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CICLO XXXV

ETHICAL EVALUATION IN WILDLIFE CONSERVATION: ART, ANIMAL-VISITOR INTERACTIONS AND EMERGENCIES IN WILDLIFE MANAGEMENT AND CONSERVATION

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Abstract

In the global biodiversity crisis, it is increasingly crucial to evaluate ethically relevant issues and consider the pluralistic nature of biodiversity conservation. Conservation ethics provides tools to perform such evaluation and assist in the decision-making processes. This Ph.D. thesis presents studies in which ethical tools are used to perform ethical evaluation and multidisciplinary assessments to approach conservation projects and wildlife management. Three different areas of application of conservation ethics are discussed: Conservation ART, animal-visitor interactions, and challenges in wildlife management during the COVID-19 emergency.

In the first area, ethical evaluation has been applied in the context of the BioRescue project, an international project in which assisted reproductive technologies (ARTs) are used in the effort to save the endangered northern white rhinoceros (*Ceratotherium simum cottoni*). Assisted reproductive technologies can make a difference in biodiversity conservation, but their application can raise ethical issues that need to be addressed. Therefore, firstly, an Ethical Matrix (EM) has been used to present a framework for the ethical analysis of the application of ART procedures in conservation. The EM, specifically built around the ovum pick-up (OPU) procedures carried out on white rhinoceros, allowed to collect ethically relevant factors to identify issues and value conflicts, evaluates complex moral scenarios where different needs, interests, and ethical concerns may conflict, and provides a template for the assessment of ART procedures in projects involving endangered species. Therefore, a new ethical evaluation tool (ETHAS) specifically developed to assess ART procedures in conservation is presented, and the first application results are reported. ETHAS, with its two checklists, provides an integrated, multilevel, and standardized self-assessment of the procedure under scrutiny, generating an ethical acceptability ranking and allowing for implementing measures to address or manage issues beforehand. ETHAS customized for OPU and *in vitro*

fertilization procedures performed on the northern white rhinoceros allowed for ensuring a high standard of procedures, improving some aspects of the communication among the projects' partners, and improving the tool itself, in order to be applied in the near future to other contexts in which ARTs are applied for the conservation of other mammal species. Finally, in the last study presented in the first section, the ethical matrix, decision tree, and Bateson's cube have been adapted to assist in the ethical analysis of a complex conservation scenarios relative to the decision regarding whether or not to continue collecting biomaterial on the oldest of the two remaining northern white rhinoceroses. By structuring these tools to implement the different value dimensions (environmental, social, and animal welfare) involved in conservation ethics, it has been possible to gather ethical pros and cons, compare the different options at stake, and establish a threshold of ethical acceptability. The application of the tools was pivotal in structuring the decision-making process and helping reach the shared, reasoned, and the transparent decision to discontinue Najin from any further oocyte collection procedures.

Conservation ethics can also assist in exploring the ethical issues concerning wildlife management during animal-visitor interactions (AVI). In this regard, Section 2 of this thesis presents studies concerning AVIs. Firstly, a participatory process has been followed with an Ethical Matrix to explore welfare and management issues related to AVIs. The inclusion of the stakeholders' perspectives allowed to record all the value demands concerning AVI and provide a map of the ethically relevant aspects involved. This map shows how the ethical acceptability of AVIs is linked to different relevant issues like animal welfare, education, and biodiversity conservation. Moreover, the results of the study highlighted the importance of cooperation among stakeholders.

Given that zoos and interactions between zoo visitors and animals play a role in conservation education, naive observers' perceptions of the emotional state of the animals could be a valuable tool to assess animal welfare. Therefore, it has been investigated how three distinct groups of people (i.e., experts, naïve adults, and children) perceived the behavior of African elephants in its emotional connotations, using free choice profiling assessment (FCP). Including children and adults in the assessment provides an idea of the emotional impact on visitors of the "elephants' experience," and the results are, therefore, potentially useful in ethical evaluations when considering the interests of visitors. Additionally, it investigated whether a difference existed between the descriptors generated by experts observing elephants in captive management and those in semi-captive management and whether similar differences could be found amongst other observers. This study suggested that the captive and semi-captive environment in which the animals are kept may influence their emotional expression. The third study presented was dedicated to the application of a protocol for the ethical assessment of AVI, the Animal-Visitor Interaction Protocol (AVIP). AVIP allows a multidisciplinary and integrated assessment of AVIs, providing a final rational ethical analysis to highlight potential conflicts of values and develop target actions. As lemur walk-in enclosures are very common in zoos, we applied AVIP to an AVI involving five ring-tailed lemurs (*Lemur catta*) hosted at Pistoia Zoo (Italy). AVIP evidenced some potential ethical concerns, but the final outcomes indicated that these conflicts were well managed, and there were no reasons to discontinue the AVI proposed to Pistoia zoo's visitors. Moreover, the first application of AVIP to animal encounters involving non-human primates confirmed its potential as a tool for the overall evaluation of AVIs, following the One Health – One Welfare approach.

The third area of research was dedicated to investigating the challenges of Italian zoological facilities during the COVID-19 emergency. In a historical moment when the pandemic has impacted everyone's lives, Zoological facilities never stopped caring for the animals they host and pursuing their educational, conservation, and research roles. A national survey has asked zoological facilities staff to give their opinion and share their experience in their daily work during and after the pandemic. Despite the different roles of the respondents, results showed that all the zoological staff agreed that the pandemic significantly impacted zoological facilities in all their sectors. In particular, results highlighted a shared concern regarding the economic damage and the educational activities carried out in different forms.

Thanks to the tools provided by conservation ethics, and the new ones developed during this Ph.D. project, it has been possible to perform an ethical evaluation of conservation projects and wildlife management. All used tools contributed to assessing and considering stakeholders' views and responsibilities and guiding the decision-making when values may conflict in biodiversity conservation.

Abstract (Italiano)

Nell’attuale crisi globale della biodiversità è sempre più cruciale valutare le questioni eticamente rilevanti e considerare la natura pluralistica della conservazione della biodiversità. L'etica della conservazione fornisce strumenti per eseguire tali valutazioni e assistere nei processi decisionali. La tesi di questo dottorato di ricerca presenta studi in cui vengono utilizzati strumenti per eseguire valutazioni etiche e multidisciplinari per valutare progetti di conservazione e gestione della fauna selvatica. Pertanto, questo lavoro di dottorato mostra tre diverse aree di applicazione dell'etica della conservazione: Conservation ART, le interazioni animale-visitatore e le sfide nella gestione della fauna selvatica durante l'emergenza COVID-19.

Nella prima sezione, la valutazione etica è stata applicata nel contesto del progetto BioRescue, in cui le tecnologie di riproduzione assistita (ART) sono utilizzate nello sforzo di salvare il rinoceronte bianco settentrionale (*Ceratotherium simum cottoni*) dall’estinzione. Le tecnologie di riproduzione assistita possono fare la differenza nella conservazione della biodiversità, ma la loro applicazione può sollevare questioni eticamente rilevanti che necessitano di essere affrontate. Pertanto, in primo luogo, è stata utilizzata la Matrice Etica (EM) per presentare un quadro per l'analisi etica dell'applicazione delle procedure ART nella conservazione. L'EM, anche se specificamente costruita attorno alle procedure di prelievo di ovociti (OPU) effettuate su rinoceronti bianchi, ha permesso di raggruppare i fattori eticamente rilevanti, identificare e valutare complessi scenari morali in cui diversi bisogni, interessi e preoccupazioni etiche possono entrare in conflitto e fornire infine un modello per la valutazione delle procedure ART in progetti che coinvolgono altre specie in via di estinzione.

In seguito, viene presentato un nuovo strumento di valutazione etica (ETHAS) specificamente sviluppato per valutare l’applicazione delle procedure ART in conservazione, e vengono illustrati i risultati delle prime applicazioni. ETHAS, con le sue

due liste checklist che lo compongono, permette di effettuare un'autovalutazione integrata, multilivello e standardizzata della procedura in esame, generando una classifica di accettabilità etica e consentendo l'attuazione di misure per affrontare o gestire eventuali problemi in anticipo. ETHAS, specificatamente customizzato per l'OPU e le procedure di fecondazione in vitro eseguite sul rinoceronte bianco settentrionale, hanno permesso di garantire un elevato standard delle procedure, migliorare alcuni aspetti della comunicazione tra i partner del progetto e migliorare lo strumento stesso al fine di essere applicato nel prossimo futuro ad altri contesti in cui le ART vengono utilizzate per la conservazione di altre specie di mammiferi.

