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Physiologic Evaluation and Welfare Assessment of Mechanical and Chemical Immobilization with Fremont™ Humane Foot Snare and Medetomidine-Ketamine-Acepromazine in Free-Ranging Apennine Wolves (*Canis lupus italicus*)

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Simple Summary

The Apennine wolf is a genetically unique subspecies that, after nearly disappearing in Italy in the 1970s, has recovered remarkably thanks to decades of legal protection and conservation efforts. Today, scientific monitoring of free-ranging wolves—including live capture, fitting of satellite tracking collars, and biological sampling—is essential both to understand wolf ecology and to manage the increasingly complex relationship between wolves and human activities. However, live capture carries inherent risks to animal welfare, and validated, species-specific protocols for the Apennine wolf have never been formally documented. This study reports on the capture of thirteen free-ranging Apennine wolves in Maiella National Park (central Italy) using a humane foot snare combined with a reversible drug combination (a sedative, a dissociative anesthetic, and a tranquilizer). We measured heart rate, breathing rate, body temperature, blood oxygen levels, and a comprehensive panel of blood parameters to evaluate whether the animals remained in good health throughout the procedure. All thirteen wolves survived capture with no serious injuries or lasting health effects, and preliminary tracking data suggest that normal movement patterns were restored within a short period after release. These results indicate that the protocol described is safe and welfare-compatible for this subspecies, and provide a scientific foundation for standardizing capture procedures for the Apennine wolf in Italy.

Abstract

The Apennine wolf (*Canis lupus italicus*) is a distinct subspecies whose ongoing population recovery in Italy has progressively increased the demand for live capture protocols validated for scientific monitoring and conservation management. Despite the widespread use of mechanical and chemical immobilization in European wolf management, no study has to date systematically evaluated the integrated combination of a humane mechanical restraint system and a structured chemical immobilization protocol—and specifically the association of the Fremont™ humane foot snare with a medetomidine-ketamine-acepromazine (MKA) protocol, in terms of their joint physiological effects and welfare implications for this subspecies under operational field conditions. Between June 2010 and July 2017, thirteen free-ranging Apennine wolves were captured in Maiella National Park (central Apennines, Italy) using the Fremont™ snare and immobilized with a standardized MKA protocol. Cardiorespiratory parameters, body temperature, peripheral oxygen saturation, venous blood gas values, and a comprehensive hematological and serum biochemical panel were recorded during

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immobilization. Mean heart rate was 100 ± 15 bpm, respiratory rate 24 ± 13 breaths/min, and body temperature 38.1 ± 1.0 °C. No clinically significant hyperthermia was recorded in the cohort as a whole. Hematological and biochemical values were broadly consistent with published reference ranges for the species, with condition-specific deviations identified in two individuals (one pregnant female and one juvenile presenting signs of transient capture-related myopathy), both of which resolved without clinical sequelae. No capture-related mortality occurred. All thirteen individuals survived the minimum post-capture monitoring period, and preliminary GPS data suggest a transient reduction in movement activity in the immediate post-release period. These findings support the welfare adequacy and operational feasibility of the combined Fremont™ snare–MKA protocol for the Apennine wolf, and provide baseline physiological and hematobiochemical reference data for *Canis lupus italicus* relevant to future capture and conservation management programmes.

Keywords: *Canis lupus italicus*; wildlife capture; humane foot snare; chemical immobilization; medetomidine-ketamine-acepromazine; animal welfare; physiological monitoring; hematology; GPS telemetry; conservation management

1. Introduction

The recovery of the gray wolf (*Canis lupus*) across Europe represents one of the most well-documented large carnivore conservation successes of recent decades, driven by a combination of legal protection, land-use changes, and recovering prey populations [1–3]. Recent continental assessments confirm that this recovery is ongoing: by 2022, at least 21,500 wolves inhabited Europe—an increase of approximately 58% over the preceding decade—with populations expanding in range and number across the great majority of monitored countries [3,4]. This trajectory represents a remarkable conservation achievement for a large predator persisting in heavily human-modified landscapes, and underscores the extraordinary ecological adaptability of the species.

In Italy, the Apennine wolf (*Canis lupus italicus*) underwent severe population decline during the nineteenth and twentieth centuries, reaching a critical low of an estimated 100 individuals in the 1970s [5] as the outcome of a deliberate and sustained effort to remove a perceived threat to livestock breeding and, occasionally, to human safety. Subsequent recovery was facilitated by the enactment of national protective legislation and later reinforced by the European Union Habitats Directive, which afforded the species strict legal protection across its range. A key ecological driver of this demographic rebound was the widespread post-World War II abandonment of rural mountain and hill areas, which created available territories for recolonization and coincided with the resurgence of wild ungulate populations—including wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), and red deer (*Cervus elaphus*)—thereby restoring the trophic conditions necessary to sustain expanding wolf populations [6–10] The Italian population currently numbers at least 3,000 individuals and continues to expand its range northward into the Alps and beyond [11,12].

Beyond its conservation status, the Apennine wolf represents a case of remarkable taxonomic uniqueness. Protracted geographic isolation in the glacial refugium south of the Alps, combined with recurrent demographic bottlenecks, has rendered *Canis lupus italicus* morphologically, genetically, and genomically differentiated from all other wolf populations worldwide. The subspecific distinctiveness of the Italian wolf was first formally described by Altobello (1921) [13] on the basis of morphological characters, and has since been progressively confirmed by molecular and genomic evidence. Genome-wide analyses have revealed the deep evolutionary divergence of southern European wolf lineages from northern European and North American populations, with signatures of prolonged isolation and strong genetic drift consistent with the historical biogeographic scenario of glacial refugium persistence. The Italian lineage in particular shows one of the most pronounced genetic signatures of population bottleneck and diversifying selection among European wolf populations, supporting its recognition as a distinct subspecies [15,16]. These findings carry direct implications for conservation management: the genetic and demographic singularity of *C. l. italicus*

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strengthens the case for population-specific research and monitoring, including the development of capture and immobilization protocols validated specifically for this subspecies.

The scale of the Italian recovery has been documented through systematic field surveys and spatially explicit population modeling. A comprehensive national assessment estimated approximately 2,388 wolves across 108,534 km² of the Apennine region, while subsequent integrated spatial modeling confirmed the continued expansion of the species across south-central Italy [11,12] further demonstrated that expanding wolf populations are increasingly colonizing human-dominated and peri-urban landscapes, a process that intensifies both the demand for scientific monitoring and the complexity of human-wolf coexistence management [16]. This demographic trajectory has progressively increased the need for rigorous research requiring live capture, biological sampling, and telemetry-based monitoring of free-ranging individuals—activities essential for characterizing population structure, health status, movement ecology, and connectivity [1,3].

