Inland Norway University

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

Nino Gardoni

Master Thesis

Assessment of arterial oxygenation and acid-base status before and during oxygen therapy in captive European bison (*Bison bonasus*) immobilized with etorphine, acepromazine and xylazine

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Abstract

Chemical immobilization of captive European bison (*Bison bonasus*) is often required for veterinary care, transport, or husbandry practices playing an important role in the reintroduction and preservation of the species. To help preventing unexpected events and reduce its negative impact we documented the efficiency and physiological effects of a combination of etorphine-acepromazine-xylazine for European bison immobilization and the benefit of oxygen therapy. Thirty-nine captive European bison were ground-darted with a combination of 1.5 mg of etorphine hydrochloride, 6.1 mg of acepromazine maleate and 20 mg xylazine hydrochloride for 100 kg of estimated weight. Arterial blood was sampled on average 20 minutes after recumbency and again 15 minutes later and immediately analyzed with a portable analyzer. At the same time heart rate, respiratory rate and rectal temperature were recorded. After the first sample we delivered oxygen intranasally at a flow rate of 1L per 100 kg of estimated weight until the end of the procedure.

Before oxygen therapy the mean P_aO_2 was 46.7 mmHg with 32 out of 33 sampled bison presenting hypoxemia. We also observed lowered values of respiratory rate, decreased pH, and mild hypercapnia consistent with the development of a mild respiratory acidosis. Oxygen therapy allowed the resolution of hypoxemia in 22 out of 32 bison but also accentuated the respiratory acidosis. Bison immobilized with higher initial dose did not require reinjection during the procedure. Additionally, we observed that lower mean rectal temperatures during the immobilization event was significantly associated with longer recovery times. For three bison presence of ruminal fluid was noticed in the mouth and nose, causing a risk for aspiration. No mortality related to the immobilizations were reported for at least two months following the procedure.

In conclusion, a combination of 0.016 $mg.kg^{-1}$ etorphine hydrochloride, 0.065 $mg.kg^{-1}$ acepromazine maleate and 0.22 $mg.kg^{-1}$ xylazine hydrochloride provides a sufficient level of immobilization for diverse common management and husbandry procedures in captive European bison. Although, it is associated with development of marked hypoxemia, a small risk of regurgitation and mild respiratory acidosis. We recommend implementing oxygen supplementation when using this protocol.

Contents

1. Introduction

Captive breeding of European bison (*Bison bonasus*, wisent) has played an important role in saving the species from extinction and still take part in the reintroduction work, the preservation of the species and for enriching the genetics of free living herds (Kaczmarek-Okrój et al., 2016; Plumb et al., 2020; Pucek et al., 2004). These breeding programs often require bison chemical immobilization and manipulation for veterinary care, transport or other husbandry practices (Castillo, 2018; Kaczmarek-Okrój et al., 2016; Krzysiak & Larska, 2014).

The European bison is one of the largest and strongest mammals in Europe (Lord et al., 2020; Pucek et al., 2004). Sharing a common ancestor with the American bison (*Bison bison*) the full separation between the two species happened around 10 000 years ago (Krasińska & Krasiński, 2013b). This genetic proximity allows to a certain extent the transposition of knowledge from one species to the other (Caulkett, 2014). European bison are nowadays mostly free-ranging or bred for re-wilding (Lord et al., 2020). Although free-ranging herds of wood bison (*Bison bison athabascae*) and plains bison (*Bison bison bison*) are increasing (Gates et al., 2010), American bison are mostly raised for commercial purposes in farms, leading to different management practice and handling methods (Krasińska & Krasiński, 2013b).

The size and strength of bison make physical restraint very dangerous for both the animal and the handler (Fowler, 2008; Kaczmarek-Okrój et al., 2016). Although hand injections in a hydraulic chute have been reported for American bison (Caulkett et al., 2000; Caulkett & Haigh, 2001; Crawford & Beckmen, 2010; L. Wolfe et al., 2017), the use of dart gun and chemical immobilization is considered as safer and highly recommended for European bison (Kaczmarek-Okrój et al., 2016; Krasińska & Krasiński, 2013a). The use of remote drug delivery system allows intramuscular injection with minimal physical manipulation of the animal which can reduce the animal's stress and physical exertion, compared to physical restraint, along with risks for veterinarians and handlers (Isaza, 2014). Yet this method also carries a risk of trauma to the animal (Cattet et al., 2006) and requires use by professionally trained personnel (Chinnadurai et al., 2016). Preclinical examination and weighing of the animal can generally not be done beforehand increasing risk of overdosing or underdosing as well as other

complications inherent to sedation (Caulkett & Arnemo, 2015; Hawkins et al., 2019). The main complications reported in American bison chemical immobilization are hypoxemia, bloat, and regurgitation (Caulkett, 2014; Caulkett et al., 2000; Harms et al., 2018). In addition, as with many other ruminant species, bison easily stress (Caulkett, 2014) which can predispose them to develop capture myopathy, a rhabdomyolysis induced by stress and muscular exertion that affects skeletal and cardiac muscles and can lead to death (Paterson, 2014). For obvious ethical reason, and because of the intrinsic value of every individual regarding the preservation of a red list species, there is a need to document European bison chemical immobilization to prevent unexpected events and reduce negative impacts.

Several protocols have been used over the years for bison immobilization. The combination of an α_2-agonist with either tiletamine-zolazepam (Castillo, 2018; Caulkett et al., 2000; Caulkett & Haigh, 2001; Chai & Petit, 2013) or ketamine (Arnemo & Kreeger, 2018; Caulkett & Haigh, 2001; Chai & Petit, 2013; Jalanka & Roeken, 1990; B. A. Wolfe, 2015) has been reported as efficient for both American bison and European bison. Azaperone and medetomidine associated with either nalbuphine (NMA) (L. Wolfe et al., 2017) or butorphanol (BAM) (Harms et al., 2018) have also been evaluated as good alternatives for American bison immobilization, and standing sedation using butorphanol and detomidine has been reported on European bison (Bouts et al., 2017). Finally, the combination of a potent opioid and an α_2-agonist have demonstrated a good efficiency in a wide range of ungulates' immobilization (Arnemo & Kreeger, 2018; Napier & Armstrong, 2014). Combination of carfentanil with xylazine has been used successfully on American bison (Arnemo & Kreeger, 2018; Kock & Berger, 1987) but carfentanil is difficult to access in Europe (European Monitoring Centre for Drugs and Drug Addiction, 2018; Expert committee on drug dependance, 2017). Currently the use of etorphine with either acepromazine (Krasiński et al., 1982; Krzysiak & Larska, 2014; Peinado et al., 1999), xylazine (Arnemo & Kreeger, 2018; Chai & Petit, 2013; Krzysiak & Larska, 2014) or both (Krzysiak & Larska, 2014) seems to be the most common for wisent immobilization (Krasińska & Krasiński, 2013a).

