspecies widespread throughout almost all the continents, the Italian or Apennine wolf (*Canis lupus italicus*), is one of them. The other wolf species known are Red wolf (*Canis rufus)*, Ethiopian wolf (*Canis simensis*) and African golden wolf (*Canis anthus)*, the inclusion of the this last two species of wolves on the family is target of a lot of controversy (Mech and Boitani 2007; Rueness et al. 2011; Koepfli et al. 2015; «O chacal-dourado de África é afinal… um lobo» 2015).

Since primordial times, the relation of the wolves and men was never a pacific one. In reality they only tolerate each other, and fear, respect and curiosity were also part of such "relation". This coexistence worsen, during the evolution of men and his change from nomad to a sedentary life. This consequently initiated the agriculture and livestock dependency. The difficulties in coexistence between man and wolf was worsened by fairy tales as "little red riding hood" and "three little pigs", writers and kings, whom caused that all the threats to wolf led direct or indirectly to the men (Caetano, Ferreira, and Mateus 2006; Wolfe and Weston 2007; Ballard 2012). Although never entirely pacific the coexistence and fascination of both parts led to the domestication of the ancestral of wolf in about 27 to 40000 years (Ballard 2012; Wolfe and Weston 2007).

Wolves are one of the most successful animals in the planet. The *Canis lupus* distribution is not only distributed in the north hemisphere with the exception of Japan, United Kingdom, Ireland, Belgium, Denmark, Luxembourg, but is also present in south hemisphere namely in Ethiopia and North and West of Africa. After the decline of the species in the 70's, nowadays the index of population abundance is considered stable and increased in some places. The wolf is a very adaptable animal that can live in a vast diversity of habitats, which include forest, deserts, swampy and arctic areas and tundra, from sea level to mountain tops. The weight, length and coat of the wolves differs according to his distribution. Regarding weight, one of the heaviest wolf is the Alaskan tundra wolf (*Canis lupus tundrarum*) with approximately 50 – 60 Kg in some males, for the other hand the smallest wolf is the Arabian Wolf (*Canis lupus arabs*), with a mean weight of 20 Kg (Malmsten 2007; Wolfe and Weston 2007; Ballard 2012; «Large Carnivore Initiative for Europe > Large carnivores > Wolf» 2015).

The wolf can be considered a very "plastic animal" because its diversity and adaptability to "food level". Usually wolves hunt medium – sized to large mammals, predominantly deer and livestock, but sometimes smaller animals such as rabbits, hares and bird species. Occasionally, wolves will scavenge at rubbish dumps and eat fruit, vegetables, insects and fish. It can also be necrophagous and in some places the domestic dog (*Canis lupus familiaris*) is a essential part of their diet (Apollonio and Mattioli 2006; Mech and Boitani 2007; Wolfe and Weston 2007; Ballard 2012).

The wolf social structure can be described as family relationship. Basic family ties promote cohesion, usually the wolf pack consist in a breeding pair and their offspring of the actual year and previous year. They possess a territory, their home range, which they defend from others packs or dispersal wolves. Food resources, prey species and abundance of prey, diseases, social competition in the pack, the ecosystem saturation as well as the presence of other packs and/or others predators, among others factors, influences pack size, which in good condition can have more than 20 individuals (Mech 1981; Mech and Boitani 2007; Wolfe and Weston 2007; Ballard 2012).

Wolves are generally a monogamous species, their breeding season varies between February to March. After a gestation period of 61 – 64 days, the female gives birth between 1 – 11 pups in a den. They stay in the den and nearby until 8 weeks of age. After this period they are presented to the remaining family members in a *rendezvous*, after an enthusiastic presentation, they will start to live above the ground, remain with their siblings who will take care of them. The socializing period is very important to acquire natural behaviors, to establish a cohesion and relation between the members. By autumn (6 – 8 months of age), the pups are large enough to follow the pack on hunts for large preys. Before this period, they are feed through regurgitation. Sexual maturity happens between 9 – 10 months (in captivity) to 5 years old, the average age is generally 2 years (in wild). This is one of the most important stages in a wolf's life, in general a wolf can either stay with his pack, become a dispersing wolf in order to find a mate and start his own family, or become part of another existing pack. There are other less common behaviors registered. Note that this dispersion phase is associated to a high mortality rate (Mech and Boitani 2007; Wolfe and Weston 2007; Ballard 2012).

Wolves show considerable variation in home range, which can go from 40 – 60 Km to 1.676 Km. Factors that influence this range vary from the size of the pack, food resources, prey abundance, presence of other packs, anthropomorphic influences, among other factors. They need to travel long distances to hunt and defend/mark they territory. Usually wolves spend about 30%of each day traveling. The most notable description about distance traveled by a wolf to find and fix in a new territory, was the wolf Slavc. He accomplished an epic 2000 Km migration across Europe from Slovenia to Italy via the Austrian Alps (Constable et al. 1998; Mech and Boitani 2007; Mattisson et al. 2013; Fabbri et al. 2014).

Across Europe, the term population is usually used regarding the wolves, they are a dynamic animal, respond to different ecological but also to different social and political rules. There are 10 wolf populations recognized in Europe: north – west Iberian (Portugal and Spain), Sierra Morena (small population in Spain), Alps (which is part some population of Italian wolves, also France, Switzerland, Austria and Slovenia), Italian peninsula, Dinaric – Balkan (Slovenia, Croatia, Bosnia & Herzegovina, Montenegro, Albania, Serbia, Greece and Bulgaria), Carpathian (Slovakia, Czech Republic, Poland, Romania, Hungary and Serbia), Central European lowlands (Germany and Poland), Baltic (Estonia, Latvia, Lithuania and Poland), Karelian (Finland) and Scandinavian (Norway and Sweden) («Large Carnivore Initiative for Europe > Large carnivores > Wolf» 2015; International Wolf Congress 2013).Nowadays it is estimated that there are 800 – 1000 individuals in the Western Alpine and Italian Apennine wolf population, nearly 100 of them live in MNP, divided into approximately 10 to 13 packs. Wolf packs in Italy, have in generally 2 – 7 members, the reproducing pair and the younger (Passilongo et al. 2010; Fabbri et al. 2014, International Wolf Congress 2013).

The Italian wolf (Figure 1), weighs an average of 25 – 35 Kg, although some males can be heavier. It measures 110 – 150 cm in body length and 50 – 70 cm in shoulder. The coat is generally grey –fulvous color, which reddens in summer. They present the lower body and facial mask lightly clear and dark bands on their back, tail tip and occasionally along the forelimbs(Apollonio and Mattioli 2006; Corpo di Polizia Provinciale 2010).

Figure 1– F3 – Martina, Apennine Wolf, photo courtesy of Antonio Antonucci, MNP.

The Italian wolf is a nocturnal hunter that feeds mainly of medium – sized animals, such as wild boar (*Sus scrofa)*, red and roe deer (*Cervus elaphus* and *Capreolus capreolus,* respectively), chamois (*Rupicapra rupicapra*) and also small animals such as hares (*Lepus spp.*), rabbits and birds. Some of their food comes from garbage, scavenger and livestock (including horses(*Equus ferus caballus*), and occasionally they consume herbs and plants for dietary fiber (Boitani 1992; Cozza et al. 1996; Capitani et al. 2004; Mattioli et al. 2004).

The relation between wolves and men is based often on an irrational cultural perception, which can be considered as a biophobia (the same as with snakes). On the other hand, worship and compassion are feelings that wolves awake in human beings. Maybe one of the biggest causes of the almost massive extinction of wolves and of the unacceptance showed by humans resides in the fact that humans unlearned how to coexist with wolves. Thus, in addition with myths and legends created by fear and incomprehension/ignorance about their biology and behavior continue to be the main threat to their existence, which cannot be contempt.

Note that the wolf is a wild animal and an excellent predator, as well as humans are. Descriptions of attacks and deaths caused by wolves, are not just myths and legends, they are real. Either because of “incompatibility” of coexistence inherent in predators (occupancy of the same temporal space and predation on the same prey in a saturated system) or because humans can be mistaken as prey (mostly children). The “bad wolf” exists, and his attacks are generally divided as defensive, by direct or indirect threat (wolf pack, territory or prey), “predatory attacks”, agonist attacks or attacks caused by diseases, namely rabies (Mech and Boitani 2007; «Wolf Attacks on Humans» 2015).

Generally wolves always try to avoid humans, and this is observed by their behavior, since their reaction in front of humans is entirely dependent on their experience or habituation to human beings (Mech and Boitani 2007).

The first approach made in the management of predators was unsuitable and culminated in the almost extinction of all the predators, inclusive the wolf in YNP. This anecdotal approach, was carried out in the belief that the "bad animals" should not be part of the “natural park’s fauna”. Since the first centuries the wolf was always seen as vile and malefic for shepherds and men that lived in mountains aftermath of the damages caused in livestock. The wolf was feared and a lot of stories and myths, involve and enhance this feeling. To worsen his situation, the Christianity also uses some metaphors with wolves, suggesting that the wolf was the evil, the Satan (Caetano, Ferreira, and Mateus 2006; Sellars 2009; «Yellowstone, Unnatural Histories - BBC Four» 2015).

The first time in history where the payment and massacre of wolves is described remote to 6th century (before Christ) by Solon, in Greece, but of course the exactly date of this persecution is not known (International Wolf Congress 2013). Direct or indirect poaching (Figure 2) and legal hunt (encouraged by payment) were the major causes of decline of this predator, however habitat loss, small and isolated populations, decrease of natural preys, among other anthropomorphic aspects contributed to the decrease or extinction of the wolf (Fabbri et al. 2014; «Life Wolfnet» 2015; «Wolfnet» 2015). On the 70's it happened a breakthrough in the life of wolves, since the wolf acquired a protection law (status) instigated by the threat of extinction. In Italy the protection of wolf happen in 1976, while in Portugal only occurred in 1988 (Boitani 1992; Caetano, Ferreira, and Mateus 2006).

Regarding United States, namely in YNP after a period of almost 60 years without wolf (as a result of their massive persecution and consequent extinction), the first real action of conservation of habitats and ecosystems of the wolf happened in 1995 – 1996, with the reintroduction of 31 wolves from western Canada (Barber-Meyer, Mech, and White 2008; Almberg et al. 2009; How Wolves Change Rivers 2014; Smith, Peterson, and Houston 2014; «Yellowstone National Park (U.S. National Park Service)» undated; International Wolf Congress 2013). Before they reintroduction, the wolves went through a quarantine period, where they were vaccinated among others aspect in order to guarantee their health, prevent health issues and increase they chances of survival, giving them the "tools" needed to build their empire (White and Garrott 2005; Barber-Meyer, Mech, and White 2008; Wittemyer et al. 2008; Ripple and Beschta 2012). The ecosystem, after wolf extinction, started to collapse. However, in a turn of events, after their reintroduction in YNP, the environment began changing, creating pressure over deer and coyotes (*Canis latrans*) they create conditions to the occurrence of an increase of the growth of some vegetation including trees (*Populus tremuloides)*, an establishment of some animal as beavers (*Castor Canadensis*), cougars (*Puma oncolor*), bears, bison (*Bison bison*), rabbits, birds as prey birds, weasels (*Mustela spp.*), badgers (*Meles meles*), reptiles, fishes and amphibious and also the behavior of the rivers was changed. The wolves also provided resources for scavenger animals, allowed the creation of new nixes and new ecosystems through trophic cascades interaction. These changes were also visible in Isle Royale but the "reintroduction" of wolf was done by themselves (Ripple et al. 2001; Wilmers et al. 2003; White and Garrott 2005; Varley and Boyce 2006; Mech and Boitani 2007; Barber-Meyer, Mech, and White 2008; Ripple and Beschta 2012;Smith, Peterson, and Houston 2014; How Wolves Change Rivers 2014).

The wolf is a protected animal in some areas of Europe and United States, while in others it is legally hunted. Nowadays it is believed that the wolf is in expansion in Europe. The conservation plans aim the reduction of wolf – livestock – human conflict through the implementation of the damage – compensation – prevention – mitigation, supplementary livestock protection and implementation of electrical fences, reduction of the phenomenon of illegal mortalities by means of reduction and/or the suppression of direct or indirect persecution on wolves, conservation and reintroduction of wild prey, dogs registration and vaccination campaigns, public awareness campaigns on large carnivores, sterilization, promotion of large carnivores as a factor contributing in ecotourism development, among other measures to optimally drive a controversial cohabitation between man and wolf («Life Wolfnet» 2015; «Wolfnet» 2015; «Início - MedWolf (POR)» 2015; International Wolf Congress 2013). Concerning herd protection dogs, it is important to refer that each country as their specific breed. In Portugal is the Serra da Estrela dog, Castro Laboreiro dog, Rafeiro Alentejano dog and cão de Gado Transmontano dog. In Italy, the herd dog used in defense of the animals is the Maremmano Abruzzese sheepdog (Figure 3).Note that these dogs are fundamental to guard the herd (generally 1 for each 50 heads), but beyond the dog, other preventive acts are important, as guarding the herd at night, using electric fences and the presence of the shepherd, which is essential and the fundamental pillar in prevention of wolf attacks («Life Wolfnet » 2015; «Início - MedWolf (POR)» 2015; International Wolf 2013).

Figure 2 – F1 – Petra entrapped in a poacher's snare, photo courtesy of Antonio Antonucci, MNP.

Figure 3 – Maremmano Abruzzese sheepdog.

On the other hand, the domestic dog and the wolf are a very close species, meaning that their reproduction is possible resulting in hybrids offspring. This is an important problem that silently threats the wolf conservation and that has increased in the last years, with the increase of free ranging dogs caused by culture, abandon or low financial condition by the owners. The real impact of the hybrids in the wolf population is not very clear, it is predicted that 10% of Italian wolves are hybrids. Regarding street dogs, they not only put the wolf in danger, but also all the wildlife and domestic animal and also human population, because of the enhance of high public health risks. Also it is important refer that hybrids usually are more aggressive and presumably have less fear from human than the wolf (Cozza et al. 1996; Mech and Boitani 2007; «Life Wolfnet» 2015; International Wolf Congress 2013).

Many efforts can be made in conservation strategies, but environment education is the most precious ally of the conservation plans, since an ideal is only changed through the children of today, whom will be the "active society" of tomorrow.

Humans frequently determine where wolves can live, and also influence their ecology and behaviour. The future of the wolf depends greatly on the values and economic interests of the people whom cohabit with wolves, which will influence their conservation (Mech and Boitani 2007).

1.3. WORK PERFORMED IN THE MAJELLA NATIONAL PARK

The action plan of MNP, involves the monitoring, management and conservation not only of the wolf but also of all life forms (animal or vegetal) that inhabit the park. Some examples of mammals are Marsican brown bear (*Ursus arctos marsicanus*), red and roe deer (*Cervus elaphus* and *Capreolus capreolus,* respectively), Apennine chamois (*Rupricapra pyrenaica ornate*) and wild cat (*Felis silvestris*), birds as golden eagle (*Aquila chrysaetos*) and rock partridge (*Alectoris graeca orlando*), reptiles as Ursini's Viper (*Viper aursinii*), amphibians as Apennine yellow – bellied toad (*Bombina pachypus*). *Galium magellense*, *Armeria majellensis*, *Cynoglossum magellense*, *Erysimum majellense*, are some examples of the diversity of the flora of the park («Majella National Park | Parco Nazionale della Majella» 2015; «Majella National Park» 2014).

Therefore, during my internship in the park, it was possible to acquire knowledge and interact with the entire ecosystem. Regarding fieldwork, every time that we went to the field, we picked up evidences and took advantage of all opportunities that nature gave us. In a short summary, I will just refer some examples of the work performed in MNP, which I had the opportunity to follow.

The topics that I will refer to in wolf monitoring, as sightings, tracking, photo and video trapping are also made for all the animals that live in the park. For example, camera trapping was used in some places, but instead of obtaining information just about one species, we obtained information regarding the entire ecosystem.

Figure 4 – Monitoring an Apennine chamois, captured in MNP.

Figure 5 – Blood sample collection from a red deer, captured MNP.

During my internship, I had the pleasure of assisting the capture of red and roe deer and Apennine chamois. Regarding these captures, the method used for these species differs from the method used in wolf captures. The animals were confined in an enclosure, the immobilization was performed just with a mixture of chemical restraint agents (to the red deer the protocol used was a Wienger Mix (xylazine with tiletamine – zolazepam combination) and for Apennine chamois and roe deer was used medetomidine, ketamine and acepromazine combination) using a CO_2 – powered rifle or a blowpipe in 3 ml lightweight plastic darts, the size and the needle used depended on the target specie. For the Apennine chamois was just necessary to use a land seine as a barrel after the animals been forced to go against the net and be minimally contained there. The aim of this captures was for translocation (Apennine chamois and roe deer) and for medical annual health control, regarding the red deer. Figure 4 shows the monitoring protocol used in the park for the chamois, while the Figure 5 demonstrated the blood sample collection for the annual health control in a red deer.

The approach and monitoring described for wolves is the same performed in all animals, with the exception of being more careful regarding the position of the animal body, ptyalism and bowel motility that can cause bloat and threaten the animal life.

The assessment of damages and decision on whether they are carried out by wild animals usually wolf and bear, or by stray dogs, is also part of the work of the park. This evaluation should be performed impartially, in order to assess the situation without having predefined ideas. The lesions observed on the "damage" will be related with the biological/ethological characteristics of the predator. In the case of MNP, the suspicions when performing a damage assessment fall upon the wolf, domestic dog and bears.

The place and the way the damages were performed (struggle sings, dragging, fence destruction, among others), can be used as a way to understand the dynamics of predation. The evaluation of the predation place, can be used as a way to understand which preventive methods can be implemented in each case.

After a complaint of an attack is made by the owner of the animal(s), the team (which varies from forestall guard, biologists or veterinarian) as to go as soon as possible to the place in order to evaluate the damage. After a general evaluation of the place of predation, a more detailed evaluation of the animal(s) is performed in order to distinguish anatomical from pathological findings, like evaluation of the *rigor mortis*, *ante – mortem* versus *post – mortem* lesions, cornea opacity, hypostasis signs, larvae contamination and/or presence of *Diptera spp*. Beetles (Angelucci et al. 2005; Ciucci and Boitani 2005; Fico, Angelucci, and Patumi 2005; Fico 1994; Nicola 2010).

A solid knowledge of biology and behaviour of each specie, is essential to be able to perform an evaluation as precise and reliable as possible. To what concerns wolves, they are excellent hunters and their attack has the characteristic of happening through an almost immediate immobilization that results in deadly and precise wounds aiming to minimize energy losses. Using medium size preys as example, like sheep and goats the lesion are mainly in the retromandibular and/or high jugular area. Sometimes, the only lesions observed are a single bite wound and only on the necropsy it is observed the muscle dilacerations, rupture of large blood vessels and of tracheal and/or laryngeal cartilages, which lead to a series of physiopathological events (cardiovascular, vasomotor and neurogenic) that culminated on a quick death of the animal. This type of predatory event is opposite to the domestic dog ones, on which multiple wounds are inflicted through the animals body in an aleatory pattern, often resulting in polytraumatic injuries. The predated animal usually suffers an agonic death which result from the post – traumatic syndrome and acute stress rather than from the attack on vital structures. Note that although predation with lesions on the posterior third can be the result of wolf and domestic dog attacks, in cases of medium sizes animals, 80% results from domestic dog attacks. Concerning big preys, the area of the wolf attack is also the neck, but in this case additional lesions are observed on the muscles of the fore and hind limbs, which results from the attack technique, that aims to overthrow the prey. Regarding bears, the animals death generally results from trauma, observing usually medullar trauma with destruction and dilacerations of intra – abdominal and/or thoracic organs as it is possible to observe in the photos of the necropsy performed in the animal of animal on Figure 6 A. Keep in mind that in the case of the bear, the evaluation of damages is not performed only on "agropecuary animals" being apiculture also a part of the damage assessment(Figure 6 B) (Angelucci et al. 2005; Ciucci and Boitani 2005;Fico, Angelucci, and Patumi 2005; Fico 1994, Nicola 2010).

Sometimes, the difficulty of distinguishing between wolf (Figure 6D) and domestic dog (Figure 6C) attacks, results on an overestimation of the wolf attacks. In cases where some evidences are found on the place (hairs, marks, faeces) it will facilitate the identification of the predator in dubious cases(Fico, Morosetti, and Giovannini 1993, Nicola 2010). Although wolves can ingest a big amount of meat per meal, the size and number of wolf pack and certain disturbances (people, cars, other packs) will influence the time that the wolf remain on the killing site. It is part of their normal behaviour to return to the killing site to finish the meal (Mech and Boitani 2007). In these cases, we can use camera trapping in order to acquire information on the predator (useful when are doubts on the damage assessment to whether it was caused by a wolf or a dog) habits, behaviour and interaction of other predators and opportunists that visit the killing site. The act of usurping food from other predators is a common habit, and sometimes there are damages caused by wolf that end up being eaten by domestic dogs, and the opposite situation is also observed. Note that in such situations, the evaluation of DNA from the dead animal may be compromised or lead to fake or wrong assumptions.

Figure 6 – Damage performed by bear in **A** in a sheep and in **B** in an apiculture; **C** – Damage performed in a horse by domestic dog and **D** – Damage performed in a cow by wolves

An effective evaluation of the damages, implementation of preventive measures (placement of electric fences, use of guard dogs, changes of pasture conduct among others) and payment of economic losses can be considered as one of the most active forms of wolf conservation.

The intervention of the Park involves not only fieldwork, but also expositions in museums that promote the fauna, flora and history of the park, local production of typical plants of the MNP, workshops, field trips, internal researches made by students, environmental education for children and adults among other no less important activities to best promote and raise awareness on nature.

2. Monitoring Methods

There are a lot of different methods that can be used in monitoring programs in wolves' conservation, as well as in other animals. The most important features to be considered are the possibility of being used in long areas and for long period of time, non invasiveness, inexpensiveness and use of analytical and modeling approaches. These methods can be subdivided in invasive and non invasive (Morton et al. 2003).

Both invasive and non invasive methods have advantages and disadvantages and the selection depends on the goal, the field characteristics and the team. Biology of the target species such as nocturnal or diurnal behavior, density population, home range and other behaviors, are factors that influence the methods used. As example, the sighting and camera trapping are the first choice in areas where howling and search tracks in snow cannot be used to confirm the presence of the wolves. After confirming the presence of the specie with non invasive techniques such as sightings, search for tracks as scats, trails, claw marks, DNA analyses (scats, hair), howling, camera trapping, usually, it is necessary to resort to more invasive methods such as the capture. Regarding invasive methods, although they present the possibility to make a continuous approach to the animal's behavior, they involves the capture of the animal, which may have an effect on his life. It's major disadvantage is associated with the displacement of people to the field (Kenward 2000; Horning et al. 2008; Dmytryk 2012; Marucco e Boitani 2012; Baratchi et al. 2013; Llaneza, García, and López-Bao 2014; «Life Wolfnet» 2015; «Wolfnet» 2015; Moskowitz and Huyett 2014; Antonucci 2014).

2.1. Non invasive methods

2.1.1. Sightings

Sighting (direct observation) is the oldest method used. Some of its advantage is the fact that can be performed by volunteers, civilians, hunters and not just by specialized people. It is particularly important for animals such as carnivores (wolves), whom are elusive and possess a large home range. Individual aspects, as age of pack and different colors in collars can be used to aid the identification of individuals in direct observations (Kenward 2000; Kindberg, Ericsson, and Swenson 2009).

2.1.2. TRACKING

The habitat and behavior of the animal may influence how easily the animal is observed. In some cases the presence of the animal is not determined by direct observation but through indirect evidences such as tracks, scat (Figure 7), urine, claw marks, trails and footprints (more easily seen in snow (Figure 8 and 9), mud or besides rivers, little ponds and streams). Tracking monitoring has some advantages such as the lack of need for equipment (which is good when the financial investment is scarce), but some knowledge and experience in "searching tracks" is necessary (Evangelista, Engeman, and Tallents 2009; Llaneza, García, and López-Bao 2014; Moskowitz and Huyett 2014).

Figure 7 – Wolf scats highlighting the size, on Gerês National Park.

Figure 8 – Wolf paw highlighting the size, photo courtesy of Antonio Antonucci, MNP.

Figure 9 – Wolf tracking, photo courtesy of Antonio Antonucci, MNP.

The wolf is a nocturnal animal and as other carnivores, it has the behavior of delimiting his territory with feces, urine and marks to define the boundaries of his home range and inform members of his own or other pack. Additionally, samples like scats, hairs and saliva can be used to perform DNA tests, measure glucocorticoids (as stress indicators on scats), obtain information about the level of parasitism and other diseases and indicate prey consumption. These are all important information for the management of ecosystem conservation (Barja, Miguel, and Bárcena 2004; Sands and Creel 2004; Mech and Boitani 2007; Bacon et al. 2011; Elbroch and Wittmer 2013; Moskowitz and Huyett 2014; Torres et al. 2015).

One alternative is the use of beads of fluorescent pigment in feces. It is generally used in small animals, not being of easily applied in wild animals. It has as a main disadvantage which is the possibility of not being ingested by the main target species. Although some studies suggest the use of food dyes, these together with telemetry are a good methods to access the "foraging behavior" of the animal, particularly in elusive animals (Kenward 2000; Giroux et al. 2012).

The kind of tracks found are influenced by the seasons and behavior. For example, on snow season it is easier to search for trails and paws, allowing to estimate the size of the pack, snow also facilitates the identification of urine samples and predation sites. Concerning behavior, there is a relationship between the intensity of wolf marking behavior and wolf reproduction that can be used as an additional information to know the location of the den. Also a “territory” inside the home range is identifiable because it is the area with highest amount of marks since it is the place more frequently used by the pack and other packs or individuals (Mech and Boitani 2007; Llaneza, García, and López-Bao 2014; Moskowitz and Huyett 2014).