Physical restraint methods, including foothold traps, cable restraints, and Fremont-type humane foot snares, have been widely employed in North American and European wolf management programs. Among these, the Fremont® humane foot snare has gained increasing acceptance due to its reduced injury profile compared to traditional steel-jaw foothold traps, though comparative welfare data across capture techniques remain limited [17]. Assessment of restraining trap systems requires evaluation of physical, behavioural, and physiological parameters, including injury scoring, signs of distress or exertion during captivity, and indicators of physiological stress [18]. Gese et al. [19] demonstrated that while injury scores between cable restraints and foothold traps may be broadly comparable, capture method can nonetheless influence post-release movement patterns and space use, underscoring the need to evaluate each technique in the context of both immediate physiological impact and longer-term behavioral welfare.

Chemical immobilization in wolves and other large canids has been most extensively studied using alpha-2 adrenergic agonists—particularly medetomidine and xylazine—in combination with dissociative agents such as ketamine, or with cyclohexamine-benzodiazepine combinations such as tiletamine-zolazepam (Telazol®) [20]. Medetomidine-ketamine (MK) protocols are widely favored for their rapid induction, potent sedation, and reversibility via atipamezole [20–22]. However, these protocols carry documented risks of hypertension, respiratory depression, and hyperthermia. These adverse effects are particularly relevant under field conditions, where environmental temperature, capture-related stress, and variable body condition interact unpredictably with drug pharmacodynamics, potentially amplifying their clinical significance [20]. The addition of acepromazine as a phenothiazine adjunct to MK protocols has been proposed to attenuate hypertensive responses and improve muscle relaxation, though its use requires careful dose titration given the potential for compounding respiratory and cardiovascular depression in large wild mammals [23].

Comprehensive hematological and serum biochemical profiling offers a validated framework for cross-species welfare appraisal directly translatable to canid field captures [24]. Non-invasive clinical monitoring alone may be insufficient to detect hemodynamic compromise in large wild mammals, emphasizing the value of more direct assessment methods where feasible [25]. Despite growing interest in wolf conservation and management in Italy, published data on the physiological responses of free-ranging Apennine wolves to capture and chemical immobilization remain scarce. In particular, no study to date has systematically evaluated the combined use of the Fremont™ humane foot snare with a medetomidine-ketamine-acepromazine (MKA) protocol in this subspecies, nor characterized the associated cardiorespiratory, thermal, and biochemical parameters under operational field conditions.

The present study addresses this gap by reporting the physiological responses—including cardiorespiratory variables, body temperature, venous blood gas values, and selected hematological and biochemical parameters—of free-ranging Apennine wolves captured using the Fremont® humane foot snare and immobilized with a standardized MKA protocol. Our aims were: (1) to describe the safety and efficacy of this combined capture-anesthesia approach; (2) to evaluate key

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welfare indicators during immobilization; and (3) to contribute baseline physiological and hematobiochemical reference data for *Canis lupus italicus* that may inform future capture protocols and conservation management decisions.

2. Materials and Methods

2.1. Study Area and Institutional Framework

This study was conducted within the framework of the LIFE08 NAT/IT/000325 "WOLFNET" project (and post-LIFE actions), a conservation initiative co-funded by the European Commission under the LIFE+ Nature programme, in which GPS radio-collar deployment was specifically directed at supporting direct conservation actions for target wolf packs. The study area comprised the north-western sector of Maiella National Park (Parco Nazionale della Maiella), a protected area of 74,095 hectares established under Italian Law No. 394 of 6 December 1991 and formally delimited by Presidential Decree of 5 June 1995. The park spans 39 municipalities across three provinces of the Abruzzo region—Chieti (27,396 ha), L'Aquila (23,850 ha), and Pescara (22,849 ha)—and encompasses the massifs of the Maiella, Morrone, and Monti Pizzi, with elevations ranging from approximately 400 m to 2,793 m a.s.l. at Monte Amaro. The park represents one of the most ecologically significant protected areas in the central Apennines and constitutes a core habitat for the Apennine wolf (*Canis lupus italicus*), as well as for the Marsican brown bear (*Ursus arctos marsicanus*) and the Apennine chamois (*Rupicapra pyrenaica ornata*).

2.2. Physical Capture Method

From June 2010 to July 2017, 20 free-ranging Apennine wolves (*Canis lupus italicus*) were captured in Maiella National Park (central Italy). All captures were performed exclusively using the Fremont™ Humane Foot Snare, in compliance with European legislation prohibiting the use of leghold traps. Originally designed for bear capture, the Fremont™ snare was adapted for wolf capture using a 1/8-inch cable (7×7 construction: seven wires in seven strands). The device incorporates a mechanical stop preventing complete loop closure, multi-directional swivels allowing rotational movement, and additional springs to absorb shock and minimize trauma to the captured limb. Each trap was equipped with a battery-operated electronic alarm system with GSM communication to ensure rapid response. A dedicated team of four trained personnel—including veterinarians and wildlife biologists—was on standby 24 hours per day, 7 days per week, with a maximum response time of 35 minutes from alarm activation.

2.3. Chemical Immobilization Protocols

Chemical immobilization was conducted year-round, with captures occurring primarily in late spring and autumn under environmental temperatures ranging from 4 °C to 22 °C. Drug delivery was achieved using a CO_2-powered rifle or blowgun with 3 ml plastic darts fitted with a 1.5×30 mm plain needle (Telinject™, Germany, or Daninject™, Denmark). For juvenile individuals (< 1 year of age), chemical immobilization was preceded by physical restraint using net and Y-pole, followed by hand-syringe injection. The injection site was consistently the large muscle mass of the hindquarters.

Three chemical immobilization protocols were employed across the 20 capture events: (i) a combination of xylazine and tiletamine-zolazepam (XTZ); (ii) medetomidine-ketamine (MK); and (iii) medetomidine-ketamine-acepromazine (MKA) [20–22]. The present study focuses on the 13 wolves anesthetized with the MKA protocol. Planned doses were medetomidine 50 µg/kg (Domitor®, Vetoquinol, Italy), ketamine 4 mg/kg (Imalgene®, Merial, Italy), and acepromazine 0.14 mg/kg (Prequillan®, Fatro, Italy), administered as a combined intramuscular injection. Actual doses delivered, calculated on the basis of individual body weight recorded at capture, are reported in the Results section. Medetomidine immobilization was reversed at the end of the procedure with atipamezole (Antisedan®, Vetoquinol, Italy) administered intramuscularly at five times the

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medetomidine dose [20–22]. Acepromazine was selected as an adjunct to the MK protocol for its sedative properties, peripheral vasodilatory action, and potential to attenuate hyperthermia and stress-related adverse effects [23].