Xylazine is an α_2-agonist producing a dose dependent sedation, analgesia and muscle relaxation that can be antagonized (Rankin, 2015). For more reliability in wildlife immobilization xylazine is often associated with a potent opioid such as etorphine (Lance,

2008). Etorphine is an extra potent μ-agonist opioid providing analgesia and dose dependent sedation (KuKanich & Wiese, 2015) that allows for a strong and rapid immobilization with a high margin of safety and efficient competitive antagonists available (Schumacher, 2008). Acepromazine is a phenothiazine often combined to an opioid for its antianxiety effects (Lamont & Grimm, 2014). The combination of those drugs potentiates their effect allowing reduced volume and mitigating some side effects of etorphine such as excitement or muscle rigidity (Lamont & Grimm, 2014; Schumacher, 2008). It remains nevertheless several risks associated with etorphine-acepromazine-xylazine immobilization of wild ungulates including bloat, ruminal tympany, regurgitation (Napier & Armstrong, 2014), hyperthermia (Kästner, 2006; Schumacher, 2008), re-narcotization (Caulkett & Arnemo, 2015; Schumacher, 2008) and cardiovascular effects (mostly bradycardia and hypotension due to xylazine) (Celly et al., 1997; Rankin, 2015). Hypoxemia associated with hypoventilation and secondary unbalanced acid-base status is also a very common complication of ruminant immobilization (Caulkett & Arnemo, 2015). Both etorphine (Pfitzer et al., 2021; Schumacher, 2008) and xylazine (Celly et al., 1997; Kästner, 2006; Read, 2003) affect blood oxygenation and their combination, with or without acepromazine, during wildlife capture has been reported to induce hypoxemia and metabolic disorders in moose (*Alces alces*) (Evans et al., 2012; Lian et al., 2014), muskox (*Ovibos moschatus*) (Lian et al., 2017), reindeer (*Rangifer tarandus*) (Risling et al., 2011), rhebok (*Pelea capreolus*) (Howard et al., 2004) and scimitar horned oryx (*Oryx dammah*) (Pearce & Kock, 1989). Prolonged hypoxemia and metabolic disorders can lead to some level of organ dysfunction (McDonell & Kerr, 2015) and affect the recovery (Caulkett & Arnemo, 2015; Risling et al., 2011). In combination with hyperthermia and an increased tissue oxygen consumption following induction hypoxemia can also increase the risk of inducing capture myopathy (Breed et al., 2019). To prevent such events it is recommended to monitor arterial blood gases and acid-base status (Ozeki et al., 2014). Improvement of oxygenation using nasal supplementation has been successfully implemented in several ruminant species (Fahlman et al., 2012, 2014; Lian et al., 2014, 2017; Risling et al., 2011) including American bison (L. Wolfe et al., 2017).

To our knowledge, only very few studies on bison have focused on potential physiological side effects of chemical immobilization (Caulkett et al., 2000; L. Wolfe et al., 2017) and none have documented the physiological effects, in terms of blood oxygenation and acid base status, of

European bison immobilization using etorphine, acepromazine and xylazine. The aims of this study are (1) to document the efficiency of captive European bison's immobilization using a combination of etorphine-acepromazine-xylazine, (2) to report its clinical and physiological effects and (3) to evaluate the benefit of oxygen therapy during the immobilization.

2. Material and method

2.1. Animals and study area

We conducted the study during six different sessions in 2013, 2014, 2017, 2019, 2021, and 2022, each event lasting one to five days, between the 18th of April and the 1st of June, at Avesta Visentpark in Sweden (60° 8.887' N, 16° 7.905' E). During immobilizations the atmospheric pressure (mean ± SD) was 754 (± 7) mmHg, and the ambient temperature was 11.6 (± 5.8) °C. The study included 39 captive bison, 25 females (64%) and 14 males (36%), 1 to 10 years old, weighing 125kg to 800kg (362 ± 144 kg, n = 28) with a higher mean weight in males (464 ± 209 kg) compared to females (328 ± 100 kg). Immobilizations were done either as part of management procedures required for bison translocation to Romania for reintroduction purposes (Kaczmarek-Okrój et al., 2016) or for husbandry procedures. Since our study was opportunistic using samples collected for monitoring immobilized captive bison, we did not require ethical approval.

2.2. Induction

Two days before immobilizations we split all animals into smaller enclosures containing two to three bison. All were fasted the morning of the immobilization event to reduce risk of regurgitation and tympany (Riebold, 2015). Individuals were isolated then immobilized one by one with a combination of 1.5 mg of etorphine hydrochloride (HCL) and 6.1 mg of acepromazine maleate per 100 kg (Large Animal Immobilon®, Novartis Animal Health, Frimley, United-Kingdom, 2.45 mg.mL^{-1} etorphine HCL, 10 mg.mL^{-1} acepromazine maleate) and 20 mg xylazine HCL for 100 kg (Rompun®, Bayer AG, Leverkusen, Germany, 500 mg). In

most cases the animal's weights were estimated ahead of time and confirmed with weighing during the immobilization using an electronic scale either the same day or three days later during loading, for those that were translocated.

The drug combination was injected intramuscularly (IM) to the hind quarter, using a Dan Inject® CO_2 injection rifle model IM, caliber 11mm and either a 1.5mL or 3mL Dan Inject® dart and 2x40 mm needle with a shortened barb. We monitored clinical signs of chemical immobilization and if recumbency was not achieved by 10-20 minutes we administered a second dose either by darting or hand injection. We adjusted the supplementary doses, from 25-100% of the first dose, based on the level of immobilization.

We recorded time from darting to the first effect of chemical immobilization and the induction time (time from darting to recumbency). Once the individual was down, we positioned it in sternal recumbency with head held up and nose pointing down, when possible, and blindfolded. In some cases, animals were pulled on a sled by a four-wheeler to a more suitable enclosure for the procedure and the recovery, leading to the large variation between individuals in timing before sampling could be done and oxygen delivered.

2.3. Arterial blood sampling and analysis

As soon as possible after recumbency and again 15 (± 4) min after the start of oxygen delivery (times since recumbency recorded as t1 and t2), we sampled arterial blood anaerobically from the auricular artery. For sampling we used a 1 mL preheparinized syringe (Portex®, Smith medical ASD Inc., Keene, USA) and a 23-gauge needle. We immediately mixed the whole blood with the anti-coagulant by rolling the syringe in the palm and discarded the two first blood drops before it was analyzed using an i-STAT®1 Portable Clinical Analyzer and i-STAT® CG4+ cartridges (Abbott Laboratories, Illinois, USA). We maintained the analyzer and the cartridges at operating temperature (16-30°C) in an insulated box filled with warmed water bottles as needed based on ambient temperatures.