2.1.3. CAMERA TRAPPING

In 1890s George Shiras III invented the first camera trap with the objective of using it in a nocturnal animal, since then, a lot of different models, with different triggers, sizes and light sources have been developed. Its evolution is such that it reaches the point of only taking pictures of the target animal (specie identify), get video directly on your computer (in the same time that are carried out), using cameras triggered with passive or activated infrared, invisible infrared flash (good to use in elusive and melanistic animals), among others. It is important to balance the advantages (maximization of the target animal triggered) with costs (O’Connell, Nichols, and Karanth 2010; Rovero and Tobler 2010; Hedges et al. 2015; “TrailMaster Infrared Trail Monitors - world leaders in infrared trail monitoring equipment. Providing game cameras and trail cameras, trail counters and traffic counters» undated).

The photo and video trapping are based on sensors triggered by movement, which command a camera where the information is recorded in a memory card. It’s a non invasive method that can provide data such as animal community metrics, population abundance indices, individual identification, number of domestic animals, anthropogenic factors and the identification of diseases such as mange (*Sarcoptes scabiei)* (O’Connell, Nichols, and Karanth 2010; Alasaad et al. 2013; Carvalho, Adania, and Esbérard 2013; Si, Kays, and Ding 2014).

Figure 10 – Camera trapping camouflage on MNP.

The localization of the camera trapping set, influences the accuracy and precision of the results. The best place to position a camera trap in the field is near the trails, dirt roads, crossing boundaries and/or near marking areas. Usually it is positioned in a tree or rock (Figure 10) and the height depends on the size of the target specie. Some authors advise the use of a lure to more easily "capture" the animal (Billodeaux and Armstrong 2005; Brown and Gegrt 2009; O'Connell, Nichols, and Karanth 2010; Dreibelbis and Locke, undated; Rovero and Tobler 2010; Moskowitz and Huyett 2014; Antonucci 2014).

Kill sites can also be a good place for camera trapping (Figure 11) as they provide important information about familiar nucleus (reproductive success, group or individual activities), behavior, health and ecology hierarchies, dimension, dynamic of members of the pack, preys and the presence of other animals such as scavengers or opportunist (Dajun et al. 2006; Miller et al. 2010; Rovero and Tobler 2010; Moskowitz and Huyett 2014; Antonucci 2014).

Figure 11 – Wolf pack photo from camera trapping, photo courtesy of Antonio Antonucci, MNP.

When positioning a camera in a potential capture place, to understand the behavior of the animal during the capture, it is advisable to build the trap apparatus only after collecting photos or video, with the aim to minimize disturbances of the catching animals (Antonucci 2014).

The drawback of this method comes from the fact that sometimes some pictures or videos aren't clear enough to identify individuals. So the choice of the location, amount of photos, seconds between them, use of more than one camera trap and the use of lures influences the amount and efficacy of information collect (Moskowitz and Huyett 2014).

According to Meek *et al*. (2014) the use of photo and video traps can influence the animal behavior, causing avoidance from camera trapping set. This has been observed in wolves and other animals such as coyotes, because of their vision and hearing capabilities. It is described that, principally in wolves, when using night cameras with flash, there was a change in behavior as changes of the travel – route, disruption of the use of kill sites and the most problematic fact the possibility to increase pups mortality by females abandoning or trying to move the litter, when the camera trapping is placed in the den sites. However it is important to remember the individual factor that can determine different reactions, despite that, it is essential to camouflage the "presence" of the camera (Sequin et al. 2003; Gibeau and Tavish 2009; Meek et al. 2014).

2.1.4. HOWLING

The howl it is one of the forms of communication that wolves use amongst the pack and between packs (reunion, social bonding, spacing and mating) or simply because they enjoy. Nowadays with the help of sonograms and other tools it is possible but it is not easy, to distinguish individual "howlings". It should be noted that there is a range of different vocalizations emitted by wolves, as howling, bark, chorus, whimper, which will differ depending on age and individual. Some features contribute to the efficiency and response of this method, for instance pack size, distance of howling, season of the year, presence or absent of pups, the nature of the stimulus (solitary wolves, wolves in a fresh kill site or pack with pups, they generally don't respond), between other situation. It is also possible to distinguish between packs because each howling is unique like a "fingerprint" (Genovesi 2002; Mech and Boitani 2007; Wolfe and Weston 2007; Passilongo et al. 2010; Llaneza, García, and López-Bao 2014; Root-Gutteridge et al. 2014).

This method is most often used in August, because it is the period when puppies are presented to the rest of the pack, in the meeting point, *rendezvous,* in order to understand breeding, namely if there is offspring born and how many. However, this is not a consensual matter, since Llaneza, García, and López-Bao 2014, defend that the howling cannot be used to estimate information concerning pups, it is more accurate to observe a territory marking, since it increases on reproductive season (Apollonio and Mattioli 2004; Wolfe and Weston 2007;International Wolf Congress 2013; Llaneza, García, and López-Bao 2014).

There are some problems related with the use of wolf howling. One is the fact that it cannot be used in places near villages, because there is the wrong common sense that this activities attract wolves to the area. The other is the difficulty (in some cases) to differentiate wolf howling from a hybrid howling (Antonucci 2014).

The use of bioboundaries or biofences like urine and faeces is described as a tool that allows the reduction of human – wolf conflict (livestock depredation). It is also described the use of translocation of problematic animals (what is assumed, is that it may be ineffective occurring in a majority of the situation, an increase of predation by pack disbanding), livestock guard animals, fladry and electronic guards. Being firstly reported by Ellies (2012), through the use of just a pack and later by Anhalt *et al*. (2014), the implementation of howling as an integrating part of biofences, in order to avoid or even disperse packs from problematic areas. The effectiveness of the application of howling in the resolution of conflicts depends on "critical resource" as the existence of pups that are incapable of accompanying the pack, kill sites and time of home sites utilization («Hunted wolves exhibit higher stress levels and social disruption, study says» undated; Godfrey and Bryant 2003; Ellies 2012; Ausband and Mitchell 2013; Anhalt et al 2014).

2.2. INVASIVE METHODS

Implant transmitters and collars are examples of invasive methods used in telemetry or radio tracking, that provide monitoring and localization information at long distances from the animal. Telemetry can be defined as the science and technology of automatic measurement and transmission of data by wire, radio, or other means from remote sources, as from space vehicles, to receiving stations for recording and analysis. Radio tracking is the process of keeping a radio or radar beam set on a target and determining the range of the target continuously. Both concepts are interconnected (Mech 2002).

The first record of telemetry dates back to 1970s by Craighead in wolves and in grizzly bears in YNP (Markham 2008; Hebblewhite and Haydon 2010; Kaczensky, Ito, and Walzer 2010; Miller et al. 2010; Recio et al. 2011).

In general, it is necessary a transmitter, a receptor (antenna) and a source of radio signal. Nowadays the most used “transmitter – receiver” in telemetry are the “conventionally” very high frequency (VHF), via satellite by transmitter signal and global position system (GPS), by signal reception. Each of these systems can be used alone or together with others, since its complementarily allows to maximize the effectiveness of wildlife monitoring (Marzluff and Millspaugh 2001; Mech 2002; Markham 2008; Miller et al. 2010).

This research field as being a target of continuous technology evolution, resulting in a constant transformation of equipment, which results in significant physical and functional changes. As for example, reduction in weight and size (which is advantageous in small mammal capture), possibility to gather information on the physiological data of the animal as heart and respiratory rates, body temperature, transmission pattern of some diseases as mange, ability to distinguish migratory and dispersal movements from mortality, as well as acquire knowledge on behaviour and behavioural interaction between species and the environment and consequences arising from its loss (Kenward 2000; Marzluff and Millspaugh 2001; Mech 2002; Morton et al. 2003; White and Garrott 2005; Evangelista, Engeman, and Tallents 2009; Eriksen and Wabakken 2009; Laporte et al. 2010; Ripple and Beschta 2012; Alasaad et al. 2013; Mattisson et al. 2013; How Wolves Change Rivers 2014; «Life Wolfnet» 2015).

The main drawback of telemetry as being an intrusive method is that it requires the animal to be captured and sometimes the intervention of the people through vehicles or by foot in the field. Also the real effects of tagging an animal aren’t yet completely understood, there are descriptions that birds, small animal and young or juveniles are the most affected by tagging. The major effects are in reproduction, grooming and feeding behaviors, diminishment of activities and in prey animals the possibility of enhance its predation. Some effects only become apparent a long time (reproduction and survivorship) after capture or with a chronic evolution, which often leads to a misinterpretation of the problem in a way that it is not considered related to the capture (Marzluff and Millspaugh 2001; Kenward 2000; Mech 2002; Wikelski et al. 2007).

The major disadvantages of telemetry are the associated costs, reduced personal in the field and problems associated with its manufacturing. Also it is important to reach an equilibrium between the sample size and the accuracy of the results, since the expensiveness of the device usually implies a reduction of the sample size but can enhance the accuracy of the results obtained (Marzluff and Millspaugh 2001; Mech 2002; Markham 2008; Hebblewhite and Haydon 2010; Miller et al. 2010).

2.2.1. TELEMETRY SYSTEMS

Since the first model a vast diversity of models has been created, aiming to a conception of a better tool that would enable the acquisition of more information with more accuracy and with reduction of the drawbacks (Kenward 2000; Mech 2002; Markham 2008).

The VHF system was the first system used to monitor animal through transmission and reception signals, by radio waves. Instead of VHF, its analogous system, the ultra high frequency (UHF) can also be used. The basis of these systems are the production of a pulse signal which reduces energy consumption in comparison with continuous signals, that is received by an antenna connected to the receptor used manually by the person on the field. The location of the animal can be obtained either by "approximation" or triangulation, in the last approach we gain in accuracy and speed but on the other hand, it is a process that needs more human and equipment resources. There are different receptor models with various signal frequencies. The most widespread antennas are Adcock (H), Loop and Yagi (as it is possible to observe on Figure 12). Antennas can be installed in the roof of a vehicle, which can have two advantages, the possibility to use bigger antennas with better reception and the location at higher positions with better signal reception. They can also be used in airplanes, boats or on fixed – site receiving stations (Marzluff and Millspaugh 2001; Mech 2002; Markham 2008; Cagnacci et al. 2010; Recio et al. 2011; Bartolommei and Francucci 2012; Parker et al. 2013).

Figure 12 – Antenna of VHF telemetry system [accessed on September 1 2015], available on http://www.edgeofexistence.org/edgeblog/?p=785.

Some of the benefits associated to VHF system is its working simplicity and lower cost (Johnson and Heard, undated; Miller et al. 2010; «Life Wolfnet » 2015).

There are some drawbacks associated with the VHF method. For example, it cannot be used as a continuous monitoring method since most of the time it needs direct human intervention, which is at least very difficult to use in a continuous monitoring. Another common problem is a decreased of the accuracy of animals location caused by the presence of natural barriers (landscape and weather) that can minimize the signal or reduce the precision. The inexperience of the persons in the field also contribute to more errors and less accuracy of this method (Marzluff and Millspaugh 2001; Kenward 2000; Mech 2002; Townsend, Ditchkoff, and Fuhlendorf 2007; Markham 2008; Bartolommei and Francucci 2012). Finally some authors defend that the number of persons and trips to the field required to monitor the animal contributes to increase the expensiveness of this method and that in the end, the costs of this system are similar to the acquisition of more modern models with GPS or other systems (Miller et al. 2010; «Life Wolfnet» 2015).

Concerning satellite devices, the system mainly used is the Argos. Its functionality is similar to the VHF, a transmitter is attached to the animal which emits an electronic signal that is received by several satellites which allows locating the animal through the triangulation method, the data obtained is sent to a base on the land (Marzluff and Millspaugh 2001; Mech 2002; Fedak et al. 2002; Cagnacci et al. 2010; Kaczensky, Ito, and Walzer 2010). Temperature, activity count, dive count, length of last dive, are examples of information obtained with this system. This method reduces the field trips though it required a higher initial investment compared to VHF system (Marzluff and Millspaugh 2001; Mech 2002; Fedak et al. 2002; Cagnacci et al. 2010; Kaczensky, Ito, and Walzer 2010).

GPS system works by a receptor device attached to the animal body, that receives signals from a group of satellites and through the use a software program, estimates the animal position. GPS telemetry has extensive use, including on small animal and birds (Mech 2002; Horning et al. 2008; Recio et al. 2011; Quaglietta et al. 2012; McMahon et al. 2014). The information obtained by “duty cycles” (ability to program radio collars to transmit only at certain times) can be stored in memory cards or received through other system. In the beginning it was necessary to recapture of the animal to retrieve the memory card, nowadays a drop off system is responsible for the fall of the collar with the memory card, without the necessity of animal recapture. The information can be received by VHF system (some systems use FM (frequency modulation) devices or a UHF modem), but this involves additional field trips. The data can also be received in a computer, using a group of satellites as the Argos, which receive the data from the animals and resends it to a computer. However this involves the payment of user charges, which are not cheap. A recent technology, which is being increasingly used relays on mobile communications networks as GSM/GPRS (cellular network standard/general packet radio system*),* GSM/SMS (short message service*)* or GMRS (general mobile radio service), to transmit the information contained on the GPS devices. The location of the animal (as well as other data evaluated as dual – axis motion sensors*)* is received by SMS, which makes the use of a satellite network unnecessary, considerably reducing the costs associated with this type of technology (Kenward 2000; Marzluff and Millspaugh 2001; Mech 2002; Fedak et al. 2002; Gervasi, Brunberg, and Swenson 2006; Gottardi et al. 2010; Tomkiewicz et al. 2010; Quaglietta et al. 2012; Stache et al. 2013).

This system allows the acquisition of more information and reconstructs the animal activity in real time, maximizing animal monitoring in a way that provides animal activity, improves animal habitat modeling and conservation, mechanisms of migration, basic ecology and conservation of wide ranging species, projecting impacts of climate changes (polar bears and the effects of environment changes in a long time in its life) and conservation impacts (wolf avoidance of humans). Despite these advantages, the landscape and the climate condition can affect the functionality of this system (Kenward 2000; Marzluff and Millspaugh 2001; Mech 2002; Rahimi and Owen-Smith 2007; Hebblewhite and Haydon 2010; Recio et al. 2011; Friebe, Zedrosser, and Swenson 2013).

The disadvantage associated to this system is its price. Though with the increasing use and demand of GPS systems, more manufactures are available resulting in the reduction of the price of this equipment. However most of the equipment have lower battery times, because of continuous input of information, which by the incorporation in the same collar of other systems as VHF or UHF reduces the information lost but enhances the costs because of the field trips necessary to monitor the animal using this systems (Marzluff and Millspaugh 2001; Mech 2002; Fedak et al. 2002; Markham 2008; Cagnacci et al. 2010; Hebblewhite and Haydon 2010).

2.2.1.1. TAGGING TELEMETRY METHODS

The device used and/or chosen depends on the purpose, weight, physiological characteristics and animal habitat (Houseknecht 1970). Telemetry devices may be external, internal (implanted) or combine an implant that is connected to an external device (Morton et al. 2003).

Surgical implants (such as subcutaneous transmitters, abdominal transmitters, or rumen transmitters) are the tagging method associated with the most problematic outcomes, because of the possibility of reduction of signal (when comparing to the external telemetry), physical impact in the animal and likelihood to cause unnatural movements (associated to the animal size, pain and anesthesia problems). Although, Horning *et al.* (2008) suggests that it is a good method, without any interfere to reproduction, with low morbidity and no mortality using otariids as example.The pros and cons of this method depends, among other factors, on the surgical abilities of the veterinary surgeon, size and specie of the animal (beavers are associated to a high mortality rate) and public opinion. Koehler and Briggs 2001, concluded that implant transmitters may provide reliable data for animals that have small home range sizes but may not be appropriate for wider – range animals (Koehler and Briggs 2001; Mech 2002; Morton et al. 2003; Horning et al. 2008).

There are several kinds of telemetry systems used in wolves, and all of them rely on the use of collars. The collar needs to be durable, comfortable and safe for the animal, withstand extreme environmental conditions, waterproof, maintain their flexibility in low temperatures, don't interfere with swallowing movements (main concern in growing animals) and others behavior movements as rub the neck. There are different models, with a diversity of sizes and systems to use in collars. In younger animal it should be used expandable (Figure 14) or breakaway collars (Koehler and Briggs 2001; Marzluff and Millspaugh 2001; Stotyn 2005; Arnemo and Fahlman 2007; Mech and Boitani 2007) .

The placement of a radio collar it is a simple task as it is possible to observe on Figure 13, but it is important to assure that the collar is well positioned, were the usual rule of thumb is that should be a gap between the collar and the neck equivalent to three finger or a hand. Also the weight of the collar compared to the animal weight, should be taken in account and generally the weight of a collar must be around 1.5 – 2 % of the weight of the animal with a maximum of 3 – 4 %, for example, for adult wolf which weigh on average from 25 to 35 Kg, generally collars that weigh between 500 and 700 g are used. The width of the collar for a wolf, is on average around 40 – 45 cm (Kenward 2000; Markham 2008, Antonucci 2014).

Figure 13 – Placement of the collar in F5 – Iris, on MNP.

Figure 14 – Juvenile wolf using an expendable collar, photo courtesy of Antonio Antonucci, MNP.

News technologies using solar panel as a complement device allowed the increase of the battery time and consequently the lengthen the time of data reception (Marzluff and Millspaugh 2001; Mech 2002; Fedak et al. 2002; Cagnacci et al. 2010; Kaczensky, Ito, and Walzer 2010).

2.3. OTHER METHODS

The evolution of science allowed the development of new forms of acquiring data and new kind of data about animal life and the interaction between them and the ecosystem. The use of robotics devices is nowadays helping the animal conservation, because of the large size of animals territory or they mobility, it is mainly used for surveillance (it is a very effective anti – poaching service, principally in endangered animals as rhinoceros) and in identification of animals (census). These are some of the uses of technology (Dunbabin and Marques 2012; Mulero-Pázmány et al. 2014).

The use of lightweight unmanned aerial vehicles or aerial kites with different sizes and forms equipped with capable sensors, that provide spatial and temporal information with a good resolution, is beginning to become a cheaper option that piloted air craft(Anderson and Gaston 2013; Iv, Pearlstine, and Percival 2006). One of the advantages associated to these devices is the possibility to study small animals and birds where biotelemetry is not so efficient because of their lightweight and the inherent difficulty associated to the miniaturization of data collector devices (Marzluff and Millspaugh 2001; Mech 2002; Ferreira and Van Aarde 2009; Göktoğan et al. 2010; Rodríguez et al. 2012; Baratchi et al. 2013; Bryson et al. 2013).

Radars, chemical sensors, microphones, infrared and thermal cameras and video devices are examples of the technology involved in the animal conservation. Note that this technology cannot be applied to all kinds of landscape because of the topography (Marzluff and Millspaugh 2001; Mech 2002; Ferreira and Van Aarde 2009; Göktoğan et al. 2010; Rodríguez et al. 2012; Baratchi et al. 2013; Bryson et al. 2013).

2.4. CAPTURE

2.4.1. THE IMPORTANCE OF CAPTURE

In conservation programs it is of vital importance to have information of good quality, not only about the animal (behavior, ecology, physiology, health and illness), but also about the surrounding ecosystem, the interaction between them and anthropogenic aspects. Some animals are easier to monitor than others, because of population densities, behavior (nocturnal versus diurnal) and terrain characteristics (Lesmerises, Dussault, and St-Laurent 2012).

The wolf is a controversial specie and as referred before in order to do its conservation is important to understand all the previously mentioned parameters as well as some specie specific parameters as family group, home range, den, *rendezvous*, reproduction, kill sites, mortality, dispersal behavior, relation with prey and livestock, interaction with street dogs and hybridization

(Marzluff and Millspaugh 2001; Genovesi 2002; Mech and Boitani 2007; Brown and Gehrt 2009; International Wolf Congress 2013).

Taking this into consideration, is essential to the use complementary monitoring methods, such as tracking, howling, photo and video trapping, aerial survey and telemetry. The choice is based on the method that enables better animal monitoring, with minimal disturbance to avoid behavior changes and to minimize false information. Usually more than one method/tool is used in order to obtain accurate knowledge and results.

Unnecessary suffering, capture or manipulation should be avoided and also the stress during approach should be minimized to a minimum. The behavior (influenced by status, age, health) and capture responses will be different between animals. Taking this into account our conduct and approach should aim to minimize injuries to the animal or to ourselves. When an animal is captured, they get enraged, fearless and in panic and we'll have a big, strong and "unhappy" animal, captive and connected in the capture set, being essential to assure that the trap will hold the tension (Spielman 1996; Elements Working Group of the Terrestrial Ecosystems Task Force 1998; Nielsen 1999; Dmytryk 2012).

One thing all animals have in common is that they learn easily. This "knowledge" can be provided by themselves or by observing other animals being captured. It has been proved that naive animals are more easily caught than animals that were captured before. Also the rates of captures decrease with time spent in one region, and the percentage of youngsters captured is often larger than adults (Editors and Arnemo 2011; Meyer et al. 2008; Logan et al. 1999, Antonucci 2014). However this is not a strict rule, it is more like a tendency and the observed behavior depends largely on social status, but in most cases it is inherent to the individual (Mech and Boitani 2007).

2.4.2. CAPTURE TECHNIQUES

There is not an universal method or technique to perform animal capture and immobilization, these should be adapted to the target species. Some factors to consider are whether, if it is a captive or free ranging animal, the terrain, the team, goals, capture situation, equipment available, the size and proximity to the animal and previous experience (Vilà and Castroviejo 1994; Tribe and Spielman 1996; Osofsky and Hirsch 2001).

2.4.2.1. NETS

Nets can be used as a single tool or to assist in the immobilization and handling of the animal, upon him capture or transport. They can also be used as a trap, to manipulate animals or to "build barricades" to lead the animal. It is available in a lot of forms and shapes and its utilization depends on the goal and the target animal. There are hoop nets, open – ended hoop nets and throw nets, each one has specific purposes. There are also land seine (a wall of netting that acts as a barrier) which can be fixed in the place or activated, bow net and Q – net (however is not very safe and usually causes injuries), whoosh net, driving, funnel and walk – in traps, dho – gaza, drop trap and project power nets (net guns, cannon nets, rocket nets, and net launchers), among others (Fowler 2008; Dmytryk 2012).

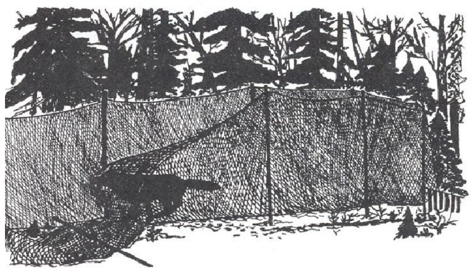

Figure 15 – Fladry and net drawing in wolf capture, adapted Okarma and Jedrzejewski 1997.

Nets aren't used in wolves as a capture method itself, but only to help in the handling. Using this method as only capture device on a wolf capture can cause unnecessary stress and several injuries as a consequence of the fight while trying to escape. To the best of our knowledge there is only one article about nets in wolf capture by Okarma and Jedrzejewsk (1997), that suggest the use of fladry and nets. After confirming the presence of the wolf pack by tracking or other method, they built an enclosure with the fladry and the nets were positioned, hidden in the vegetation, the beaters walking and "chase" the pack in the direction of the nets, as it is possible observe on Figure 15. They defend the use of this technique instead the foot hold traps, because it is less stressful, safe and effective. However it cannot be applied to all the kinds of landscape and in light of by the few captures performed, the kind and severity of injuries during from capture are unknown (Okarma and Jedrzejewski 1997; Dmytryk 2012; International Wolf Congress 2013).

2.4.2.2. BOXES AND CAGES TRAPS

Box and cage traps are more applied to small carnivores or felids as a capture method, to translocation or after the capture upon the recovery of the animal whenever necessary. Boxes or cage traps have a lot of different models, sizes and shapes, they are typically applied as "humane traps". They are typically rectangular with one opening in one of the sides, but there are several descriptions of models with two openings. The mechanism of closure involves a level were the animal needs to step on (others mechanism are available) and stay captured inside the box (West, Heard, and Caulkett 2007; Dmytryk 2012).

It is important to make a regular vigilance or use other devices as video surveillance or alarm system to prevent injuries in the captured animal, during the struggle when is trying to escape (West, Heard, and Caulkett 2007; Dmytryk 2012).

2.4.2.3. TRAPS

Firstly, it is important to note that not all the traps can be used legally to capture in Europe, but the main information about this subject was achieved in America where the law and methods are different.

I'm just reviewing some of the traps most used in wildlife principally in carnivores as wolf, but a lot of different marks and models are available in the market.

2.4.2.3.1. TRAPS TYPES

There are a lot of types of foothold traps (off set traps as rubber pad (Figure 16) and teeth jaws (Figure 17) and Belisle®, Aldrich® and Fremont® foot snare), but the major aspects to consider when selecting a foothold trap is what repercussions can result to the animal (West, Heard, and Caulkett 2007; Association of Fish and Wildlife Agencies Furbearer Technical Work Group 2009).

Figure 16– Foot hold trap with rubbed paw jaws [accessed on September 1 2015], available on http://www.ruralking.com/duke-trap-3-cs-pad-rubber-jaw-coyote-474.html.

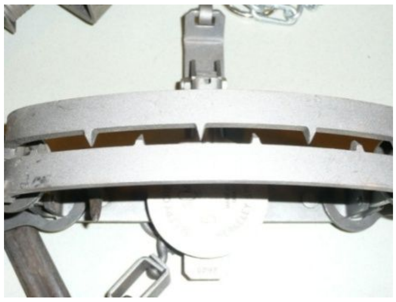

Figure 17 – Foot hold trap with teeth jaws, [accessed on September 1 2015], available on http://animalcrueltyidaho.org/.