2.4. Clinical Monitoring

All immobilization procedures were conducted at or near the trap site, or in a sheltered handling area on a covered pick-up truck during adverse weather conditions. Body weight was determined at the handling site using a digital dynamometer scale. Once loss of consciousness was confirmed, all procedures—including clinical monitoring, blood sampling, biometric measurements, and radio-collar fitting—were conducted with the animal maintained in right lateral or sternal recumbency, as determined by the individual's clinical condition and logistic requirements at the handling site. Clinical parameters were monitored throughout the immobilization period using a portable multiparameter monitor and/or a stethoscope and a digital thermometer., and included heart rate (HR), respiratory rate (RR), peripheral oxygen saturation (SpO_2), and rectal temperature (T). Physiological parameters were recorded at minimum three times per animal: T1: within 15 minutes of complete induction, i.e., the finding of loss of consciousness / the moment of starting the handling; T2: within the following 15 minutes; T3: within the subsequent 15 minutes or immediately before antagonist administration. Statistical summaries are based on per-animal means, each representing the mean of three recordings obtained at these standardized intervals. All individual values were retained in the analysis. Condition-specific clinical findings observed in individual animals are discussed separately in the relevant sections. For SpO_2, only values recorded prior to supplemental oxygen administration were included in the analysis; oxygen supplementation was performed in four individuals (M1, F4, M5, F7), in all cases after 30 minutes from induction. All wolves were fitted with GPS/GSM radiocollars (Followit®, Lindesberg, Sweden, or Vectronic®, Berlin, Germany), programmed with an intensive monitoring protocol to allow continuous assessment of animal health status, activity, and survival in the post-capture period. For each captured individual, the minimum confirmed post-capture survival period was determined through continuous GPS/GSM radiocollar tracking. In cases where collar functionality ceased prior to the end of the monitoring period, survival was further documented through alternative methods, including field recovery of injured (individual F6) or deceased animals, and detection via camera trapping. This multi-method approach allowed the establishment of minimum survival periods for all 13 individuals regardless of collar operational status.

2.5. Hematological, Biochemical, Venous Emogas and Serological Analyses

Blood samples were collected from the jugular or cephalic vein during immobilization and processed within 24 hours of collection at the IZSAM Central Laboratory (Istituto Zooprofilattico Sperimentale Abruzzo e Molise—Teramo, Italy). Complete blood count (CBC) was performed on whole blood samples using a cytochemical automated analyser (procedure IZS TE S5 B2.1.6 SOP003-Rev0-07). Parameters assessed included white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), haemoglobin distribution width (HDW), platelet count (PLT), mean platelet volume (MPV), and differential leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells, LUC). A core panel of serum biochemical parameters was analysed at IZSAM according to procedure IZS TE S5 B2.1.6 SOP001-Rev1-07. Aspartate aminotransferase (AST/GOT), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT/GPT), amylase, and alkaline phosphatase (ALP) were measured by kinetic assay; blood urea nitrogen (BUN) and glucose by enzymatic assay; uric acid, cholesterol, and creatinine by enzymatic-colorimetric assay; triglycerides by kinetic-colorimetric assay; total protein, albumin, total bilirubin, and calcium by colorimetric assay. Additional biochemical parameters—specifically globulins (GLOB), phosphorus (PHOS), potassium (K^+), and sodium (Na^+)—were

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analysed at the Wildlife Research Center laboratory of Maiella National Park using the Vetscan® VS2 analyzer (version 2.1.5; Zoetis, formerly Abaxis, Inc., Union City, CA, USA; Comprehensive Diagnostic Profile rotor #500-0038). Venous blood gas analysis was performed in four individuals presenting potentially critical clinical conditions. Analysis was conducted in the field immediately after blood collection using a handheld blood gas analyzer (i-STAT 1®, Abbott Laboratories, Abbott Park, IL, USA) with CG4+ and EC8+ cartridges. Arterial blood sampling was not feasible under the logistic constraints of field capture operations; venous samples were therefore obtained as the available alternative. Of the parameters measured, venous pH and lactate concentrations were selected for reporting as the most clinically relevant indicators of acid-base status and metabolic stress in the context of capture and chemical immobilization. In order to evaluate any animal exposure to potential pathogens able to influence the health status, antibodies against canine protoparvovirus 1 (CPV), canine adenoviruses (CadVs), canine distemper virus (CDV), Canid alphaherpesvirus 1 (CHV), *Ehrlichia* spp., and *Leptospira* spp. were measured as previously described [24,25].

2.6. Ethical Statement

All capture and immobilization procedures were carried out under authorization from the Italian Ministry of Environment (permit No. 026416, 22 June 2009, extended by permit No. 46090, 2014), issued pursuant to Presidential Decree No. 357/1997, which transposes Council Directive 92/43/EEC (Habitats Directive) into Italian national law. Ministerial authorization was granted on the basis of a formal technical opinion issued by ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale), the Italian national authority competent for wildlife conservation and research. In the Italian regulatory framework applicable during the study period, the ISPRA technical opinion encompasses both conservation and animal welfare considerations, and is functionally equivalent to the Institutional Animal Care and Use Committee (IACUC) approval or Institutional Review Board (IRB) statement required in other national jurisdictions.

All procedures were conducted in accordance with current Italian and European legislation on the protection of wildlife, and in compliance with Council Regulation (EEC) No. 3254/91 of 4 November 1991, which prohibits the use of leghold traps within the European Union and establishes humane trapping standards for species listed in Annex I of that Regulation, within which the grey wolf (*Canis lupus*) is included.

2.7. Data Availability and Use of Generative AI

The dataset supporting the results of this study is available upon request from the Wildlife Research Center of Maiella National Park—Veterinary Service (simone.angelucci@parcomajella.it). In the preparation of this manuscript, the authors used Claude (Anthropic, claude.ai) to assist in English translation, scientific writing, and formatting. All content was reviewed, verified, and validated by the authors, who take full responsibility for the accuracy and integrity of the published work.

3. Results

3.1. Animal Demographics and Capture Conditions

Thirteen free-ranging Apennine wolves (*Canis lupus italicus*) were captured and immobilized with the MKA protocol between June 2010 and July 2017 in Maiella National Park. The cohort comprised 6 males and 7 females, of which 10 were adults (4 males, 6 females) and 3 were juveniles (2 males, 1 female). Mean body weight was 29.52 ± 5.22 kg (range: 22.0–38.3 kg) in adults and 16.60 ± 2.92 kg (range: 13.5–19.3 kg) in juveniles.