For the arterial blood gas and acid base analysis the partial pressure of oxygen in arterial blood (P_aO_2), the partial pressure of carbon dioxide (P_aCO_2), pH and lactate were measured while the

base excess (BE) and bicarbonate (HCO_3^-) were calculated. We measured rectal temperature at the sampling time and used it for correction of blood gas and pH value for both samples (Haskins, 2015). Based on temperature corrected values we defined hypoxemia as mild (P_aO_2 = 60 - 80 mmHg), marked (P_aO_2 = 40 - 60 mmHg) or severe (P_aO_2 < 40 mmHg). We defined hypocapnia as P_aCO_2 < 30 mmHg and we considered hypercapnia as mild for P_aCO_2 = 50 – 60 mmHg, marked for P_aCO_2 = 60 – 80 mmHg and severe for P_aCO_2 > 80 mmHg. We defined acidemia as pH < 7.35 and considered it as marked for pH < 7.2.

To assess the adequacy of gas exchange within the lungs we calculated later the alveolar-arterial PO_2 gradient ($P_{A\text{-}a}O_2$) based on the alveolar gas equation:

(1) $P_AO_2 = F_iO_2 (PB – P_{H2O}) – (P_aCO_2/RQ)$

Where P_AO_2 is the alveolar oxygen tension, F_iO_2 is the fraction of inspired oxygen (0.21), PB the barometric pressure (recorded with the i-Stat® analyzer), P_{H2O} the saturated vapor pressure for water at 38.1°C (50 mmHg) (Haskins, 2015) and RQ the respiratory quotient (assumed to be 1 for herbivores (Schmidt-Nielsen, 1997)). Values of $P_{A\text{-}a}O_2$ > 15 mmHg were considered as indicators of impaired oxygen exchange (Fahlman, 2014). We did not calculate $P_{A\text{-}a}O_2$ after oxygen insufflation, because the F_iO_2 was then unknown (Haskins, 2015).

2.4. Monitoring and animal handling

After the first arterial blood sample we positioned a cannula made of soft flexible plastic in the nostril and advanced to the level of the medial canthus of the eye and secured by tape (Fahlman, 2014). A flow of 100% oxygen concentration was delivered by oxygen cylinder through this cannula at a flow rate of approximately 1 $L.min^{-1}$ for every 100 kg based on the estimated weight. We recorded time when the oxygen was started.

We monitored several physiological parameters throughout the immobilization and recorded these just after recumbency and 10-15 minutes later. We evaluated the level of immobilization and muscle relaxation based on palpebral reflex, jaw tone and muscle relaxation. We monitored heart rate by direct auscultation with a stethoscope or by palpation of the auricular artery. We assessed the capillary refill time and the mucus membrane color from the gingiva,

the respiratory rate by counting thoracic elevation and we measured the rectal temperature by using a digital thermometer.

During the immobilization a long-acting neuroleptic was administered to the animals that were scheduled for translocation as a part of the re-wilding program. We used 1 mL per 100kg of Cisordinol-Depot® IM (Lundbeck Pharma A/S, Valby, Danemark, zuclopenthixol decanoate 200 $mg.mL^{-1}$). The onset of action of this neuroleptic is about a week (Holz & Barnett, 1996; B. A. Wolfe, 2015) so we do not consider that it might interfere with our study.

2.5. Reversal

When all the procedures were done, we discontinued oxygen supplementation, and removed all the monitoring equipment. Immediately after we antagonized the immobilizing agents with 1.35 mg diprenorphine (Large Animal Revivon®, Novartis Animal Health, Frimley, United-Kingdom, 3 $mg.mL^{-1}$ diprenorphine) per 1 mg etorphine HCL and 1 mg atipamezole HCL (Antisedan®, Orion Pharma Animal Health, Turku, Finland, 5 $mg.mL^{-1}$ atipamezole HCL) for every 8 mg xylazine HCL IM. We recorded the time when O_2 delivery was stopped and times of antagonist administration, first sign of recovery and standing. We defined the recovery time as the time between the antagonist administration and the time the animal was standing. Animals were observed regularly during the 24 hours following immobilization for adverse effects.

2.6. Statistical analysis

2.6.1. Sample size

Due to the opportunistic aspect of the study, we conducted the analyses on different sample sizes. Motive of removal from analysis were either the absence of sample or sample contamination, missing weight, discontinuity during oxygen delivery, early reversal for safety reason or use of alternative reversal agent (naltrexone). For every analysis we report the sample size (n).

2.6.2. Need for reinjection

We calculated the summary statistics (reported as mean ± SD (range)) for the total doses of etorphine HCL, acepromazine maleate and xylazine HCL administered through the whole procedure.

To study the relation between the need for reinjection and the dose injected with the first dart we split the bison into two groups depending on whether they had received only one injection or several injections. Then we modelled the dose injected with the first dart as a function of the group they belong to with a simple linear model. The full results of the regression analysis are reported in Appendix 1.

2.6.3. Descriptive analyses of the physiological variables

We calculated the summary statistics for each physiological variable (i.e., rectal temperature, pulse rate, respiratory rate, PaO_2, $PaCO_2$, pH, HCO_3, base excess (BE) and lactate) for both samples (t_1 and t_2). The only exception is the $P_{A\text{-}a}O_2$ that has been calculated only for the first sample.

2.6.4. Change in physiological parameters between t1 and t2

The normality of the distribution for each parameter was assessed both graphically and by using a Shapiro-Wilk test on the value from both samples and from the difference between the two measurements. For normally distributed data we used a two-tailed paired *t*-test to analyze the variation between the first and the second sample. For those that violated the normality assumption we used the non-parametric Wilcoxon signed rank test for paired data. For every comparison we reported the mean difference ($\bar{x}_{t2\text{-}t1}$), the T- or V-statistic of the test with the degree of freedom (T_{df} and V_{df}) and the p-value (*p*).

2.6.5. Immobilization time

We calculated the summary statistics for duration of different steps of the immobilizations. To assess factors that can potentially affect the recovery time we used gamma generalized linear models with log-link function. To reduce the risk of overfitting, regarding our sample size and because of the presence of collinear covariates, we first modelled the recovery time as a function of either the mean P_aO_2 of the procedure (calculated as the sum of P_aO_2 at t_1 and t_2 divided by two), the mean rectal temperature (calculated as the sum of T at t_1 and t_2 divided by two), the procedure time (defined as the time in minutes from the moment the animal is down to the time the antagonist is administered), the total dose of etorphine HCL received or the total dose of xylazine received (both in $mg.kg^{-1}$). Then, we applied a forward selection method based on the small sample corrected Akaike Information Criterion (AICc). In case of $\Delta AICc < 2$ between two models we chose the one with the lowest degree of freedom according with the principle of parsimony (Appendix 2). We reported in the results the sample size, the regression coefficient (β), and the 95 percent confidence interval (95% CI) for the best model and after back-transformation of its coefficients using the exponential function to help its interpretation. The full results of the regression analysis are presented in Appendix 1.

All the statistics were done using the software R, version 4.1.2 (R Core Team, 2021). For all the analyses the level of significance was set to 0.05. For each model, we verified the assumptions by using the DHARMa residuals diagnostic tools from the package DHARMa (Hartig, 2021).