Each trap has advantages and disadvantages. In a brief description, the off set traps require a space of 3 to 6 mm between the jaws after closure, with the objective of minimizing compression. Some of these traps possess certain types of jaws that inflict a lot of injuries, as teethed jaws and should not be used. Others have a rubber pad to minimize the pressure upon closure. The foot snares have a closure trap system, which activates upon an animal touch, which in the same time, the pressure system activates the closure of the jaws on the animal's paw. Traps as Aldrich® and Fremont® foot snares (Figure 18), upon the animal paw pressure, causes the closure of a loop. This system respond very quickly mostly through a spring mechanism, which has the adequate pressure and strength to close the loop. These models are used to capture bears, wolves, cougars and foxes. An "intermediate trap" is the Belisle® foot snare (Figure 19). This trap has the same structure as the off set traps but its closure mechanism does not hold so much pressure allowing the animal to release themselves from it. In addition, this trap has a loop capable of upholding the animal captured. This trap is used more often on bears, wolves, coyotes, foxes (*Vulpes vulpes)*, lynxes (*Lynx spp.*) and bobcats (*Lynx rufus)* (Phillips, Gruver, and Williams 1996; Onderka, Skinner, and Todd 1990; Ballard, Franzmann, and Gardner 1982; Powell and Proulx 2003; Zemlicka et al. 1997; Zemlicka and Bruce 1991; Sahr and Knowlton 2000; West, Heard, and Caulkett 2007; Association of Fish and Wildlife Agencies Furbearer Technical Work Group 2009).

Figure 18 – Fremont® trap.

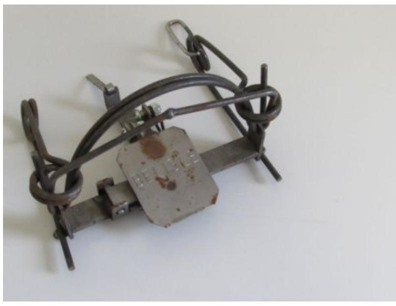

Figure 19 – Belisle® trap.

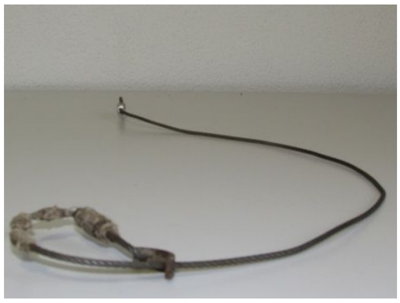

Figure 20– Snare.

Regarding the Belisle® advantages, it has a very quick closure system and does not require any concerns about the orientation and positioning of the trap. Thanks to the structure of the trap, it allows sufficient movements, so that the wolf is able to disarm the jaws of the trap and remain arrested in the capture system by the snare. The disadvantages are the dimensions of the hole necessary to bury it, the low capacity (strength) to release the jaws in case of captured juveniles or non target species with a greater probability of injuries (this is more evident if the paw tension is not adequate). On the other hand, the Fremont® trap does not require a big pit, however it is necessary to orientate the trap very well and its closure mechanism is slower than to the Belisle® (Dmytryk 2012; Antonucci 2014).

These types of trap meet all the standards of the Agreement on International Humane Trapping Standards(AIHTS) (Antonucci 2014).

2.4.2.3.2. SNARES

Snares can be used alone as a "simple" trap or can be part of a more complex mechanism that constitutes the trap, as seen in Figure 20, this last situation is the most common.

Snares are the oldest, and in some way, the most common capture method. In Alaska and other countries where the use of traps for fur is legal and widely spread, they are frequently used in killing traps for wolves and other animals capture for fur, but they can also be used to capture animals without killing them, for which is necessary to engage a "loop stop" such as a ferrule or steel nut, onto the cable (Ballenberghe 1984; Elements Working Group of the Terrestrial

Ecosystems Task Force 1998; Logan et al. 1999; West, Heard, and Caulkett 2007; Association of Fish and Wildlife Agencies Furbearer Technical Work Group 2009; Antonucci 2014).

Snares have the advantages that are economical, lightweight and more selective since they are associated with reduced possibility of non target animals' capture. The purpose of the loop stop is to prevent the complete closure of the snare, which will reduce the injuries caused by constriction of body areas resulting on reduction of blood supply, necrosis and even death. It is underlying to put the loop stop in such way that when the snares' loop is closed, it matches the diameter of the paw or chest of the wolf (in this case). This also decreases the possibility of non target species capture, as foxes. In some models of snares it is possible to add diverters or antenna wires to prevent ungulate capture, since the animal touches this and passes on avoiding placing the head in the snare. There are a lot of methods and forms to put the snares, depending on the dynamic of the species or pack (in case of wolves), goal and the amount of animals likely to be captured (Ballenberghe 1984; Elements Working Group of the Terrestrial Ecosystems Task Force 1998; West, Heard, and Caulkett 2007; Association of Fish and Wildlife Agencies Furbearer Technical Work Group 2009; Antonucci 2014).

There are characteristics of the snare, as the kind of cable, the size of wire and the kind of strands used, the lock of the snare, swivels, springs, break away advices, which will influence the outcome of capture (death or alive animals), the capture of non target species and the presence and severity of the injuries caused (Ballenberghe 1984; Alaska Trappers Association 2007; West, Heard, and Caulkett 2007; Association of Fish and Wildlife Agencies Furbearer Technical Work Group 2009).

In my opinion, an interesting but anti ethical approach is that of the neck snaring, which cannot be used in wolves, but can be used in foxes and coyote in Canada. This is caused by the conservation status (least concern) and because foxes and coyotes are game animals. The authorizations of this kind of traps (snares) legally, can improve its illegally utilization associated to facility in acquisition (Government Alberta 2013).

Figure 21 – Collarum® trap, [accessed on September 1 2015], available on http://www.snareshop.com/products.asp?dept=202.

There is a type of snare, the Collarum® (Figure 21) that can be used on all carnivores for restrain or capture by the neck, principally in foxes, coyotes and wild dogs. The mechanism action is activated when the animal is trying to take out the lure, by a projection of a non – choking cable loop over a canine's head and around their neck. The injuries inflicted by this trap are considered minimal, but is a controversial subject. This trap is very secure to humans and domestic animals (Vilas 2015; Mech and Gese 1992; Dmytryk 2012; West, Heard, and Caulkett 2007; Association of Fish and Wildlife Agencies Furbearer Technical Work Group 2009; «Collarum® | Wildlife Control Supplies» 2014).

2.4.2.3.3. OTHER TRAPS

A different kind of trap described by Kunkel *et al*. (1991) and Mech and Gese (1992), is the Wild Link Capture Collar®, this can be described as a "normal" collar to use in telemetry with the same functions, but with a supplementary function namely anesthetic darts that can be fired through remote command. The disadvantage of this trap is lower battery time. This capture method implies a great control of animal movements since after sedating the animal, it is necessary to administer a supplementary dose of drugs in order to handle and monitor the animal (Kunkel et al. 1991; Mech and Gese 1992; Mech 2002).

2.4.2.4. TRAP PREPARATION

Traps need to be prepared before their set for capture. Capturing wolves is much more difficult than other carnivores, since "the rules are stricter". The first step is to check every part that composes the chosen "trap" (Alaska Trappers Association 2007; Antonucci 2014).

Wolves have an acute senses of smell and if they feel or smell something strange and or new on their trail they go away and avoid returning to the place (this is more observed on adults, since young are generally less prudent). Some authors refer that wolves aren't afraid of human smells, but are particularly suspicious of unnatural smells like plastic fuels. So the next step prior to start the capture season is to make “human” smell imperceptible. The strategy is different between the use of a first – hand trap or an used one. When a new trap is bought (or other constituents) it is necessary to boil it to remove the plastic, iron and other odors. In order to achieve this, the constituents of the capture set should be boiled using caustic soda, with leaves and soil or cold wash. In the case of a second hand trap, the cleaning process can be made with water and dirt (Alaska Trappers Association 2007; Antonucci 2014; Anhalt et al. 2014).

After this step, we should avoid touch anything without protection like gloves and arms cuffs (Alaska Trappers Association 2007; Antonucci 2014).

2.4.2.5. CAPTURE SETTING

The choice of the capture set is important in order to prevent animal injuries not just in the time of the capture but also when the animal is released. So it is important to choose places as far away from traffic areas, dangerous falls, precipices, cliffs, rivers and lakes as possible but also is important to use places with a good access by car (because of the need to transport necessary equipment) and with telephone coverage (because of alarm system by GSM) (Nielsen 1999; Osofsky and Hirsch 2001; Arnemo and Ahlqvist 2006; «Life Wolfnet » 2015; Government Alberta 2013).

To assemble the capture set natural (Figure 22) or unnatural places can be used. The natural places are usually places marked by wolves or kill sites and are preferably regarding the wolf behavior. Wolves generally return to the “oldest places” as mark places and are less suspicious and afraid. These places represent benefits since make the capture much probable (Alaska Trappers Association 2007; Antonucci 2014).

Boundaries between two or more packs, are very good places to set up the capture set, because in natural boundaries a large amount of animals crosses the sites and since it is a familiar location where wolves are usually less alert. The problems about marking places are that its location usually crosses between dirt roads and trails that are often frequented by humans.

Also, most of the times, this places don't offer the features required to place the traps, to anchor them properly, don't have telephone coverage, easy accesses, safety, between others (Barja, Miguel, and Bárcena 2004; Alaska Trappers Association 2007;International Wolf Congress 2013; Moskowitz and Huyett 2014).

Figure 22 – Capture setting activated emphasizing the importance of a good camouflage, on MNP.

Sometimes a natural place is not found, and it is necessary to adapt the terrain to assemble the capture set, taking in account that we should not disturb the place and should minimize evidences of our presence.

One of the first descriptions of an attempt to decrease injuries during the capture was the used of tranquilizers trap devices (TTDs) coupled to foot traps, that can be used in wild species and dogs. The use of this device took into consideration the behavior that the animals have when captured. This system uses drugs as propiopromazine hydrochloride or diazepam (trap tab). The TTDs are described in a large amount of mammals, as foxes, bobcats, coyotes as well in wolves, among others. In some studies it was observed that the addition of ascorbic acid to the propiopromazine hydrochloride increased its efficiency, since the ascorbic acid helped in degradation of it (Balser 1965; Zemlicka and Bruce 1991; Zemlicka et al. 1997; Sahr and Knowlton 2000; Savarie et al. 2004; Government Alberta 2013).

The problem associated of TTDs utilization is the difficulty to control the final result in light off the variable amount of drug ingested, the presence or absence of food in stomach. But the major disadvantage is associated to the overdose in non target animals, although this is a controversy subject (Savarie, Fagerstone, and Schafer Jr 1993; Sahr and Knowlton 2000; Byrne and Allen 2008).

2.4.2.6. ANCHOR AND DRAB SYSTEM

The anchor may be mobile or fixed/stationary. The choice is up to the team and is influenced by the method and trap used. The movable anchors (Figure 24), drag hook (and consequently traps), aren't as good because the animal will drag the trap and the anchor until the anchor grips in some place during the struggle. This can cause injuries to the animal and the animal may drag the trap to a very distant place from the original capture set location (Logan et al. 1999; Alaska Trappers Association 2007; International Wolf Congress 2013). This kind of capture cannot be used in Europe.

Figure 23 – Example of a fixed natural anchor, photo courtesy of Antonio Antonucci, MNP

Figure 24 – Mobile anchor and trap [accessed on September 1 2015], available on, http://www.centuryleisure.com/hunting/hunting5.html

The fixed anchors (Figure 23) have enough strength to guarantee that the animal cannot move from the trap site. The chain or iron cable with spring (to endure the pressure caused by the wolf during their fight to escape), function better when it measures between 1 – 1.5 m, since its just the perfect distance to anchor the snare until our fix point. Trees, bush and rocks may be used depending on the characteristics of the trap site (Alaska Trappers Association 2007; Antonucci 2014).When trees or bush are chosen it is important that they are alive since they need to have adequate strength. When natural anchor points are not present, an artificial one can be built (Alaska Trappers Association 2007; Antonucci 2014).

Another type of fixing artificial anchor that can be used is duckbills, which is easy to install. The downside is that in the end of the capture season it is necessary to dig a hole to remove it, or as happens most of the times, it remains in its place (to eventually be used in future catches)(Alaska Trappers Association 2007; In conversation with Helena Rio Maior and Monia Nakamura, 2015).

2.4.2.7. "ATTRACTIVE"

After assembling the capture set, it is necessary to put something to attract our target specie to the place. There are four types of different attraction strategies: decoys, food baits, scented or olfactory lures and audio lures (described in coyotes and birds). The choice depends on the target specie, capture set and personal preference (Dmytryk 2012).

Concerning the utilization of decoys, in my opinion the use of living decoy is not ethical, because of the unnecessary stress and excused death of the prey animal.

Kill sites represent natural baits and can be detected when wolves have radio collars, from the clusters given by the GPS system or by livestock depredation. We can also look for the presence of other opportunist animals as ravens and magpies, to localize the kill site or carcass. Most times in proper conditions, wolves can eat the entire prey in two hours (although this can be affected by the number of individuals, previous hunt, presence or absent of disturbance factors like other animals, cars or humans), which might difficult the use kill sites (Alaska Trappers Association 2007; Fröhlich et al. 2012; Tambling et al. 2012; Bacon et al. 2011; «Wolf Country, wolves as hunters, survival» 2015).

The carcass of dead animals killed by traffic or by livestock depredation can also be used. The use of this last kind of decoy possesses a problem because of the risk of spreading Bovine Spongiform Encephalopathy (BSE), meaning that not all "free" livestock carcass can be used as a result of the implementation of law EC Reg.1774/2002. Another problem of the use of this kind of decoys is the fact that it does not attract just the target specie (wolves), but also other opportunist animals such as foxes, wild board, bears and domestic dogs (Alaska Trappers Association 2007; Fröhlich et al. 2012; Tambling et al. 2012; Bacon et al. 2011; «Wolf Country, wolves as hunters, survival» 2015; Antonucci 2014; Moskowitz and Huyett 2014).

Bluefish conserves are an unnatural source of bait that can be used, it has the advantage that can be easily transported but as the same disadvantages described previously about other bait food, as attracting non target species.

Figure 25 – Artificial olfactory lures [accessed on September 1 2015], available onhttp://www.traps.com.au/lures.htm

Figure 26 – Lure of wolf scats on MNP.

Figure 27 – Wolf urine marking, photo courtesy of Antonio Antonucci, MNP.

The olfactory lures can also be subdivided in natural (Figure 26 and 27) or unnatural (Figure 25). Unnatural ones are bought and they are available in a lot of forms and types. It can have the smells similar to scats and urine, can be hormones taken from the glands mostly present in paws/feet, skin, anal sacs, ears (controversy), back and tail and even sexual hormones in the reproductive period, sometimes not just from the target specie but also mixed with others as coyotes and badgers. Most of them are very efficient and easily acquired than going to the field in search of natural ones. However,(in my opinion) their use may be a little bit unethical, since it was collected from wild wolves and other animals that were hunted (Mech 1981; Wolfe and Weston 2007; Mech and Boitani 2007, Moskowitz and Huyett 2014).

Natural lures can be obtained in the field, as urine (more easily found in snow season) and scats (in some cases the marked place can also be used to assemble the capture set). Another lure that can be used are natural markings. These can be grass, soil with leaves and little wood branches that the animal touched with its paws or other body area in order to mark places (because of the presence of glands). It is important to hand them carefully, specially scats, applying always the basic principles of biosafety use of biological samples collection to avoid the risk of contamination, since carnivorous feces are a widespread means of contamination, mostly those of wild animals. The fresher and with more organic material the better, it is also important to avoid freezing them (Alaska Trappers Association 2007;West, Heard, and Caulkett 2007; Association of Fish and Wildlife Agencies Furbearer Technical Work Group 2009).

2.4.2.8. ALARM SYSTEM

The alarm system is an important mechanism that helps to increase the efficiency with little effect for the animal since it decreases the time that the animal stays captured. This has the advantages of minimizing the stress (which can lead to hyperthermia), reducing injuries as self – mutilation or as a result of the friction of the snare, chain and all the components of the trap or the capture set as bush, trees and stones in the paw but also on the mouth, tongue and gums (will be discussed in more detail later) (Ballenberghe 1984; Mech 2002; Arnemo and Ahlqvist 2006; Dmytryk 2012).

Nowadays there are a lot of different models of alarm systems. The first one created was based on a VHF system, this kind of alarm need a person to stay nearby the capture set and listen the VHF frequency in order to understand if anything changed and if an animal had been captured. This wasn't an easy method because of the need of human resources in permanent alert, in order to receive the information with antennas from VHF radio transmission. But still is a good system in places where it is not possible to implement another alert system (Mech 2002; Dmytryk 2012; Campbell and Griffith 2015).

A big leap forward was the arrival of the GMS alarm system. This alarm sends a message (SMS) to the numbers that were previously recorded in the system. Some devices may also send a MMS. The first requirement to the use of this tool is phone coverage, considering the effects of weather adversities as low clouds, fog, rain and storm. In order to try to overcome this effect, booster antennas and/or international cards may be use. The alarm system is activated when the wolf (or other animal) is captured, this occurs because the alarm system is connected through the fishing wire to the trap and to "the box of system alarm" by a magnet. When the trap is activated, it pulls the wire removing the magnet and the alarm system sends a message to the number that was previous put on the memory card (Néill et al. 2007; Woodford and Robley 2011; Campbell and Griffith 2015; Antonucci 2014).

The problem of this kind of devices is poor intrinsically function caused by malfunction of the device, which depends on the model used. After the activation of the trap it is necessary to go on a daily bases to the set to confirm that the alarm system is functional, the capture set is unchanged and that no animal has been trapped. We should not rest assure just because an alarm system was installed, as the system may fail with severe consequences to the trapped animal (Néill et al. 2007; Dmytryk 2012; Campbell and Griffith 2015; Antonucci 2014).

In situations where the alarm system is not used, as a choice or by economic factors, it is necessary check the capture set once or twice a day, which means that in case that an animal gets caught, they don't remain trapped more than 12 to 24 h which will have implications on the immobilization level as well as on the physiological level (Campbell and Griffith 2015; Government Alberta 2013; Antonucci 2014).

2.4.3. PROBLEMS CAUSED BY THE CAPTURE

The capture of an animal is not an innocuous procedure, even in healthy animals. The complications and the injuries that can result from it depend on certain aspects like if the caught animal is or not the target specie, the kind of trap and chemical protocol or the anesthetic event itself. The presence of lesions can be inherent to individual characteristic or can be a secondary to the capture event (Figure 28), as trauma or myopathy. The real effect of the capture sometimes cannot be seen in the capture moment, so it is important to monitor the animal in the days after the capture and to intervene if necessary (Nielsen 1999; Fahlman 2005; Arnemo and Ahlqvist 2006; Cattet and Boulanger 2008; Fahlman et al. 2008).

Mortality rate should be less than 2%. This mortality rate is commonly used to evaluate the efficiency of the capture only in the capture "moment". However Cattet and Boulanger 2008claim that mortality rate during the capture process is of little value, since it does not account for the animals that died after release. The mortality can be related directly to the effects of the drug, as respiratory depression, hyperthermia, shock or the trauma of the impact of the dart in the muscle tissues or due to misplacement of dart (pneumothorax). And can also be secondary to the capture process as capture myopathy, separation of the offspring from the mother, problems with radio collars or implantable transmitters. The evaluation of landscape topography and the capture set are also important to decrease injuries and mortality (Nielsen 1999; Fahlman 2005; Arnemo and Ahlqvist 2006; Cattet and Boulanger 2008; Government Alberta 2013).

Figure 28– Wolf captured by a foot hold leg, [accessed on September. 1 2015], available on http://thepoliticalenvironment.blogspot.pt/2013/10/wisconsins-state-sanctioned-wolf-hunt.html.

The goal is to reduce all the risk factors, both primarily and secondary causes that may contribute to threat the animal's life. Firstly it is important to avoid capturing pups, nursing or lactating females (Government Alberta 2013).

The literature refers that the probability of a non target animal capture (mammals or bird) might be between a large interval from0 to 67 %, depending on the type of lure used (meat enhances the possibility of capturing the diversity of animal that lives in the territory), paw tension and kind of trap (laminated or other foothold traps, mixed trap, snares). Albeit, it is important that the trap is sufficiently strong to avoid the target animal escape (Powell and Proulx 2003; Mallonée and Joslin 2004; Alaska Trappers Association 2007; Frame and Meier 2007; Gusset et al. 2008;Turnbull, Cain, and Roemer 2011; American Veterinary Medical Association 2008; Government Alberta 2013). In our experience, in eleven trap activation only two was by the target specie (wolf).

When a non target specie is captured, the procedures are the same as a target specie, so it should be performed a physical restraint or a chemical immobilization to access the animal health and application of adequate treatment if needed, after which the animal should be released in the place, unless it needs to be recover in medical facilities (Government Alberta 2013).

The capture outcome affects not only the non target specie, also compromising the target animals. The real impact of the capture procedures in the life of the animal is not very understood, more studies are needed (in my opinion) (Godfrey and Bryant 2003).

A study performed in otters (*Lutra lutra*) refers that the utilization of the alarm system reduced the amount of time between the capture of the animal and the arrival of the team to the capture set, which decreased the severity and the amount of injuries inflicted by the capture on the animal (Néill et al. 2007; American Veterinary Medical Association Animal Welfare Division 2008).

Although there is little published information, about the influence of the capture after animal release, there is described behavior disorders and decrease of fitness. To increase the accuracy of the information of the animal's behavior and mobility it is suggested the used of the telemetry information after two weeks of the animals release (Mech 2002).

2.4.3.1. INJURIES

Although the capture of wild animals has been performed for many years, injuries or death casualties are rarely documented. Regarding capture injuries, there is a deficiency of long term information because after release, it is impossible to monitor the evolution of the process, at least at a fine level, since sometimes with radio tracking and photo trapping we can only see if the animal is alive or not and if apparently recover completed or not. The studies performed on traps used in the capture, are related to the injuries and are mostly described in the United States, where different legislation is present about the kind of traps used. Additionally these studies aren't performed only on wolves. The physical injuries can be described as an indicator of "potential stress and struggle" during the capture. The capture of animals can present a degree of stress that varies in its duration and intensity, which affect direct and/or indirectly the animal welfare. It may cause anxiety (caused by olfactory stimuli, metallic sounds as a result of the struggled or because of the reduced mobility), lesions on the caught member (ischemic wound that may evolve to necrotic culminating with the loss of function among other, increase the risk of infectious diseases caused by the immunosuppression, among others. Usually, lesions are "measured" in a short period, since physical lesions and blood findings from the animal (which varies according to time extent of time the animal is captured). The real long time effect is unknown, because of the lack of studies on such subject (Houben et al. 1993; Sahr and Knowlton 2000; Powell and Proulx 2003;Cattet, Stenhouse, and Bollinger 2008; Marks 2010).

Figure 29– Mouth injuries during the capture in M1 – Orphento, photo courtesy of Antonio Antonucci, MNP.

Figure 30– Paw lesion during the capture in F5 – Iris, on MNP.

When an animal is captured, they try to escape. Usually the behavior observed are attempts of running, jumping, chewing and biting. During its struggle, the injuries can be caused by the trap or by the vegetation around the capture set. The trap influences injuries inflicted on the animal, generally, snares namely, chest snares cause more aggressive injuries and can culminate with the death of the animal, than mixed traps. The location of injuries is linked to the body part that is caught in the trap (feet, leg, chest or neck) and on the mouth because of the fighting behavior in an attempt to escape, but can have other locations. The injuries observed can differ from simple erosion, wounds, cuts or edema to profound and severe wounds and cuts, which can end with fractures or loss of teeth, loss or fractures of fingers, footpad and leg, between others. Figures 29 and 30 demonstrated some examples of lesions caused by the capture event in wolves. These can compromise the fitness or animal life, such as loss of teeth, fractures, amputation and even death (Ballenberghe 1984; Marks 2010; Frame and Meier 2007; Arnemo and Ahlqvist 2006; Savarie et al. 2004; Onderka, Skinner, and Todd 1990).

Concerning foot hold traps, the use of traps with paddle jaws, decrease the injuries compared to other traps. A study performed in coyotes concluded that using a mixed trap Fremont®, decrease the amount of injuries observed. Note that a study performed in foxes, observed that although the captured method influenced the lesion, other factors as the time the animal remains in the place negatively influences the severity of the lesions (visible or not) and as well as the protocol used and the presence of concomitant diseases (Onderka, Skinner, and Todd 1990; Savarie, Fagerstone, and Schafer Jr 1993; Phillips, Gruver, and Williams 1996; Shivik and Gruver 2000; Arnemo and Ahlqvist 2006;Cattet et al. 2006; Frame and Meier 2007; Darrow et al. 2009; Marks 2010; Turnbull, Cain, and Roemer 2011; Barber-Meyer and Mech 2014; American Veterinary Medical Association Animal Welfare Division 2008).

There are two major causes of death described in the literature, hyperthermia and capture myopathy (CM). Hyperthermia is one of the key factors to the development of CM, associated with other factors, as extreme exertion, struggle or stress and the chemical immobilization (Nielsen 1999; Cattet et al. 2003; Montané et al. 2003; West, Heard, and Caulkett 2007; Cattet and Boulanger 2008; Fahlman et al. 2008; Government Alberta 2013). Regarding hyperthermia, the temperature enhance is induced not only by stress but also by physical activity. Cattet and Boulanger 2008also suggest a relationship between hyperthermia and muscular exertion in leg hold snares in captured grizzly bears. In this study it is described the CM as a probable cause of death. However when comparing captures performed by pursuit or by physical restrain as a trap, pursuit captures implied a major risk of hyperthermia (Nielsen 1999; Cattet et al. 2003; Montané et al. 2003; Cattet and Boulanger 2008). A study performed in wolves tried to relate rectal body temperature during capture with the survival time of the animal after being released and monitored. Barber – Meyer *et al.* (2014) did not found any evidence of relation between these two aspects, but note that the duration of high temperatures was not considered (Barber-Meyer and Mech 2014).

CM is a non – infectious and metabolic disease that can occur in different situations and that is also described in domestic and wildlife animals captures, which processes a significant morbility and mortality. It was originally related only to ungulate animals, however, nowadays there are descriptions of CM in primates, ursids, canids, felids, marsupials, birds, rodents, mustelids cetaceans, pinnipeds, and also in fish and amphibians (Wallace, Bush, and Montali 1987; Fowler 1993; Hartup et al. 1999; Nielsen 1999; Cattet et al. 2003; Montané et al. 2003; Ignasi et al. 2006; Herráez et al. 2007; Arnemo and Ahlqvist 2006; Roe and Spraker 2012; Herráez et al. 2013; West, Heard, and Caulkett 2007).