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Table 1. Captured wolves between June 2010 and July 2017 in Maiella National Park with Fremont and MKA protocol.

Wolf Code MNP	Age at capture	Date of Capture	Weight (Kg)	Min. Post-Capture Survival Per.
F1	25 months	18/06/2010	28	18 months
F2	18 months	02/12/2010	25	48 months
M1	8 months (*juv.*)	17/01/2011	13,5	8 months
F3	7 months (*juv.*)	11/11/2011	17	7 months
M2	24 months	05/05/2012	38,3	24 months
M3	18 months	15/11/2012	27	48 months
M4	23 months	06/04/2013	33,1	9 months
F4	48 months	08/04/2013	29,4	11 months
M5	6 months (*juv.*)	11/10/2013	19,3	4 months
F5	18 months	23/11/2014	31	26 months
M6	12 months	04/05/2016	36,4	15 months
F6	25 months	03/06/2017	25	57 months
F7	14 months	11/07/2017	22	17 months

The mean time elapsed between trap activation and drug administration was 78.00 ± 53.95 minutes (range: 36–240 minutes). The extended maximum interval was attributable to a single case in which a malfunctioning GSM alarm system delayed detection. With the exception of one adult female that required a second dart, all individuals were fully immobilized following a single intramuscular injection.

3.2. Anesthetic Protocol and Drug Doses

Planned doses were medetomidine 50 μg/kg, ketamine 4 mg/kg, and acepromazine 0.14 mg/kg. Mean doses actually delivered are reported in Table 2. Adults received medetomidine 0.052 ± 0.005 mg/kg, ketamine 4.400 ± 0.474 mg/kg, and acepromazine 0.142 ± 0.037 mg/kg. Juveniles received medetomidine 0.059 ± 0.016 mg/kg, ketamine 3.989 ± 0.824 mg/kg, and acepromazine 0.149 ± 0.040 mg/kg.

Table 2. Mean ± SD doses (mg/kg) of medetomidine, ketamine, and acepromazine administered to adult and juvenile Apennine wolves immobilized with the MKA protocol.

	Medetomidine (mg/kg)	Ketamine (mg/kg)	Acepromazine (mg/kg)
Adults (n = 10)	0.052 ± 0.005	4.400 ± 0.474	0.142 ± 0.037
Juveniles (n = 3)	0.059 ± 0.016	3.989 ± 0.824	0.149 ± 0.040

3.3. Physiological Parameters During Immobilization

The first clinical sign of drug effect was loss of head posture ("head down"), occurring at a mean of 8.60 ± 2.95 minutes in adults and 6.00 ± 2.00 minutes in juveniles. Full induction was reached at a mean of 17.30 ± 6.36 minutes in adults and 10.00 ± 5.29 minutes in juveniles. Mean respiratory rate was 24 ± 13 breaths/min (range: 13–60). Mean heart rate was 100 ± 15 bpm (range: 84–126 bpm). Mean body temperature was 38.1 ± 1.0 °C (range: 36.0–41.0 °C). Mean SpO_2 (n=12) was 88 ± 11% (range: 66–97%). No case of clinically significant hyperthermia requiring active cooling was recorded in the cohort as a whole. Two individuals (F4 and F7) presented potentially critical clinical findings as detailed in Section 3.5.

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3.4. Recovery Timeline

Atipamezole was administered at 0.4 mg/kg at a mean of 75.90 ± 16.15 minutes post-induction in adults and 62.67 ± 15.50 minutes in juveniles. In juvenile individuals, voluntary head movements were first observed also prior to atipamezole administration, at a mean of 52.00 ± 19.36 minutes post-induction. In adults, voluntary head movements were first observed at a mean of 79.30 ± 19.84 minutes post-induction. Return to stable four-legged posture occurred at a mean of 97.40 ± 27.80 minutes in adults and 75.33 ± 23.69 minutes in juveniles. Autonomous departure from the handling site was recorded at a mean of 108.90 ± 33.14 minutes in adults and 87.33 ± 29.48 minutes in juveniles. No fatalities or severe adverse events occurred during immobilization or recovery.

3.5. Hematological Parameters

Blood samples for hematological analysis were available from 12 of 13 immobilized wolves. Results are summarized in Table 3. Mean values: RBC 7.0 ± 1.1 × 10^{12}/L, hemoglobin 175.1 ± 26.2 g/L, hematocrit 47.8 ± 9.0%, MCV 68.8 ± 5.7 fl, WBC 16.0 ± 5.4 × 10^{9}/L, platelets 244.5 ± 111.2 × 10^{9}/L. Differential counts showed neutrophil predominance (73.2 ± 12.2%). No stress leukogram, significant anemia, or thrombocytopenia was identified. Individual F7 presented hematocrit of 68.2%, consistent with hemoconcentration secondary to dehydration.

Table 3. Hematological parameters (mean ± SD, min and max) recorded in free-ranging Apennine wolves (n = 12/13) immobilized with the MKA protocol.

Parameter	Mean	SD	Min	Max
RBC (×10^{12}/L)	7.0	1.1	5.5	8.9
Hemoglobin (g/L)	175.1	26.2	122.0	217.0
Hematocrit (%)	47.8	9.0	33.3	68.2
MCV (fl)	68.8	5.7	59.9	77.6
MCH (pg)	25.1	3.3	22.0	31.3
MCHC (g/L)	370.1	69.9	282.0	492.0
RDW (%)	20.0	8.7	12.7	35.3
HDW (g/L)	300.4	103.0	159.0	550.0
Platelets (×10^{9}/L)	244.5	111.2	131.0	526.0
MPV (fl)	9.8	3.0	3.7	14.7
WBC (×10^{9}/L)	16.0	5.4	8.2	23.3
Neutrophils (%)	73.2	12.2	45.9	85.8
Lymphocytes (%)	14.6	7.5	7.5	26.8
Monocytes (%)	6.9	6.6	3.6	27.4
Eosinophils (%)	6.4	3.8	0.8	12.3
Basophils (%)	0.8	0.6	0.1	2.5
LUC (%)	0.2	0.2	0.0	0.8

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RBC: red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; HDW: hemoglobin distribution width; MPV: mean platelet volume; WBC: white blood cell count; LUC: large unstained cells.

3.6. Serum Biochemistry and Venous Blood Gas Analysis

Serum biochemical results are summarized in Table 4. Mean total protein was 68 ± 10 g/L, albumin 31 ± 6 g/L, globulins 47 ± 14 g/L. Hepatic enzyme activities: ALT 79 ± 56 IU/L, AST 161 ± 177 IU/L, ALP 92 ± 49 IU/L. Mean creatinine 79.23 ± 28 µmol/L, BUN 12.48 ± 6 mmol/L, glucose 5.27 ± 2 mmol/L. Individual F4 (pregnant female) exhibited elevated total protein and reduced albumin consistent with pregnancy-associated hypoalbuminemia. Individual F7 showed markedly elevated AST (699 IU/L) and ALT (195 IU/L), indicative of transient capture-related myopathy.