Nell'ultimo studio presentato nella prima sezione, la matrice etica, l'albero decisionale e il cubo di Bateson sono stati adattati per assistere nell'analisi etica di un complesso scenario relativo alla decisione se continuare o meno la raccolta di biomateriale sul più anziano dei due rimanenti rinoceronti bianchi settentrionali, Najin. Strutturando questi strumenti per implementare le diverse dimensioni di valore (ambientale, sociale e benessere animale) coinvolte nell'etica della conservazione, è stato possibile raccogliere pro e contro, confrontare le diverse opzioni e stabilire una soglia di accettabilità etica. L'applicazione degli strumenti è stata fondamentale per strutturare il processo decisionale e aiutare a raggiungere la decisione condivisa, ragionata e trasparente di sospendere Najin da qualsiasi ulteriore procedura di prelievo di ovociti.

L'etica della conservazione può anche aiutare ad esplorare le questioni etiche riguardanti la gestione della fauna selvatica durante le interazioni animale-visitatore (AVI) che si svolgono nelle strutture zoologiche. A questo proposito, la Sezione 2 di questa tesi presenta studi riguardanti le AVI. In primo luogo, è stato seguito un processo partecipativo con una matrice etica per esplorare le questioni di benessere e gestione relative alle AVI. L'inclusione delle prospettive delle parti interessate ha permesso di registrare le *value demands* riguardanti le interazioni e fornire una mappa degli aspetti eticamente rilevanti. Questa mappa mostra come l'accettabilità etica degli AVI sia collegata a diverse questioni etiche rilevanti come il benessere degli animali, l'educazione e la conservazione della biodiversità. Inoltre, i risultati dello studio hanno evidenziato l'importanza della cooperazione tra le parti interessate.

Dato che le strutture zoologiche e le interazioni tra i visitatori degli zoo e gli animali svolgono un ruolo nell'educazione alla conservazione, la percezione dello stato emotivo degli animali coinvolti da parte dei visitatori (osservatori non esperti) potrebbe essere uno strumento prezioso per valutare il benessere degli animali. Pertanto, è stato studiato come tre gruppi distinti di persone (esperti, adulti e bambini) hanno percepito il comportamento degli elefanti africani nelle sue connotazioni emotive, utilizzando il free choice profiling (FCP). L'inclusione di bambini e adulti nelle valutazioni fornisce un'idea dell'impatto emotivo dell'interazione con gli elefanti sui visitatori e i risultati sono, quindi, potenzialmente utili nelle valutazioni etiche quando si considerano gli interessi dei visitatori. Inoltre, questo studio ha permesso di indagare se esistesse una differenza tra i descrittori generati da esperti che osservavano gli elefanti gestiti in ambiente controllato e quelli gestiti in semi-cattività, e se tali differenze potessero essere riscontrate anche tra gli altri gruppi di osservatori. I risultati di questo studio hanno suggerito che l'ambiente in cui sono gestiti gli animali (cattività/semi-cattività) può influenzare la loro espressione emotiva. Il terzo studio presentato riguarda l'applicazione di un protocollo per la valutazione etica dell'AVI, l'"Animal-Visitor Interaction Protocol" (AVIP). AVIP consente una valutazione multidisciplinare e integrata delle AVI, fornendo un'analisi etica razionale finale per evidenziare potenziali conflitti di valori e sviluppare azioni mirate. Poiché i lemuri gestiti all'interno di recinti *walk-in* sono molto comuni nelle strutture zoologiche, l'AVIP è stato applicato per valutare una *walk-in* che ospita cinque lemuri dalla coda ad anelli (*Lemur catta*) presso il Giardino Zoologico di Pistoia (Italia). L'AVIP ha evidenziato alcune potenziali preoccupazioni etiche, ma i risultati finali hanno indicato che questi conflitti sono ben gestiti e non risultano motivi per interrompere l'AVI proposto ai visitatori dello zoo. Inoltre, la prima applicazione di AVIP per valutare attività di interazione che coinvolgono primati non umani ha confermato il suo potenziale come strumento per la valutazione complessiva degli AVI, seguendo l'approccio One Health – One Welfare.

Il terzo ambito di ricerca è stato dedicato allo studio delle sfide che le strutture zoologiche italiane hanno dovuto affrontare durante l'emergenza da COVID-19. In un momento storico in cui la pandemia ha avuto un impatto sulla vita di tutti, le strutture zoologiche non hanno mai smesso di prendersi cura degli animali che ospitano e di svolgere il proprio ruolo educativo, di conservazione e di ricerca. Tramite un sondaggio nazionale è stato chiesto a tutto il personale delle strutture zoologiche di esprimere la propria opinione e condividere la propria esperienza e sfide nel lavoro quotidiano durante e dopo la pandemia. Nonostante i diversi ruoli degli intervistati, i risultati hanno mostrato che tutto il personale zoologico concorda sul fatto che la pandemia ha avuto un impatto significativo sulle strutture zoologiche in tutti i loro settori. In particolare, i risultati hanno evidenziato una preoccupazione condivisa per quanto riguarda il danno economico e le attività educative.

Grazie agli strumenti forniti dall'etica della conservazione e quelli nuovi sviluppati durante questo progetto di dottorato, è stato possibile effettuare nuove valutazioni etiche dei progetti di conservazione e della gestione della fauna selvatica. Tutti gli strumenti utilizzati hanno contribuito a valutare e considerare le opinioni e le responsabilità delle parti interessate e a guidare il processo decisionale ogni qualvolta i valori etici nella conservazione della biodiversità potrebbero entrare in conflitto.

General Introduction

The alarming decline in the number of mammals, birds, fish, reptiles, amphibians, invertebrates, and plant species indicates that the sixth phase of mass extinction has already begun. The Anthropocene era, characterized by humans' overexploitation of limited natural resources, has already driven 22% of all mammalian species close to being lost forever (Ceballos et al., 2015; Hildebrandt et al., 2021).

To help biodiversity conservation, scientists must be prepared to preserve the few remaining habitats and species. According to the International Union for Conservation of Nature (IUCN, 2013) guidelines, conservation in the wild is the ultimate objective. However, actually, *in-situ* conservation is not always more feasible. Due to the global biodiversity loss, many species of mammals became extinct in the wild, have low individual numbers, or are fragmented and in isolated populations. In all these situations, captive breeding approaches are strategies that are always more recommended (Bath & Sofi, 2021). Therefore, regardless of being *in-situ* or *ex-situ*, the population of wild species must be managed in order to preserve them and their genetic heritage.

The design and implementation of biodiversity policies in wildlife management have to deal with multiple, and sometimes conflicting, values and ethical positions. This is more evident when there is the need to make quick decisions under uncertain social-ecological conditions and the issue has high social visibility (Colloff et al., 2017). Given the current biodiversity crisis, and the need to quickly take actions to preserve biodiversity, conservation ethics enable us to engage with multiple ethical issues and values to reduce injustices and consider the multidisciplinary nature of biodiversity conservation (Cortes-Capano et al., 2022). As biodiversity conservation can intersect various dimensions of value, and there are different and sometimes conflicting ethical approaches to conservation (Gamborg et al. 2012; Biasetti & de Mori 2019), conservation ethics helps address questions of what, how, and why should be conserved, guiding actions and decisions and directing decisions over values, stakes, and potential conflict (Baard, 2021). The current thesis, by adapting existing tools from other fields or developing new ones, and using a multidisciplinary approach, presents a combination of ethical tools to approach biodiversity conservation and wildlife management in both *in-situ*, *ex-situ*, and emergency scenarios. Through the application of the tools, it has been possible to structure the processes to approach biodiversity conservation and wildlife management, provide an organized framework for gathering relevant information in conservation projects, and analyze the available options by engaging the stakeholders.

In the first part of this thesis the ethical evaluation of Conservation ART is addressed, and in particular ART applied to save the critically endangered Northern White Rhinoceros (NWR, *Ceratotherium simum cottoni*) in the context of the BioRescue project. Defined as any procedure or technique that involves the handling of gametes or embryos with the final aim of achieving reproduction (Hildebrandt et al., 2021), ART represents a crucial and important strategy for biodiversity conservation as it can make the genetic management of populations more efficient and increase the number of individuals per generation. ART procedures range from hormone monitoring and administration, ovum pick-up (OPU), semen collection (SC), in vitro fertilization (IVF), artificial insemination (AI), and embryo transfer (ET) (Hildebrandt et al., 2021). In recent years, classical ART has been combined with more sophisticated and advanced laboratory procedures that utilize genetic material from somatic cells to generate offspring such as intracytoplasmatic sperm injection (ICSI), somatic cell nuclear transfer (SCNT), inner cell mass (ICM) exchange, and stem cell-associated techniques (SCAT) - the so-called aART - and with cryopreservation techniques (Hildebrandt et al., 2021). Therefore, Conservation ART are all those ART and aART used to assist in establishing self-sustaining populations for reintroduction or as a genetic reserve and have been highlighted as a strategic technology for saving critically endangered or practically extinct species (Bolton et al., 2022; Hildebrandt et al., 2021; Gómez et al., 2004).