CM is characterized as a degenerative or necrotizing damage to skeletal and cardiac muscle associated with physiological imbalances after extreme muscular activity, trigged by fear, exhaustation and stress during trap, pursuit, capture, restrain and transport. Predispositions factors may be genetic or acquired (Cattet et al. 2003; Ignasi et al. 2006; West, Heard, and Caulkett 2007). Features that contribute to CM are specie (prey species are the most affected, regarding birds the most affected are the long – legged wading birds), environment (extreme environment condition, including temperature, rain and humidity, as well jeopardize associated with landscape topography), capture – related factors (capture method, injuries resulting from the capture, capture time), other diseases (increase of susceptibility, as example heartworms compromise cardiovascular system), signalment (pregnant, young and old animals are more susceptive), nutrition (animals with pre – existing vitamin E or selenium deficiency may be a predisposition factor), drugs (opioids drugs are the agents more associated to CM, caused by its side effects as excitement, spontaneous movements, muscle rigidity, hypoventilation, catecholamine release and hyperthermia) (Fowler 1993; West, Heard, and Caulkett 2007). There are theories that relate CM with the prey – predator relationship, since it is associated to a relatively quick and painless death of the prey and allows the predator to save a large amount of energy West, Heard and Caulkett 2007quoting Spraker *et al*. (1993).

The pathophysiology of CM is associated with the duration and intensity of the physical effort expended by an animal during the capture attempt. It involves three primary components: perception of fear, sympathetic nervous and adrenal systems and muscular activity. CM can be classified as temporal syndromes as hyperacute, acute, subacute and chronic or can be described concerning the clinical findings and the pathologic events in four syndromes: capture shock syndrome (can occur during the immobilization or moments after capture, the clinical signals usually observed are elevated body temperature, weak thready pulses and death), ataxic myoglobinuria syndrome (most common, the clinical signal can be observed after several hours or even days post – capture, as mild to severe ataxia, torticollis and myoglobinuria), ruptured muscle syndrome (the clinical signs are evident only 24 to 48 h after the capture event, generally is observed marked drop in the hindquarters and hyper – flexion of the hock because of unilateral or bilateral rupture of the gastrocnemius muscle) and delayed – peracute syndrome (is the rarest syndrome and happens in animals that are in captivity for a period of 24 h, the animals does not show any manifestation until is disturbed, after a quick stress and fight response to try escape, they abruptly stay down for a few moments and die (Wallace, Bush, and Montali 1987; Fowler 1993; Hartup et al. 1999; Nielsen 1999; Cattet et al. 2003; Montané et al. 2003; Arnemo and Ahlqvist 2006; Ignasi et al. 2006; Herráez et al. 2007; West, Heard, and Caulkett 2007; Roe and Spraker 2012; Herráez et al. 2013). Moreover the best prevention is reduction of the chase, capture time, handling and transporting to the minimum, avoiding the capture pregnant or lactating females, a good monitoring approach and the selection of an efficient chemical immobilization (Nielsen 1999; Fowler 2008;Government Alberta 2013).

Regarding injury severity, some authors are trying to create tables in order to increase accuracy in the classification of observed injuries. There are different tables and each clinician can use and adapt the one that works better for him. Ballenberghe 1984 created a classification of four classes, class I – slight foot and/or leg edema with no lacerations and no evidence of broken bones or dislocated joints, class II – moderate edema with a skin laceration 2.5 cm or less long, bones and joints as in class I, class III – skin laceration greater than 2.5 cm long with visible damage to underlying tissues, tendon intact, bone breakage limited to one phalanx or metacarpal and class IV – various combination of deep, wide laceration, severed tendons, broken metacarpals, broken radius and ulna bones and joint dislocation of the leg. The conclusion was that neck snares in most of times are the cause of wolf deaths, making it important avoid its utilization. Younger individuals present an increase of injuries compared with adult wolves. They also concluded that the real effect of these injuries (broken teeth, missing feet, severed tendons and poorly healed bones) is unknown, but there is a reduction of "fitness and shortened life span", which cannot be evaluated because the real long term impact of the capture in unknown. Summarizing, if all wolves captured present lesions (with different severity levels), the capture will affect their performance and it is important to use all methods available in order to minimize the consequences of the capture to a minimum (Ballenberghe 1984; Kuehn et al. 1986; Onderka, Skinner, and Todd 1990).

Kuehn *et al*. (1986) created another kind of table also subdivided in 4 classes "class 1 – no visible damage, class 2 – cuts smaller than 2.5 cm in total, class 3 – cuts bigger than 2.5 cm or 1 injured phalanx or metacarpal, class 4 – 2 or more injured metacarpals or phalanges or radius or ulna". In this study it was observed more injuries from classes 3 and 4 in pups. It was also observed a lot of injuries in teeth, mostly pre molars and milk teeth (Kuehn et al. 1986; Frame and Meier 2007).

Since not only healthy animals are trapped, but also some sick animals, mainly if we use food bait in the trap, it is important to access this situation and give them the proper solution. If a more labor intense therapy is required the animal is put in a proper enclosure in order to be released after the treatment (Marks 2010; Kuehn et al. 1986).

Figure 31 – Amputated wolf [accessed on September 1 2015], available on http://feralfront.com/index.php?topic=1755922.0

Sometimes capture (or poaching), causes irreversible lesions on members which may need amputation (Figure 31). Considering such situations in addition to the fact that wolves are a predator specie, a decision must be made between releasing the animal or maintaining it in captivity. There are reports of wolves released after an amputation of a limb. These reports concluded that there are no evidence that the amputation affected the lifestyle of the animal, however the number of studies on this subject is very scarce and some cases had short time monitor. One of these cases concerns a Mexican wolf (*Canis lupus baileyi*) that was released but because of livestock depredation, it was necessary to recapture him and transfer him to a captive enclosure. In the case of an Iberian wolf (*Canis lupus signatus*), Trevo and a female Red wolf it was verified that their mobility was not affected and that they presented a large dispersion behaviour. In the case of the female, after this dispersion alone, she was accepted into her former pack, whereas Trevo continued his dispersion in search for a territory on the other side of the boundary, in Spain (Rodrigues 2015;Turnbull, Cain and Roemer 2011;«Three Legged Wolves Roam and Thrive in New Mexico Wild |Tripawds Blogs | Three Legged Dog Cat Amputation Pet Cancer Support Information Help Advice» 2015).

One of the major decisions during wildlife capture, concerns the animal future. Is it ethical or human to put an animal in an enclosure for the rest of his life? This is a very controversy subject because some conservationist defends the importance to have an "ambassador/diplomat" animals, based on the belief that when people can behold and/or touch an animal they can feel more affinity and empathy with the species. On the other hand, is it more humane and ethical to euthanize an animal just because they cannot be released back to the wild? Or simply to release them and let "nature due its job"? Is it the conservation of the specie or the awareness of the people what really matters in the benefit of the individual? It is important to not forget the conservationist point view of the specie and the utility of their genetic heritage. There is a description about a female wolf, Tenino, which was captured by helicopter and placed in captivity as a management way of problems (predation) that she caused to livestock. Tenino displayed symptoms that were similar to those of humans with post traumatic stress disorder. These symptoms included hypervigilance, exaggerated startles, generalized fear, avoidance, looking up behaviors and arousal. The effect of wild animal captivity is the subject of an increasingly number of studies and preoccupation. There are many reports on behavior abnormalities and environmental enrichment. However, even the best condition and environment enrichments are not their real habitats. This is particularly important in animals that need a big territories as well as solitary ones (Onderka, Skinner, and Todd 1990; Powell and Proulx 2003; Gusset et al. 2008; Mech 1981; Mech and Boitani 2007; Mech and Cluff 2010; Mallonée and Joslin 2004; American Veterinary Medical Association Animal Welfare Division 2008).

The real effect of the capture in the animal's life is not completely understood. More research is necessary in order to improve capture methods into the least the invasive possible and minimize its effects in the life of the animal.

3. CHEMICAL IMMOBILIZATION

3.1. BRIEF HISTORY

Chemical restraint is closely linked with wildlife capture, they complement and influences each other.

Today the main concern is to determine the type of approach and immobilization protocols to be used (Osofsky and Hirsch 2001; Tribe and Spielman 1996).

Capturing wildlife is part of our history and evolution, for many reasons as human subsistence, enrichment, sport, curiosity, entertainment or enlightenment. The use of drug delivery systems as blowpipe darts, arrows or spears have been reported with the use of natural poisons (animals and plants) by aboriginal hunters from South America, Asia, Africa and Australia (Fowler 2008; Nielsen 1999).

Only in the mid – 50 is that there was an increase of interest in the use of chemical immobilization in the scope to wildlife conservation (Fowler 2008; Nielsen 1999).

Safe, effective and reversible are the aims of the drugs used in any protocol. The chemical immobilization, if correctly executed, is not a harmless situation, it can be used as a first approach to capture (example: capture using a helicopter as shooting platform) or may be used as a "complement" to the animal captured in a trap (Tribe and Spielman 1996; Larsen et al. 2002; Arnemo and Fahlman 2007; Editors and Arnemo 2011).

3.2. RESOURCES EMPLOYED

There are different delivery drug systems available, from, the most basic method, which is oral chemical restrain, to the complex system of projectile syringes or darts. The oral chemical restrain is not used on wildlife animals because it is impossible to control the amount of drug ingested, its effects depend on the quantity of food present in the stomach and when regarding the use of bait with the drug, there are the possibility that other species rather than the target specie eat the drug, among others factors that will influence the final outcome (Martinez and Papich 2009). The hand – held syringe is not also frequently used because of the proximity that the process requires, which can unnecessary enhance the stress on the animal and also can compromise our security. The pole syringe (stick syringe) can be used with the help of a net and a y – pole (Figure 33) mostly in younger animals, but can also be an effective method to adults usually with a catch pole (ketch – all pole) (Figure 32) as helper if the operator has experience (Tribe and Spielman 1996; Fowler 2008; Kreeger and Arnemo 2012; Nielsen 1999; International Wolf Congress 2013).

Figure 32– Catch pole [accessed on September, 1 2015], available on http://www.livetrap.com/index.php?dispatch=categories.view&category_id=638.

Figure 33 – Y pole [accessed on September, 1 2015], available on http://animal-care.com.au/index.php/vmchk/Poles/ACES-Y-

The blowgun/blowpipe is a popular technique invented many centuries ago. Some cultures use blowpipes with poison to hunt animals (Nielsen 1999; Mattos et al. 2007). The advantages associated with this approach are the infliction of minimal damage impact in the animal tissues, silent projection and absence of mechanical parts nor maintenance, it can be purchased or build by oneself. There are different models with very different lengths, 1 to 3 m that can deliver the drug to 10 – 15 m of distance. It has a low velocity when comparing to delivery systems that are more advanced and the trajectory of the dart is greatly influenced by the wind, which culminates in a progressive decrease in the accuracy of the immobilization towards the end of the effective range. It is a technique that needs some experience from the operator. It is mainly used in "small animals", because of the small drug volume capacity (0.5 – 3 ml) of the darts (Tribe and Spielman 1996; Kreeger and Arnemo 2012; Nielsen 1999; West, Heard, and Caulkett 2007).

For wildlife captures, the use of power projected syringes was a revolutionary and very useful technique. For longer distances then those where we typically use the blowpipe (10 to 15 m), we can use a special rifle or a special pistol (Figure 34), the first one usually for longer ranges (up to 50 m) and the later for short range (less than 20 m). These guns use special syringes, often adapted only to the respective gun brand, that deliver the drug when the needle crosses the skin by a compressed air system or by a small explosive charge (Tribe and Spielman 1996; Fowler 2008; Kreeger and Arnemo 2012; Nielsen 1999; «Life Wolfnet» 2015; «Wolfnet» 2015).The same technique can be used with the crossbow. It is useful because is a silent and exact weapon. The biggest disadvantage of this technique is the need of surgical removal of the arrow (Fowler 2008; «Life Wolfnet» 2015; «Wolfnet» 2015). There are also descriptions using an arrow and bow in leopards and lions. This technique is very efficient, silent, cheaper, with a greater accuracy when used by a person with experience, as the people that live in the place that frequently used this method to hunt and can be used in a distance up to 30 m of the animal. With the joint of a dart in the arrow, this method started to be a hopeful choice to use in shy animals and when the pursuit by foot is necessary to capture them (because of the terrain characteristics). However, care should be taken with the force applied, since the force used in the bow to capture has to be smaller than the one used in hunting (Stander et al. 1996).

Figure 34 – Performing the chemical immobilization using a CO_2 – powered rifle, photo courtesy of Antonio Antonucci, MNP.

Figure 35– Example of immobilization dart, photo courtesy José Manuel Almeida

After a brief description of the delivery system that can be used, note that on the whole, the drugs can be supplied in two ways, by a syringe or a dart.

There are a lot of syringes available, with different models and sizes. In this case, the injection can be applied manually or by a pole syringe (Nielsen 1999; Kreeger and Arnemo 2012; West, Heard, and Caulkett 2007). The animal needs to be contained (cage, trap …) to make the administration (Nielsen 1999; West, Heard, and Caulkett 2007).

Concerning the darts, nowadays is the most common approach used in chemical immobilization of wildlife animals. The darts can be classified as: two chambered compressed gas darts (examples are, the blow darts and molded nylon, chambered plastic darts), power explosive powder dart (which have two types: modular powder explosive powered dart and prefabricated powder explosive darts) and in miscellaneous dart types (can be subdivided in aluminum two chambered compressed gas, chemical powered dart and spring powered dart) (Nielsen 1999; West, Heard, and Caulkett 2007).

The base of the dart has a similar constitution in all the darts, but the kind of needle, material (plastic, aluminum), discharge mechanism to inject the drug (pressurized or butane, explosive discharge mechanism, spring, among others) and being disposable or not differs. The dart can be subdivided in 4 pieces, as it is observed on Figure 35. Using the two – chambered compressed gas darts as example: a needle is placed in the anterior chamber with the objective to penetrate the animal skin and inject the drug. There are different sizes and forms, generally the hole is not in the tip but on the body of the needle and a silicone sleeve is necessary to occlude the hole. The dart body is divided into two chambers by a movable, rubber syringe plunger in the center. The drug is placed in the anterior chamber while in the posterior chamber air is introduced with a help of a syringe, creating a positive pressure. The last constituent of the dart is the tail piece which works as a flight stabilizer, to make the route as straight as possible (Nielsen 1999; Kreeger and Arnemo 2012; West, Heard, and Caulkett 2007; Miller and Fowler 2011).

Another kind of remote delivery system described is the solid drug pellet system. The idea is to use a solid, lightweight, absorbable bullet. This method was used in the first captures on 1950s using gallimine (neuromuscular blocker, acts in acetylcholine receptor) and nowadays it is used on various materials as hydroxypropyl cellulose that dissolves and absorb into the muscle. Its utilization is not exclusive to wild animals but also to livestock to perform remote administration of vaccines and other medication. The problem associated to its use in wild animals is the absorption time, which is longer when compared to other delivery system used (Saldanha and Howlin 1989; Nielsen 1999; Cattet et al. 2006).

In this kind of approach it is essential to choose the right time to make a clear shot. The wind and the distance to the animal influences the dart trajectory and consequently its accuracy. It is important to analyze all factors that can interfere with the delivery system and can hurt the animal, in order to minimize or control them (Nielsen 1999;Cattet et al. 2006; Angerer 2010).

The dart preparation can be done in the field, just prior to the delivery and taking in account the specific weight and condition of the animal in the trap, or different darts for different situations can be prepared previously in the clinical facilities. Choosing one or the other approach depends on the operator preferences (Nielsen 1999; International Wolf Congress 2013).

3.3. CURRENT DRUGS USED IN WILD ANIMALS

Wildlife management and conservation are relatively new concepts, which associated with the variety of situations and species involved in chemical restraint leads to the fact that this is a subject were we still don't have a well established "cooking book". The exact date of the first wolf capture for scientific proposes is not accurately established, but the first descriptions of wolf capture vary between before 1970 in captivity and before 1974 in wild wolves (Ballard, Franzmann and Gardner 1982 quoting Seal *et al.* 1970 and Mech *et al.* 1974) (Ballard, Franzmann, and Gardner 1982).

The perfect drug for chemical restraint does not exist, so the goal is to choose a drug, or a combination of drugs. The use of a balanced combination of drugs allows, not only to reduce the individual doses, but also to minimize its side effects (Tranquilli, Thurmon, and Grimm 2007). Some aspects are important in the drug choice such as potent but with small volume of dose, high therapeutic index, mechanism of action, fast induction time, providing a deep and constant anesthesia and with minimal effect on the cardiovascular and respiratory systems, have a minimum impact and not cause alteration/lesion in the tissues after an IV or IM administration, such as necrosis. Another important aspect in relation to the drug, is that can be mixed with others in the darts/syringe without pharmaceutical incompatibilities, i.e., causing changes in the drug properties (Tribe and Spielman 1996; Nielsen 1999; Kreeger and Arnemo 2012; Tranquilli, Thurmon, and Grimm 2007; West, Heard, and Caulkett 2007; Miller and Fowler 2011).

The choice of the drug, delivery system used and handling will depend in the animal species, size, physiological condition, the preference and experience of the veterinarian, topography of the landscape and weather (Tribe and Spielman 1996;Osofsky and Hirsch 2001; Arnemo and Fahlman 2007; Malmsten 2007; Editors and Arnemo 2011).

“Animal factors” that include both physical and physiological aspects as the specie, gender, age, weight, stress, health, among others, can influence direct or indirectly the protocol and dose used. A more stressed animal and/or a pregnant female probably will need more dose than a sick animal (Nielsen 1999; Fowler 2008).

The most common drugs used in animal restraint are tranquilizers/sedatives such as phenothiazines, benzodiazepines, alpha adrenergic, cyclohexamines and opioids (West, Heard, and Caulkett 2007; Tranquilli, Thurmon, and Grimm 2007;Kreeger and Arnemo 2012; Nielsen 1999).

The ideal anesthetic recovery should be rapid, complete and stress free for the animal. This is particularly important in wild animals, because of the scarce resources in the field (Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010). That is why the implementation of protocols with drugs that can be quickly reversed with an antagonist is common practice in wild life chemical restraint. Some examples of drugs and its antagonist are medetomidine and dexmedetomine reverted by atipamezole, midazolam and diazepam reversed with flumazenil and using naltrexone as antagonist of opioids agonists (Tranquilli, Thurmon, and Grimm 2007; Muir et al. 2013).

It should be noted that conduct and dose protocol used should be different when it comes to capture captive or wild wolves(Vilà and Castroviejo 1994).

3.3.1. SEDATIVES

Sedatives are a group of agents that cause central nervous system (CNS) depression with consequent decrease in animal excitement and activity and may even cause somnolence, but without inducing and maintaining a state of sleep, which is typical of hypnotic drugs (Brunton, Chabner, and Knollman 2011). Of course, this division is sometimes more theoretical then real because some sedatives, with increasing doses, can induce sleep, unconsciousness and even death after fatal cardiorespiratory arrest (Brunton, Chabner, and Knollman 2011).

Sedatives can be subdivided in phenothiazines, butyrophenones, benzodiazepines and alpha 2 agonists (Tranquilli, Thurmon, and Grimm 2007).

3.3.1.1. PHENOTHIAZINES

Phenothiazines are used in human medicine as antipsychotic drugs and are classified as neuroleptics. However, in chemical restraint they are used mainly by their tranquilizer and sedative effects. These drugs are broadly used in veterinary medicine, mostly associated to pre anesthesia (Boyd, McDonell, and Valliant 1991; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Bell et al. 2011; Muir et al. 2013).

Phenothiazines are dopamine receptor (D1 and D2) antagonist in the CNS (Muir et al. 2013; Seymour and Duke-Novakovski 2007; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009; Grimm, Tranquilli, and Lamont 2011; Saponaro et al. 2013; Zapata and Hofmeister 2013). The sedative (the first signal observed, that starts very slowly) and anxiolytic effects are dose dependent up to a threshold level, with an increase in side effects with increasing dosage (Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009; Monteiro et al. 2009).

This group provide mild to moderate tranquilization and sedation, while analgesia is a controversial effect associated with these agents (Monteiro et al. 2008; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009; Monteiro et al. 2009; Hoffmann et al. 2012; Muir et al. 2013).

The first description of a protocol with phenothiazines in wolves used promazine alone or in combination with phencyclidine or with etorphine (Ballard, Franzmann, and Gardner 1982; Fuller and Kuehn 1983). It was observed that the best results were obtained by the combination with etorphine, which add the advantage to have an antagonist (diprenorphine), however a larger dose was needed (Ballard, Franzmann, and Gardner 1982). Protocols with associations with phencyclidine, ketamine and etorphine are also used to decreased the excitement and convulsions observed (Tobey and Ballard 1985).

The death of four wolves chased by helicopter, at the time of persecution was described with etorphine and acepromazine combination (Tobey and Ballard 1985). Tobey and Ballard 1985suggested avoiding this protocol in captures by helicopter and high temperatures. Deaths using etorphine alone or phencyclidine with promazine are also reported, possibly caused by hyperthermia (Alford, Burkhart, and Johnson 1974; Tobey and Ballard 1985). It is important to refer that its quick and potent effect, make a good choice to use in wild animals, but some attention is necessary because of the side effects namely in thermoregulation and in respiratory system (Alford, Burkhart, and Johnson 1974). Due to necessary precaution in handling the etorphine by humans, small LD_{50} (median lethal dose) and for being a drug under a compulsory license in addition to the fact that phencyclidine stopped being commercially available, the combination of other drugs was necessary (Nielsen 1999; Ballard, Franzmann, and Gardner 1982; Fuller and Kuehn 1983; Papich and Riviere 2010).

3.3.1.1.1. ACEPROMAZINE

Acepromazine is the phenothiazine most frequently used in veterinary medicine and it's the safest. It is an acetyl derivative of promazine. It is supplied as a yellow solution or in tablet (Bufalari et al. 1997; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Fowler 2008; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009; Papich and Riviere 2010; Muir et al. 2013).

One of the great advantages of this drug is related to the decrease in the volume dose of other agents (Bufalari et al. 1997; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009).

The pharmacokinetics so far is still unknown in wolves, and the domestic dog must be used as a model. It can be used by IV, IM, SC or oral route. It is described a peak of 1 – 3 min after IV administration, in IM route the peak is observed past 15 – 20 min and 60 min in oral administrations (Fowler 2008; Monteiro et al. 2008; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009; Monteiro et al. 2009).

Acepromazine has a large plasma protein binding (> 99%), it is metabolized by the liver and unconjugated and conjugated metabolites are excreted by urine. The effects of acepromazine may be still observed within 4 to 8 h, but the residual effects may remain for 48 h or more in geriatric animals and animals with liver diseases. When compared with other drugs, as alpha 2 agonist, acepromazine alone cause a less reliable and quality sedation (Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Muir et al. 2013).

Regarding cardiovascular system, it is observed a decrease of arterial blood pressure, specially using high doses and when used alone, but usually it is not observed a real hypotension in healthy dogs (Bufalari et al. 1997; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Monteiro et al. 2008; Monteiro et al. 2009; Bell et al. 2011; Saponaro et al. 2013; Zapata and Hofmeister 2013; Salla, Bennett, et al. 2014). It is also observed bradycardia, because of the antagonist effect in alpha – 1 adrenoceptors and depression of the vasomotor centre within the hypothalamus with consequent vasodilation (Boyd, McDonell, and Valliant 1991; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Monteiro et al. 2008; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009; Monteiro et al. 2009; Bell et al. 2011; Saponaro et al. 2013; Zapata and Hofmeister 2013). Eventually, a tachycardia can be induced by the hypotension (Muir et al. 2013; Monteiro et al. 2008; Monteiro et al. 2009; Saponaro et al. 2013). It is also described that acepromazine can have an anti arrhythmogenic effect (under halothane anesthesia). There are descriptions as an useful agent against premature ventricular contraction and ventricular fibrillation induced by catecholamines, however it is a controversial effect. Some authors defend that this anti –arrhythmogenic effect is secondary to the antagonist effect in alpha 1 – adrenergic receptors in the heart with consequent increases of the dose of catecholamines, as epinephrine, which becomes the cause and not the prevention of ventricular arrhythmias (Dyson and Pettifer 1997; Monteiro et al. 2007; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Monteiro et al. 2008; Monteiro et al. 2009; Muir et al. 2013).The result of acepromazine in pulse rate is unpredictable (Monteiro et al. 2009).

In the respiratory system it is observed bradypnea, but respiratory rate can also be unchanged or only slightly changed (Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Monteiro et al. 2008; Monteiro et al. 2009; Zapata and Hofmeister 2013; Muir et al. 2013).

Acepromazine can cause hypothermia that is amplified by the vasodilatation(Muir et al. 2013; Seymour and Duke-Novakovski 2007; Monteiro et al. 2008; Monteiro et al. 2009).

Acepromazine has an anti – emetic effect by depression of chemoreceptor trigger zone and vomiting center, which is particularly useful to counteract the emetic effect induced by opioid drugs (Monteiro et al. 2008; Fowler 2008; Monteiro et al. 2009; Bell et al. 2011; Muir et al. 2013).

Another advantage associated with acepromazine is muscle relaxation, which is very important when associated with drugs that cause muscular rigidity, such as ketamine (Muir et al. 2013; West, Heard, and Caulkett 2007). On the other hand, this can be a disadvantage because its effect on relaxation of pharyngeal muscles, reduces lower esophageal sphincter tone, delays gastric emptying and may increase the incidence of gastric reflux. This effects can be present using acepromazine alone or associated with opioids (Tranquilli, Thurmon, and Grimm 2007).

It can induce small changes in blood pH, decreases in hematocrit (20 to 30 % with 30 min of acepromazine administration) and inhibit platelet aggregation (but appear not to alter the hemostasis in normal dogs) (Tranquilli, Thurmon, and Grimm 2007; Zapata and Hofmeister 2013).