3. Results

During the six sessions from 2013 – 2022, we immobilized 39 bison. The average dose used was 16 ± 3 (11 – 24) $\mu g.kg^{-1}$ of etorphine HCL and 65 ± 12 (45 – 97) $\mu g.kg^{-1}$ of acepromazine maleate combined with 0.25 ± 0.09 (0.17 – 0.48) $mg.kg^{-1}$ of xylazine HCL IM (n = 26). For 25 bison (64%) the dose injected with the first dart provided a sufficient level of immobilization for the whole procedure while 14 (36%) required 1 to 3 reinjections to complete the procedure. Among those animals, 5 (12.8%) received extra doses of only etorphine/acepromazine, 1 (2.6%) received an extra dose of only xylazine and 8 (20.5%)

received both. Bison that required extra doses received significantly smaller initial dose of etorphine (n = 26; β = - 0.006; 95% CI [- 0.004, - 0.008]) and xylazine (n = 26; β = -0.04; 95% CI [- 0.06, - 0.01]) from the first dart, than the bison not requiring extra doses (*Figure 1*).

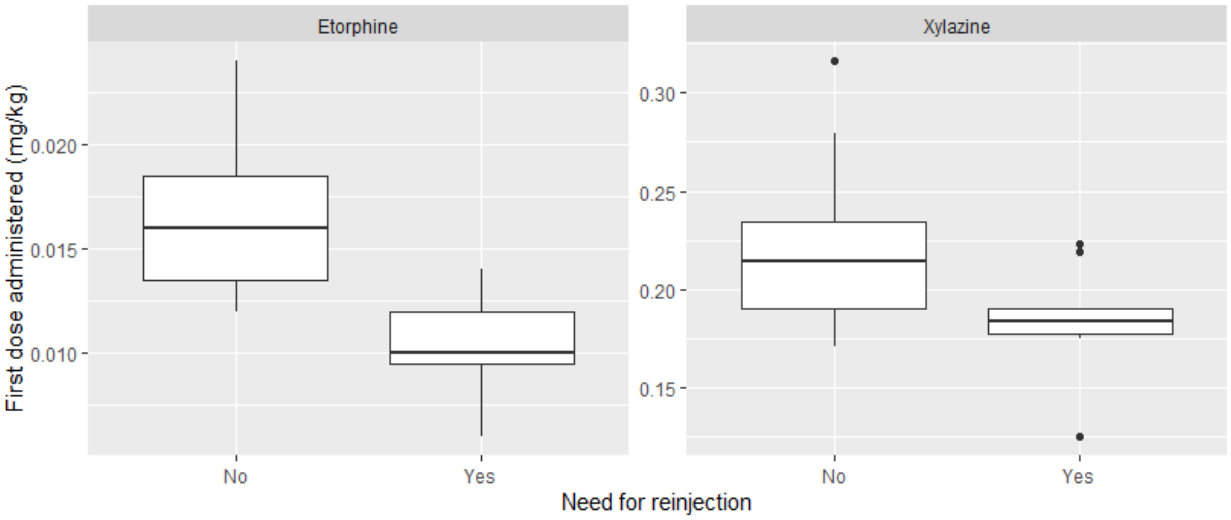


Figure 1. Dose of etorphine and xylazine administered with the first dart injection for individuals successfully immobilized with one dart and did not need reinjection (No) and for animals that needed reinjection (Yes).

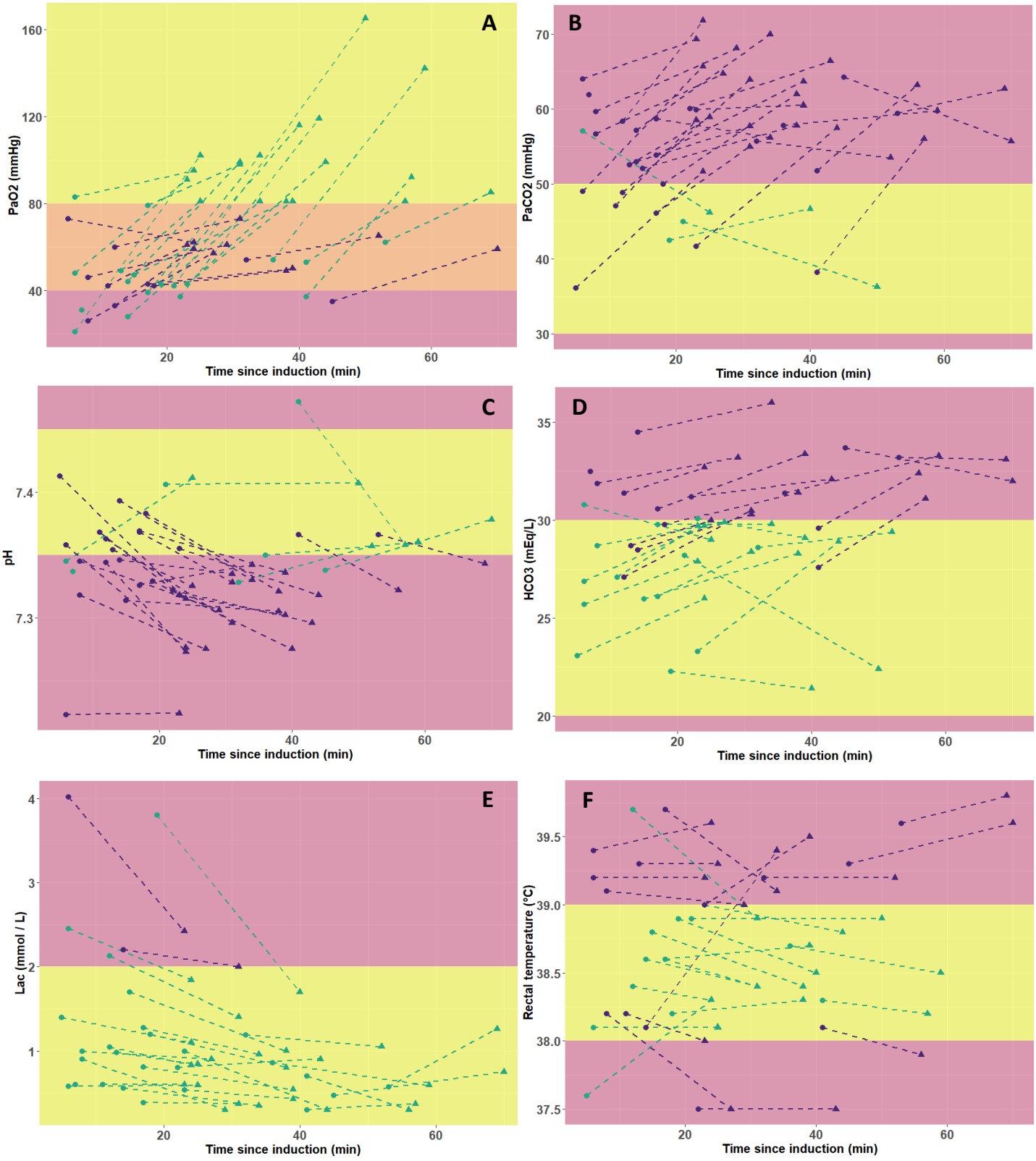
We successfully analyzed the initial blood gas sample for 33 bison. Due to either problem with oxygen delivery or sampling issues, we conducted the second blood gas analysis on 30 individuals. We compared the two samples for the 29 bison where both results were available. The physiological parameters and blood gases measured before and after the start of O_2 administration are reported in Table 1. Before the start of oxygen delivery, the mean P_aO_2 was 46.7 ± 14.1 (21 – 83) mmHg. Thirty-two bison (97%) presented a P_aO_2 < 80 mmHg (21 – 79) among those nine (27%) had a P_aO_2 < 40 mmHg, 16 (55%) a P_aO_2 between 40 – 60 mmHg and 5 (15%) a P_aO_2 between 60 - 80 mmHg. Hypercapnia was present in 27 bison (46 – 64 mmHg). The average $P_{A\text{-}aO2}$ calculated was 48.8 ± 13.6 (14.6 – 73.3) mmHg with 32 bison presenting potential venous admixture. The pH was between 7.30 and 7.35 for 15 bison and equal to 7.22 for one. The lactate was 1.22 ± 0.91 (0.30 – 4.02) $mmol.L^{-1}$, and the bicarbonate concentration was 28.7 ± 2.9 (22.3 – 34.5) $mmol.L^{-1}$.