ART has a long history of success, for both domestic and non-domestic mammal species (Mastromonaco & Songsasen, 2020; Comizzoli & Holt, 2019; Herrick, 2019; Martin-Wintle et al., 2019; Roth & Swanson, 2018; Howard et al., 2016). However, the use of ART in wildlife conservation may encounter technical challenges: ART procedures need species-specific optimization and refinement, which, in turn, depends on the knowledge of the reproductive biology of the species involved (Herrick, 2019). Moreover, the use of conservation ART raises several ethical issues (de Mori et al., 2021). Conservation ART is usually less established and, in some cases, more demanding for the subjected animals than ART performed on domestic animals. Moreover, some Conservation ART and all Conservation aART are more complex and more challenging in terms of equipment and veterinary expertise than ART performed in livestock. Finally, by redrawing the boundaries of the concept of reproduction—and, in some cases, of extinction—Conservation ART can have a social and scientific impact that must be scrupulously considered. Therefore, the ethical evaluation of procedures in the context of Conservation ART is crucial.

An example of a Conservation ART project is the “BioRescue” project. This international project, founded by the German Federal Ministry of Education and Research (BMBF), led by the Leibniz Institute for Zoo and Wildlife Research (Leibniz-IZW) and composed by Czech Dvůr Králové Zoo, Avantea srl, Max Delbrück Center for Molecular Medicine (MDC), Kyushu University, Padova University and other additional international partners (biorescue.org) is composed of an interdisciplinary team which aim to save the critically endangered Northern White Rhinoceroses (Saragousty et al., 2016) from extinction.

Northern White Rhinoceros has a long history of conservational unsuccess. After years of *in-situ* protection, this iconic herbivore of the Democratic Republic of Congo in 2008 was declared "extinct in the wild" by the International Union for Conservation of Nature (IUCN) (Emslie, 2020). Given the breeding difficulties of the NWR housed in zoological facilities, in December 2009, only six northern white rhinos existed worldwide. In the context of the "Last Chance to Survive" breeding program, four of them were transported to Ol Pejeta Conservancy in Kenya. However, even the translocation program was unsuccessful. The translocated animals did not reproduce, and after the death of Sudan in 2018, the last known NWR male, only two females are still alive: Najin and Fatu. Fatu is the daughter of Najin, and Najin is the daughter of Sudan, and they both live in Kenya.

The BioRescue project's ultimate aim is therefore to establish a self-sustaining and genetically healthy northern white rhino population to be reintroduced into the wild. To accomplish this goal, the BioRescue team developed and implemented a new approach that combines assisted and advanced assisted reproductive technologies, in addition to the two established main strategies of habitat protection and classical *ex-situ* conservation programs. This approach allows the employment of biomaterial of live and deceased individuals in the form of cryopreserved gametes (Hermes et al., 2018), and skin samples for fibroblast cultures that, via induced pluripotent stem cell transformation, can subsequently be used for the in vitro production of artificial gametes.

Since its start on June 24, 2019, the BioRescue team successfully applied ART procedures to Najin and Fatu. The procedures foresee GnRH stimulation, anesthesia, and transrectal ultrasound-guided oocyte recovery (Hildebrandt et al., 2018). With intervals of a minimum of 3 months, from the first ovum pick-up procedure on August 22, 2019, the animals undertook 7 more OPU procedures. The procedures brought the development of 14 embryos, stored in liquid nitrogen, ready to be transferred into a Southern White Rhinoceros (SWR, *Ceratotherium simum simum*) surrogate mother in the near future. To accomplish BioRescue's goal, cooperation with zoological facilities is also essential. As SWRs also have reproductive problems, the cooperation ensures that ART procedures are refined and optimized, creating a win-win situation for Southern and Northern white rhinoceros, and allowing to collect and preserve as many genetic materials as possible from the individuals. Therefore, ovum pick-up, embryo transfer, and semen collection procedures have also been performed in SWR held in European zoological facilities since the BioRescue project start.

Section 1 of this thesis presents a dedicated toolkit to support ethical analysis, responsible decision making and monitoring of ART procedures by adapting existing tools from other fields and devising new ones for the BioRescue project. Firstly, it is presented a framework for the ethical analysis of the application of ART procedures in conservation based on the Ethical Matrix (EM) and its application to discuss a specific case study—ovum pick-up (OPU) procedures performed on the northern white rhinoceros (Biasetti et al., 2022a). The Ethical Matrix (EM, Mepham, 1996) is a conceptual tool for the ethical analysis of value-complex scenarios. Initially developed in the field of food ethics (Mepham, 1996), it has been revised and adapted to suit the specific scenarios of conservation (Biasetti & de Mori, 2019; Biasetti & de Mori, 2021). The EM helps decision-makers reach responsible and defensible decisions by summarizing the moral interests involved. Therefore, through the EM, it is possible to identify the stakeholders' value demands according to different principles of common morality (well-being, autonomy, fairness), map the ethically relevant aspects involved in the issue at stake, and anticipate the various potential value conflicts. Following a bottom-up approach (Mepham, 1996; Mepham et al., 2006), an EM describing the OPU procedures in endangered species such as NWR has been developed. Secondly, the Ethical Self-Assessment Tool (ETHAS) is presented (de Mori et al., 2021). ETHAS has been developed and applied during the Ph.D. period to monitor the general protocols of conservation ART procedures and to evaluate their implementations on non-human mammals. ETHAS consists of two checklists, one to be filled in before starting the procedures, the other before any implementation, by different stakeholders involved in the procedures. Therefore, the tool reports the degree of compliance with the conservation ethics desiderata and highlight any failure to meet critically important ones. As the two checklists can be customized for different procedures and taxa, we customized and applied ETHAS to monitor each implementation of procedures (i.e., OPU, IVF, ET) involving white rhinoceros in the context of the BioRescue project from an ethical point of view. Therefore, the pilot version and a brief case study reporting the first applications of the tool both on NWRs and SWRs are presented (de Mori et al. 2021). Finally, in Section 1 the adaptation of three decision-making tools to the ethical analysis in biodiversity conservation is presented (Biasetti et al., 2022b). Besides the already mentioned Ethical Matrix, the Decision Tree (DT) and Bateson's Cube (BC) have been adapted to structure and assist participatory decision-making processes on ethically sensitive issues: to decide whether to continue to employ Najin as a donor of biomaterial or discontinue her from this role in the project. Decision Trees are flowchart-like schemes used to predict outcomes. The DTs highlight probabilistic events that can influence a course of action, the related options, and possible results. Therefore, DT identifies the choices available and allows for the identification of the ethical best outcome in the face of uncertainty. The Bateson's Cube - developed by Bateson (1986) – in its original form allows the assessment of the ethical acceptability of scientific research involving animal experimentation (Driscoll & Bateson, 1988). By displaying the possible combinations between scores attributed along three dimensions representing ethical criteria, the BC is a model for decision-making. The three adapted tools, by structuring the decision-making process in a standardized and repeatable form, have been used in a participatory decision-making, making the reasons behind the final choice explicit and transparent (Biasetti et al., 2022).

As evidenced above, zoological facilities by managing thousands of animal species, even endangered ones, represent essential places for both *in-situ* and *ex-situ* conservation. Zoological facilities – zoos, aquariums and other animal facilities - host and manage animals with the aim of promoting conservation, education, and research activities. To accomplish their goals, zoological facilities have increased the option of wild animal encounters for their visitors (D'Cruze et al., 2019). These activities have considerable appeal and can be a powerful way to educate and increase awareness of nature and conservation issues, as well as having educational and fundraising functions for conservation projects. Encounters, like Animal-Visitor Interactions (AVIs), involve visitors that interact with program or ambassador animals at a distance closer than allowed in usual circumstances (D'Cruze, et al., 2019; de Mori et al., 2019a). They range from low and medium proximity to close proximity AVIs, where visitors can even pet, feed, or ride wild animals (Acaralp-Rehnberg, 2019). Studies show that AVIs can have an impact on human welfare and health (both visitors and staff), animal welfare and well-being, and biodiversity conservation. However, these impacts could be either positive and negative or neutral (Fernandez & Chiew, 2021; Learmonth et al., 2021; Mellor et al., 2020; Godinez & Fernandez, 2019; Sherwen & Hemsworth, 2019; Cole & Fraser, 2018; Wolfensohn et al., 2018; Higham & Shelton, 2011). In fact, if not properly managed, these activities could affect both animal and human welfare and health, transmit incorrect conservational and educational messages, and have negative impacts on the conservation of the species involved (Biasetti et al., 2020; Reynolds & Braithwaite, 2001). Therefore, AVIs must be closely monitored to understand the implications on animal welfare, conservation, and people. This field of research is make difficult because of the many variables involved—the type of AVI, the species involved, the individual characteristics of the animals and their position on the wild-captive continuum, the type of facility, the management practices occurring, etc. For this reason, AVIs also need to be investigated from an ethical standpoint, considering the different value dimensions relative to people, animals, and biodiversity and how they relate (Learmonth, 2020; Moorhouse et al., 2017). The need for a multidisciplinary, multidimensional ethical analysis of AVIs is made even more urgent by the proliferation of these activities. Section 2 of the current thesis presents studies performed to assess animal-visitor interaction activities that occurs in South African and Italian animal facilities following different approaches.