An abnormal behavior and/or response to the administration of acepromazine described in small animals is increase in anxiety and development of restlessness and occasionally aggression. Disorientation, ataxia and attempts to escape can also be observed. These effects result from paradoxical excitement (extrapyramidal effect) that can cause akinesia (inability to initiate movement) and akathisia (inability to remain motionless) particularly in aged animals (Muir et al. 2013).

There is no specific antagonist to this drug, but with the use of a suitable stimulus we can temporally reverse the CNS depression (Fowler 2008; Tranquilli, Thurmon, and Grimm 2007; Zapata and Hofmeister 2013; Muir et al. 2013).

In an excited animal a larger dose might be required (Muir et al. 2013).

The use of acepromazine in chemical immobilization protocols of wolves is not widely adopted. There are description of protocols that use acepromazine, ketamine and medetomidine in captivity red wolves (Sladky et al. 2000), these and buthorphanol in captivity gray wolves (Valerio et al. 2005) and in Italian wolves (Guglielmini 2006), with ketamine in wild Ethiopian wolves (Sillero-Zubiri 1996) and also associated with etorphine(Tobey and Ballard 1985).

3.3.1.2. BUTYROPHENONES

Butyrophenones are a class of drug that acts in the same way as phenothiazines by blocking the dopamine receptors (D2), but also have some effect in alpha – 1 adrenergic, histaminic and cholinergic receptors. The sedative effect can be used in behavior condition and decreasing “spontaneous motor activity”, higher doses might result in extrapyramidal effects (Tranquilli, Thurmon, and Grimm 2007; West, Heard, and Caulkett 2007; Fowler 2008; Papich and Riviere 2010). It is referred as a good class of drugs to use in wild animals, because it has a safe margin, minimal effects in cardiovascular system and in thermoregulation (Nielsen 1999). An antagonist drug is not available for this class (Nielsen 1999; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010).

Haloperidol, droperidol and azaperone, are examples of drugs from this class.

Azaperone is used as a single drug in domestic animals, is mostly restricted to swine. In wild animals, there are many combinations that can be used, some examples are combination with alpha adrenergic, dissociatives and opioids drugs, among others. Rhinoceros, elephant and other ungulates are animals in which this drug is most often used. The use of azaperone in some marine animals is also described. It causes little effects on respiratory system, but has a high influence in cardiovascular system and in thermoregulation, inducing bradycardia, hypotension and hypothermia. The duration of the effect remains during 2 – 3 h after IM administration (Nielsen 1999; Tranquilli, Thurmon, and Grimm 2007; West, Heard, and Caulkett 2007; Fowler 2008; Papich and Riviere 2010).

Droperidol is a very good anti – emetic acting in chemoreceptor trigger zone, can be used with the same class of drug described with azaperone, but the most widespread description is with fentanyl which is available as a commercial combination (Innovar-vet®). It is used mostly in small animals with little applications to wild animals (Nielsen 1999; Tranquilli, Thurmon, and Grimm 2007; West, Heard, and Caulkett 2007; Fowler 2008; Papich and Riviere 2010).

Haloperidol is a neuroleptic of longer – acting duration, it is used in aggressive or excitable capture animals, newly captured animals, translocation and in releases in unfamiliar area. Overdoses cause hypotension. Administration in herbivores animals can induce aggressiveness. Haloperidol can't be used with opioids class drugs (Nielsen 1999; Tranquilli, Thurmon, and Grimm 2007; West, Heard, and Caulkett 2007; Fowler 2008; Papich and Riviere 2010; Yang et al. 2011).

3.3.1.3. BENZODIAZEPINES

Benzodiazepines are sedative – hypnotics that are usually used in chemical restraint as weak tranquilizers. They bind to the GABA (gamma – aminobutyric acid) receptor increasing the conductance to the chlorine ion which leads to a hyper – polarization of the neuron membrane and hence reducing the neuron sensibility to a threshold potent (Papich and Riviere 2010; Seymour and Duke-Novakovski 2007; Muir et al. 2013; Grimm, Tranquilli, and Lamont 2011). Besides this CNS effect they are also good muscle relaxants, have minimal cardio respiratory effects and have a good safety margin (West, Heard, and Caulkett 2007; Muir et al. 2013). Some adverse effects described are restlessness, vocalization and aggression caused by fear (Seymour and Duke-Novakovski 2007; Muir et al. 2013). The most used benzodiazepines in wildlife chemical restrain are diazepam, midazolam and zolazepam, the later usually used in combination with tiletamine, a dissociative anesthetic. The effects of this class of drugs can be reversed using the antagonist flumazenil (Seymour and Duke-Novakovski 2007; Muir et al. 2013; Grimm, Tranquilli, and Lamont 2011).

3.3.1.4. ALPHA 2 AGONISTS

The alpha 2 agonists can induce a deep sedation, which is useful in chemical restrain and also a good analgesia (Papich and Riviere 2010). Alpha 2 agonists are the most used group of sedatives in veterinary medicine and include several molecules from which xylazine, detomidine, medetomidine, are the ones more widely used in veterinary practice (Cullen 1996; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010). One great advantage in this group is the existence of their antagonists, yohimbine, tolazoline and atipamezole, which can easily revert their effects which increase the safety of alpha 2 agonist use, which is particularly important in the field chemical restraint (Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010).

Alpha 2 agonists produce their effects trough binding to alpha adrenergic and imidazoline receptors (Bousquet et al. 1999; Papich and Riviere 2010; Kurko et al. 2014). The alpha 2 adrenergic receptors are present in different body structures and are subdivided in 2A (or 2D or 2A/D), 2C and 2B (Calzada and Artiñano 2001; Badino, Odore, and Re 2005; Talukder et al. 2009). The binding to the 2A alpha receptor, which is a G protein – coupled receptor, is responsible for the sedative effect (Khan, Ferguson, and Jones 1999; Badino, Odore, and Re 2005; Cormack, Orme, and Costello2005). The stimulation of imidazoline receptor is linked to hypotension and anti arrhythmogenesis (Bousquet et al. 1999; Murrell and Hellebrekers 2005; Papich and Riviere 2010).

The most common alpha 2 agonists effects are hypotension, bradycardia and respiratory depression among others (Kreeger et al. 1988; Nielsen 1999;Kreeger and Arnemo 2012). Due to prolonged sedation and the unpredictable recover, xylazine is not a drug of choice for use alone in wild carnivorous, but as it can be antagonized by yohimbine, tolazoline or atipamezole, becomes a viable option when combined with other drugs (Fuller and Kuehn 1983; Kreeger et al. 1987; Kreeger et al. 1989; Nielsen 1999). It is important to note that yohimbine is only effective as xylazine antagonist, having no reversal effect in cyclohexamines drugs as ketamine (Kreeger and Seal 1986).

The chemical immobilization with xylazine and ketamine is widely used in various animals, like all the carnivores, including wolves (Fuller and Kuehn 1983; Kreeger and Seal 1986; Kreeger et al. 1987; Kreeger et al. 1988; Nielsen 1999; Sladky et al. 2000). The addition of atropine, an anticholinergic agent, to xylazine alone or in combination with ketamine is also mentioned. The addition of atropine to the protocols have multiple applications as it stabilizes heart rate (i.e., bradycardia and atrioventricular block induced by overdose of xylazine), decreases ptyalism, induces bronchodilation, among others (Nielsen 1999). Holtz, Holz and Barnett 1994, described tachycardia in protocols using atropine, ketamine and medetomidine, while bradycardia, arrhythmias and ptyalism, are refer in a group of wolves using just ketamine and medetomidine without atropine (Holz, Holz, and Barnett 1994; Nielsen 1999).

Kreeger *et al*. (1988), describe that the use of ketamine and xylazine protocols is beneficial in painful and long procedures. If additional doses of ketamine are necessary, the recovery time will be greater and problematic (Kreeger et al. 1988). Bradycardia and hypertension are also described with ketamine and xylazine protocols (Kreeger et al. 1987). Using the same protocol (ketamine xylazine) in red wolves, it was observed hypertension, tachycardia, the induction and recovery times were longer and some ataxia was occurred. This unpredictable recovery can contribute to enhance the mortality in the field (Sladky et al. 2000).

3.3.1.4.1. MEDETOMIDINE

Medetomidine is supplied as a mixture of two enantiomers, dexmedetomidine and levomedetomidine (1:1), the first one, the active enantiomer, is responsible by the effects seen with the use of medetomidine and second enantiomer does not have effects at therapeutic levels (Kuusela et al. 2000; Kuusela et al. 2001).

As far as we know there are no pharmacokinetic studies with medetomidine in wolves, but once dogs are a close related specie, they can be considered as a model for wolves. With a dose of 40 µg/Kg IV, medetomidine has a half – life of 0.96 h and after 40 min the dog is still moderately sedated (Kuusela et al. 2000). It is eliminated mainly by liver metabolization, in the first phase is hydrolysis by cytochrome P_{450} isoenzymes, followed in a second phase by glucuronidation, with the inactive metabolite being excreted in the urine (Kuusela et al. 2000; Tranquilli, Thurmon, and Grimm 2007; Duhamel, Troncy, and Beaudry 2010; Muir et al. 2013; Flaherty 2013a).

The effects of medetomidine are dose dependent (Simon, Romváry, and Mora 1989; Vainio, Vähä-Vahe, and Palmu 1989; Valerio et al. 2005). The cardiovascular effects can be divided in two phases, the first is peripheral, with vasoconstriction and increased blood pressure, followed by a central phase with a marked depression of sympathetic tone with sedation, analgesia and muscle relaxation (Valerio et al. 2005). One of the most important effect is in the cardiovascular system, which include sinus bradycardia, hypertension and atrioventricular blocks (AV blocks) (Cullen 1996; Bettschart-Wolfensberger et al. 1999; Murrell and Hellebrekers 2005; Tranquilli, Thurmon, and Grimm 2007; Costa 2011; Flaherty 2013a; Flaherty 2013b). The administration of atropine or glycopyrrolate, an anticholinergic drugs, can minimize effects as bradycardia, hypertension and AV blocks, albeit it utilization is controversial (Cullen 1996; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010; Flaherty 2013a; Flaherty 2013b; West, Heard, and Caulkett 2007).

All the organs and systems are more or less affected by this drug, such as respiratory depression rate (cyanosis can be observed in some dogs), emesis, decreased bowel motility, polyuria, myometrium contraction in the last trimester of pregnancy, hyperglycemia (by activation of receptors in beta cells of pancreas resulting in an inhibition of the release of insulin), increase of intracranial pressure, decrease of catecholamine levels and incapacity of thermoregulation with hypothermia, caused by direct suppress in the thermoregulatory center but also secondary to muscle relaxation and reduced shivering (Cullen 1996; Bettschart-Wolfensberger et al. 1999; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007;Muir et al. 2013; Papich and Riviere 2010; Arnemo et al. 2013; Flaherty 2013a; West, Heard, and Caulkett 2007).

In literature there are referred some factors as stress, fear, excitement and pain that can change or delay the effect of medetomidine (Tranquilli, Thurmon, and Grimm 2007). It can be used IM or IV with the first signs observed 15 – 20 min after administration (Arnemo et al. 2013). Medetomidine induce a reduction in catecholamines and in cortisol levels, which in turn reduce the stress response to trauma, which is very important in wild animal capture (Cullen

1996; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010; West, Heard, and Caulkett 2007). Another cardiovascular effect of medetomidine is vasoconstriction which can interfere with monitoring parameters, namely the capillary refill time and pulse oximetry that can provide erroneous results (Costa 2011; Flaherty 2013b).Medetomidine is the most used alpha 2 agonist used in small animals and it is also used in wild animals, including wolves (Tranquilli, Thurmon, and Grimm 2007; West, Heard, and Caulkett 2007). Tranquilli, Thurmon and Grimm 2007, refer that medetomidine and acepromazine are the only sedatives reliable to use in dogs.

A lot of protocols are described with the use of medetomidine alone or with other drugs such as ketamine (Sladky et al. 2000; Arnemo et al. 2013), butorphanol alone or with ketamine (Sladky et al. 2000), diazepam (Larsen et al. 2002), tiletamine and zolazepam (Valerio et al. 2005; Arnemo e Fahlman 2006; Malmsten 2007; Editors and Arnemo 2011), ketamine and acepromazine (Sladky et al. 2000).

Medetomidine dose can be reduced when in combination with other drugs, which will decrease its side effects (Murrell and Hellebrekers 2005; Seymour and Duke-Novakovski 2007).

3.3.1.4.2. ATIPAMEZOLE

Alpha 2 adrenergic agonist can be reversed with atipamezole, yohimbine, idazoxan, tolazoline and MK – 467 (Cullen 1996; Ambrisko and Hikasa 2003; Tranquilli, Thurmon, and Grimm 2007; Seymour and Duke-Novakovski 2007; Talukder and Hikasa 2009; Papich and Riviere 2010; Honkavaara et al. 2011; Restitutti et al. 2012; J. Honkavaara et al. 2012; Salla, Restitutti, et al. 2014).

Atipamezole is a medetomidine specific antagonist and since medetomidine is widely used, the use of atipamezole is also frequent. It acts at central and peripheral level with high selectivity to the alpha 2 adrenoceptors, with the same affinity to 2A, 2B and 2C receptors as yohimbine, almost 100 times more affinity to 2D and without affinity to others receptors, unlike yohimbine, as serotonergic, cholinergic, dopaminergic, GABA, muscarinic opioids and benzodiazepines receptors (Holz, Holz, and Barnett 1994; Dyson and Pettifer 1997; Larsen et al. 2002; Tranquilli, Thurmon, and Grimm 2007; Seymour and Duke-Novakovski 2007; Talukder et al. 2009; Papich and Riviere 2010; Restitutti et al. 2012; Arnemo et al. 2013). This specificity decreases the side effects caused by the atipamezole (Papich and Riviere 2010). As consequence of receptor specificity associated with atipamezole, it is described that some animals need supplementary doses to achieve a complete reversion and are others cases of some animals that become resedated later (Papich and Riviere 2010).

It can be used by IV and IM, however the IM route is more widespread and safer to use. The IV administration needs to be performed very slowly and because sometimes can culminate with death it is advised to only use this route in cases of emergency. Cases of cardiac collapse after IV administration are described, possibly by the preservative methylparaben that result in a decrease of blood pressure caused by the increase of the release of histamine, the other possibility is that the use of a large dose in patient with peripheral vasoconstriction, with a consequent bradycardia and an abrupt increase in vagal tone resulting in collapse. Quick IV administration can also be the cause death as a consequence of a quick reverse of vasoconstriction, without sufficient time to the sympathetic nervous system to act and increase cardiac output resulting in a severe hypotension (Ambrisko and Hikasa 2003; Tranquilli, Thurmon, and Grimm 2007; Seymour and Duke-Novakovski 2007; Fowler 2008; Papich and Riviere 2010).

The atipamezole should only be administered 30 min after the start of chemical immobilization. There is not pharmacokinetic studies available in wolves, so we will use the domestic dog as a model. After the atipamezole administration a plasmatic peak is obtained at 25 min, the half – life elimination is about 1h in canine plasma and recover is obtained in 5 min and in 10 min the animal is already walking. Resedation can happen 30 to 60 min after administration (Cullen 1996; Ambrisko and Hikasa 2003; Tranquilli, Thurmon, and Grimm 2007).

Generally it is described that the dose of atipamezole used in wildlife animals should be 4 to 6 times the dose of medetomidine used (Pettifer, Dyson, and McDonell 1996; Cullen 1996; Ambrisko and Hikasa 2003; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010; Grimm, Tranquilli, and Lamont 2011). Papich and Riviere 2010, refer that atipamezole has a safety margin and healthy dogs can tolerate 10 times the prescription dose (Papich and Riviere 2010).

Some effects of medetomidine as: sedation, depression, analgesia, anxiolysis, hypothermia and some cardiovascular effects as hypertension and bradycardia are reversed by atipamezole, albeit not all the effects in cardiovascular system can be reverted (Cullen 1996; Pettifer, Dyson, and McDonell 1996; Tranquilli, Thurmon, and Grimm 2007; Fowler 2008; Talukder et al. 2009; Papich and Riviere 2010). It is also described the capacity of antagonizing the diuretic effect, decrease of specific gravity and osmolality of urine, increase levels of insulin, creatinine, sodium, potassium and chloride, decrease of atrial natriuretic peptide and the release of catecholamines. Advantages of using the atipamezole instead of another antagonist is that it is a safe drug that cause a quick return to consciousness used alone or with other drugs (Cullen 1996; Ambrisko and Hikasa 2003; Talukder et al. 2009; Papich and Riviere 2010; Restitutti et al. 2012).

The response observed is dose dependent and the observed signals of overdose are excitement, panting, trembling, vomiting, mild diarrhea and sclera injections. Other adverse effects that can occur are vasodilation, hypotension, tachycardia, respiratory depression, ptyalism, increase of intracranial pressure, delirium, apprehension and aggression, over – alertness and/or excitement and absence of analgesia (Ambrisko and Hikasa 2003; Fowler 2008; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010; Grimm, Tranquilli, and Lamont 2011; Arnemo et al. 2013). Arnemo *et al.*(2013), report in one wolf absence of response after atipamezole administration with the recovery happening only after 8h of darting (Arnemo et al. 2013).

Concomitant administration of atipamezole and anticholinergics should be avoided due to enhance heart rate (Tranquilli, Thurmon, and Grimm 2007).

Arnemo *et al.* (2013), described in wolves captured by helicopter, a decrease of awaken time in protocols made with medetomidine – ketamine, using atipamezole 22.4 to 40 min compared with protocols that use tiletamine – zolazepam that has a “recovery” that can vary between 6 to 8 h(Arnemo et al. 2013). Depending on the dose of atipamezole, the recovery time can vary from 5 to 20 min, with a larger dose, less time is required to achieve the recuperation (Ambrisko and Hikasa 2003). Atipamezole is often described as being associated with a “smooth” and “good quality” recover from chemical restraint (Seymour and Duke-Novakovski 2007).

A lot of different protocols with different doses are described using atipamezole as antagonist in wolves (Holz, Holz, and Barnett 1994; Larsen et al. 2002; Sladky et al. 2000; Fowler 2008).

3.3.1.5. DISSOCIATIVE AGENTS

The dissociative agents don't cause a general depression of the CNS, they only block ascending transmissions related to parts of the CNS linked with conscious and unconscious functions, inducing a cataleptic state, with open eyes, slow nystagmus, hypertonus and reflex movements from skeletal muscles and with poor analgesic properties (Tranquilli, Thurmon, and Grimm 2007).

Dissociative agents are noncompetitive antagonists of glutamate, an excitatory neurotransmitter, at N – Methyl – D – aspartate (NMDA) receptor resulting in depression activity in some CNS structures. Additionally it is known that they have effect in cholinergic, sigmaergic, opioidergic receptors and mono – aminergic uptake systems (Malmsten 2007; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Sinner and Graf 2008; Chen, Shu, and Bayliss 2009; Persson 2013; Potter and Choudhury 2014; Muir et al. 2013).

Dissociative agents are arylcyclohexylamines derivatives and include drugs like ketamine, tiletamine and phencyclidine (Tranquilli, Thurmon, and Grimm 2007).

3.3.1.5.1. KETAMINE

Ketamine is widely used not only in the clinic for small animals for the last 40 years but also in wildlife animals (Kreeger and Seal 1986; Sladky et al. 2000; Ward et al. 2006; Sarrau et al. 2007; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Fowler 2008; Arnemo et al. 2013; Casoni, Spadavecchia, and Adami 2014; Muir et al. 2013).

Ketamine is a phencyclidine derivate with a racemic composition of two stereoisomers S(+) and R(-) ketamine in equal proportion (Papich and Riviere 2010). It is available as a lyophilized white powder that needs to be reconstituted (Seymour and Duke-Novakovski 2007; Duque et al.

2008; Bergadano, Andersen, Arendt-Nielsen, and Spadavecchia 2009; Casoni, Spadavecchia, and Adami 2014).

Ketamine can be administered trough: IV, IM, SC or intraperitoneal, oral, rectal, transnasal, intra – articular and intradermal route (Seymour and Duke-Novakovski 2007; Gomes et al. 2011; Potter and Choudhury 2014).

Until now there is not any pharmacokinetic study in wolves, but using dogs as a model, ketamine has a fast distribution in the tissues and the effects can be seen after 10 – 15 min of a parenteral administration, but with a short effect (Seymour and Duke-Novakovski 2007; Fowler 2008).

The recovery is generally violent and rough, with muscle hypertonicity and ataxia that sometimes can end with convulsions (Fowler 2008; Tranquilli, Thurmon, and Grimm 2007; Mair et al. 2009; Li et al. 2012). Occasionally prolonged recovery and drowsiness, becoming hypersensitive to noise, light and handling is associated to repeated doses (Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007).

The half – life of intravenous ketamine is 61 min (range: 44 – 77 min). Ketamine is converted by demethylation in the liver to norketamine, that has one – third to one – fifth the activity of ketamine and is excreted in the urine (Seymour and Duke-Novakovski 2007; Fowler 2008; Gianotti et al. 2014; Potter and Choudhury 2014).

The ketamine effect is caused by the depression in the CNS (Valerio et al. 2005; Gomes et al. 2011) and can also modulate spinal "wind – up" effect reducing the nociceptive pain (Seymour and Duke-Novakovski 2007; Bergadano, Andersen, Arendt-Nielsen, Theurillat, et al. 2009).

The advantage of using ketamine with other drugs is that the volume of drug can be reduced which causes less side effects (Valerio et al. 2005; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Fowler 2008; Arnemo et al. 2013; Muir et al. 2013).

There are no ketamine antagonists available (Fowler 2008; Ueyama et al. 2008).

Some side effects observed are hyperexcitability, catalepsy, increased muscle tone (Fowler 2008; Seymour and Duke-Novakovski 2007; Mair et al. 2009) and in one study realized by Mair *et al.* (2009), it was observed abnormal behavior as lip licking and head swaying. At cardiovascular level there is tachycardia, hypertension and increase of cardiac output. However, in case of "severely compromised animals" or in associations with other anesthetic agents it can be observed a cardiovascular depression. A transient hypotension may be observed in cases of administration of high doses of ketamine (Seymour and Duke-Novakovski 2007). The effect in respiratory system floats between minimal or medium effects (Seymour and Duke-Novakovski 2007; Li et al. 2012; Muir et al. 2013), maybe the most important effect observed with the utilization of ketamine and other dissociative agents is after a minimal respiratory depression an “apneustic pattern of breathing” (Seymour and Duke-Novakovski 2007; Fowler 2008; Mair et al. 2009). An important side effect of ketamine is ptyalism (Bergadano, Andersen, Arendt-Nielsen, Theurillat, et al. 2009; Lee et al. 2010)and in some cases the increase of respiratory secretion, which can cause severe anesthetic problems as upper airway obstruction (Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007). To prevent or control this effect it can be administrated an anticholinergic agent as atropine (Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Fowler 2008). In some cases there were observed mydriasis, nystagmus and the presence of all the reflexes as palpebral, ocular, laryngeal, pharyngeal, pinnal and pedal reflex with the administration of this dissociative drug (Seymour and Duke-Novakovski 2007; Fowler 2008; Lee et al. 2010; Muir et al. 2013).

Ketamine is not used alone, mainly by his poor muscle relaxation and cardiovascular effects (Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Mair et al. 2009; Ueyama et al. 2008; Li et al. 2012; Kovalcuka et al. 2013; Muir et al. 2013). The use of ketamine subsequent to the administration of promazine was used to immobilize both captive and free ranging wolves as well as the association with xylazine in captivity (Fuller and Kuehn 1983).

Its use is widespread in wildlife animals and a lot of associations between this drug and others are described in wolves (Malmsten 2007; Fowler 2008), as xylazine (Fuller and Kuehn 1983; Kreeger and Seal 1986; Kreeger et al. 1987; Sladky et al. 2000), medetomidine (Sladky et al. 2000; Arnemo and Fahlman 2007; Arnemo et al. 2013), medetomidine and acepromazine (Sladky et al. 2000), medetomidine and butorphanol (Sladky et al. 2000; Larsen et al. 2002), medetomidine, butorphanol and acepromazine (Valerio et al. 2005) and with tiletamine – zolazepam and acepromazine (Sillero-Zubiri 1996), are some of the examples.

A study performed in red wolves describes the combination of ketamine and medetomidine as safe, with less side effects such as hypertension and a decrease in induction time and recovery compared to other protocols that use xylazine instead medetomidine (Sladky et al. 2000). But tachycardia is a side effect referred using this protocol (Sladky et al. 2000). With this protocol Arnemo *et al*. (2013), described a good muscle relaxation, palpebral reflexes present and the animals were completely immobilized after one dart, with an induction time longer to adults than juveniles. Some side effects associated with this protocol were emesis (only during the recuperation phase) and hyperthermia (possibly the cause of two deaths) (Arnemo et al. 2013). It is recommended to monitor closely the recuperation in free ranging wolves (Arnemo et al. 2013).

Due to some side effects reported at the level of the cardiovascular system when using ketamine – medetomidine combinations, association with other drugs to this protocol was proposed. Sladky *et al*. (2000), reports combinations of two other drugs to this protocol, in one describes the association of acepromazine and in the other the association of butorphanol (Sladky et al. 2000) with the best results with this last molecule (Sladky et al. 2000). The incorporation of butorphanol lead to a good sedation and muscle relaxation, decreased heart and respiratory rate, deepened the anesthesia and also facilitates intubation (Sladky et al. 2000). Hypertension (but less than with the others groups) and transient hypoxemia related effect, were also referred in this protocol (Sladky et al. 2000).

Larsen *et al.* (2002), tested the efficacy of medetomidine and butorphanol in captive red wolves and concluded that the use of this protocol or the addition of diazepam are a good choice for use as a chemical immobilization with a deep sedation plane and prolonged immobilization time (Larsen et al. 2002). The side effects observed in protocols used by Larsen *et al.* (2002) were hypertension, metabolic acidosis, hyperthermia and tachycardia (butorphanol, ketamine and medetomidine group) and bradycardia (butorphanol, diazepam and medetomidine protocol). All of these drugs were antagonized with a suitable antagonist. The authors concluded that these protocols are a good choice and can be used in wildlife, but more research is necessary, because of the side effects described(Larsen et al. 2002).