Table 4. Serum biochemistry parameters (mean ± SD, min and max) recorded in free-ranging Apennine wolves (n = 12/13) immobilized with the MKA protocol.

Parameter	Mean	SD	Min	Max
Total Protein (g/L)	68	10	59	90
Albumin (g/L)	31	6	16	38
Globulins (g/L)	47	14	32	74
Glucose (mmol/L)	5.27	2.0	2.0	7.0
ALT (IU/L)	79	56	26	195
ALP (IU/L)	92	49	49	195
AST (IU/L)	161	177	52	699
Creatinine (µmol/L)	79.23	28.0	42.0	133.0
BUN (mmol/L)	12.48	6.0	4.0	23.0
Amylase (IU/L)	202	51	133	340
Total Bilirubin (µmol/L)	3.70	1.0	2.0	5.0
Cholesterol (mmol/L)	4.00	1.0	3.0	5.0
Triglycerides (mmol/L)	0.48	0.3	0.0	1.0
Uric Acid (µmol/L)	70.76	14.0	54.0	101.0
Calcium (mmol/L)	2.26	0.3	2.0	3.0
Phosphorus (mmol/L)	1.38	1.0	1.0	3.0
Sodium (mmol/L)	144	11	125	157
Potassium (mmol/L)	4.18	1.0	3.0	5.0

ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BUN: blood urea nitrogen.

Venous blood gas analysis was performed in 6 of 13 captured wolves (F2, M3, M4, F4, M6, F7). The remaining 7 individuals could not be sampled due to logistical constraints inherent to field capture conditions. Venous pH ranged from 7.221 to 7.429 (mean ± SD: 7.386 ± 0.064). Four of six individuals (F2, M3, M4, M6) presented pH values within or immediately adjacent to the canine venous reference range (7.35–7.45) [26]. Two individuals (F4 and F7) presented pH values below 7.30 (7.274 and 7.221, respectively), indicating mild to moderate venous acidosis at the time of sampling. Venous lactate ranged from 1.02 to 3.49 mmol/L (mean ± SD: 1.50 ± 0.97 mmol/L). Four individuals (F2, M3, M4, M6) showed lactate values within the reference range (0.43–2.10 mmol/L) [26] and similar to those previously reported in healty dogs (<2.5 mmol/L, <2.6 mmol/L) [27,28]. The two individuals presenting lower pH values (F4 and F7) also showed the highest lactate concentrations (3.23 and 3.49 mmol/L, respectively), consistent with a transient stress- and exertion-related lactic acidosis at the time of capture.

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Table 5. Venous blood gas parameters measured at capture in free-ranging Apennine wolves (*Canis lupus italicus*, n = 6) immobilized with the MKA protocol and restrained with the Fremont™ Humane Foot Snare.

Wolf Code MNP	pH	Lactate (mmol/L)
F2	7.429	1.05
M3	7.397	1.13
M4	7.420	1.05
F4	7.274	3.23
M6	7.410	1.02
F7	7.221	3.49
Mean ± SD	**7.386 ± 0.064**	**1.50 ± 0.97**

Reference range (canine venous): pH: 7.35–7.45; Lactate: 0.43–2.10 mmol/L (Bachmann et al., 2018).

3.7. Serological Analyses

Serological investigations were carried out on 12 individuals. Ten animals were positive for CDV with antibody titers ranging from 1:4 to >= 1:256. Nine animals were positive for CadVs with antibody titers ranging from 1:4 to >= 1:256. Eight animals were positive for CPV with antibody titers ranging from 1:16 to 1:256. Five animals were positive for CHV with antibody titers ranging from 1:8 to 1:32.

3.8. Physical Examination and Injury Assessment

Physical examination revealed minimal trauma. A total of 61.5% of wolves (8/13) showed no detectable foot lesions. The remaining 38.5% (5/13) exhibited first-degree excoriations without deep tissue involvement. Reversible distal limb edema was observed in 30.8% of individuals (4/13). No skeletal or tendinous injuries were recorded. Minor oral mucosal lesions were noted in 46.2% of individuals (6/13), attributable to snare-biting behavior prior to chemical immobilization.

4. Discussion

4.1. Capture Method and Pre-Induction Stress Management

The Fremont™ Humane Foot Snare proved effective for the capture of both adult and juvenile Apennine wolves, confirming its applicability across age classes in field conditions. The mean time elapsed between trap activation and drug administration was 78.00 ± 53.95 minutes, an interval that, despite its duration, was not associated with critical physiological disturbances in the majority of captured individuals. This observation has important implications for the understanding of capture-related stress in large canids.

Direct observation via camera traps positioned at capture sites revealed a characteristic behavioral pattern: following initial restraint, wolves engaged in organized, goal-directed escape attempts—struggling against and biting the snare cable—but remained relatively calm during the period preceding the arrival of the capture team. A marked increase in behavioral stress was consistently observed at the moment wolves detected the approaching vehicle and operators, with the peak stress response occurring during the final approach phase. This field observation is consistent with the hypothesis that the dominant driver of capture-associated physiopathology in wild mammals is not the duration or intensity of physical exertion per se, but rather the psychological component of the stress response—specifically, the perception of an approaching "predator" [20,23]. This hypothesis finds support in comparative capture studies across taxa. In impalas (*Aepyceros melampus*), the magnitude of body temperature rise during chemical immobilization was shown to be determined primarily by the psychological fright response experienced during capture, rather than by the pharmacological properties of the immobilizing drugs [29]. The duration of consciousness—from darting to recumbency—emerged as the key variable influencing thermal response, with stress-induced hyperthermia appearing to be centrally regulated via an elevated

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thermoregulatory set-point, rather than driven solely by catecholamine-mediated vascular and metabolic effects.

The practical implication for field operations is clear: minimizing the duration and intensity of anthropogenic stimulation from first detection by the animal through to complete anesthetic induction is the single most important welfare intervention available to the capture team. Based on our experience, the approach to the trapped animal must be immediate and decisive, drug delivery must be rapid and efficient, and operators must withdraw as soon as possible after injection, minimizing visual and auditory stimuli during the induction phase. The use of night-vision equipment to monitor induction at distance is recommended where feasible, and repeated approach attempts before 15 minutes post-injection should be avoided. These procedural recommendations are consistent with published guidance on minimizing exertional and psychological stress during wildlife capture [17,20,23].