In Southern Africa, mostly in South Africa, Botswana, Zimbabwe, and Zambia are widespread facilities offering wild animals experiences (i.e., elephants, lions, cheetahs, etc.,). Visitors, with previous fee payments, can access these facilities and closely interact with the animals. AVIs proposed in these facilities range from training demonstrations, hand-feeding, walking with, brushing & bathing them, and riding. During the interaction programs, visitors may learn about the animals' biology, ethology, and conservation issues, in a recreational way. Making a parallelism with interacting programs taking place in many western zoos, it can be argued that, if conducted in certain ways, even these programs can be powerful educational tools to convey cognitive and emotional messages about conservation and wildlife (Moore 2020). In particular, African elephant AVIs have been met with huge popularity and demand by tourists. In most of the facilities offering elephant AVIs, the host elephants are a result of the culling operations which took place in 1990s. Some young elephants of the culled herds were spared, taken out of the wild, and sent to training centers, which became the first elephant facilities. However, recently facilities offering elephant AVIs have become the object of public scrutiny, especially the ones offering elephant back riding (Grotto et al. 2020); The public debate was recently fueled by some associations, which abandoned constructive criticism, declaring these interactions unacceptable (SATSA, 2019). Little information actually exists on whether and how AVI activities affect elephants' welfare (Grotto et al. 2020) and there is no agreement on a standard protocol for elephant welfare assessment (de Mori et al., 2019b).

In fact, most of the research on elephant welfare has been done on zoo animals: hence caution should be used in ‘transferring’ welfare assessment protocols developed for zoo animals (such as Yon et al., 2019) to conspecifics held in different conditions, like in South Africa (de Mori et al., 2019b).

Therefore, firstly, the value demands concerning Animal-Visitor Interaction in South Africa have been mapped using an EM. The EM has been first populated through a top-down approach and then refined throughout a participatory process. The methodology mentioned above allowed us to consider a wide range of perspectives and include contextual variables from the South African scenario in the analysis. The participatory process was composed of a workshop based on ethical reasoning, in which 18 participants discussed management and welfare issues regarding AVIs. Moreover, two online surveys were used – one for the visitors of South African animal facilities that offer AVIs, and one for the staff who daily deals with animals involved in AVIs - to include their direct perspective in the process. The data collected with the participatory process allowed us to build a detailed EM for AVIs, and to define the concepts representing the stakeholders’ interests (Muzzo et al., 2023).

Subsequently, it has investigated how three distinct groups of people differing in age and knowledge of the species (i.e., children, non-expert adults, and experts) perceived the behaviours of African elephants in their emotional connotations during different moments of the elephants day – including AVI – including both South African and Italian facilities. In this project, qualitative behavioral assessment (QBA) (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001), which measures how animals respond to their environment following the 'whole-animal' approach, has been used. The QBA has been shown to be able to assess an animal's affective state and welfare quickly, reliably, and non-invasively (Minero et al., 2016) in several animal species and management conditions (Rose et al., 2019; Minero et al., 2018; Walker et al., 2016; Phythian et al., 2016; Wickham et al., 2012). By asking to focus on the dynamic expressivity of the behavioral demeanor, it has been requested to 37 observers to generate their descriptors characterizing the emotional expressivity of the animals using free choice profiling assessment (FCP). This procedure is potentially valuable for ethical evaluations when considering the interests of visitors. In fact, this assessment running with children and adults - i.e., zoological facilities' primary visitors - provides an idea of the emotional impact on visitors of the "elephants' experience." The descriptors generated by the expert group also allowed the investigation of possible differences between the captive and semi-captive management and whether similar differences could be found among the other observers' groups (Pollastri et al., 2021).

Finally, the multidisciplinary and integrated approach provided by the Animal-Visitor Interaction Protocol (AVIP; de Mori et al., 2019a, Normando et al., 2018) has been followed to assess an AVI involving five ring-tailed lemurs (*Lemur catta*) hosted in a walk-in enclosure at Giardino Zoologico di Pistoia (Pollastri et al., 2022). Walk-in enclosures allow particular AVI where visitors can experience close proximity to wild captive animals without physical barriers. Studies have shown that the closeness with the animals provided by this type of enclosure that allows seeing animals in a more naturalistic way generate positive effects on visitor attitudes (Goodenough et al., 2019; Mun et al., 2013). Several animal species are managed in walk-in enclosures but commonly host non-human primates, particularly lemurs (Sherwen et al., 2015). Studies found that viewing lemurs is attractive to visitors (Goodenough et al., 2019; Jens et al., 2012; Tofield et al., 2003; Webster, 2000). However, research that assessed this kind of AVIs focused on a few welfare parameters, or visitors' effects and exhibit design, or educational contents, without fully exploring the undesirable outcomes (Spooner et al., 2021). The Animal-Visitor Interaction Protocol has been applied for the first time in this kind of AVI. AVIP, following WAZA guidelines (WAZA, 2020; Mellor et al., 2015) and a "One Health, One Welfare" approach (oie.int), throughout six steps, provides a rational ethical analysis to highlight potential conflicts of values of the AVI under assessment and suggests actions to optimize both animal and human well-being and safety, conservation education, and wildlife conservation. Specifically, AVIP foresees behavioral and physiological assessments, together with Animal Welfare Risk Assessment, to evaluate the effects and consequences of AVIs on animal welfare and health. Moreover, it foresees the evaluation of the impact of AVIs on the safety and welfare of visitors and changes in visitors' attitudes towards animals and conservation issues, as well as their education and experience in terms of motivation and expectations. The results obtained with the first assessments are compared with the content of an Ethical Matrix representing the ideal situation for all stakeholders to perform the ethical assessment and interpret and discuss the results. Finally, a final checklist provides an explicit result of the evaluation process, by which strengths and weaknesses of the AVI under assessment can be identified.

The last section of this thesis, section 3, investigates how the care and management of wild animals housed in zoological facilities have been affected during the COVID-19 pandemic. During the pandemic period, the main activities regularly carried out by zoological structures (i.e., research, conservation, and education) were forced to undergo significant changes (Bandoli et al., 2021; Gili et al., 2021) to be able to carry on their mission. Furthermore, during the pandemic period, despite the zoo gates being closed for several months, the care and management of the animals continued. Therefore, profound changes have been applied to continuously provide the animals with all the necessary care, safeguarding the health and welfare of the animal and the staff. It should be noted that Italian zoological facilities mainly depend on visitor ticket fees and that revenues from interaction programs generally impact resource availability. The absence of visitors has therefore created a critical lack of financial support for the zoological structures, which have had to find alternative ways to receive support from society. After a national meeting in June 2021 in which Italian zoological facilities shared and discussed the main difficulties encountered during the months of closure and the strategies put in place to deal with them, in collaboration with the Unione Italiana dei Giardini Zoologici ed Acquari (Italian Union of Zoos and Aquariums, UIZA), a national survey was launched. Through the survey, addressed to all the zoological facilities staff (i.e., directors, veterinarians, keepers, etc.), we aimed to examine how the pandemic impacted animal management, the professional activity of the staff, and in general, the challenges that zoological facilities faced (Pollastri et al., 2022 – *sub*) during and after the lockdown period.

All the studies presented in this Ph.D. thesis have a shared aim: to assess wildlife management through different ethical frameworks and tools. Therefore, the sections show three areas of application of the ethical evaluation: an ethical evaluation of the application of Conservation ART, studies concerning the ethical assessment of animal-visitor interactions, and a study dedicated to understanding the challenges in wildlife management during the COVID-19 emergency. Despite the differences in wildlife management typologies, the ethical evaluation needs to be addressed when dealing with both *in-situ* and *ex-situ* conservation.

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Section 1. Wildlife management and ethical evaluation of Conservation ART

1.1. Ethical Analysis of the Application of Assisted Reproduction Technologies in Biodiversity Conservation and the Case of White Rhinoceros (*Ceratotherium simum*) Ovum Pick-Up Procedures

Adapted from:

Biasetti, P., Hildebrandt, T. B., Göritz, F., Hermes, R., Holtze, S., Galli, C., Lazzari, G., Colleoni, S., Pollastri, I., Spiriti, M.M., Stejskal, J., Seet, S., Zwilling, J., Ngulu, S., Mutisya, S., Kariuki, L., Lokolool, I., Omondo, P., Ndeereh, D. & De Mori, B. (2022a). Ethical Analysis of the Application of Assisted Reproduction Technologies in Biodiversity Conservation and the Case of White Rhinoceros (*Ceratotherium simum*) Ovum Pick-Up Procedures. *Frontiers in Veterinary Science*, *9*.