Table 1. Arterial blood gas and clinical parameters from captive European bison during chemical immobilization using etorphine-acepromazine-xylazine delivered by dart-injection rifle from ground. [*]: temperature corrected values; [o]: significant difference after oxygen supplementation. t_1 and t_2 correspond to the time from when the animal is down to respectively the 1st and the 2nd sample. Δ_t is the time between the first and the second sample. For every parameter the mean ± SD (range) and the number of animals (n_t) are reported. The p-values are the results of paired tests between t_1 and t_2 for each parameter on n individuals. [1]: two-tail paired t-test; [2]: Wilcoxon signed rank test.

		Before O_2			**During O_2**		
		t_1: 19.6 ± 12.1 (5 - 53) minutes (n = 38)		Δ_t: 17.9 ± 4.1 (11 - 29) minutes (n = 34)		t_2: 38.3 ± 13.3 (23 - 70) minutes (n = 34)	
Variable	*Unit*	n_{t1}	*Mean ± SD (min – max)*	*Trend*	n_{t2}	*Mean ± SD (min – max)*	*p-value (n)*
Pulse rate	Beats.min^{-1}	39	54 ± 8 (32-76)	=	29	52 ± 9 (32-72)	0.313[1] (n = 28)
Respiratory rate	Breath.min^{-1}	39	11 ± 8 (2 - 36)	=	29	9 ± 4 (4 - 16)	0.177[2] (n = 28)
Rectal temperature	°C	39	38.7 ± 0.6 (37.3 – 39.7)	=	32	38.7 ± 0.6 (37.5 – 39.8)	0.442[2] (n = 31)
pH [*a]		33	7.35 ± 0.04 (7.22 – 7.47)	↘	32	7.33 ± 0.05 (7.22 – 7.46)	= 0.001[1] (n = 29)
P_aO_2 [*a]	mmHg	33	46.7 ± 14.1 (21 – 83)	↗	32	90.2 ± 28.3 (49 - 165)	< 0.001[1] (n = 29)
P_aCO_2 [*a]	mmHg	33	52.7 ± 7.2 (36.1 - 64.3)	↗	32	59.0 ± 8.2 (36.3 - 71.8)	< 0.001[1] (n = 29)
$P_{A\text{-}aO2}$	mmHg	33	48.8 ± 13.6 (14.6 – 73.3)				
HCO_3^- [a]	mmol.L^{-1}	33	28.7 ± 2.9 (22.3 – 34.5)	↗	32	30.2 ± 3.4 (21.4 – 36)	0.002[2] (n = 29)
BE	mmol.L^{-1}	33	7.3 ± 11.0 (-3.0 – 36.0)	=	32	4.0 ± 3.3 (-5.0 – 10.0)	0.851[2] (n = 29)
Lactate [a]	mmol.L^{-1}	32	1.23 ± 0.91 (0.30 – 4.02)	↘	32	0.85 ± 0.54 (0.30 – 2.42)	0.001[2] (n = 28)

After 15.7 ± 3.5 (10.0 – 23.0) min (n = 28) with 12 ± 5 (7 – 32) mL.kg^{-1}.min^{-1} of oxygen supplementation (n = 20), the P_aO_2 ($\overline{x}_{t2\text{-}t1}$ = 41.3 mmHg; T_{28} = 7.0 ; p < 0.001), P_aCO_2 ($\overline{x}_{t2\text{-}t1}$ = 6.2 mmHg; T_{28} = 4.2; p < 0.001) and bicarbonate levels ($\overline{x}_{t2\text{-}t1}$ = 1.5 mmol.L^{-1}; V_{28} = 339 ; p = 0.002) increased significantly while pH ($\overline{x}_{t2\text{-}t1}$ = - 0.03; T_{28} = -3.68; p = 0.001) and lactate ($\overline{x}_{t2\text{-}t1}$= - 0.38 mmol.L^{-1}; V_{27} = 45.5; p = 0.002) decreased significantly *(Figure 2).* Of the 30 bison sampled after oxygen supplementation 10 presented hypoxemia,

30 hypercapnia, 23 acidemia, and one elevated lactate levels. No significant differences were found for pulse rate, respiratory rate, and temperature before and after oxygen supplementation.