4.2. Anesthetic Protocol: Induction, Physiological Parameters, and the Role of Acepromazine

Mean induction times of 17.30 ± 6.36 minutes in adults and 10.00 ± 5.29 minutes in juveniles are consistent with published data for MK-based protocols in free-ranging gray wolves and other large canids [20]. The shorter induction times in juveniles likely reflect differences in body mass, metabolic rate, and behavioral response to handling [30,31]. In juvenile individuals, voluntary head movements were observed prior to atipamezole administration, suggesting spontaneous partial recovery from immobilization before antagonist delivery. This finding is consistent with the more rapid pharmacokinetic clearance expected in younger and lighter individuals.

The MKA protocol produced stable anesthesia with no cases of clinically significant hyperthermia in the cohort as a whole. Drew and Struthers (2025) [30], in a retrospective analysis of 490 captures in Idaho, demonstrated that ketamine-medetomidine was associated with significantly higher initial body temperatures than tiletamine-zolazepam, with hyperthermia (≥41 °C) occurring in 13.7% of summer and 26.7% of winter aerial captures. Initial hyperthermia was also documented in captive red wolves anesthetized with MKA and other alpha-2-ketamine combinations by [22].

The mean body temperature recorded in our cohort (38.1 ± 1.4 °C, range: 35.8–40.7 °C) was notably lower than the baseline values reported for free-ranging gray wolves at rest (39.7 ± 0.08 °C) and during short-duration exertion (40.2 ± 0.14 °C), and well below the hyperthermia threshold of 41 °C. While multiple contextual factors preclude any definitive attribution of this finding to a single variable, two elements are likely to have contributed. First, the strict field management protocol—with immediate approach, rapid drug delivery, and prompt withdrawal of personnel during the induction phase—was specifically designed to minimize the duration and intensity of psychoemotional stimulation, which is recognized as a primary driver of stress-induced hyperthermia in captured wildlife [29]. Second, the peripheral vasodilatory properties of acepromazine, through alpha-1 adrenergic blockade, may have promoted cutaneous heat dissipation, potentially contributing to the favorable thermal profile observed. The relative contribution of each of these factors cannot be determined from the present dataset, and further controlled studies would be required to disentangle their respective roles.

The use of medetomidine carries an inherent risk of ventilation-perfusion mismatch. Alpha-2 adrenoceptor agonists cause pulmonary vasoconstriction, raising pulmonary arterial pressure and altering regional blood flow distribution. In a frightened, physically active animal, the cascade—psychological stress → exertional myopathy → lactic acidemia → hypoxemia → hyperthermia → tissue damage—represents the core pathophysiological risk in large carnivore capture [20,23,30]. In our cohort, mean SpO_2 of 86 ± 10% (range: 65.5–97.0%) indicates that hypoxemia was present in a subset of individuals, consistent with the V/Q mismatch expected with medetomidine-based immobilization.

The cardiorespiratory data from [22] in captive red wolves provide the most directly comparable published reference for our MKA cohort. Heart rates in the MKA group ranged from 87.6 to 102.4 bpm across measurement intervals, values closely comparable to our mean of 100 ± 15 bpm. The

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absence of blood pressure monitoring in our field study represents a limitation: future captures should incorporate non-invasive blood pressure assessment where logistically feasible, as emphasized by Morelli et al. (2020) [31]. Comparative data from Talukdar & Raina (2023) [32] on Tibetan wolves (*Canis lupus chanco*) and Gutema et al. (2018) [33] on African wolves (*Canis lupaster*) further confirm the importance of thermal monitoring in all alpha-2-ketamine field protocols.

4.3. Hematological and Biochemical Parameters: Baseline Reference Values and Individual Cases

To our knowledge, this study provides the first systematic report of hematological and serum biochemical reference intervals for free-ranging Apennine wolves (*Canis lupus italicus*) captured with a humane foot snare. The values obtained were broadly consistent with published reference data for gray wolves from southcentral Alaska [34], Minnesota, Alaska [35], Scandinavia [36], captive wolves [22], and Iberian wolves captured with leg-hold snares [37]. Hematological findings did not reveal stress leukograms, significant anemia, thrombocytopenia, dehydration, or clinically relevant elevations in muscle enzymes in the majority of individuals. However, the interpretive value of the absence of a stress leukogram requires contextual qualification. Two distinct leukocyte responses to stress must be distinguished: the rapid catecholamine-mediated physiologic leukocytosis, which develops within minutes and resolves within 30–60 minutes of the stimulus, and the corticosteroid-mediated stress leukogram, which requires 4–8 hours to develop and peaks at 6–8 hours after glucocorticoid release [38–40]. Neither response would be expected to be detectable at the time of sampling in this study: the catecholamine response had already resolved by the time blood was collected following anaesthetic induction, and the corticosteroid-mediated pattern had not yet had sufficient time to develop within the mean handling duration of this study (autonomous departure from the handling site was recorded at a mean of 108.90 ± 33.14 minutes in adults and 87.33 ± 29.48 minutes in juveniles). Additionally, physiologic leukocytosis from catecholamines is known to be particularly weak and clinically undetectable in adult dogs, rarely producing values outside the reference interval in this species [38]. Accordingly, the absence of leukogram changes reflects the sampling timeframe rather than the absence of a stress response per se, and should be interpreted with this limitation in mind. The absence of other hematological indicators of systemic compromise nonetheless provides supportive, if partial, evidence for the welfare adequacy of the combined capture-anaesthesia protocol under the conditions described. Two individuals presented condition-specific findings. Individual F4, an adult female captured in mid-pregnancy, exhibited the highest respiratory rate (60 breaths/min) and heart rate (126 bpm) in the cohort. These values are consistent with the physiological adaptations expected in mid-to-late gestation: progressive uterine distension reduces functional residual lung capacity, increases respiratory work, and elevates cardiac output requirements. The biochemical profile of F4, elevated total protein with reduced albumin, is consistent with the gestational hemodilution-associated hypoalbuminemia documented in dogs from mid-pregnancy onward [41,42], and analogous to that described in other mammalian species during gestation.

The venous blood gas values recorded in individual F4 (pH 7.274, lactate 3.23 mmol/L) represent the most marked acid-base deviation in the cohort. According to current definitions, hyperlactataemia is classified as a mild to moderate increase in lactate concentration (2–5 mmol/L) without concurrent metabolic acidosis, whereas lactic acidosis is defined as a persistently elevated lactate concentration, typically exceeding 5 mmol/L, in association with metabolic acidosis (pH < 7.35). By these criteria, the values recorded in F4 fall within the range of mild to moderate hyperlactataemia with concurrent mild acidaemia, and remain below the thresholds defining lactic acidosis. The internal consistency between the pH and lactate values confirms their analytical reliability. These findings are therefore attributable to the combined physiological demands of capture stress and advanced pregnancy, and are not indicative of a pathological condition. Critically, post-capture GPS monitoring confirmed that F4 successfully delivered and raised four pups in the subsequent summer, demonstrating the absence of lasting reproductive impact from the capture event.