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Abstract

Originally applied on domestic and lab animals, assisted reproduction technologies (ARTs) have also found application in conservation breeding programs, where they can make the genetic management of populations more efficient, and increase the number of individuals per generation. However, their application in wildlife conservation opens up new ethical scenarios that have not yet been fully explored. This study presents a frame for the ethical analysis of the application of ART procedures in conservation based on the Ethical Matrix (EM), and discusses a specific case study—ovum pick-up (OPU) procedures performed in the current conservation efforts for the northern white rhinoceros (*Ceratotherium simum cottoni*)—providing a template for the assessment of ART procedures in projects involving other endangered species.

Introduction

Assisted reproduction technologies (ARTs) offer increasingly important opportunities for biodiversity conservation (1–3). Originally applied mainly on domestic and lab animals, ARTs have found usage also in conservation breeding programs, where they can enhance the genetic management of populations, and increase the number of offspring per generation. More elaborate and costly techniques, advanced assisted reproduction technologies (aARTs) not commonly employed on livestock and laboratory animals, may even spark hope for the survival of taxa that are functionally extinct or at the verge of extinction (4, 5).

However, the application of ARTs in biodiversity conservation opens up new ethical scenarios that have not yet been fully explored. Like any other technology capable of redefining the boundaries of extinction (6), ARTs question the very idea of conservation we want to pursue and the values it needs to convey. Moreover, ART applications may have ethically relevant consequences—on conservation projects, on the people involved or otherwise affected, and on the animals on which they are performed—that should be carefully discussed.

The ethical assessment of the involved procedures is an integral and crucial part of the ethical assessment of conservation projects (7). Here, we propose a frame for the ethical analysis of ART procedures in conservation using the Ethical Matrix (EM), and we discuss a case study based on ovum pick-ups (OPUs) performed for the current conservation efforts of the northern white rhinoceros (NWR, *Ceratotherium simum cottoni*, Lydekker, 1908).

The NWR is a subspecies of the white rhino (*Ceratotherium simum*, Burchell, 1817) of which only two females remain (8), and whose fate is irremediably tied to the recovery and manipulation of the existing biomaterials. It should be noted, however, that the entire

Rhinocerotidae family, consisting of five extant species— white rhinoceros, black rhinoceros (*Diceros bicornis*, Linnaeus, 1758), Sumatran rhinoceros (*Dicerorhinus sumatrensis*, Fischer, 1814), Javan rhinoceros (*Rhinoceros sondaicus*, Desmarest, 1822), and the greater one-horned rhinoceros (*Rhinoceros unicornis*, Linnaeus, 1758)—is currently under severe threat due to habitat loss and persistent poaching (9). In particular, black, Sumatran, and Javan rhinoceros are critically endangered—with the latter two species reduced to small (>80 individuals and 46–66 individuals, respectively) dwindling populations (10,11). Moreover, even the less endangered taxon—the southern white rhinoceros (SWR, *Ceratotherium simum* simum, Burchell, 1817)—while "only" near threatened in the wild (12), does not have self-sustainable captive populations (13). It is likely that, among other strategies, future conservation efforts of rhinoceros will resort to ARTs (5). While new technologies like stem cell- associated techniques and *in vitro* follicular growth (5) may eventually ensure a stable supply of gametes without the need for *in vivo* collection, in the near future, procedures like OPU and semen collection will presumably remain the only viable methods to obtain the necessary biomaterial for *in vitro* embryo production. It is necessary, then, to analyze the ethical issues associated with these interventions.

The purpose of this study is, therefore, three-fold: (i) to provide a methodology for the ethical analysis of ART procedures in conservation projects; (ii) to use this methodology to assess the OPU procedures performed in the case study; (iii) to provide a template for the assessment of OPU procedures in other projects involving white rhinoceros or other members of the rhinocerotidae family.

Materials and methods

Assessing ARTs in Conservation Projects

In human medicine, ARTs are usually defined as those procedures or treatments in which both the male and female gametes or embryos are manipulated *in vitro* to achieve pregnancy (14). In contrast, in veterinary medicine, the catalog of ART is normally broader, including, for instance, artificial insemination (15–21), cloning via somatic cell nuclear transfer (3, 22–25), and gamete production from induced pluripotent stem cells (3, 5). Following this broader use, the term ART will hereinafter be applied to any procedure involving, in one or more of its stages, the manipulation of reproductive cycles, gametes, or embryos with the final aim of producing a new individual.

With biodiversity conservation, we mean, instead, those scientifically grounded activities aimed at managing natural environments, ecosystems, wildlife, flora, biotic process, and, more generally, the whole biosphere with the end of maintaining and, eventually, restoring, the natural diversity of life on our planet and its evolution processes at all biological levels—from the ecosystem to genes. Biodiversity conservation is an ethically significant activity since it preserves the source of different kinds of values, both instrumental and non-instrumental.

Applications of ARTs in livestock, laboratory animals, and wildlife usually differ in their goals. In livestock and laboratory animals, ARTs are primarily used to maximize the offspring from genetically desired individuals. Producing large numbers of individuals with certain recurring genetic characteristics is instead generally neither useful nor desirable in the context of wildlife conservation. Rather, the goal of what could be termed "conservation ARTs" is to assist in the establishment of self- sustaining populations for reintroduction or as a genetic reserve. ARTs can contribute to this goal in two complementary ways. They can help increase the number of individuals in each generation, by expanding the opportunities and chances for achieving pregnancy. Moreover, they can improve the genetic management, by facilitating the breeding between spatially separate animals without the need for translocation, and by reintroducing into the gene pool those individuals who, for various reasons, are incapable of mating or breeding— including dead individuals whose suitable biomaterials have been cryopreserved.

Ethical analysis is crucial when conservation ARTs are involved. ART procedures in wildlife, for instance, are usually less established and—in some cases—more demanding for the subjected animals than those performed on the domestic animals. Moreover, given the different goals, some of the techniques used in conservation are more complex, as well as more challenging in terms of equipment and veterinary expertise required, than those normally employed for livestock. Finally, by redrawing the boundaries of the concept of reproduction—and, in some cases, of extinction—conservation ARTs can have a social and scientific impact that must be scrupulously considered.

The Frame for the Ethical Analysis of Conservation ARTs

Ethical analysis permits us to determine whether a procedure is acceptable according to certain standards of value and to identify the critical issues that need to be addressed before its implementation. This should not be confused with the assessment of the project, or with the assessment of the specific implementations of the procedure. In the first case, the focus is much broader. In the second case, there is the need to include the various contextual variables in the evaluation. In both cases, however, the ethical analysis of the procedures provides a fundamental support: as an essential part of project assessment, and as a backbone for the assessment of implementations.

Carrying out a comprehensive ethical analysis of a specific conservation ART procedure means identifying and gathering numerous relevant factors beyond the technical and scientific details of its execution. The procedure has to be considered in the context of the project it is part of, and in the broader perspective of biodiversity conservation. Moreover, as conservation activities take place at the crossroad between different value dimensions (26), the procedure has to be evaluated in its wider effects on animals and people, that is, beyond its mere conservation value.

The factors to be considered for conducting a thoughtful ethical analysis of conservation ARTs can be grouped into five categories. One category revolves around the immediate context of the procedure, that is, around the project it belongs to, its goals, the probability of achieving them, and the values they convey. Some questions to be raised in this regard are as follows: What are the goals of the project? Have success criteria been clearly defined? How reasonable are the chances of success of the project according to these criteria? What is the conservation value of the project? What other values are brought forward by the project? In case of failure, would the project still lead to some kind of valuable advancement (ecological, scientific, social, etc.)? An exhaustive answer to the above questions would require a detailed analysis of the overall project, and is therefore not feasible when assessing a procedure. However, it is still necessary to have a sufficiently defined picture of the ultimate reasons why the procedure is undertaken, as this provides the context for assessing eventual critical aspects.

Moreover, it is necessary to focus on the role of the procedure in the project and its effectiveness in reaching the assigned goals. What purpose does the procedure serve in the project? Is the success of the procedure a key part of the project? Can there be alternatives in case of failure? Is it the most effective way to perform the task assigned? Have the alternatives been considered? How has the procedure been chosen? Besides the reasons for efficiency, the effectiveness of a procedure is a central issue where ethically relevant risks or costs are present. Moreover, the reasons that led to the inclusion of the procedure into the project should also be made explicit and examined to detect eventual biases.

The procedure must also be analyzed beyond its immediate contribution to the project. This means investigating its possible value beyond its effectiveness in carrying out the specific goal of the project. For instance, what is the scientific value of performing the procedure? Can it lead to scientific and technological improvements? Does it establish or refine protocols that could be employed in other biodiversity conservation projects? Can carrying out the procedure have a positive impact on the welfare of the animals involved? Can it have a positive social effect of some kind, for example, by promoting knowledge transfer or capacity building? While procedures do not happen in a vacuum, meaning that their implementation always happens in a project, the project itself may not exhaust their usefulness. Answering the above questions permits us to extend our understanding of the possible merits of the procedure beyond its instrumental value for the project.