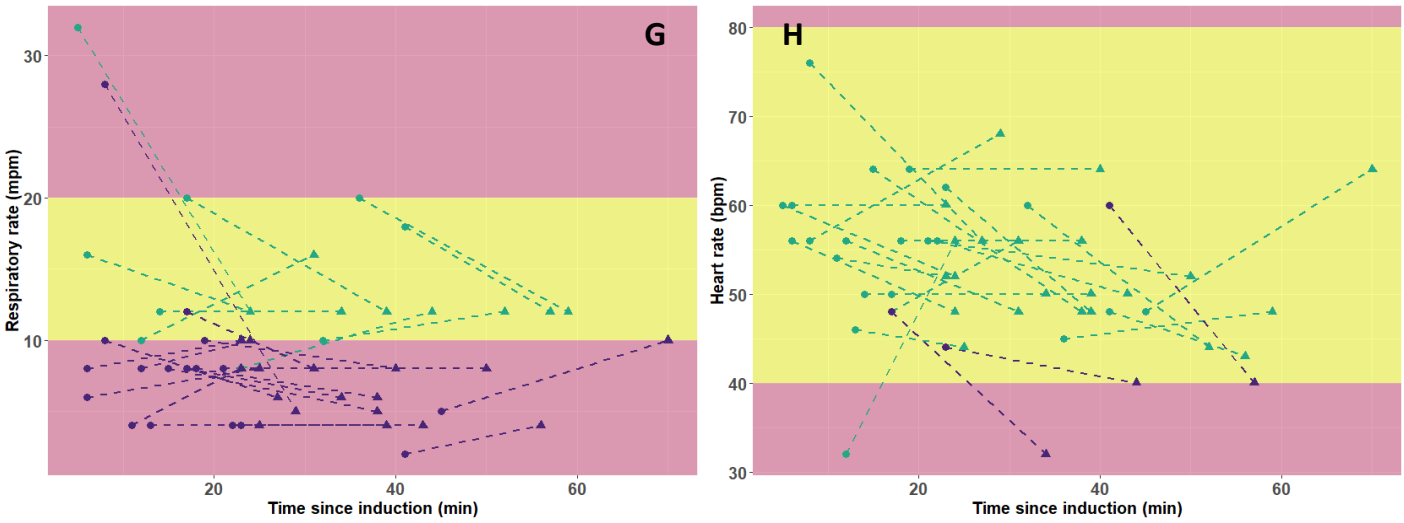


Figure 2. Presentation of P_aO_2 (A), P_aCO_2 (B), pH (C), bicarbonate blood concentration (D), lactate blood concentration (E), rectal temperature (F), respiratory rate (G), and heart rate (H) through time. The round corresponds to the first sample and the triangle to the second. The dash line corresponds to a hypothetical linear variation of the parameter between the two sample. Purple symbols correspond to bison that did not reach physiological values during oxygen supplementation. Green symbols correspond to bison that stayed into or reached physiological range during the procedure. Background colours indicate physiological range (green) and non-physiological values (orange and red).

The induction time, procedure time and recovery time are reported in Table 2.

Table 2. Induction, procedure, and recovery times (recorded in minutes) for captive European bison immobilized with etorphine and xylazine

Time (minutes)	***n***	***Mean***	***SD***	***Range***
Induction	37	6.6	4.7	1 – 22
Procedure	33	49.1	13.5	24 – 71
Recovery	34	11.4	7.5	2 – 38

The recovery was negatively associated with the mean rectal temperature during the procedure (n = 33; β = 0.62; 95% CI [0.44, 0.86]) *(Figure 3)*. We did not identify a significant association between recovery time and mean P_aO_2, procedure time, etorphine or xylazine dose. During all the immobilization events three individuals presented ruminal fluids without solid matters in the mouth and the nose during the procedure and two had rough recoveries characterized by either hyperexcitation or important difficulty to recover balance. No mortality was reported for at least two months post procedures that can be related to it.

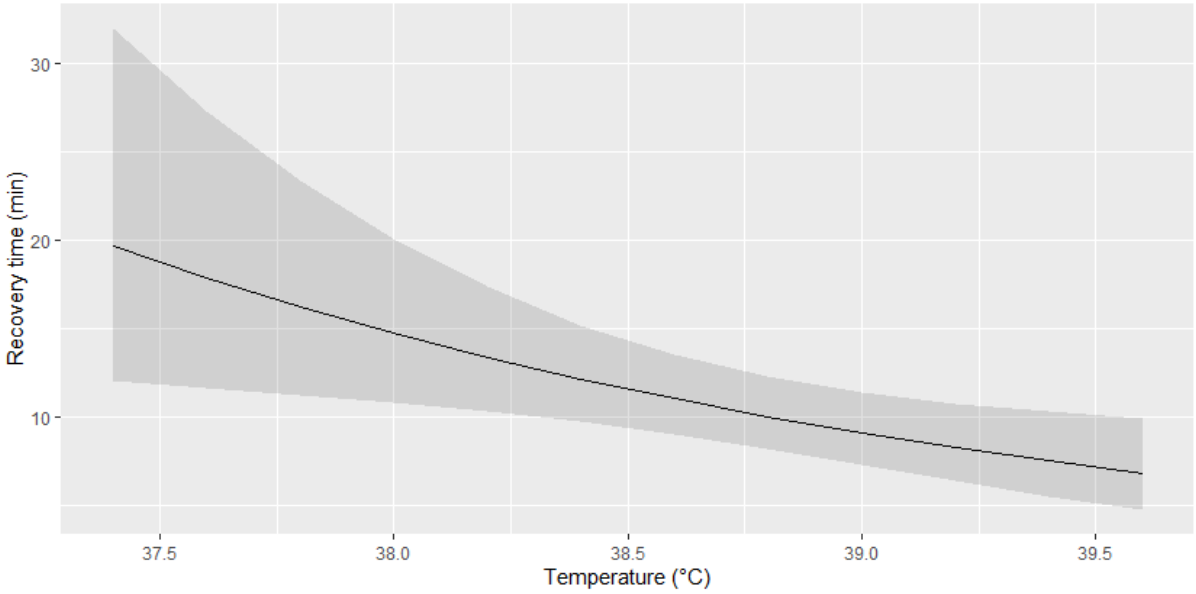


Figure 3. Predicted time from antidote administration to animal standing as a function of the mean rectal temperature recorded through the immobilization procedure of captive bison. The dark grey area around the curve correspond to the 95% confidence interval.

4. Discussion

Here we present a protocol for chemical immobilization of captive European bison using a combination of etorphine-acepromazine-xylazine. Our main results indicated faster induction times and recumbency when using one dart with higher initial dose of etorphine and xylazine, severe hypoxemia alleviated using nasal oxygen insufflation, and slower recovery times for animals with decreasing body temperatures. Lowered respiratory rate, decreased pH and mild hypercapnia also characterized a risk of respiratory acidosis induced by chemical immobilization and possibly intensified by oxygen therapy.

The average dose of etorphine required was similar to what was previously reported for European bison (15 µg.kg^{-1}) but we used slightly higher dose of xylazine (0.025 mg.kg^{-1} instead of 0.020 mg.kg^{-1}) (Arnemo & Kreeger, 2018). Also, we observed that when we underdosed the first dart, due to the difficulty to accurately estimate the weight, we more often had to reinject part of the initial dose either to reach or extend a safe level of immobilization. Although lower dosage for this combination of drugs have been reported to provide safe immobilization on European bison (Krzysiak & Larska, 2014), underdosed animals are at risk for handlers since

bison suddenly can be aroused. Additionally, delayed induction time is potentially associated with overexcitation and stress following an underdosing can increase the risk of trauma (Caulkett, 2014; Schumacher, 2008), capture myopathy and lead to higher effective dose (Caulkett & Arnemo, 2015). Etorphine has a relatively high therapeutic index (9263 in mice) (Alford et al., 1974) and doses up to 30 – 50 $\mu g.kg^{-1}$ on European bison have been reported without associated mortality or observed severe side effects (Peinado et al., 1999). Xylazine presents a more narrow safety margin, but reports show dosages exceeding three to ten times our dosage in several ruminant species without mortality (Young & Whyte, 1973). Those relatively broad safety margins, together with the availability of specific antagonists allowing a quick reversal in case of emergency for both drugs (Lamont & Grimm, 2014), lead us to recommend personnel working with European bison to rather dose the animal on the slightly higher end, rather than aiming for the minimal effective dosage risking underdosing and re-darting.