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Individual F7, a 13-month-old female, presented hematocrit of 68.2% and markedly elevated AST and ALT activities, findings indicative of hemoconcentration secondary to dehydration and transient capture-related myopathy. Elevated transaminase activities following capture stress and muscular exertion in wild canids have been documented previously, with studies reporting increased LDH levels in hyperthermic wolves [30], elevated CK levels in trapped Iberian wolves [37], and generally higher enzyme activities in free-ranging versus captive wolves [35]. In F7, the pattern of enzyme elevation in the absence of other systemic abnormalities is consistent with a self-limiting, capture-stress-mediated event. The elevated LDH values observed in hyperthermic Idaho wolves by Drew and Struthers [30] provide a useful comparative benchmark, confirming that CK, AST, and LDH are established markers of exertional rhabdomyolysis in the capture medicine literature.

Venous blood gas data, available for 6 of 13 captured individuals, indicate that frank acidosis did not develop in the majority of assessed cases. Four of six wolves presented pH values consistent with physiological compensation, while two individuals (F4 and F7) showed pH values below 7.30 accompanied by elevated lactate concentrations (3.23 and 3.49 mmol/L, respectively). This pattern is consistent with a transient, exertion- and stress-related lactic acidosis, typically observed in wild canids subjected to physical restraint and chemical immobilization, and is not considered indicative of a pathological anaesthetic complication in the absence of concurrent cardiovascular or respiratory signs.

The lactate values recorded in the two outlier individuals warrant contextual interpretation. Elevated perioperative lactate in wild wolves has not been systematically characterized in the field literature; however, Mattaliano et al. (2023) [43], reporting on three captive grey wolves undergoing prolonged general anaesthesia for dental procedures, documented intraoperative venous acidosis (pH 7.10–7.33) and electrolyte disturbances including severe hyperkalaemia (up to 8.8 mmol/L), associated with prolonged recumbency, use of α2-adrenoceptor agonists, and extended anaesthetic duration. Although the clinical scenario described by those authors—captive wolves under isoflurane maintenance for 5.5–10 hours—differs substantially from the short-duration field immobilization reported here, their findings underscore the importance of monitoring acid-base and electrolyte parameters in wolves undergoing anaesthesia, and highlight the potential for hyperkalaemia-associated bradyarrhythmia as a time-dependent complication of medetomidine-based protocols. In the present cohort, immobilization duration was consistently brief, and no cardiovascular complications were recorded in any individual, suggesting that the risk factors identified by Mattaliano et al. (2023) [43], namely prolonged anaesthetic time and sustained α2-adrenoceptor agonist exposure, were not operative under field conditions.

The absence of arterial blood gas capability in the field precluded assessment of PaO_2, $PaCO_2$, and derived parameters such as alveolar-arterial oxygen gradient, which would have allowed more precise characterization of respiratory function during immobilization. Acquisition of portable arterial blood gas capability is strongly recommended for future wildlife capture protocols, as it would substantially enhance physiological monitoring and contribute to the growing evidence base on anaesthetic safety in free-ranging protected animals.

Serological results indicate previous exposure to common canid pathogens, mainly CDV, CadVs and CPV, as already reported in other free-ranging wolf populations [44–46]. Given the small sample size, these findings should be interpreted primarily as an indicator of pathogen circulation rather than as evidence of a major impact on the health status of the captured wolves. This interpretation is further supported by direct investigations performed in two wolves, which confirmed the circulation of CPV-2b and CAV-2, as previously described by Di Francesco et al. (2019) [24]. The limited positive results for Leptospira spp. and Canid alphaherpesvirus 1 further suggest occasional exposure.

4.4. Physical Trauma and Welfare Assessment

Physical injuries associated with restraining trap systems have been systematically categorized by Proulx (2022) [18], who elaborated an Injury Score System based on International Mammal Trapping Standards, distinguishing direct injuries, caused by the mechanical action of the snare, from

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indirect injuries resulting from the animal's behavioral response to capture. The injury profile observed in this cohort is consistent with the lowest severity categories of this system. Using a modified foot injury [47], 61.5% of wolves (8/13) presented no excoriations, and the remaining 38.5% showed only superficial cuts less than 2.5 cm in total (Grade 2), without skeletal or tendinous involvement. These findings contrast markedly with early baseline data on steel foothold traps, in which Van Ballenberghe (1984) [48] documented severe foot and leg injuries (Class III–IV) in 41% of adult captures, including cases of bone fracture and near-amputation of distal limbs—injury categories entirely absent in the present cohort.

These results confirm that direct injuries are minimal or negligible when: (i) the snare incorporates springs and swivels that absorb tension and allow rotational movement; (ii) the trap is positioned at sites whose topographic and vegetational characteristics do not predispose to traumatic impact; and (iii) the capture team reaches the trap site within the protocol-defined response time of 30 minutes from activation, substantially reducing both direct and indirect injuries and the systemic responses that can compromise animal welfare [17,18]. It should be noted that the mean time elapsed between trap activation and drug administration recorded in this study (78.00 ± 53.95 minutes; range: 36–240 minutes) does not reflect the response time to the trap site, but rather the total duration of a multi-step operational sequence that follows arrival at the site: visual assessment of the restrained animal, estimation of body weight and calculation of drug dosages, approach, and—depending on age class—either dart delivery by pole syringe or blowpipe in adults, or mechanical restraint with Y-pole and net followed by manual injection in juveniles. These procedural steps are operationally necessary and inherent to safe and welfare-compliant immobilization in complex mountain terrain. Oral mucosal lesions, observed in 46.2% of individuals, reflect purposeful snare-directed behavior rather than panic-driven trauma, and are a function of the duration of conscious restraint prior to drug effect. This proportion is consistent with the 46% rate of tooth, lip, and gum injuries reported by Van Ballenberghe (1984) [48] in wolves captured with steel foothold traps, suggesting that oral mucosal involvement represents an intrinsic feature of mechanical conscious restraint across device types, rather than a specific indicator of compromised welfare. In the European regulatory context, Gethöffer et al. (2022) [49] documented the feasibility of net box traps as an alternative capture method. The Fremont™ snare, operating within the framework of European humane trapping standards, represents a pragmatic and welfare-compatible alternative where its use is legally permissible.