Valerio *et al.*(2005) and Guglielmini *et al.* (2006), described a protocol in captivity wolves using acepromazine, butorphanol, ketamine and medetomidine, using atipamezole as antagonist (Valerio et al. 2005; Guglielmini 2006). The maintenance of anesthesia is provided with isoflurane. Cardiovascular abnormalities were reported in some wolves as first and second degree atrioventricular block and sinus arrhythmia during the maintenance with isoflurane (Valerio et al. 2005; Guglielmini 2006). Valerio *et al.* (2005),propose that best dosages and drug protocols should be studied and rethought to use as a chemical immobilization in wolves (Valerio et al. 2005).

As far as we know, there is just one description of a protocol similar to ours in literature. This protocol was performed in seven captivity red wolves, with 0.04 mg/Kg of medetomidine, 2 mg/Kg of ketamine and 0.01 mg/Kg of acepromazine and using atipamezole (0.2 mg/Kg) as the antagonist agent (Sladky et al. 2000). Given that the wolves were in captivity some actions were carried out to obtain better efficiency, food was withdrawn 24 h before capture. The intubation was performed in all the wolves but wasn't made supplemental oxygen to simulate field conditions (Sladky et al. 2000).

The results reported by Sladky *et al.* (2000), were: modest hypocapnia and a slight increase in respiratory rates, body temperature was high initially, but decreased during the handling and by the end of treatment was between 38.7 – 39.1°C. In some cases it was found a fast, "smooth and complete" recovery observed after administration of antagonist drug.

3.3.1.5.2. TILETAMINE – ZOLAZEPAM

A combination of cyclohexylamine and benzodiazepines are commercially available, tiletamine and zolazepam (Telazol®, Zoletal® and Zoletil®) (Nielsen 1999; Seymour and Duke-Novakovski 2007; Papich and Riviere 2010). This mixture causes a cataleptoid – dissociative effect, which is similar to the one observed in ketamine but three to four times more potent (Nielsen 1999).

Its use in free ranging wolves goes back more than 20 years and is still used in wild and in captive wolves and is described as safe and effective agent to use in chemical immobilization. This combination is not only described in gray wolves, but also in Iberian wolf subspecies and others species such as Ethiopian wolf (Kreeger et al. 1989; Vilà and Castroviejo 1994; Sillero-Zubiri 1996; Arnemo and Fahlman 2007; Editors and Arnemo 2011). Despite the use tiletamine – zolazepam being relatively safe in gray wolves, the lack of a tiletamine antagonist will delay the complete recovery, which poses some risks like hypothermia, attacks from other wolves, poaching, drowning and traffic accidents (Arnemo et al. 2013). As a consequence it is recommended that the animals are placed in a “local shelter” for three to four hours(Sillero-Zubiri 1996). Flumazenil may be used to reverse the zolazepam (Nielsen 1999).

The induction time and the duration of the effect is dose dependent (Nielsen 1999; Seymour and Duke-Novakovski 2007). Some side effects observed are hypertension, tachycardia, respiratory depression, muscle rigidity, vomit, vocalization, salivation, among others (Nielsen 1999; Malmsten 2007; Seymour and Duke-Novakovski 2007; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010). The animal species influences the observed effect. In dogs the tiletamine effects exceeds that of zolazepam (Nielsen 1999; Seymour and Duke-Novakovski 2007; Papich and Riviere 2010). A lower amount of tiletamine – zolazepam is necessary in captive wolves and "calm" animals compared to wild wolves and animals that are stressed or excited, presumably also influences the observable physiological response (Vilà and Castroviejo 1994). Advantages of using tiletamine – zolazepam, is that it can be reconstituted in a small volume, its absence or minimal side effects and has a safety margin (Sillero-Zubiri 1996; Tranquilli, Thurmon, and Grimm 2007). Regarding the disadvantages, it is associated with prolonged recoveries, short life after reconstituted and as referred, lacks an antagonist. It is recommend to make a closely temperature monitoring (Sillero-Zubiri 1996; Tranquilli, Thurmon, and Grimm 2007).

If after administration of tiletamine – zolazepam the animal is not sedated enough to allow handling, it is necessary to administer medetomidine in order to reduce stress and ease handling (Arnemo and Fahlman 2007; Malmsten 2007; Editors and Arnemo 2011; Arnemo et al. 2013). It is described that this combination contributes to a smaller volume of tiletamine – zolazepam necessary as well as a decrease of side effects on mean arterial blood pressure, cardiac and respiratory frequency (Malmsten 2007). Others studies suggest the use of ketamine instead of medetomidine with tiletamine – zolazepam or additional doses of tiletamine – zolazepam (Kreeger et al. 1989; Sladky et al. 2000). Less recovery time and a decrease in side effects was observed with the combination of tiletamine – zolazepam – ketamine (Kreeger et al. 1989). Tiletamine – zolazepam combination with ketamine and acepromazine were also tried, but there was an increase in induction and recovery times(Sillero-Zubiri 1996).

3.3.1.6. OPIOIDS AGENTS

Opioid drugs are a vast class of agents that can be obtained from opium or through synthetic production. They act troughμ (morphine),κ (kappa), δ (delta) and NOP (nociceptin orphanin) receptors (Waldhoer, Bartlett, and Whistler 2004; Tranquilli, Thurmon, and Grimm 2007; Papich and Riviere 2010). This class has a broad therapeutic use as potent analgesics (antinociception), sedatives and tranquilizers, in chemical restraint, for inhibition of gastrointestinal motility, as antitussives and as adjunctive use for general anesthesia. Not all species have similar responses to opioids. The main side effects are respiratory and cardiac depression, excitation, emesis and nausea. A lot of drugs are available being the most used opioids: morphine, hydromorphone, codeine, meperidine, butorphanol, buprenorphine, fentanyl, etorphine, sufentanil, among others. Opioids can be used alone or in conjunction with other molecules. There are specific reversing agents to opioids, with naloxone being the most widespread, other available reversal opioids agents are nalmefene (unspecific), naltrexone (to carfentanil) and diprenorphine (more used to revert the effects of etorphine) (Tranquilli, Thurmon, and Grimm 2007; Seymour and Duke-Novakovski 2007; Papich and Riviere 2010; Nielsen 1999; Miller and Fowler 2011).

Not all drugs referred are used in combination. Some protocols described in wolves reported the use of etorphine as the only immobilizing drug (Kreeger et al. 1988), however Nielsen *et al.* (1999), noted that it should not be used as the only option for excited or stressed animals because it will affect the induction time and will prolong the action.

Combinations of alpha 2 adrenergic agonists with opioids are referred in the literature as having the same advantages described in association with alpha 2 agonists with dissociative agents (Nielsen 1999). These two classes have drugs capable of reversing the effects (Kreeger et al. 1989; Nielsen 1999). Other associations described to immobilize wolves (in captivity) are protocols with xylazine and butorphanol, being observed bradycardia and decrease of respiratory rate (Kreeger et al. 1989). This protocol is reversed with naloxone and yohimbine, resulting in a quick recovery with enhance of respiratory and heart rate(Kreeger et al. 1989). Other association was performed using sufentanil citrate with xylazine instead of butorphanol (Kreeger e Seal 1990).

Figure 36– Induction time**,** photo courtesy of Antonio Antonucci, MNP.

Kreeger e Seal 1990, compared three opioids in wolves and concluded that etorphine and carfentanil can be used and have similar performance regarding induction and recovery times, but that sufentanil poses some risks. The use in wolves results from the balance between the cost, amount of volume necessary to immobilize and comparison with other available drugs on the market (Kreeger e Seal 1990).

3.4. INDUCTION TIME

The induction time can be described as the interval between the time from the administration of anesthetic protocol to the moment that the animal is stationary and the approximation is possible (Figure 36), in order to initiate all processes needed during capture of animal (Nielsen 1999; Fowler 2008). Usually, the objective is to achieve 10 - 15 min induction time (Kreeger, Terry, and Arnemo 2012; Fowler 2008).

In a normal situation of "induction" it is observed a decrease of the struggle, lowering of hindquarters or of the motion, that starts to progress cranially to the forelimbs and head. Generally after we observe that the animal is immobilized and with head down we can make the first approach to the animal. This is described as "normal" but there are differences depending upon observation of drug parameters, specie, gender, age, physiologic and individuals parameters (Kreeger, Terry, and Arnemo 2012).

The drug as well as the delivery system used, physiologic and individual aspects but also factors relating to the landscape and weather have influence in the induction time (Nielsen 1999).

Choosing a well vascularized muscle with a thin layer of fat, while avoiding injuries, will reduce the induction time, since the drugs are more readily removed by blood flow (Nielsen 1999; Kreeger and Arnemo 2012). Taking this into account the hindquarters, shoulder and neck are considered the best options as inoculation points (Nielsen 1999; Kreeger and Arnemo 2012). Regarding dose, the literature suggests that it is preferable to underestimate weight and consequently the dose, than giving another subsequent dose to attain the desire effect (Nielsen 1999).

If the induction time is prolonged, some of the complications can be hypothermia or hyperthermia worsened by some drugs, cardiac arrest, respiratory disorders as consequence of the animal struggle (although this parameters are more important during handling) (Fowler 2008; Kreeger and Arnemo 2012).

Factors as thick layer of fat usually in winter time, uptake food and water intake may influence the speed of drug absorption and can cause some complications such bloat, regurgitations, vomit and aspiration pneumonia (Nielsen 1999; Kreeger and Arnemo 2012; Tranquilli, Thurmon, and Grimm 2007; Fowler 2008).

The induction phase is potentially dangerous for the animal, so the situation should be constantly evaluated and the best decisions should be made without any delay. The topography and vegetation can be a risk factor when the animal start to fall to the ground, since he can be recumbent in a bad position, with compression of the airways or the head can become in a lower position than the body, which can led to an aspiration pneumonia or can pose a traumatic risk. That's why we should always be prepared to reverse the induction or to treat injuries in the animal (Nielsen 1999; Kreeger and Arnemo 2012).

3.5. APPROACH TO THE ANIMAL

After the chemical immobilization is achieved, a member of the capture team should approach the animal trough the back as fast as possible, but keeping the noise and the movement at a minimum. After reaching the animal, with the aid of a stick he should poke the animal to confirm the immobilization status and decide if additional doses are needed (Nielsen 1999; Kreeger and Arnemo 2012; Fowler 2008; Tranquilli, Thurmon, and Grimm 2007).

After confirming that the animal is properly immobilized the wolf should be repositioned. Several approaches are described, but in general it is important to position the head at a higher level than the body, adopt a sternal or lateral decubitus position. In winter it is important to use a blanket or other isolation material below and/or under the animal. In summer it is necessary to place the animal in the shade avoiding direct sunlight (Nielsen 1999;Kreeger and Arnemo 2012; Fowler 2008; Tranquilli, Thurmon, and Grimm 2007).

Some anesthetic drugs like ketamine induce a "wake up" effect on eyes during sedation. In this case in particular, but in all the cases is general, it is important to cover the eyes to avoid injury, additionally an ointment such as artificial tears or saline solution can also be used to protect and hydrate the eye. We can also bind the animal ears, but can't forget to pull the plug after the handling (Nielsen 1999; Kreeger and Arnemo 2012; Fowler 2008; Tranquilli, Thurmon, and Grimm 2007).

3.5.1. HANDLING

If the animal can't be totally immobilized it can be dangerous. If during the approach we encounter an emergency case is firstly necessary to deal with the emergency in question and after stabilization/resolution, make the best possible monitoring, as well as manage it as best as possible (Nielsen 1999; Kreeger and Arnemo 2012; Fowler 2008; Tranquilli, Thurmon, and Grimm 2007).

Images of the captured animal can be of a great help to compare with other photos and videos or collected from camera traps, thus contributing to a better understanding of the individual and their interaction with the pack or the environment, specially when the animal has unique marking. Besides videos and photos of the whole body, detailed photos of teeth, injuries and particular makings should be made (Stein, Fuller, and Marker 2008; Hernandez-Blanco et al. 2013; «Tammie Matson » 10 Things You May Not Know About African Wild Dogs» 2015; Hedges et al. 2015).

During handling period, if available, a radio collar is attached to the neck of the animal to follow up after release.

The general animal state as body condition, clues or signal of diseases are observed in order to balance the possibility of making some sort of medical treatment and/or prevention. It is also an opportunity to collect biologic samples of blood, hair, feces or other suitable ones.

3.5.2. DATA COLLECTION

Captured animals represent an excellent opportunity to collect, not only physiological data (performed through monitoring, from the samples collected and measurements), but also about animal behavioral and its interaction with the ecosystem (by radio collar) (Nielsen 1999; Cattet et al. 2006; Cattet and Boulanger 2008).

3.5.2.1. BIOMETRY

Wolf biometric parameters are already standardized for this species and usually it is measured at least the ears, body, tail, forelimb, hindquarter and paw length and neck and body circumferences (Figure 37). Teeth area also subject to observation (Figure 38) since they give important information regarding age alongside animal coat, nipple and testis size, the teeth is a important and a precise way to predict the age of the wolf, by distinguishing between milk teeth and adult dentition (it is in place at about 6 – 7 months of age), and through tooth wear charts. The measure of the age is not always easy because of the wear of the teeth depends in the type of food preys and food availability. The wolf is a very plastic animal, he can eat the garbage or be a scavenger. The presence of some lesions in teeth combined with season variation can complicate the age determination. But can be subdivided in pups (until 4 to 6 months), juvenile and adults, since the age of passage from juvenile to adult varies and sometimes the sexual maturity is used to distinguish this two classes (which may vary between 9 mouths to 5 years). Other method described is through the dispersion age (1 or 2 years). The scale used should be adapted to each situation (Landon et al. 1998; Mech 2006; Mech and Boitani 2007; International Wolf Congress 2013).

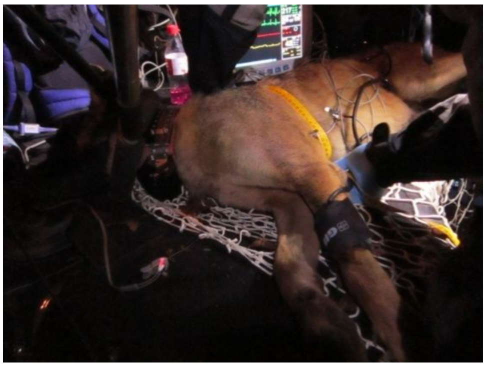

Figure 37 – Biometric of body circumferences during the capture of F5 – Iris, on MNP.

Figure 38 – Distance between wolf canine teeth, photo courtesy of Marco Carafa, MNP.

3.5.3. MONITORING PARAMETERS

Figure 39 – Monitoring parameters during the capture, photo courtesy of Antonio Antonucci, MNP.

Figure 40 – Monitoring parameters during the capture in F5 – Iris, on MNP.

Due to the conditions of the field, which can sometimes be harsh it is necessary to make a precise, intensive monitoring, as seen on Figure 40 and with the largest number of possible parameters. The basic tools to make an accurate monitoring are the stethoscope, thermometer and pulse oximetry. The ideal equipment is a multiparameter portable monitor, which has the advantage of facilitating our work, giving us recording information of various parameters in simultaneous, however its price make it unavailable in many situations.

The evaluation and monitoring of basic but essential parameters, enables interpretation of the animal's response to the capture and protocol used.

The data obtained during this procedure in reality does not end here, as it will continue to be monitored through the radio collar.

3.5.3.1. STETHOSCOPE

Stethoscope is a widely and vital tool to use in veterinary medicine, not just in cardio respiratory systems (Figure 39), but is also used in the gastrointestinal system. We can quickly access the heart and respiratory rate as well as some abnormalities in the rhythm (cardiovascular system) or in sound (respiratory system). It is an indispensable tool in chemical immobilization, in order to verify the action of drugs on the animal. Through a careful monitoring, it is possible to respond more quickly to any abnormality in these systems.

3.5.3.2. THERMOMETER

The continuous evaluation of body temperature is crucial, because it is very easy to be confronted with a life threatening situation of hypo or hyperthermia, both as a consequence not only of atmospheric conditions, but also induced by the chemical restraint protocol.

3.5.3.3. PULSE OXIMETRY

Is a non invasive method of continuously measuring hemoglobin saturation (SPO_2), its function is based on the different light absorption spectrum of oxyhemoglobin and reduced hemoglobin. Hypoxaemia occurs when the SPO_2 value is less than 90% (West, Heard, and Caulkett 2007; Clarke and Trim 2013).

3.5.3.4. ELECTROCARDIOGRAM

The electrocardiogram evaluates the electrical conduction of the heart which is measured with positive and negatives electrodes that "form" different leads. The aim of this technique is to measure the propagation and the formation of the heart impulse (Martin 2007). A metaphorical description of the cardiovascular system, the heart is represented as a pump of which the blood vessels are the pipes. So in order of the pump to works, it is necessary that under normal conditions, the cells of sinoatrial node (SA node) act as a pacemaker, generating at regular intervals an impulse, which goes forward to the atrioventricular node (AV node). The continuous depolarization of the bundle of His (subdivided in right and left bundle branches) and finishing in the Purkinje fibers. A new impulse consequently a new cardiac cycle is ready to start in the SA node (Martin 2007; Ettinger and Feldman 2010).

For a good interpretation of the electrical conduction it is necessary and indispensable to correctly position the animal. The animals are preferably placed in a right lateral recumbent and the limbs should be held perpendicular to the body (Ettinger and Feldman 2010). The number of electrodes and its positioning will depend on the type of equipment used, and nowadays there are a lot of different veterinary specific models with different specifications (Fuentes, Johnson, and Dennis 2010).

The goal is to obtain the deviation (I, II, III, aVR, aVL and aVF). These leads vary in morphology because of the reading from positive or negative electrodes and the position where the electrode is attached. The lining obtained in different derivations presents various morphologies depending on the position and on the electrical charge of the leads. As an example the lead II, the more widespread used, reads the electrical response from the left hindlimb where is the positive electrode to the right forelimb that has the negative electrode attached (Fuentes, Johnson, and Dennis 2010).

In a brief description the P wave is the result of auricular depolarization, the QRS complex is from ventricular depolarization, namely Q wave is the first part of depolarization of ventricle, the R wave is the ventricular myocardial mass depolarization and the S wave is the depolarization of basilar and the T wave is the ventricular repolarization (Martin 2007; Fuentes, Johnson, and Dennis 2010).

3.5.3.5. SAMPLES COLLECTION

The sample collection is also part of the animal monitorization, since it allows to afterwards evaluate the animal’s health status. Blood samples are collected from the animal in order to obtain their analytical blood profile, biochemistry and antibody titulation for infectious diseases. Fur, faeces, urine, saliva among other biological samples may also be collected depending on developing researches and investigations.

3.5.4. RECOVERY TIME

During the recover, since the animals is not fully awake, it is ataxic, disorientated, eventually with an extra weight from the GPS collar, it cannot be left unattended as there are various risks, such as the proximity of rivers, slope, possibility of predation by others animals but also the pharmacologic effect of the chemical immobilization, which can put the life of the animal in danger (Nielsen 1999; Fowler 2008; Kreeger and Arnemo 2012).

Before the administration of the antagonist drug it is important decide if the wolf should wake up “alone” in the field or if it is better to use a “transporting box” and just release the animal when it is “completely awake”. The anesthesia, monitoring parameters, experience of the team, field and other features are used in order to choose the better solution, since it is a very subjective action.

Figure 41 – F5 – Iris recovery after a capture in a box, on MNP.

When the animal is placed to recover in a wooden box (Figure 41), the antagonist is administered and the animal is left inside it, until the animal is “completely awake" and has the conditions to be released without any threat to his life. During the period that the animal is in the box, the procedure is the same, as during the handling phase, so he will be monitored and respiratory frequency should be recorded and observed the movement (ears, tail or other) and positioning (sternal position, sitting or standing) observed. Even when the animal is left in the field without any restriction, it should also be monitored, but of course the parameters evaluated will be less by practical reasons. If any abnormality is detected during the recovery, we can only step in if is in the box.

3.5.4.1. RELEASE

After all the efforts are made, for the best way to carry on the monitoring/animal handling, it is time to the animal release the wolf (Figure 42). The choice of the place of the capture becomes especially important at this stage, since the animal cannot be released in a place with humans, highways, urbanizations, abyss and so on (Nielsen 1999; Kreeger and Arnemo 2012; Malmsten 2007). Although some authors argue that livestock sites should be avoided, because of the fact that the animal has just been through as upper stressful situation and in the first few days will have a reduction in their fitness, it will be "simpler and easier" to predate a confined animal than a wild prey which will led to human conflicts (Turnbull, Cain and Roemer 2011). A study was performed in Mexican wolves where they evaluated the risk of predation associated to wolves injuries, including leg amputation. The authors did not find a direct relationship between the lesions in captured wolves and the increase in livestock predation (Turnbull, Cain and Roemer 2011).

The real impact of the capture in the wolf is not very known and described. The use of radio collar can provide information about this, but until nowadays there is not literature regarding the effects of it in wolves. In a study performed on pumas (*Puma concolor*) it was noted a decrease of mobility during the two days that followed the capture. However for our admiration the capture did not affect the mobility of the female pumas, as affect the males. The possible cause that Logan *et al*. (1999) described to this difference between males and females is the fact that male home range is bigger than female's, which means they have to walk longer distances. This associated to lesions acquired during the capture may discourage males, influencing their mobility. It is important to note that with this study, it was observed some "learned behaviors" of how to remove the foot loop. It was observed that females possess a bigger propensity to life threatening lesions than males since they struggles longer (Logan et al. 1999). Some authors suggest that because of the presence of the collar after a capture, the database of the first weak cannot be considered regarding as normal behavior (Mech 2002) The impact of the capture was also studied in bears. It was described a decrease of mobility and alterations of behavior. The effect observed depended on the day, season, reproductive period and gender. This behavioral alteration can affect the body condition, decline the food searching, as well as under shelter/den time or even increase the time spent on it (Cattet and Boulanger 2008).

More studies and database build are necessary to better understand the impact of wild animal capture in their normal behavior.

The animal suffers a great deal of stress when captured. It is in a situation of "fight or flight". In my opinion this stress, fear, residual effects of the chemical immobilization, injuries, weight gain and discomfort (caused by the collar) that the animal has when "wakes up”, contributes to this behavioral alteration observed.

Figure 42 – Release of a wolf after capture, photo courtesy of Antonio Antonucci, MNP.

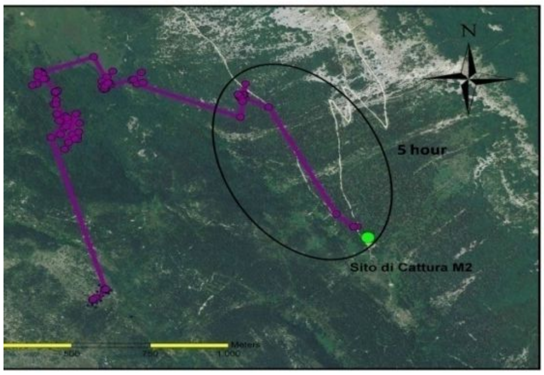

Figure 43 – Tracing of M2 – Salle movements during the monitoring period by GPS reception, photo courtesy of Antonio Antonucci, MNP.

One of the priorities in captures of wildlife animals is the animal safety. The aim is to chose the best capture method, which includes trap, delivery systems, chemical immobilization in accordance to the specific situation that we have, so that the final option is the one that has less impact on animal safety and behavior (Osofsky and Hirsch 2001; Editors and Arnemo 2011). Concerning wolves, they live in packs, the capture can influence their social status, as hinder the return to the pack, or decreased or increasing their social status. The real implications of the wolf's capture and other animals is still not fully understood, it is essential to carry out studies in order to improve our performance in the wild.

3.5.4.2. POST CAPTURE MONITORING

Post capture monitoring can be done trough the radio collar, which gives us a lot of precious and accurate information not only about the home range of wolves, territory, social pack, individual, rendezvous, dens and killing sites, but also about individual wolves behavior (Figure 43), dispersal and social, reproductive and nursing time, among others. Besides the use of radio collar, there are other monitoring methods that can give us important information as photo and video trapping, howling, direct visualization, tracks, all of them in conjunction contribute to better understand the wolf behavior and its interaction with the ecosystem where it lives and to more effectively act in its conservation.

4. MATERIAL AND METHODS

The study was conducted in Majella National Park, in the protected area of central Apennines in Italy. It has an area of 740.95 Km² ranging from 200 to 2800 m above sea level. The landscape is composed by two thirds of woodland and one third of scrubland («Majella National Park | Parco Nazionale della Majella» 2015). It is an area of great flora diversity, where meadows and beech forest are the most widespread landscapes. Even today, agriculture and livestock are important activities in this region and MNP has a long history of evolution and coexistence, not always peaceful, between man, his activities and wildlife. MNP is very rich in fauna and include species as red and roe deer, chamois, wolves, bears, wild cat, polecats (*Mustela putorius*), otters, golden eagles (*Aquila chrysaetos*), alpine choughs (*Pyrrhocorax graculus*), red – billed choughs (*Pyrrhocorax pyrrhocorax*), ursini vipers (*Vipera ursinii*), spectacled salamanders (*Salamandrina terdigitata*), Apennine yellow – bellied toads (*Bombina pachypus*), among others («Majella National Park» 2014; «Majella National Park | Parco Nazionale della Majella» 2015).

The objectives of the wolf capture is to better understand the behavior and wolf ecosystem, that is achieved not only by the measures taken during handling, the samples collected, but also and more importantly trough the GPS collar that is placed in the wolf. With the GPS it is possible to obtain information that allow a better understand of predation, socioecology, sleeping habits and even death of the animal among other valuable data, that otherwise would be impossible or more difficult to obtain (Mech 2002; Mech and Boitani 2007).

After the adhesion of Italy to Berne Convection in 1979, the protection of some species as wolves (*Canis lupus,* Lineu, 1758) became essential and the methods used to capture them to scientific projects aiming its conservation began to be regulated by law.