Special attention should also be paid to the risks and costs associated with the procedure itself. What are the known risks of performing the procedure? Who is responsible? Can the procedure harm the welfare of the animals involved? Does it put at risk their lives? Are there risks for people? What could be the repercussions in case of failure? Are there any negative side effects to consider in case of success? As veterinary interventions, conservation ARTs invariably entail some risks during their performance as well as before and after (translocation, handling, restraining, recovery, etc.). These risks should be investigated and their distribution among the different involved stakeholders should be made clear, since this, alongside the distribution of benefits, is important to evaluate the acceptability of the procedure.

The last category of ethically relevant factors focuses on how the procedure fits into the values and worldview of public opinion and conservationists. Does the procedure raise public concerns? Are there any groups that particularly oppose it? Why? How does the procedure match or challenge the various existing perspectives on biodiversity conservation? Public opinion can be skeptical of the project and the employed procedures. Sometimes this is just due to lack of involvement or inadequate information. However, in other cases, the reasons can be more substantial: the unfair distribution of the costs and benefits of the project among the people and communities involved; there is distrust for the individuals or the institutions carrying out the project; the goals and the methods of the project conflict with the shared values, etc. Similarly, uses of conservation ARTs may challenge the tenets of some conservation philosophies. A careful analysis of the factors in this category allows for the anticipation of potential conflicts so that it should be possible to take countermeasures.

Gathering Factors Through the EM

Table 1 summarizes the necessary factors to be considered for analyzing the applications of conservation ARTs. Some factors (i.e., the goals of the project, feasibility, and the effectiveness of the procedure) can be retrieved from the description of the project itself. Other factors must instead be identified by analyzing the procedure from an ethical standpoint. To achieve this goal, a specific ethical tool—the EM—can be applied.

Table 1. Relevant factors for the ethical analysis of conservation ARTs.

Group	Factors to be investigated	Examples of associated questions
1. Context of the procedure	• Goals of the project • Values conveyed by the project’s goals • Feasibility of the project	• What are the goals of the project? • Have success criteria been clearly defined? • How reasonable are the chances of success of the project according to these criteria? • What is the conservation value of the project? • What other values are brought forward by the project? • In case of failure, would the project still lead to some kind of advancement (ecological, scientific, social, etc.)?

2. Role of the procedure in the project	• Value of the procedure for the project • Effectiveness	• What purpose does the procedure serve in the project? • Is the success of the procedure a key part of the project? • Can there be alternatives in case of failure of the procedure? • Is it the most effective way to perform the task assigned? • Have alternatives been considered? • How has the procedure been chosen?
3. Value of the procedure beyond the project	• Scientific value • Conservation value • Animal welfare value • Social value	• What is the scientific value of performing the procedure? • Can it lead to scientific and technological improvements? • Does it establish or refine protocols that could be employed in other biodiversity conservation projects? • Can carrying out the procedure have a positive impact on the welfare of the animals involved? • Can it have a positive social effect of some kind, for example by promoting knowledge transfer or capacity building?
4. Risks and costs of the procedure	• Known risks of the procedure, and their distribution • Costs of failure of the procedure • Negative side-effects of the procedure in case of success	• What are the known risks of performing the procedure? • On who do they fall? • Can the procedure harm the welfare of the animals involved? • Does it put at risk their lives? • Are there risks for people? • What could be the repercussion in case of failure?

		• Are there any negative side-effects to consider in case of success?
5. Views on the procedure	• Public opinion's views on the procedure • Conservationists' views on the procedure	• Does the procedure raise public concerns? • Are there any groups that particularly oppose it? • Why? • How does the procedure match or challenge the various existing perspectives on biodiversity conservation?

The EM permits us to unpack and analyze the ethically relevant aspects involved in a complex scenario, reorganizing them into a transparent and comprehensible picture of value demands. Originally developed by Mepham (27) for the ethical assessment of technologies and policies in agriculture and food processing, the EM has since been applied in many other fields—including veterinary medicine (28, 29), forestry (30), aquaculture (31, 32), assessment of human–animal interactions (33, 34), management of contaminated agricultural ecosystems and radioactive waste (35, 36), and conservation (37).

The EM embraces a pluralistic ethical approach. Cells from the first column of the EM list stakeholders. Cells from the first row list three general ethical principles, influential, recognized, and shared tenets of ethical reasoning and common morality such as wellbeing, autonomy, and fairness (38, 39). Intersecting cells list the value demands for the stakeholders derived from the general ethical principles.

The EM specifically tailored for conservation (40) includes three categories of potential stakeholders: ecological entities, individual animals, and people. Table 2 recaps the general value demands generated by applying the ethical principles on these categories of stakeholders.

The methodology of the EM is to apply the general template on a specific case, first by identifying the stakeholders involved, and then by applying the general ethical principles in order to derive the value demands.

Table 2. General EM.

	Wellbeing	**Autonomy**	**Fairness**
Ecological entities	Conservation	Freedom from human intervention	Equal treatment in relation to conservation
Animals	Health and functioning Absence of negative affective states and allowance of positive ones	Living natural lives and expressing species-specific behaviors	Equal treatment in relation to welfare
People	Psychological and physiological welfare Sustainable social, economical, and cultural welfare	Freedom of choice Capacity to exercise the various fundamental aspects of one's own persona Self-determination	Equal and fair treatment

The Case Study: OPU on White Rhinoceros

As a case study, we analyzed the OPU procedures performed in the recent conservation efforts to save the NWR. The case appears interesting due to several reasons. It is rather complex, with many ethically relevant issues packed together; it involves many stakeholders and multiple value dimensions, with a variety of potential value conflicts;

the ART techniques employed in the project have the potential to redefine the boundaries of wildlife reproduction and extinction.

The most peculiar aspect of the case is that the NWR has been declared "functionally extinct" (8). From ~2,230 individuals in 1960 (41), the wild population of NWR has been reduced, mainly by poaching, to a few individuals by the 1980s, and presumably erased sometime after 2007 (8). During the same years, the small population kept in zoos proved to be not self-sustainable. White rhinoceros have a low reproductive rate in captivity (42). Despite various breeding attempts, only four NWR offspring were ever known to be born in this way (at the Dvu˚r Králové Zoo). Since the death of the last male Sudan in 2018, two females, both living at Ol Pejeta Conservancy in Kenya, have become the lasts of their kind. They are Najin, aged 32, and her offspring Fatu, aged 21.

The current conservation efforts for the NWR by the BioRescue project—an international consortium coordinated by the Leibniz Institute for Zoo and Wildlife Research in Berlin— combine aARTs and stem cell-associated techniques (43). Frozen semen from five NWR males is available, and the stored tissue could be used in the future to produce gametes by using novel technologies. Due to severe reproductive pathologies, both the remaining females cannot carry to term a pregnancy. In the case of the older female, this is due to tendon problems in the hind legs; in the case of the younger, this is due to the uterine pathology of an unknown origin. The only current way to "de- doom" the taxon is to collect their oocytes to create embryos using intracytoplasmic sperm injection (ICSI) to be transferred into SWR recipient cows.

The first point to be made here is that, despite the possible similarities, this conservation effort must not be confused with an attempt at de-extinction. De-extinction can be defined as the process of bringing back an extinct taxon (6), and it can be divided into two categories: the de-extinction of recently extinct taxa, and the de-extinction of species that had gone extinct hundreds or thousands of years ago, and whose significant ecological relationships have now disappeared ["deep de-extinction"; (44)]. Both the categories raise specific conceptual and ethical challenges (45). While de-dooming a functionally extinct taxon like the NWR may resemble a case of de-extinction in the first, non-deep, sense—in both scenarios the original ecological context still exists—the two differ in a decisive aspect, that is, generational continuity. Generational continuity cannot be recreated through de-extinction, and this may constitute in some taxa both an ecological and ethical issue: ecological, as some behaviors and functions can only be acquired through interaction between adults and juveniles; and ethical, as animal welfare may be harmed by the absence of these behaviors and functions. For these reasons, it makes sense to keep conceptually distinct the actions of de-dooming a functionally extinct taxon and de-extinguishing a vanished taxon. In the case of NWR, since only two females remain, one of which had no offspring—the generational continuity is at least partially impoverished. Nevertheless, it has not disappeared altogether, and SWR individuals can be used as proxies for NWR adults to transmit those behaviors that are known to be similar between the two subspecies, such as reproductive behaviors (46).