Hypoxemia is generally considered as one of the main concerns during American bison immobilization because of its severity and its frequent occurrence (Caulkett, 2014). Mild to marked levels of hypoxemia was previously reported in American bison immobilized with different drug combinations (xylazine-tiletamine-zolazepam (XZT), P_aO_2 = 53 mmHg (Caulkett et al., 2000) ; medetomidine-tiletamine-zolazepam (MZT) , P_aO_2 = 58 mmHg (Caulkett et al., 2000) ; BAM, P_aO_2 = 62 mmHg (Shury et al., 2008) ; NMA , P_aO_2 = 64 mmHg (L. Wolfe et al., 2017)). Several causes can lead to its development such as hypoventilation or drug induced intrapulmonary problems (ventilation–perfusion mismatch, shunt, or diffusion impairment) (Fahlman, 2014). In large mammals, the recumbency is known to influence the development of hypoxemia through the pressure applied by abdominal viscera on the diaphragm leading to ventilation/perfusion mismatch on the lung (McDonell & Kerr, 2015). In our study all animals were positioned in sternal recumbency and, although it is considered as favoring a better oxygenation than lateral and dorsal recumbency in several species (Fahlman et al., 2016; Gleed & Dobson, 1988; Morkel et al., 2010), it probably added to the development of hypoxemia (McDonell & Kerr, 2015). Our immobilization protocol included the administration of etorphine and xylazine, both known to affect the respiratory system (Rankin, 2015), and associated with hypoxemia during other ruminants immobilizations (Evans et al., 2012; Howard et al., 2004; Lian et al., 2014, 2017; Pearce & Kock, 1989; Risling et al., 2011).

Etorphine is reported to decrease responsiveness of central chemoreceptors to the variation in P_aCO_2 (Lamont & Grimm, 2014) inducing a dose dependent respiratory depression in ruminants resulting in a decreased respiratory rate and tidal volume (Pfitzer et al., 2021; Schumacher, 2008). A similar effect of xylazine on central responsiveness to CO_2 is reported (Lamont & Grimm, 2014). Also, α_2-agonist are reported to induce more severe hypoxemia in sheep than other species by increasing venous admixture and through the development of a pulmonary oedema (Celly et al., 1997; Kästner, 2006). Currently most of these studies have been carried out on sheep, however new research about the underlying mechanisms tend to suggest that it can also affect other ruminant species (Abouelfetouh et al., 2021). Former studies on ungulates have suggested venous admixture and hypoventilation to potentially be major contributors to hypoxemia during immobilization using etorphine and xylazine (Evans et al., 2012; Fahlman et al., 2016; Lian et al., 2014). In our study we observed an elevated alveolar-arterial PO_2 gradient for 30 out of 31 bison indicating some venous admixture (Haskins, 2015). Likewise, we observed a lowered mean respiratory rate (physiological range: 10 - 20mpm ; Christopherson et al., 1979; Jaczewski, 2000) and a mild hypercapnia (physiological range: 30-50 mmHg; B. A. Wolfe, 2015) that reflects hypoventilation (McDonell & Kerr, 2015). Therefore, the development of hypoxemia in our study likely results from the observed hypoventilation and venous admixture induced by the drugs administered and the sternal recumbency.

After intra-nasal oxygen delivery, we observed a significant increase in mean P_aO_2 and values reaching physiological range for 20 out of the 30 bison sampled. Non-invasive oxygen therapy is recognized as a quick, easy and safe method to improve arterial oxygenation in animals that experience ventilation–perfusion mismatching and hypoventilation (Fahlman, 2014; Mosley, 2015) and former studies reported its successful implementation in several ungulate species (Evans et al., 2012; Fahlman et al., 2012, 2014; Howard et al., 2004; Lian et al., 2014, 2017; Risling et al., 2011). The remaining presence of hypoxemic bison after oxygen treatment can be explained by the fact that we used a relatively low delivery flow. Our average flow rate was 12 $ml.kg^{-1}.min^{-1}$, resulting from studies in other species where 10 $ml.kg^{-1}.min^{-1}$ (1 $L.min^{-1}$ per 100 kg) has been sufficient to relieve the hypoxemia (Lian et al., 2014, 2017), while recommended flow for American plains bison average 10-15 $L.min^{-1}$ (Caulkett, 2014). Using a higher nasal insufflation rate would allow a better flush of the anatomical dead space,

increasing the available oxygen concentration and volume for inhalation, and would better match with the inspiratory flow of bison, leading to an increased fraction of inspired oxygen (Dunphy et al., 2002; Wilson et al., 2006; Zimmerman et al., 2013). Therefore, although the respiratory rate, the breathing pattern, and the delivery method also contribute to the efficiency of oxygen therapy (Fahlman, 2014; Floriano et al., 2022; Zimmerman et al., 2013), we strongly recommend to further investigate the benefit of higher delivery rates on the treatment of hypoxemia during European bison immobilization. We observed a significant increase of P_aCO_2 during oxygen therapy. Partly because of the hypoventilation, and not using mechanical ventilation to compensate the drug induced depression of receptors sensitive to P_aCO_2, the hypercapnia continue to increase with time in immobilized animals (Fahlman, 2014). Besides, oxygen delivery can increase the degree of hypoventilation in immobilized ruminants (Paterson et al., 2009; Risling et al., 2011). This would be explain by the combination of the Haldane effect, resulting in decreased affinity of hemoglobin for CO_2 in oxygenated blood, and an impairment of hypoxic pulmonary vasoconstriction (Kavanagh & Hedenstierna, 2019). Mild hypercapnia, such observed in our study, is reported to be generally well tolerated in ruminants (McDonell & Kerr, 2015) and can actually have some beneficial effect during the immobilization by supporting the cardiovascular function, through a positive inotropic effect and vasoconstriction, and by favoring the release of oxygen from hemoglobin to the tissue (Fahlman et al., 2014). Yet severe or prolonged hypercapnia might result in deleterious effects on cardiovascular and neurological function including increased intracranial pressure, impaired myocardial contractility, narcosis and coma (Daly, 2015). Furthermore hypercapnia is also responsible to the development of respiratory acidosis (Johnson & Autran de Morais, 2012). In our study the mild hypercapnia observed is associated with lowered mean pH value and respiratory acidosis in several bison. The pH levels observed here are similar to those reported for American bison immobilizations (Shury et al., 2008; L. Wolfe et al., 2017) as well as in other ungulate species (Lian et al., 2017; Pearce & Kock, 1989; Risling et al., 2011). The increased mean level of bicarbonate observed probably resulted from a metabolic compensation to respiratory acidosis (Johnson & Autran de Morais, 2012). In our study, acidemia and hypercapnia remained within a mild range. Nevertheless, severe acidemia can result in serious detrimental effects on cardiovascular function affecting myocardial contractility, arterial blood pressure and predisposing to arrhythmia and ventricular

fibrillation (DiBartola, 2012). This must be considered when increasing oxygen flow rates, and careful titration with blood gas analyses are recommended. The low lactate values are likely reflective of calm inductions (Boesch et al., 2011; Crawford & Beckmen, 2010; Haga et al., 2009).