Capture procedures and animal handle were designed taking into account the European legislation that avoids the capture with leg hold traps (EEC) No 3254/91 of 4 November 1991. So the capture need an authorization based on the applicable legislation for this subject, as regulation implementing Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, and the subsequent Presidential Decree March 12, 2003 n.120, also according to the ethic and welfare concerning of Europe Council Decision 98/487/EC of July 13, 1998, Council Decision 98/142/EC of 26 January 1998, Council Regulation (CR) No 3254/91 of November 4, 1991 and subsequent amendments. The authorization was subjected to assessment of evaluation by the ISPRA (Institute for Environmental Protection and Research) and approved by the Ministry of Environment of Italy (Fur Institute of Canada 2013; Internation Wolf Congress 2013; «Conservation of wolves in Europe - Wolves and Humans Foundation» 2014; Antonucci 2014).

Captures were performed from June 2010 until December of 2014, mostly during the late spring and autumn. During this period 10 animals (a juvenile female, two juvenile males, four adult females and three adult males) were captured for the first time. From these animals an adult female and a juvenile male were re – captured: the adult female because she was entrapped in a poacher's snare, the juvenile male because he was recovered in an enclosure for rehabilitation and reintroduced in the same place of capture after 4 weeks (International Wolf Congress 2013).

Before the assemble of a capture set, it was necessary to check what was the best place to install the capture set looking for places where we could find more signs of wolf presence. Techniques like siblings, photo and video traps, howling and search for tracks as scats, urine, marks and damages caused by wolves, were used to decide which sites would be best to assemble the capture set. With the gaining experience through collecting additional data there was an improve our trap sites.

All needed equipment and materials checked regarding its operationality and passed through a cleaning process, the equipment was brushed with soil, while scrubbing with a sponge, followed by thorough wash with water. The equipment was left to dry on sunlight and it was scrubbed with vaseline. The second step in the preparation of the trap was to leave all the components of the capture set during a period of 10 days in a box with leaves, branches in particular with needles and pine cones to remove the smell.

Wolves were captured using a mixed trap namely a Fremont® Humane Foot Snare. This is a trap made for bear capture, but has been well adapted to wolf (1/8 inch cable size, 7x7, seven wires in 7 strands), other constituents of the trap are snare, anchor and alarm system

(International Wolf Congress 2013; Antonucci 2014). The trap was buried in the field and a very careful cleaning job was done in order to disturb as little as possible the environment in our set.

Another component of our trap was the snare. The presence of a “loop stop” aims to reduce the constriction of the wolf's paw and subsequently minimize injuries, this “loop stop" has a mechanism that makes it impossible to open when closed, which prevents the escape of the animal. In the loop of the snare was also put vetrap® to decrease the contact between the wire and the skin of the animal, with the same purpose that the stop was used. This snare was attached to the trap so that when the animal activated the trap caused the closure of the snare.

The anchor used in MNP was fixed. In some cases, we took advantage of the landscape by using trees, shrubs or rocks. Other times it was necessary to make our one anchors. The important thing was to disturb the place at a minimum level. As anchor, chains with swivel or cable connection with damping spring can be use. The anchor cannot be long, because the longer it is, the greater the space the wolf has to boost fight. The chain can have one strong swivel or two small swivels. An important aspect when we put the swivels in the chain, is to leave neither a small nor big space between the little “chains” because this is what's going to support the pressure of the wolf when they struggle to free themselves.

In all trap system was used an alarm system, with the aim to minimize as much as possible the stress and injuries. The idea is to reach the capture site in less than an hour.

We can use some guide sticks as some branches of *Rosa Canina,* or other bushes or tree branches, creating a little “passage” to the animal to be captured more easily.

During two to seven days the trap stayed inactivated with a little amount of stones to fill the cavity or an irregularity on the ground. When it was activated the scats (our bait) were added placed.

The traps were verify every day at the middle of the day if all the components of the trap worked well, make rearrangements to the capture set or if necessary replaced the lure.

Immediately prior to animal snare release and manipulation, it was delivered a mixture of chemical restraint agents using a CO_2 – powered rifle or blowpipe and 3 ml lightweight plastic darts fitted with 1.5x30 mm plain needle (Telinject). Chemical restraint in young animals (less than 1 years old), was made with a hand – syringe injection after immobilizing them with a net and y – pole. In all animals the injection site was the large muscles of the hindquarters, the semimembranosus and semitendinosus (International Wolf Congress 2013).

The dart preparation was made previously to the capture. Three different darts were prepared. It is considered that the weight of young wolves is 15 – 20Kg, females or small adult' males 20 – 30Kg and male adults 33 – 36Kg.

The protocol used in the chemical immobilization was a mixture of 0.05 mg/Kg of medetomidine (Domitor®), 4.29 mg/Kg of ketamine (Imalgene®) and 0.15 mg/Kg of acepromazine (Prequillan®). There were two exceptions to this protocol regarding the first wolf captured (F1 – Petra) in whom we used a combination of 1.5mg/Kg of xylazine (Rompum®), 10mg/Kg of tiletamine-zolazepam (Zoletil®) and 0.05 mg/Kg of acepromazine (Prequillan®), and in one juvenile wolf (M5 – Orphento) the protocol didn't include acepromazine, but only 0.041 mg/Kg of medetomidine (Domitor®) and 3.19 mg/Kg of ketamine (Imalgene®).

Induction, handling and recovery time were monitored, total capture time were registered and physiologic parameters checked. Achieved all the condition, the measures and parameters performed, the reverse drug, 0.04mg/Kg of atipamezole (Antisedan®) was administered.

A subcutaneous microchip implant was placed in the right neck region. It was also done, as routine, a preventive administration of ivermectin (*Ivomec*® 0.5mL/25kg SC), amoxicillin (*Clamoxyl® LA* 15mg/Kg SC) and dexamethasone (*Dexadreson*® 2mg/mL IM).

Wolves were divided in young animals aged less than one year and adults using the wear of the teeth.

DNA analysis was also performed to generate a "family tree" and to differentiate hybrids from wolves.

A basic but essential monitoring, was performed manually using a stethoscope and instrumentally with a multiparameter portable monitor (R, STAR 80000 B). The parameters monitored during the handling were heart rate, respiratory rate, SPO_2, temperature, NIBP (Noninvasive Blood Pressure) and an ECG. Our objective was to repeat the measurements in each animal three times, however, since we were making a field study with wild animals this was not always possible and there were animals where the number of measurements was lower and others where we had the opportunity to obtain a large number of measurements. Concerning the ECG, it was performed with the animals positioned in right lateral recumbency, with the legs in a perpendicular position to the body and slightly parted. Five electrodes (alligator clips) were positioned in the animal body (2 electrodes (one for each limb) attached proximal to the elbow, 2 other on the shoulder and one precordial to the chest) wetted with 70 % isopropyl alcohol. The information obtained was measured in a paper at a speed of 25mm/sec. The mean values were obtained from ECG performed in F4 – Alpha and F5 – Iris. It is important refer that F4 – Alpha was a pregnant female, and this two wolves compared to the other wolves captured were more stressed and with a lighter sedation.

Blood samples were collected by venipuncture with vacuntainer needle of the left cephalic or left saphenous veins. Blood was collected to two tubes, one with EDTA and another with heparin. After centrifugation of the EDTA tube, 0.3 ml of supernatant were put in two small tubes and were frozen at - $20^{o}C$ until analyzed by Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" IZSAM – Sezione di Pescara. Hematologic parameters measured included white blood cell (WBC), RBC (red blood cell), hemoglobin (HGB); hematocrit (HCT) or packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), hemoglobin distribution width (HDW), platelets (PLT), mean platelet volume (MPV), neutrophils (Neut), lymphocytes (Lymp), monocytes (Mon), eosinophils (Eos), basophils (Bas) and large unstained cells (*LUC*). Blood biochemistry parameters measured included aspartate transaminase (AST), triglycerides (Triglyc), total proteins (TP), albumin (Alb), globulin (Glob), gamma – glutamyltransferase (GGT), amylase (Amy), alanine aminotransferase (ALT), blood urea nitrogen (BUN), cholesterol (Chol), glucose (Glu), total bilirubin (TB), creatinine (Creat), alkaline phosphatase (ALP), calcium (Ca^{2+}), phosphorus (P^+), sodium (Na^+), potassium (K^+) and uric acid (UC).

Analytical methods used for serum biochemistry measurements in F5 – Iris were GGT – kinetic (k), creatinine – kinetic – colorimetric (k-c), glucoses – enzymatic (e), total proteins – colorimetric (c), blood urea nitrogen – e, cholesterol – enzymatic – colorimetric (e-c), alanine aminotransferase – k, total bilirubin – c, calcium – c, uric acid – e – c, triglycerides – k – c, albumin –c, aspartate transaminase – k and amylase – k. Regarding the hematologic parameters, they were performed by flow cytometer.

Injuries were rated in four classes based on Kuehn *et al.* (1986) work additionally some parameters as: little hemorrhagic lesions of the oral mucosa, tongue or gingival, broken teeth, reversible edema of foot, subcutaneous muscle laceration, tendon or ligament laceration, joint luxation, fracture and amputation (Kuehn et al. 1986; Angellucci 2014).

The statistic analyses performed to all the parameters was a simple statics analysis using the mean and standard deviation of all the parameters and measures of the wolves captured.

Data were checked in relation to the normality of distribution (Shapiro – Wilk test) and for homoscedasticity (Bartlett's test or the Levene's test). The data parameters that full field one – way analysis of variance (ANOVA) basic requirements were compared regarding sex using ANOVA followed by the T – test. The "time of administration after trap alarm", "head down" and "induction time" parameters and "time antagonist administration", "recuperation time", "standing" and "away" parameters were subjected to a simple bivariate linear regression analysis. The statistical analysis was made with JMP 10 (Zar 2010).

5. Results and Discussion

We have data of ten wolves captured in the MNP which are the sample that we analyzed.

5.1. Weight

	Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
Total Weight (Kg)	28.70	5.64	19.64	38.30	19.30	8
Female Weight (Kg)	28.70	2.55	8.88	31.00	25.00	4
Male Weight (Kg)	30.05	8.18	27.22	38.30	19.30	4

Table 1– Weigh in adult captured wolves.

Regarding weight M3 – Martina and M1 – Jakob were excluded from the statistical analysis, M3 – Martina because she was a subadult and the later was excluded because he presented an underweight value for a sub adult individual, because of his bad physical condition, which made it necessary to proceed to his hospitalization for treatment.

Our measures gave us an average weight for female 28.70 Kg and 30.05 Kg for male, which are in accordance with Apollonio and Mattioli 2006, that refers 32.80 Kg to males and 29.30 Kg to females (Passilongo et al. 2010; Apollonio and Mattioli 2006).

After performing a statistical analysis with JMP program, we obtain a non – significant statistical difference in weight between males and females with a p value greater than 0.05. Considering the fact that ours sample (n) is small and that it is described differences between males and females, namely in dogs, we cannot discharge the possibility that in a larger sample this difference would have statistical significance.

5.2. Chemical restraint

A different protocol was used to M5 – Orphento, due to logistical problems, wasn't administrated acepromazine. So this animal was excluded from this result.

	Average	Standard deviation	Coefficient of variation %	N
Medetomidine (mg/Kg)	0.05	0.01	16.42	9
Ketamine (mg/Kg)	4.42	0.45	10.19	9
Acepromazine (mg/Kg)	0.15	0.04	27.18	9

Table 2 - Drugs and doses used in all captured wolves.

On average, our wolves were chemical restrained with a combination of 0.05 mg/Kg of medetomidine, 4.42mg/Kg of ketamine and 0.15 mg/Kg of acepromazine.

Considering the only paper that uses the same protocol as ours, although the dose of medetomidine was similar to ours, Sladky *et al.* (2000) used half the dose of ketamine and fourteen times less acepromazine. This may be explained by the fact of the red wolf is a different specie and/or because they are in captivity (so less amount of drug was necessary to make the immobilization) (Vilà and Castroviejo 1994; Nielsen 1999; Fowler 2008).

The exact dose administered in F5 – Iris is unknown. F5 – Iris was a wolf rather "wild" that moved a lot, making the chemical immobilization more difficult, as a consequence it was required three attempts with darts (previously prepared for the an average weights of 18, 26 and 35 Kg). The darts rebounded rendering impossible to know the exact dose administrated. In an attempt to deepen the anesthetic plan, an IM administration of medetomidine and ketamine was performed. There are possibilities of error in the dosage of the administered drugs.

5.3. INDUCTION AND APPROACH TO THE ANIMAL

The main goal after the activation of the alarm system is to arrive at the capture place as soon as possible, which may vary between 15 – 45 min (depending on the location of the capture set). Upon arrival, the presence or absence of an animal is verified. The body weight and general physical state of the animal are evaluated in order to perform chemical immobilization.

The wolf M3 – Martina is, according to our classification criteria, a juvenile (less than 1 year old) however, she will be included in the adult group regarding chemical restraint parameters, hematology and blood biochemistry. This decision is based in the fact that literature on wolves does not present an universal classification on age classes and because of the fact that some parameters have not been studied on wolves, being necessary to use the reference values of the closest phylogenetic specie, the domestic dog and that on this specie reference values for juvenile domestic dogs consider animals with a maximum of 3 months and M3 – Martina was older then that.

M5 – Orphento and M1 – Jackob will be excluded from induction time, head down, antagonist time, monitoring parameters and recovery time. The M5 – Orphento because was administrated a different protocol and M1 – Jackob had an "unhealthy" condition.

5.3.1. TIME OF ADMINISTRATION AFTER TRAP ALARM ACTIVATION

	Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
All animals (min)	77.00	27.72	36.00	110.00	24.00	8
Females (min)	82.00	32.28	39.37	110.00	24.00	5
Males (min)	55.00	22.61	41.10	92.00	51.00	3

Table 3 – Mean time since the alarm system was activated until chemical restrain administration.

The mean time since the alarm system was activated until we arrive to the capture set and approach the animal to administer the anesthetic protocol was 77.00 min. Note that both females (F4 – Alpha and F5 – Iris) struggles until it was possible to administer the immobilization protocol which increased the interval.

The time necessary to make the administration of the protocol was an average of 82 min in females and 55 min in males. This difference can be caused by individual factors namely physical and psychological, social structure (pack member or dispersal) and weather (Nielsen 1999; Kreeger and Arnemo 2012).

5.3.2. "HEAD DOWN" (AFTER ADMINISTRATION TIME)

	Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
All animals (min)	8.00	2.33	29.12	11.00	3.00	8
Females (min)	8.00	0.89	11.18	8.00	6.00	5
Males (min)	9.00	4.16	46.26	11.00	3.00	3

Table 4 – Head down in all captured wolves.

"Head down" was achieved 8 min (mean) after administration on all animals (9), been around 8 min for females and 9 min for males.

5.3.3. INDUCTION TIME (= HANDLING TIME)

	Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
All animals (min)	13.00	6.00	46.14	23.00	8.00	8
Females (min)	14.00	6.50	46.40	23.00	8.00	5
Males (min)	11.00	5.69	51.69	19.00	8.00	3

Table 5 – Induction time in all captured wolves.

The handling (induction time) of the wolves were possible 13 min after drug delivery which are in line with Sladky *et. al.* (2000), whom presented an induction time of 11.1 min. Concerning the class females and males, the mean of induction time achieved was 14 and 11 min, respectively.

In animals, which took longer to make the drug administration, more time was required for the animal to have the "first signal" of immobilization (head down) which in turn influenced the increase of induction time, observed in the females group. Stress may be in the origin of the difference between females and males, since more time was necessary to the administration to at least one of the females (F5 – Iris) and consequently stayed more time in a stressful situation, contributing to enhance the time of induction time. In a study performed in pumas, was observed that the females struggle more than the males, in the capture (Logan et al. 1999).

There are descriptions consistent with this information regarding deer, where a higher dose was necessary to anesthetize females than males (Tsuruga et al. 1999) and as referred previously in pumas, which was observed a bigger struggle/stress response in females than in males (Logan et al. 1999).

Using the JMP software as the basis of our statistical analysis, it was performed an analysis of the “head down” and the “induction time” with the time that the wolf was captured, comparing between female and male classes. According to our data, the greater the “waiting time” between the capture and the administration of chemical immobilization, the greater the time of “head down” and consequently of the “induction time”. It was observed the absence of statistical significance to “head down”, however was detected the existence of statistical significance between the “induction time” (compared to the time of administration of the drug) and headdown, independently of the gender of the animal, even to an n as reduced as ours.

Concerning this result, we can conclude that it is necessary to change the approach to the administration time and/or administration technique in order to minimize the induction time, which will contribute to minimize struggle and stress (and all the cascade of novice events that precedes) as well as reduce the possibilities of lesions.

5.4. MONITORING PARAMETERS

Unfortunately it was not possible to obtain data for all parameters on all wolves. The limitations allays associated with a field work, with the additional problem that this study was made in an endangered top predator with all that implies. It is our opinion that the non publication of these would represent a loss of information on such an emblematic specie. The statistical analysis using JMP software was performed to the classes HR, RR and T^{o}C, exploring the possibility of differences between male and females (it was only analysed these parameters because of being the parameters with the bigger amount of data). For the parameters analyzed we did not found any statistical difference between males and females.

		Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
	HR (bpm)	23.25	14.74	63.38	60.00	13.50	8
	RR (rpm)	101.00	16.02	15.86	129.00	84.13	8
	T (°C)	38.05	1.35	3.56	40.70	36.45	8
	SPO_2 (%)	89.00	2.52	2.83	91.00	86.00	3
NIBP (mmHg)	**SABP**	213.50	ND	ND	ND	ND	1
	MABP	182.50	ND	ND	ND	ND	1
	DABP	167.50	ND	ND	ND	ND	1

Table 6– Monitoring parameters in all captured wolves.

Our monitoring parameters will be compared with Sladky *et al*. (2000) article which the only one with the same immobilization protocol (medetomidine – ketamine – acepromazine) as ours. Not all the parameters measured by us are described in the article and it is important to refer that the capture method used was different from ours and was performed on captivity red wolves.

Stress response is individual and unique (Cockrem 2013). HR is usually the most common stress indicator (Montané et al. 2003). The HR and RR of the wolves captured were 101.00 bpm and 23.25 rpm, respectively. These values are similar to the ones described by Sladky *et al.* (2000), which are 93.30 bpm (HR) and 22.60 rpm (RR).

The temperature measured by Sladky *et al*. (2000) in red wolves (39.67 °C) was higher than ours (38.05°C). Sladky *et al*. (2000) refers that all their wolves developed hyperthermia after immobilization (39.7 to 40°C) with a subsequent decline during handling (38.7 to 39.1°C) (Sladky 2000). A possible factor that contributed to the difference between studies is the capture method used, the weather and the time that the animal stayed in the immobilization box and how the induced stress response affected their thermoregulation.

At individual level, the wolf that showed the most marked differences in the monitoring values (maximum value), was the F4 – Alpha, who was pregnant. She struggled a lot during induction, which complicated her sedation and in consequence contributed to a superficial anesthesia. It was only possible to monitor HR, RR and T °C, which were all elevated, which is compatible with the stress and superficial anesthesia. It is important to avoid the anesthesia of pregnant females, but when we don't have another choice cautions must be taken, not only concerning the female, but also the implications in the foetus. Drugs with high protein bound should be avoided as they have an increased potential for toxicity and because albumin and glycoprotein are decreased in neonates. Literature refers physiological changes of heart and respiratory rate, NIBP, cardiac output, renal blood flow and glomerular filtration rate on pregnant canids, which will influence the availability and elimination of drugs (Shirley et al 2001; Blanco et al. 2012; Lopate 2012).

Concerning our anesthetic protocol, it is described that phenothiazines should be avoided in pregnant animals as they can cause neonatal CNS depression. Ketamine is considered a safer alternative, however it may originate depression (due to its accumulation in the placenta) in puppies in cases of caesarean section and may result in increased intrauterine pressure and consequent premature labor. Decrease of myometrium contraction happens upon utilization of low concentration off medetomidine and the opposite effects (increase of myometrium contraction) occurs with high doses. The administration of medetomidine is linked to fetal abnormalities in the first trimester and myometrium contraction in the last trimester of pregnancy (Shirley et al 2001; Kustritz 2009; Papich and Riviere 2010; Feldman, Nelson, Reusch and Scott-Moncrieff 2014). These drugs should be administrated carefully in pregnant animals (Shirley et al 2001; Papich and Riviere 2010; Flaherty 2013b).

Regarding the wolf captures, unfortunately it is impossible to predict if the captured animal will be or not pregnant, we can only minimize the stress and capture time decreasing the possibilities of problems to the mother or puppies.

A slight difference of hemoglobin oxygen saturation (SPO_2) is observed between our data (89.00 %) and Sladky *et al.* (2000) (92.46 %). It was not possible to measure this parameter in all wolves. Hypoxaemia occurs when the SPO_2 value is inferior to 90% (West, Heard, and Caulkett 2007; Clarke and Trim 2013). In our opinion the result of SPO_2 does not reflects the real value. Firstly, we are in field conditions and sometimes a slight animal movement or ours (during our approach) can remove the probe of place, also the amount of saliva can influence the movement of the probe. Other factors that can led to an unsuccessful reading by the pulse oximetry are the shape of the sensor, thickness of the tissue where the sensor is placed and the presence of pigments (Clarke and Trim 2013).

Regarding blood pressure, our values and Sladky *et al.* (2000) were respectively: systolic arterial blood pressure 213.50 and 167.60 mmHg, mean arterial blood pressure 182.50 and 139.58 mmHg and diastolic arterial blood pressure 167.50 and 121.60 mmHg. However, our data is based in only one animal, the wolf F5 – Iris, and she struggled a lot during capture, with little anesthetic depth during handling and voluntary movements all of which may have caused in our data. Another possibility is a misplaced cuff. The other parameters (HR, RR and T°C) lie within the limit reference values.

5.5. RECOVERY TIME

The antagonist used to reverse the chemical restraint was atipamezole (Antisedan®) at a 0.04 mg/Kg dose. Its administration occurred upon the end of monitorization of the vital parameters and animal general physical condition, implementation of preventive measures, placement of radio collar and sample collection.

	Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
Antagonist administration (min)	65.00	12.65	19.46	90.00	45.00	8
Standing (min)	72.00	29.41	40.84	130.00	48.00	8
Away (min)	85.00	35.52	41.79	155.00	54.00	8

Table 7 - Monitoring antagonist effects in all captured wolves

Our mean time of administration of antagonist was 65 min. Our time corresponds to elapsed time from administration of chemical immobilization to the standing. The standing time for red wolves were 16.6 min, this time can be calculated minus the time the antagonist is administered until the time when the animal is standing. We obtained less time than Sladky *et al.* (2000) that is 7 min. But it is important to refer that our wolves only went away 20 min after the antagonist administration.

Using the software JMP, as statistical analysis, it was verify, a connection between the time of handling and the recovery time. With more time the wolf was influenced with the effect of the CNS depressor drugs more time is required to the animal recover ($p=0,0089$ [$p<0,01$]), to standing ($p=0,0255$ [$p<0,05$]) and consequently goes away ($p=0,0255$ [$p<0,05$]).

5.6. BLOOD SAMPLE PARAMETERS

As far as we know, there are no publications available on hematological and biochemical parameters of Italian wolf, so our values will be compared with data published about the Iberian wolf, Scandinavian gray wolf, Alaska gray wolf and gray wolves worldwide (*Canis lupus)* (Butler, Ballard, and Whitlaw 2006; Mech and Boitani 2007; Thoresen, Arnemo, and Liberg 2009; Santos et al 2014). To those parameters not available in the wolf, our data will be compared with the information available on the closest phylogenetic specie, the domestic dog.

Although M5 – Orphento was subjected to a slightly different chemical restraint protocol, we included his values in the statistical analysis since this fact is not likely to influence haematological and blood biochemical parameters. Young wolf data was also included in this analysis since according to in work done with dogs, there is no age related difference in haematological and blood biochemistry values in animals three or more months (Willard and Tvedten 2012). The value of the blood parameters of M1 – Jackob was excluded from this data. Also, since there were no statistical significance differences between the female and male classes regarding haematological and blood biochemical parameters, the final values result from male and female data aggregation with the exception of potassium.

5.6.1. HEMATOLOGY PARAMETERS

	Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
RBC (10^12/L)	6.19	0.90	14.53	7.97	5.52	9
Hgb (g/L)	176.00	24.34	13.83	194.00	122.00	9
PCV/HTC (L/L)	0.44	0.06	13.09	0.53	0.33	9
MCV (fl)	67.00	5.90	8.80	77.60	59.90	9
MCH (pg)	23.30	3.76	16.15	31.30	22.00	9
MCHC (g/L)	369.00	78.18	21.19	492.00	282.00	9
RDW (%)	16.90	9.25	54.75	35.30	14.10	9
HDW (g/L)	3.32	1.02	30.70	5.50	1.60	9
PLT (10^9/L)	269.00	114.09	42.41	526.00	140.00	9
MPV (fl)	9.10	3.37	37.05	14.70	3.70	9
WBC (10^9/L)	18.45	5.44	29.50	23.27	9.00	9
Neut (10^9/L)	14.12	4.50	31.85	18.00	4.13	9
Neut (%)	75.90	12.68	16.71	85.30	45.90	9
Lymp (10^9/L)	2.25	1.49	66.32	6.11	0.97	9
Lymp (%)	11.60	7.83	67.51	26.80	7.50	9
Mon (10^9/L)	0.97	0.58	59.50	2.47	0.52	9
Mon (%)	4.70	7.56	160.85	27.40	3.80	9
Eos (10^9/L)	0.94	0.85	90.42	2.86	0.02	9
Eos (%)	6.70	3.80	56.70	12.30	0.08	9
Bas (10^9/L)	0.10	0.07	71.79	0.23	0.02	9
Bas (%)	0.80	0.69	86.71	2.50	0.08	9
LUC (%)	0.10	0.10	103.51	0.30	0.10	8

Table 8 – MNP wolf hematological values.

All the parameters which were possible to compare with the existing literature on wolves are within the reference intervals. Note, that it was not possible to compare the HDW and LUC parameters on wolves (Constable et al. 1998; Butler, Ballard, and Whitlaw 2006; Mech and Boitani 2007; Thoresen, Arnemo, and Liberg 2009; Santos et al 2014). Using the domestic dog as a model, our LUC parameter is within the reference interval to this specie. Regarding HDW, the medium of our values (3.32 g/L) is slighter than the reference interval (1.6 – 2.7 g/L). Considering the change on the HDW, it is advised to perform a manual count of the blood smear and check the existent of variations in erythrocyte morphology (which was not performed) (Willard and Tvedten 2012; Ettinger and Feldman 2010).