In vivo oocyte collection in rhinoceros is a relatively new intervention. The full procedure as it is performed currently in white rhinoceros involves ovarian super stimulation, full anesthesia, and transrectal ultrasound-guided oocyte recovery (17, 43, 47). In the addressed context, the procedure has been conducted regularly (albeit with at least 3 months of lapse) in the two remaining NWR females (48). Table 3 recaps the results of the seven procedures that have been executed so far in NWR. Overall, the procedure has been rather successful in Fatu, with 95 oocytes collected in seven OPUs between 2019 and 2021, which have been used to produce a total of 13 embryos. The procedure has been less successful with Najin presumably due to her age and health, and the partners in the project have decided to discontinue performing OPU on her in 2021. Although this choice further reduces the gene pool available for embryo creation, it was preferred over other options after carefully considering the ethical and scientific elements involved (49). At the same time, SWR oocytes are also collected from females across European zoos, in order to establish the technology also for this taxon and to synergistically support the research related to the project.

Table 3. Results of OPU and ICSI on NWR.

	Najin		Fatu	
	Oocytes	**Embryos**	**Oocytes**	**Embryos**
1. (08/22/2019)	5	0	5	2
2. (12/17/2019)	3	0	6	1
3. (08/18/2020)	2	0	9	0
4. (12/13/2020)	0	-	14	2
5. (03/28/2021)	-	-	21	4
6. (07/06/2021)	-	-	17	3
7. (10/25/2021)	-	-	23	1
Total:	**10**	**0**	**95**	**13**

Results

Building Up the EM

Following the proposed methodology, an EM for the procedure has been developed (Table 4) using the template provided in Table 2. The stakeholders included in the EM are biodiversity, the individual females subjected to the procedure, and all people involved in the project.

The level of resolution of the EM could be increased by adding more stakeholders or breaking down the existing ones into more specific items. It could be possible, for instance, to break down biodiversity into the different rhino species and the ecosystems involved or to add to the list the NWR calves born as a result of the project, the conservationist community, etc. Such a high-resolution EM would be especially useful to analyze the whole project in detail. However, since the goal is to assess a specific procedure, increasing the resolution of the EM is neither necessary nor desirable.

Table 4. EM for OPU in NWR conservation efforts.

	Wellbeing	**Autonomy**	**Fairness**
Biodiversity	Conservation • NWR has a historical-naturalistic value. • NWR has an ecological value. • Cryobanking is a good conservation strategy per se (collect now or regret later). • Refining through application the OPU procedure may open new ways for the conservation of other taxa. • Incidents or complications during the procedure could damage the image of the project and of conservation ARTs in general.	Freedom from human intervention • Conservation ART may be deemed a technofix. • Conservation ART may lead to moral hazard. • Conservation ART may be deemed hubristic	Equal treatment in relation to conservation • Charismatic animals like rhinoceros receive a disproportionate amount of attention. • However, conservation of the NWR could benefit the conservation of other less charismatic species. • The opportunity costs of the project do not fall on more traditional conservation efforts, including conservation of other rhino taxa.
Rhino females subjected to the procedure	Health and functioning. Absence of negative affective states and allowance of positive ones • Some aspects of the procedure may harm the animals according to these dimensions of welfare. More specifically: ovarian superstimulation, anesthesia, transrectal puncture all bear a	Living natural lives and species-specific behaviors • The procedure increases the possibility for some of the animals involved to express social behaviors currently not accessible.	Equal treatment in relation to welfare • The animals involved are treated like a means for the conservation of their taxon. • However, they receive extra veterinary screening and care.

	possible risk of side-effects.		
People	Psychological and physiological welfare • Affective value for people caring for the animals. Sustainable social, economical, and cultural welfare • Economic value of the animals. • Ecotourism.	Capacity to exercise the various fundamental aspects of one's own persona • The procedure is an opportunity for professional growth, knowledge transfer, and capacity building. • NWR may have eudaimonistic (aesthetic, scientific, and reverential) value for people. • NWR may have transformative value for people. • NWR may have existential value for people.	Equal and fair treatment • Costs and benefits of the procedure should be distributed equally, and compensation given whenever this is not possible.

Biodiversity

The three basic value demands for biodiversity are (refer to Table 2): (i) conservation (under wellbeing); (ii) freedom from human intervention (under autonomy); (iii) and equal treatment in relation to conservation, without bias grounded on human preferences (under fairness).

From the standpoint of conservation, at least three values can be attached to the goals of the project, that is, bringing the NWR population back to a viable level—attaining demographic security and stability (50)—and subsequently reintroducing the taxon into the wild. The first two values are the historical and the naturalistic values of the subspecies—being a unique and irreplaceable product of the evolutionary process which would be lost for purely anthropogenic reasons. The third value is the ecological value of this taxon. Mega-herbivores are important ecosystem engineers whose contribution to shaping their environment cannot be replicated by smaller herbivores (51). White rhinoceros make no exception to this rule, and their presence can make a difference in preserving the African savannah ecosystem (52, 53). Reintroducing the NWR would then be a way to restore and maintain the ecological relationships that are now lost.

The OPU procedure has also an additional conservation value which is independent from the succes or the failure of the project. Due to the mounting extinction crisis (54), cryobanking biomaterial from endangered taxa has become an important conservation goal (19, 55), following the imperative to collect now, or regret later (5). Moreover, by carrying out the procedure, it is possible to collect technical and scientific data for developing OPU protocols in other rhino taxa, or even in other large mammals, expanding in this way the opportunities for their conservation. However, accidents during the procedure could damage the image of the project.

From the standpoint of freedom from human intervention, this procedure, like other conservation ARTs, could be considered a negative example of "technofix" that is, the use of a technology to reverse the outcomes of morally problematic activities (in this case, poaching and habitat loss) leaving intact the causes (56). Similarly, the methodology of the project could be accused of making wildlife decline overly mundane, by providing, at least in theory, an "easy" way to revert the phenomenon. This could create a moral hazard, which, in turn, could help further accelerate the extinction crisis. Finally, applications of conservation ARTs to de-doom the functionally extinct taxa may be accused to be an aggressive form of conservation, through which we attempt to forcefully impose our scheme and solutions on reality, following a hubristic attitude which has already been shown to be a part of the problem and not of the solution.

Considering equal treatment in relation to conservation, the question may be raised as to why concentrate so much effort and resources on one rhino subspecies when there are so many other endangered taxa. Rhinoceros are among the most charismatic animals (57), and this may be an explanation, albeit one that clearly expounds a bias. However, there are good reasons for not considering the choice of the NWR as unfair. Rhinoceros can serve as umbrella and flagship species (58), meaning that the reintroduction of the NWR could foster the conservation of other less charismatic species (59). Furthermore, as previously mentioned, the refinement of conservation ART protocols could open new opportunities for the conservation of other rhino taxa or even other large mammals.

In fact, one of the advantages of this project is that its opportunity costs do not fall on other more traditional conservation endeavors, including other rhino conservation efforts. This is because it draws on funds allocated for biotechnology, and does not make use of the money collected for conservation of other rhino taxa.

Females Subjected to the Procedure

Table 2 lists three basic value demands for the females subjected to the procedure: (i) health and functioning and absence of negative affective states and allowance of positive ones (under wellbeing); (ii) living natural lives and species-specific behaviors (under autonomy); (iii) equal treatment in relation to welfare (under fairness). This captures the multidimensional nature of animal welfare (60) and should help in gathering useful elements for the assessment relative to the risks and costs of the procedure and its value beyond the goals of the project.

Regarding the first value demand, OPU on rhinoceros is a relatively new intervention, and, as such, there is no specific and systematic investigation of its effects, immediate or prolonged, on any of the previously defined criteria of animal welfare. An overall evaluation can nevertheless be attempted, starting with some considerations to be extrapolated from similar (yet not analogous) interventions performed on other species. OPUs have been performed regularly on domestic animals in the recent decades. *In vivo* oocyte collection was first performed on cattle via laparoscopy (61), and, a few years later, transvaginal ultrasound-guided follicle aspiration was introduced (62, 63). Today, laparoscopic OPU is still used in small ruminants, such as sheep and goats (64), while transvaginal ultrasound-guided OPU has become the standard for cattle, buffalo, and horses (65, 66). Applications of these methods to exotic species were first performed in the mid-nineties (67), starting with zebras (68), and llamas (69).

Transvaginal ultrasound-guided OPU procedures are regularly repeated in the same cattle and buffalo cows twice per week (66, 70–72), as this is the frequency that assures the best yield of the oocytes (65). Horses can be subjected to OPU procedures on a biweekly schedule (73). The effects of the procedure and of its steady repetition in cattle, buffalo, and horses concerning the reproductive and productive capacities of the treated animals are well-documented (65, 70, 74–76).

In this regard, there is a general consensus that OPU procedures, even when reiterated regularly and for prolonged periods of time, do not have particularly adverse side effects. Studies with a stronger focus on criteria relative to the minimization of unpleasant affective states, partly caution this optimism, highlighting some invasive aspects of the OPU procedure. While repeated transvaginal punctures seem not to provoke the signs of short- and long-term stress neither in cattle (77), nor in buffalo cows (78), other possible sources of welfare impairments are nevertheless present, namely the possibility of minor ovarian alteration, and, most importantly, the negative physiological and behavioral responses to the epidural anesthesia administered during the procedure (79, 80). Studies on the reaction of horses to transvaginal ultrasound-guided OPU in terms of pain and discomfort are few and less systematic (24), but possible negative side-effects of the procedure have been reported (81–83).