In our study we observed increased recovery time for individuals with the lower rectal temperature. This can possibly be explained by a slowdown of drug metabolism in animal with low body temperature (Ko & Krimins, 2014; Pottie et al., 2007). We recorded rectal temperatures close to the average rectal temperature reported for European bison (38.5 ± 2°C (Christopherson et al., 1979) ; 38.7 °C (Hawley & Peden, 1982)) as well as relatively low mean recovery time (11.5 ± 7.8 min), close to that reported for XZT combination (11.8 ± 9.7 min; Caulkett et al., 2000), although higher than that for BAM (4.9 ± 2.8 min ; Harms et al., 2018), NMA (4.0 ± 1.1 min ; Wolfe et al., 2017), carfentanil-xylazine (4.1 ± 1.6 min ; Kock et al., 1987) and MZT (1.7 ± 0.8 min ; Caulkett et al., 2000) combinations. Unlike what has been previously described in reindeer for this protocol (Risling et al., 2011) we did not identify a significant relationship between hypoxemia and the recovery time but it might be related to the lower measurement rate of P_aO_2 in our study that didn't allow an accurate averaging of P_aO_2 through the immobilization.

By using this combination, we obtained an efficient and reversible immobilization for all the 39 bison included in our study. This protocol provided a quick induction with a mean induction time (6.8 ± 4.7 min) lower than those reported for ground darted American bison using BAM (10.8 ± 7.3 min ; Harms et al., 2018), NMA (11.5 ± 1.3 min ; Wolfe et al., 2017) or carfentanil-xylazine (14.2 ± 2.9 min ; Kock et al., 1987) combination and close to XZT (4.1 ± 0.97 min) and MZT (7.5 ± 2.11 min) protocoles (Caulkett et al., 2000).

Regurgitation is a well-known side effect of both opioids and α_2-agonists (Arnemo et al., 2014). In our study no bison regurgitated per se, but three animals presented a small amount of ruminal fluids in the mouth and the nose out of the 39 immobilized, making it a non-negligible risk associated to our protocol. The presence of ruminal content in mouth and nose is considered a major concern during immobilization as it can lead to aspiration and consequent pneumonia (Caulkett, 2014). To reduce this risk it is recommended to fasten animals when possible and to positioned the animal in sternal recumbency with the mouth and nose pointing

downward (Napier & Armstrong, 2014). In case of aspiration long acting antibiotics with a broad spectrum should be administered to prevent the development of aspiration pneumoniae (Arnemo et al., 2014).

Between 2008 and 2022, 189 immobilizations of European bison were carried out, including 60 for loading the animals onto transport vehicles, following the same protocol that we described (without acepromazine in some cases) (S. Björck, personal communication, April 6th, 2022). The absence of mortality or obvious clinical symptoms reported post immobilizations are an encouraging output to our study reflecting a sufficient degree of safety associated to our protocol. However, immobilization remains a stressful event with a cost for the animals that can accumulate with other sources of stress and affect the animal's general well-being and potential translocation success (Dickens et al., 2010; Teixeira et al., 2007). Additional studies documenting the efficiency and repercussion of this protocol and other drug combination protocols would therefore greatly benefit to the overall knowledge and safety regarding European bison immobilization. Based on our experience we suggest that further monitoring of cardiovascular parameters such as blood pressure might help to better assess the real impact of our protocol. Likewise, assessing the effect of different flow rate as well as the use of supplementary drugs such as vatinoxan (Adam et al., 2022) or serotonin agonist (Pfitzer et al., 2019) could help to better mitigate the side effects of the immobilization drugs.

5. Conclusion

Our results indicate that the combination of etorphine, acepromazine and xylazine provides sufficient level of immobilization for captive European bison for diverse common management and husbandry procedures. Yet, the immobilization process is associated with the development of mild to severe hypoxemia, a small risk of regurgitation and mild respiratory acidosis potentially accentuated by oxygen therapy. Our recommended dose is 0.016 mg.kg^{-1} etorphine HCL (i.e., 0.015 mg.kg^{-1} etorphine base) and 0.22 mg.kg^{-1} xylazine HCL since we found that this dose induced recumbency and sedation using just one dart.

When using this protocol, we recommend fastening the animal before immobilization and to position them in sternal recumbency for the procedure. Considering the large benefit on

hypoxemia compared to the mild level of respiratory acidosis we recommend implementing oxygen supplementation and a close monitoring of respiratory parameters. Further studies are required to properly assess a more accurate flow rate that would properly balance the resolution of hypoxemia.

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Appendix 1

Appendix 1: Results of the regression analyses for the best models. The variable called "several injections' group" corresponds to a categorical variable with two level: one is the group of bison that have required more than one injection during the whole procedure and the other one is for those that got only one injection.

Parameter (unit)	Model family (link)	Sample size	variable (unit)	Estimate	95% CI	p-value	r^2
Recovery time (min)	Gamma (log)	33	Intercept	20.96	[8.20, 33.86]	0.005	0.13
			Temperature (°C)	-0.48	[-0.81, -0.15]	0.011	
Etorphine start dose (mg/kg)	Gaussian (identity)	26	Intercept	0.16	[0.015, 0.018]	< 0.001	0.52
			Several injections' group	-0.006	[-0.008, -0.004]	< 0.001	
Xylazine start dose (mg/kg)	Gaussian (identity)	26	Intercept	0.22	[0.20, 0.24]	<0.001	0.20
			Several injections' group	-0.04	[-0.06, -0.01]	0.022	

Appendix 2

Appendix 2: Model selection using forward selection method based on the small sample corrected Akaike Information Criterion (AIC) to assess factors that can affect recovery time. The compared models are gamma generalized linear models with log-link function. The best model for each step is in bold.

Parameter	Variable	N	df	AICc	ΔAICc
Recovery time	**Rectal temperature**	**17**	**2**	**110.4**	**0**
	Procedure time	17	3	112.5	2.1
	Etorphine total dose	17	3	113.1	2.7
	Intercept	17	3	115.3	4.9
	Xylazine total dose	17	3	116.9	6.5
	Mean P_aO_2	17	3	117.7	7.3
Recovery time	Temperature	17	3	110.4	0
	Temperature + Etorphine total dose	17	4	111.7	1.3
	Temperature + Procedure time	**17**	**4**	**111.7**	**1.3**
	Temperature + Xylazine total dose	17	4	112.9	2.5
	Temperature + Mean P_aO_2	17	4	113.7	3.3