5.6.2. BLOOD BIOCHEMISTRY PARAMETERS

	Average	Standard deviation	Coefficient of variation %	Maximum	Minimum	n
TP (g/L)	65.00	12.28	18.89	90.00	59.00	7
Alb (g/L)	29.00	5.83	20.11	35.00	16.00	7
Glob (g/L)	46.00	19.77	42.99	74.00	32.00	4
Glu (mmol/L)	5.13	1.73	33.68	7.05	2.28	8
ALT (IU/L)	50.50	59.82	118.45	188.00	27.00	6
GGT (IU/L)	6.05	3.27	54.09	7.00	0.00	4
ALP (IU/L)	114.00	55.09	48.33	195.00	53.00	7
AST (IU/L)	69.00	27.95	40.51	116.00	52.00	5
Creat (µmol/L)	70.72	31.22	44.15	132.60	44.20	7
BUN (mmol/L)	10.71	5.86	54.72	21.06	3.57	8
Amy (IU/L)	199.00	60.76	30.53	340.00	163.00	7
TB (µmol/L)	5.13	0.86	16.67	5.13	3.42	4
Chol (mmol/L)	3.93	0.76	19.44	5.25	3.33	5
Triglyc (mmol/L)	0.52	0.10	20.16	0.59	0.32	5
UC (mmol/L)	65.43	9.41	14.37	77.33	53.54	5
Ca^{2+} (mmol/L)	2.36	0.23	9.58	2.83	2.20	6
P^{+} (mmol/L)	0.74	0.87	117.17	2.42	0.58	4
Na^{+} (mmol/L)	153.00	3.30	2.16	157.00	150.00	4
K^{+} (mmol/L)*	4.60	0.14	3.07	4.70	4.50	2

Table 9– MNP wolf blood biochemical values.

*1 – parameters obtain only in females.

The results of biochemistry of all individuals captured in MNP, lied within reference limits to other gray wolf subspecies namely gray wolf, Alaskan wolf, Scandinavian wolf and Iberian wolf (Constable et al. 1998; Butler, Ballard, and Whitlaw 2006; Mech and Boitani 2007; Thoresen, Arnemo, and Liberg 2009; Santos et al 2014).

Note that there is a lack of information in biochemistry since the n vary among parameters.

5.7. ELECTROCARDIOGRAM

An electrocardiogram was performed on all captured wolves, however we only retained information about two wolves. Although the sampling size is small, we are going to analyze the data observed.

The following values correspond to the ECGs performed on two female wolves, the F4 – Alpha and F5 – Iris. It is important to refer that F4 – Alpha was pregnant and that these two individual wolves manifested a higher stress response and a lighter sedation when compared to the majority of wolves captured.

The ECG of both animals, as observed in Figure 44 revealed alterations on rhythm and wave morphology. It was detected an arrhythmia in F5 – Iris (Figure 44 A) (possibly a respiratory sinus arrhythmia (RSA), however as the tracing is short it is not possible to establish a correct diagnosis) and a tachyarrhythmia in F4 – Alpha (Figure 44 B) with irregular and sometimes biphasic P waves. Both animals presented a deep S wave (more prominent on the F4 – Alpha), T waves higher than usually and an elevation of ST segments is observed.

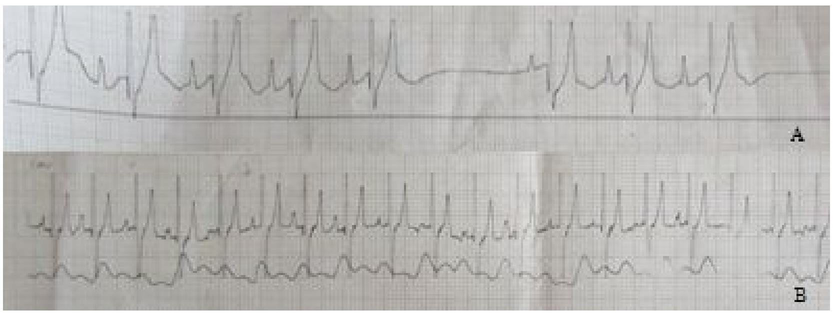

Figure 44 – A – F5 – Iris ECG; B – F4 – Alpha ECG.

In conclusion, F4 Alpha processed a higher HR, S waves and QT intervals with smaller P wave amplitude as well PR intervals. When HR is relatively high, the ECG alterations can be explained by an adaptation of the heart to its rate (Table 10). The atrial and ventricular depolarization (P and R wave, respectively), are influenced by the heart rate. If there is an increase of the HR, P waves will be smaller as the impulse and the atrial depolarization will be faster, and the contraction of the ventricles will be quicker, which can be observed by a decrease in the height of R wave. This will consequently influence the PR and QT intervals, given that the impulse propagation will be faster (PR interval) and the duration between ventricular repolarization and depolarization (QT interval) will be shorter (Martin 2007; Tilley et al. 2008; Ettinger and Feldman 2010; Fuentes, Johnson, and Dennis 2010; Diniz et al. 2013; Oyama, Kraus, and Gelzer 2013).

Wolf	HR (bpm)	P wave amplitude (mV)	P wave duration (sec)	R wave amplitude (mV)	S wave amplitude (mV)	T wave amplitude (mV)	PR interval duration (sec)	QRS complex duration (sec)	QT interval duration (sec)
F 4 - Alpha	145.34	0.25	0.04	1.2	1.2	1	0.08	0.8	0.24
F5 - Iris	83.13	0.6	0.05	1.4	0.67	1.4	0.23	0.8	0.32
Constable et al. (1998)	151+/-7	0.46 +- 0.05	ND	1.69 +/- 0.08	0.30 +/- 0.08	ND	0.0945 +/- 0.005	0.0742 +/- 0.0028	0.2433 +/- 0.0066

Table 10–ECG parameters in two wolves (F4 – Alpha and F5 – Iris) and wild wolf ECG parameters from Constable *et al.* (1998).

The changes observed in HR of F4 – Alpha can be explained by her pregnancy since it is common to observe differences in monitoring parameters upon such conditions namely in cardiovascular effects as HR and blood pressure. These variations are usually observed in canine pregnant females and it is estimated that it aims to rise the cardiac output in order to supply nutrient and blood flow to the fetus (Blanco et al. 2012; Lopate 2012; Shirley et al 2001).

To the present state of our knowledge, there is only one description of wolf ECG. Constable *et al.* (1998), measured differences between ECGs of wild and captivity wolves and concluded that the lifestyle of the animal influences him tracing. The article refer that this alteration can be explained by the cardiac chamber enlargement and hypertrophy evidenced in ECG, since they showed a propensity to an "increased QRS and QT interval duration, a tendency towards increased Q, R and S wave amplitudes in all leads, and a significant decrease in heart rate". This ECG evidences were observed in wild wolves, this changes weren't observed in captivity animals possibly because of the decrease in mobility as a consequence of the limited space available (Constable et al. 1998). The protocol used to anesthetized all wolves (captivity and free ranging wolves) was tiletamine – zolazepam. To perform the IM administration was used a pole syringe in captivity wolves after cornered in each enclosure, regarding wild wolves they were persecuted by helicopter (Constable et al. 1998).

Comparing to our results, the P wave amplitude is very similar, HR and amplitude of R wave have smaller values than reported by Constable *et al.* (1998). Regarding the others parameters (PR and QT interval, QRS complex duration and S waves) our results have higher values.

Domestic dog literature describes a normal amplitude for P wave of 0.4 mV (Tilley et al. 2008; Ettinger and Feldman 2010; Fuentes, Johnson, and Dennis 2010; Oyama, Kraus, and Gelzer 2013). On F5 – Iris it was observed an increase of P wave amplitude (0.5 – 0.7 mV) (Table 10) (Constable et al. 1998). Literature points out pulmonary disease "P pulmonary" (right pulmonary enlargement) as its possible cause, in the absence of heart diseases. It is also referred in the literature that "variation of P wave height is a normal finding in the dog and a manifestation of alterations in vagal tone" (Atkins 2007; Tilley et al. 2008; Ettinger and Feldman 2010; Fuentes, Johnson, and Dennis 2010; Oyama, Kraus, and Gelzer 2013). Taking this account, one of the possibilities of the change on the amplitude of P wave, in our wolves, could be associated to changes in the vagal tonus and not necessarily the result of a pulmonary disease. Although pulmonary worms, cannot be excluded, because none tests were performed in our wolves.

The changes observed in the morphology of P wave (in F4 – Alpha) may be described as a "wandering pacemaker", which may coexist with RSA. As the heart rate varies with nodo SA, there are cyclic changes in the shape and amplitude of the P waves. The cause of these changes is attributed to the shifting of the pacemaker within the SA node induced by changes in vagal tone and it is termed "wandering pacemaker within the SA node" (Willard and Tvedten 2012).

Literature describes normal duration of QRS complex as less than 0.06 sec in domestic dogs and, as described above, 0.07 sec in wild wolves (Constable et al. 1998; Tilley et al. 2008; Ettinger and Feldman 2010; Fuentes, Johnson, and Dennis 2010; Oyama, Kraus, and Gelzer 2013), thus, our values were higher than both references (0.08 sec).

There are descriptions relating the enhance of QRS complex duration to the enlargement of left ventricle and right or left bundle branch block (Tilley et al. 2008; Ettinger and Feldman 2010; Fuentes, Johnson, and Dennis 2010; Oyama, Kraus, and Gelzer 2013).

A study conducted on maned sloths (*Bradypus torquatus*) detected that the presence of gastric content influences the heart position through compression/oppositions of the diaphragm which resulted on changes of QRS complex. Since we are working with wild animals, the time of their previous feeds is unpredictable (Silva, Duarte, and Costa 2005; Diniz et al. 2013). Using the Indian wolf (*Canis lupus pallipes*) as a model, the average of feeding intervals was 3.6 $^{+}/_{-}$ 0.7 days and the average consumption/wolf/day was 1.8 $^{+}/_{-}$ 0.3, which he often eats on only one meal (Jethva and Jhala 2004). Meaning that there is a chance that, like in maned sloths, the gastric distension influences the position of the heart and consequently the ECG trace.

The S wave represents depolarization of the basal sections of the ventricular posterior wall and interventricular septum. The S wave is defined as the first negative deflection following the R wave in the QRS complex. Occasionally, under normal condition, there may be described a widening of entire QRS complex and consequently of the S wave. It is described as normal on some monkey species (Richig 2013). However, it may also occur in cases of right ventricular hypertrophy and no treatment is advised (Tilley et al. 2008; Ettinger and Feldman 2010; Willard and Tvedten 2012; Richig 2013). It is our opinion that the changes of the S wave amplitude are consequence of an increase in the duration of QRS complex.

The major ECG differences observed concerns T waves. This wave represents the most rapid period of ventricular repolarization, during which potassium briskly leaks out of the cell (Ettinger and Feldman 2010; Willard and Tvedten 2012; Richig 2013). Our T wave had an amplitude of 1.2 mV and 1 mV, in F5 – Iris and F4 – Alpha, respectively (Table 10). Literature does not refer values to this wave in wolves. Thus, using the domestic dog as a model, the amplitude of the T wave should not exceed one – fourth the amplitude of the R wave or one – fourth the amplitude of the Q wave, if Q wave is greater than R wave, or 0.5 mV to 1.0 mV (Tilley et al. 2008; Ettinger and Feldman 2010; Fuentes, Johnson, and Dennis 2010; Oyama, Kraus, and Gelzer 2013). The morphology of T waves in small animals is very variable (positive, negative or biphasic) and the diagnostic value of T wave changes is very limited. T wave abnormalities are associated to electrolyte abnormalities, myocardial hypoxia, being these the two the most common causes, however it may also be the consequence of interventricular conduction abnormalities, drug toxicity (example digoxin), ventricular enlargement, increased circulating catecholamines, among others (Tilley et al. 2008; Ettinger and Feldman 2010; Oyama, Kraus, and Gelzer 2013). On the other hand, there are descriptions that refer that changes of the T wave are considered pathological only when detected in consecutive ECGs, associated to modification of other ECG waves and intervals, accompanying clinical signs and/or related to the administration of drugs or intoxications (Martin 2007; Diniz et al. 2013).

As referred previously both wolves struggled since we arrived at the place until the chemical immobilization was performed, the anesthesia plan wasn't deep enough and some movements were observed during the handling.

Fear, stress and struggle during the capture event, simultaneously to the muscular activity, may have been the trigger for a succession of events. During a capture episode, anaesthesia inhibits the "muscle pump" resulting in a decrease on muscle blood flow. This is mostly observed on striated muscle, but also on cardiac muscle, and, as a consequence of the animal struggle, there is a release on the release of catecholamines (by activation of CNS and adrenal system) and increase of muscle activity. As a result of the running out of aerobic metabolism energy on the ischemic tissues, anaerobic metabolism initiates causing an increase of lactic acid, additional muscle hypoxia, culminating on cellular lesions with release of its components, namely potassium. This increase in potassium concentration in blood, results in hyperkalemia (Murray and Fowler 1993; Nielsen 1999; Marks 2010; West, Heard, and Caulkett 2007).The increase of potassium produces an increase of cellular membrane permeability to this ion, mainly during repolarization. One of ECG findings related to hyperkalemia is the increase of T wave amplitude, which presents as a prominent and tended/spiked wave. These waves are also associated to myocardium hypoxia (which is also presented by an increase of ST segment declivity) and ventricular enlargement. However, the concentration at which these changes are noted is variable, usually when potassium levels exceeds 5.7 to 6.0 mEq/L, T waves become peaked with a narrow base, P wave amplitude decreases, and the P – R interval prolongs. If potassium is very high, QRS complexes widen and P waves disappear (atrial standstill) and with further elevation in potassium, conduction delays leading to ventricular fibrillation and ventricular asystole (Martin 2007; Tilley et al. 2008; Ettinger and Feldman 2010; Richig 2013).There is also described the manifestation of natural occurring hyperkalemia on catecholamine releasing situations, specifically fear and pain (Richig 2013).

Unfortunately we didn't measure potassium in the current study for this two females, so we cannot confirm or reject the hyperkalemia hypothesis. In the future this should be a situation to explore.

Modifications of ST segment may not be associated to pathological conditions, but to situations of increased myocardium oxygen demand as anxiety, exercise or anaesthesia (Ettinger and Feldman 2010; Richig 2013). Athletes have higher vagal tones than resting persons, the vagal activity may affect depolarization and repolarization, regarding the ECG findings is represented by ST segment elevation (Hong et al. 2015). Changes in the ST segment are also associated to hypokalemia, but considering the observed changes in the ECG, it is not compatible with hypokalaemia. This etiology is excluded (Tilley et al. 2008; Ettinger and Feldman 2010).

Ventricular enlargement is generally associated to an increase in fitness on humans and sled dogs (Constable et al. 1998; Hinchcliff et al. 1997). Apart from ventricular enlargement, there is also described alteration in ECG of athletic humans, such as sinus bradycardia, sinus arrhythmia, first and second – degree block. Regarding repolarization and abnormalities observed in humans, it is detected a J – point and ST – segment elevation. This apparent "abnormalities" are associated to normal adaptation of the heart to the activity. Cardiac output increases five fold in order to facilitate O_2 delivery to exercising muscles, however during resting HR is slower. These cardiac changes in cardiac output are only achieved through an enhance of cardiac electric activity. During exercise, there is a need for an increase of cardiac output, which is only possible through an increase of electrical activity and the influence of large stroke volumes (Gati and Sharma 2012; Hong e al. 2015). Constable *et al.* (1998) detected a ventricular enlargement on ECG evaluation of wild wolves and correlated it with their life style (Constable et al. 1998; Tilley et al. 2008; Ettinger and Feldman 2010; Richig 2013).

An increase of T wave amplitude and over position of S – T segment is described on 2.5 to 14 % of humans without being associated to cardiac or extracardiac pathologies. However it may be also associated to acute pericarditis, acute coronary insufficiency, hyperkalemia and acute myocardial infarction (Diniz et al. 2013; Lazzoli et al. 2002). Exercise and low heart rates are associated to higher T waves. This discrepancy is caused by regional dispersion of repolarization regarding usually low resting heart rates begin with T wave amplitudes that are higher, possibility due to increased vagal – mediated (because vagal modulation of cardiac transient outward potassium current, which affect the repolarization) (Hong et al. 2015). In the "Quantification of T Wave Shape Changes Following Exercise" paper the T wave amplitude initially increased rapidly on cessation of exercise, in practicality all humans (16 of 20) (Langley, Di Bernardo, and Murray 2002).

There are descriptions of T wave amplitudes of 45 % of R waves on racing horses, 46% on raccoon (*Procyon lotor*), 50 % on rabbits and of the same height of R wave in agoutis (*Dasyprocta punctata*) (Diniz et al. 2013). There are reports that T wave amplitude is associated with myocarditis or hyperkalemia, only when higher than 1.4 mV in horses (Diniz, Michima, and Fernandes 2011). Body anatomy influences the ECG, as well individual species, so it is important have this in consideration during the ECG perform and evaluation (Richig 2013)

Kitchin and Neilson 1972, observed that on horses ECG the period that followed exercise was accompanied with an increases of T wave amplitudes, decreases of ST segment duration and slope and concluded that this could result from alterations of sympathetic tone, since on the active exercise period stopped, T waves presented a decrease on their amplitude (Kitchin and Neilson 1972). In extrapolation to our situation, our wolves presented a moment of active exercise during the struggle of the capture, perhaps if the chemical immobilization had bigger duration it would be possible to see a decrease in T waves.

Regarding the electrolytic values, note that it is not only the increase in potassium that causes changes in the hearts electrical functionality, but also other ions as calcium, phosphorus and sodium, among other. Changes in heart functionality are sometimes associated to changes in more than one ion and/or as a consequence of primary disease or other diseases combined (Tilley et al. 2008; Ettinger and Feldman 2010; Richig 2013). The calcium, sodium and phosphorus are within the reference values of wolves, regarding F4 – Alpha, however on F5 – Iris it was only possible to evaluate calcium, which was also within the reference values to the specie.

Phosphorus is mainly stored on bones, in its inorganic form, it is crucial to the maintenance of the structure of hard tissues as bone and teeth, of cell membrane and of energy supply. The increase and decrease of phosphorus is generally associated to renal, gastric and muscular changes. There is not described any ECG evidence in phosphorous abnormalities, similar to the ones observed on F5 – Iris ECG, and for this reason this ion is excluded as a possible cause for these changes (Tilley et al. 2008; Ettinger and Feldman 2010; Richig 2013).

Changes of sodium levels are generally associated to loss of water and the increase of sodium intake in the case of hypernatremia, occurring the opposite on hyponatremia. The clinical signs are generally associated to changes on neurological level and also may occur lethargy, anorexia, vomiting, weakness, incoordination, disorientation, seizures and coma (these last clinical signs are mainly associated to hyponatremia). It is not associated to specific changes at the ECG level, being its effect more marked when associated to the presence of other electrolytic changes (hyperkalemia, hyponatremia, hypocalcaemia, also associated to metabolic acidosis), namely changes on T wave. However, as F4 – Alpha presented normal sodium levels and the same changes as F5 – Iris, this cause is refuted. There are other ions but are not associated to significative cardiovascular changes, mainly as the ones referred (Tilley et al. 2008; Ettinger and Feldman 2010).

The simpatic stimulation and consequent release of catecholamine, causes myocardial cell damage, producing necrotic areas, even in non – ischemic hearts, which contributes to cardiac ischemia, resulting on arrhythmias. Note that, one of the causes of this simpatic overstimulation is fear and anxiety (Schömig 1988; Schömig 1990; Slavíková, Kuncová, and Topolcan 2007; Tilley et al. 2008; Ettinger and Feldman 2010).

Presumably, the deviations observed on the ECG do not correspond to a pathologic condition, but to a mixture of physiological reaction of the animal to capture, as fear, stress or simply the struggle (exercise) and effect of sedation. Note that these changes are just assumptions on our part, since it was not possible to reach to any conclusion because of the lake of data.

However, considering the lack of information on wild animals, namely the wolf, we considered these, relevant alterations, that required a report, so that later could be included on future investigations with a bigger n.

5.8. CAPTURE INJURIES

The table that was used to calculate the percentage of injuries was a modified scale of Kuehn *et al*. (1986) with addition of some parameters as referred previously on this document (Kuehn et al. 1986; Frame and Meier 2007; Angelucci 2014).

In the first nine wolves captured it was observed that only four animals possessed class 2 injures. Regarding mouth lesion, the same number was observed with little hemorrhagic lesions of the oral mucosa, tongue or gingival and one animal presented reversible edema in the foot.

During my internship, captures were performed throughout a month period. Two wolves were captured, one of them wasn't actually caught, since he only activated the trap and escaped, which was confirmed by the camera traps.

The F5 – Iris presented some light injuries: few erosions in the mouth as she tried to release herself after being caught. The injuries observed were: cuts < 2.5 cm in total in the right front leg in the medial region of metacarpal phalanx junction (Figure 45), little erosion less of 2.5 cm in the right hind limb in the medial zone between the heel and tarsal - 2 circular injuries of 1 cm (Figure 46), small cuts in left footpad between the digital pads and erosion in the abdominal/inguinal region one of 5 – 6 cm and other with 2 cm of length (Figure 47).

With the help of camera traps installed in the place of the capture set, it was possible to observe that, as seen in the other captures, F5 – Iris struggled in the first 5 – 15 min and after this agonic phase remained quiet until the time of our arrival, when she started to struggle again until the chemical immobilization produce effect.

Figure 45 – Right from leg in the medial region of metacarpal phalanx junction – little erosion less of 2.5 cm on F5 – Iris, on MNP

Figure 46 – Right hind limb in the medial zone between the heel and tarsal – 2 circular injuring of 1cm, on F5 – Iris, on MNP

Figure 47 – Erosion in the abdominal/inguinal region – one bigger of 5 – 6 cm and another with 2 cm of length, on F5 – Iris.

Sometimes the activation of the trap was observed, but no animal was captured. The observations of the size of the loop left and the presence of marks left by the non target animal permitted to confirm the presence of foxes, as example. Another non target animal observed was a cow that passed by and activated the capture set, but didn't got caught. With the help of a camera trapping in one of the capture sets, a domestic dog was identified. All these were non target species and none of them were captured because they can release themselves easier due to specific measures taken by biologists in order to decrease this type of incidents.

During the period of time before my arrival at MNP, it was only carried out the capture of two non target animals, one wild boar and a red deer and no injuries were observed.

6. CONCLUSION

The conservation of wildlife animals and of nature itself it is one of the objectives of modern society, being sometimes involved in much controversy and disagreement, but also of will of fight and change the much threatened “wilderness”. The loss and destruction of habitat, pursuit (hunt and traffic) of animal and climate changes are, in my opinion, the main threats to wildlife.

The possibility of performing my internship in a wild natural park, apart from being a personal and professional enriching experience, allowed the acquisition of knowledge of a reality much different from the one that I was used to, regarding wildlife, conservation and veterinary medicine actions as well as park management and ecotourism. It strengthen the importance of a multidisciplinary approach and team in a widespread area itself. As it was expected, the human – wolf conflict, exist alongside his distribution. In the same way it is also object to legends and persecution, but also of fascination and conservation.

Unfortunately, the area of wildlife medicine still presents an “emptiness” regarding the academic – professional, civic and of course, infrastructural levels and means needed to perform an effective professional work. In most centres/parks, there is a lack of means. Professionals have to deal on a daily basis with frustrating situations, in which is necessary to balance the “importance” of the animal with the resources available and other animals.

The reality I witnessed on the park differs from the Portugal’s reality and with the one I was used to interact. I contacted with the discrepancy of the economic and infrastructural character, since in my opinion, there is a misuse/mismanagement of the infrastructures available on the park, which could be used in a more advantageous way, in order to function as a rehabilitation centre. This would enrich even more the excellent role that the park is having in the conservation of wild fauna.

I was confronted with the fact that intervention of the veterinarian is on occasion complicated by matters of the logistical and bureaucratic levels, which sometimes renders it impossible to act in a more active way. This was one of the reasons of being captured only one wolf during my internship.

Regarding wildlife capture, there is not a single approach to every situation, not only the capture itself but also the animal specie, age and gender, administration method, protocol, health, monitorization, release, topography of the landscape and the aim of the capture. In this line of thought, the choice must be based on animal ethics and welfare questions. Note that the choices differs with each particular case.

Taking into consideration the obtained results with such a small sample, in my opinion some changes should be considered so that in the future, the performance and the results might improved, mainly regarding capture and vital signs monitorization. Surmise that capture itself is a stressful procedure to the animal and it was observed that the longer the animal struggled, the bigger is going to be the induction time and consequently greater the changes and repercussions to the animal. The methods used in the chemical immobilization administration, was CO_2 – powered rifle, and on the F5 – Iris case, it was observed some difficulty in performing a successful administration of the protocol. Other approaches that may be used are the Y – pole, pole catch, nets and/or pole syringe or blow pipe. Regarding monitorization, it was not possible to control the basic parameters on all animals, however in this case, it cannot be forgotten that we were not in a controlled environment. Within the possibilities, we should try to always monitor at least three parameters per animal, thru a better communication and attribution of tasks. Regarding ECG, in order to confirm or refute the possibility of changes which we attributed to stress and struggle responses, it should be performed the capture of stray dogs with the same capture and administration methods, immobilization protocol and monitorization time, in order to understand if the changes could be caused be the capture time and/or protocol used.

The role of the veterinarian has to start being dignified by the meaning and difference of its action at the wildlife conservation level. Being essential a multidisciplinary approach, the implementation of partnerships, an increase of financial support to centres and research projects, as well as professionalization of the involved parties on this area and more importantly to reinforce the awareness, civility and relevance of this area.

All earthlings have equal right to life and are not only an important part of an ecosystem, but also as an individual. When fighting for nature, we fight for ourselves.

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