Although systematic analysis of post-release movement data falls outside the scope of the present study and will be addressed in a dedicated publication, preliminary GPS observations available for a subset of captured individuals allow a qualitative characterization of the immediate post-release behavioral response, presented here for contextual completeness. A preliminary qualitative analysis of GPS tracking data available for three individuals (M2, F2, F3) provides initial, necessarily tentative insights into this behavioral pattern. Given the very limited sample size, no statistically robust inference can be drawn, and the following observations should be interpreted exclusively as preliminary descriptive data warranting systematic investigation in future studies. In all three cases, active displacement from the capture site ceased within approximately two hours of release, after which the animals alternated brief relocations of 300–1,800 m with extended stationary periods lasting 1.5 to 21 hours. The ratio of active to total GPS fixes in the first 48 hours post-release was consistently low across individuals (19/96 for M2; 15/48 for F2; 33/96 for F3), reflecting a predominance of immobility over directed movement during this early post-capture window. Notably, in all three cases the animals did not return immediately to the core of their established home range but instead selected refuge sites at intermediate distances from the capture location, suggesting a pattern of sequential short-range relocations toward perceived safe areas rather than a single directed return movement. The potential behavioral significance of this pattern, while again emphasizing its preliminary nature given the sample of three individuals, may be better appreciated in the context of baseline movement data available for wolves in the central Apennines. Under normal ranging conditions, resident wolves in this region travel an estimated mean of approximately 27 km

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per night at average speeds of 2.5 km/h [50], within annual home ranges averaging 104 ± 24 km² for pack members [51]. More directly comparable baseline data are available from 24-hour continuous GPS monitoring sessions conducted on individuals belonging to the same Maiella population between June 2010 and May 2011, yielding mean daily travel distances of 10.8 km (n = 6 sessions) for individual F1, 13.3 km (n = 3 sessions) for individual F2, and 5.5 km (n = 4 sessions) for individual M1 [52]. These values, obtained with the same GPS methodology and in the same population, provide a direct reference against which the post-release displacements of 600–4,400 m recorded in the first 48 hours for M2, F2, and F3 can be qualitatively evaluated, indicating a reduction in daily ranging activity of approximately one order of magnitude relative to normal movement levels. In the same population and study period, in a manuscript currently under peer review [53] reported seasonal home ranges averaging 138.6 km² in summer and 67.6 km² in winter for resident individuals, and notably excluded the first 10 days of post-capture GPS data from home range analyses in order to minimize biases attributable to post-capture behavioral disruption, explicitly acknowledging this as a systematic source of movement pattern alteration requiring methodological correction.

While these observations are insufficiently powered to constitute a formal demonstration, they suggest the hypothesis that the post-capture quiescence documented in this subset may reflect a predominantly psycho-emotional stress response, an adaptive behavioral strategy in which the animal minimizes energetic expenditure and exposure while recovering from the aversive capture experience, rather than a pharmacological or orthopedic effect. This interpretation is further supported by Gese et al. (2019) [17], who observed that even wolves presenting no or low injury scores at gross examination showed measurable delays in resuming normal space use, attributing this finding to subcutaneous injuries, including edematous swelling, tissue maceration, and tendon involvement, not detectable by external inspection alone. The absence of macroscopic lesions of Grade 3 or above in our cohort, combined with the post-release quiescence observed in these three individuals, points to a predominantly behavioral rather than orthopedic mechanism of activity suppression. This interpretation aligns with current understanding of stress-coping behavior in social carnivores, including wolves specifically, in which capture has been documented to elicit both physiological and behavioral stress responses [37], and warrants systematic investigation in future studies with adequate sample sizes and standardized GPS fix intervals across all captured individuals.

Taken together, the survival data reported in Table 1, the absence of capture-related mortality across all 13 individuals, the hematological and serum biochemical findings, which, with the notable exception of the two individuals discussed above, did not reveal parameters of clinical concern and remained within or close to reference ranges documented for the species, and the post-release behavioral pattern documented in this subset collectively substantiate the welfare adequacy of the combined Fremont™ snare–MKA protocol under the field conditions described.

5. Conclusions

The wolf capture programme described in this paper represents a significant research and conservation initiative that has inaugurated, in Italy, a new era of scientific investigation on the Apennine wolf. The data obtained from GPS radiocollars have enabled detailed characterization of home range dynamics, movement patterns, activity rhythms, social relationships among pack members, and wolf-human interactions. Live capture has therefore been indispensable for establishing an adequate conservation and management framework, and remains so today for supporting specific management actions aimed at addressing the challenges arising from an increasingly complex wolf-human interface, a complexity that is itself a direct expression of the success of conservation efforts implemented over the past five decades.

This study has compiled the information necessary to document a combined mechanical and chemical immobilization protocol that, on the basis of current evidence, appears to be the most appropriate approach given the ecology and ethology of the Apennine wolf. All available parameters have been systematically analyzed to assess compliance with animal welfare standards for the

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individuals subjected to capture and monitoring. In doing so, this work opens both a necessity and an opportunity: to improve field monitoring systems and patient support protocols, with the aim of preventing the onset of pathological conditions or, in the worst case, capture-related mortality that could arise as an unintended consequence of scientific research activity.

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Institutional Review Board Statement: All capture and immobilization procedures were carried out under authorization from the Italian Ministry of Environment (permit No. 026416, 22 June 2009, extended by permit No. 46090, 2014), issued pursuant to Presidential Decree No. 357/1997, which transposes Council Directive 92/43/EEC (Habitats Directive) into Italian national law. Ministerial authorization was granted on the basis of a formal technical opinion issued by ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale), the Italian na-tional authority competent for wildlife conservation and research. In the Italian regu-latory framework applicable during the study period, the ISPRA technical opinion en-compasses both conservation and animal welfare considerations, and is functionally equivalent to the Institutional Animal Care and Use Committee (IACUC) approval or Institutional Review Board (IRB) statement required in other national jurisdictions. All procedures were conducted in accordance with current Italian and European leg-islation on the protection of wildlife, and in compliance with Council Regulation (EEC) No. 3254/91 of 4 November 1991, which prohibits the use of leghold traps within the European Union and establishes humane trapping standards for species listed in An-nex I of that Regulation, within which the grey wolf (Canis lupus) is included.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the results reported in this study are not publicly archived but are available upon reasonable request from the corresponding author. All raw data are stored at the Wildlife Research Center, Majella National Park, Via del Vivaio, 65023 Caramanico Terme (PE), Italy, and may be requested by contacting simone.angelucci@parcomajella.it.

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Abbreviations

The following abbreviations are used in this manuscript:

MKA	Medetomidine. Ketamine—Acepromazine

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