In general, the OPU procedure on rhinoceros is related to those practiced on horses and cattle (43, 47). Horses, in particular, being members of the order *Perissodactyla* like rhinoceros, are considered good models due to their taxonomic relatedness. However, two crucial differences between the specific procedures complicate any possible linear comparison: the transrectal instead of transvaginal approach, and the full anesthesia.

The length of the reproductive tract, and the impossibility of palpating the ovaries through the rectum, make the transvaginal approach unfeasible in rhinoceros (except for the Sumatran rhinoceros). Since the classic laparoscopic approach is equally unfeasible (47), OPU in rhinoceros is performed transrectally (84). This raises issues of limited sterility of the procedure and of the possibilities of infection. Indeed, even if restricted to a single penetration of the rectal wall, OPU in rhinoceros still poses a minimal risk of bacterial contamination of the puncture needle even after a prior thorough cleaning and disinfection of the rectum (47).

Moreover, safe immobilization and full anesthesia are required to perform the OPU procedure in rhinoceros. Full anesthesia prevents unexpected movement, limiting the risk of injuries both to the animal and to the people carrying out the operation, yet it poses its relevant risks of complications. Standard anesthesia protocols in rhinoceros are etorphine hydrochloride-based (85). Some of these protocols have been reported to be suitable for weekly (86) and bimonthly (87) anesthetization of the same animals—a black rhinoceros and a greater one-horned rhinoceros, respectively. Nevertheless, anesthesia in general, and the use of etorphine-based protocols in particular, have been associated with many potential and possibly fatal complications, including aspiration, respiratory depression, hypoxemia, hypertension, pulmonary shunting, and ventilation/perfusion mismatch (88–91). Moreover, etorphine can be very dangerous to people, and cases of accidental exposure, while very rare, are reported in the literature (92, 93).

Transrectal oocyte retrieval is preceded by ovarian stimulation. The ovarian stimulation protocols administered to the animals employ Histrelin, a slow-release GnRH analog. The GnRH analog is injected every other day either three or four times before the OPU procedure. Captive white rhinoceros are known to suffer from various genital tract pathologies, most likely favored by long non-reproductive periods (94). Hormonal stimulation could potentially contribute to the progression of these pathologies.

From the standpoint of the second value demand, that is, the possibility of living natural lives and expressing species-specific behaviors, the procedure, by contributing to the success of the project, could be evaluated positively, at least for the two NWR females, as it may provide them, in the medium term, with a chance for expressing some parts of their behavioral repertoire which are currently not accessible. White rhinos form cow-calf and cow-adolescent pairs, which are typical groupings in the social structure of the species, with no need for males to rearing a calf (95). This means that there is a concrete possibility that the remaining females could establish social bonds with the newborn NWR. In this regard, it is important to note that, although both Najin and Fatu were born in captivity, they were accompanied during their earlier lives by several other captive-born as well as wild-caught NWR, and had, in this way, enough opportunity to learn social behaviors from conspecifics. Although it is not possible to determine a priori to what degree the normal social structure of the species can be recovered from this bottleneck of two individuals, returning the population to viable numbers could allow its members to cultivate a wider range of species- specific social behaviors.

On the other hand, from the standpoint of the third value

demand, equity regarding welfare would require managing similar animals in the same manner. This is violated as soon as the animal is subjected to a procedure that could cause stress, discomfort, and even, in the worst cases, harm, without any direct and substantial benefit. However, while it is undeniable that in the procedure animals are mainly treated as a means for a goal—the collection of oocytes—which is only tangentially tied to their wellbeing, it is equally true that they receive much more veterinary screening and care than what constitutes the norm for white rhinoceros in captivity. Given the particular vulnerability of captive female rhinoceros to reproductive tract pathologies, such as tumors (42, 94, 96, 97), this is not an aspect to consider lightly.

People Involved in the Project

Table 2 lists three basic value demands for people involved in the project: (i) psychological and physiological welfare and sustainable social, economical, and cultural welfare (under wellbeing); (ii) freedom of choice, capability to exercise the various crucial aspects of one's own persona, as well as self- determination (under autonomy); and (iii) equal and fair treatment (under fairness). This should help in gathering useful factors for the assessment relative to the context of the procedure, of its value beyond the project and of its risks and costs.

Considering the first value demand, it is important to note that several people—keepers, veterinarians, caregivers—have regular, if not daily, contact with the animals involved, and may have built affective bonds with them. It may be expected that these people will be especially concerned for the safety of the animals during the procedure.

A second aspect to note is that the animals involved have a certain economic value, which could be reduced in case of complications during the procedure. At the same time, communities living in the area of the eventual reintroduction of the NWR could benefit from the success of the project, as it could create new opportunities for ecotourism.

Concerning the second value demand, the possibility of performing the procedure can be both an opportunity for professional growth and, given the international nature and the cutting-edge technologies of the project, an occasion for knowledge exchange and transfer. Re-establishing a self-sufficient population of NWR and reintroducing it could also promote several kinds of values linked to our fulfillment as individuals (98, 99).

Indeed, majestic animals like rhinoceros can be sources of aesthetic value, scientific value, reverential value, and transformative value—meaning with this latter, the capacity of producing powerful and even life-changing experiences. Moreover, even just knowing that the NWR has been saved from extinction can be important for many people (the so-called existential value of biodiversity), even if they cannot directly experience or benefit from this.

Concerning the third value demand, a requirement should be that costs and benefits of the procedure be distributed equally, and compensation should be given whenever this is not possible.

Discussion

Factors for the Assessment

Along with the results from the project description, the value demands listed in the EM can be used to gather the factors for the ethical analysis frame presented before. Table 5 shows the outcome of this process.

Table 5. Factors for the ethical analysis of OPU procedures in white rhinoceros.

Category	Factors to be investigated	Description
1. Context of the procedure	• Goals of the project • Values conveyed by the goals • Feasibility	• The ultimate goal is to create a self-sustaining population of NWR to be reintroduced into the wild. This will be the ultimate criterion of success of the project. • Such a goal conveys several form of value: - Historical, naturalistic and ecological value directly tied to saving the NWR from extinction and reintroducing it. - Welfare value, for giving to the two remaining NWR the chance to exercise social behaviors currently not accessible. - Economic value, tied to the opportunity for ecotourism. - Transformative value for people, as encounter with NWR could lead to life-changing experiences. - Eudaimonistic (aesthetic, scientific and reverential) value, as encounter with NWR could lead to significative experiences. - Existential value, as people could still find valuable the existence of the NWR even without directly experiencing it. • It is not possible to establish with absolute certainty that the project is inevitably destined to

		succeed due to the limited access to biomaterial and the cutting-edge technology it requires. • The scientific and conservation values fulfilled by the refinement of protocols could still be realized even in case of failure of the project.
2. Role of the procedure in the project	• Value of the procedure for the project • Effectiveness	• Performing the OPU procedure is needed to collect the necessary oocytes for refining the ICSI and ET protocols, defining embryo quality standards, and creating NWR embryos. For this reasons, it is a key part of the project. • While gamete production from somatic cell associated-techniques can perform a crucial complementary role to the OPU procedure, techniques are still in the process of being adapted to rhinoceros. • The OPU procedure has shown to be rather effective, with 95 oocytes retrieved so far from a single NWR female, Fatu, in 7 interventions, and 13 embryos created via ICSI (see Table 3).

3. Value of the procedure beyond the project	• Scientific value • Conservation value • Animal welfare value • Social value	• Beyond its instrumental value for the project, the procedure conveys several other forms of value: - Scientific and conservation values for cryopreserving biomaterial from an endangered taxon and refining new protocols that could be used for projects involving other taxa. - Welfare value, as extra veterinary screening and care is provided to the animals involved. - Social value, by fostering knowledge transfer and the development and strengthening of links between people, groups and institutions dedicated to conservation.
4. Risks and costs of the procedure	• Known risks, and their distribution • Costs of failure • Negative side-effects in case of success	• Some parts of the procedure (ovarian superstimulation, anesthesia, transrectal ovarian puncture) may lead to complications that could harm the animals involved. • Negative repercussions in case of complication could be: animal welfare impairment; economic damage to the owners; suffering to people who had established bonds with the animal; damage to the image for the project and for the entire conservation world.

5. Views on the procedure	• Public opinion's views on the procedure • Conservationists' views on the procedure	• Conservation ARTs may be accused of being a technofix, of creating a moral hazard, and of being hubristic.

Context of the Procedure

The ultimate goal of this conservation effort is to create a self- sustaining population of NWR to be reintroduced into its still existing natural habitat. Establishing a population with these characteristics is, therefore, the ultimate success criterion of the project. This goal conveys many kinds of values: from the historical and naturalistic to the ecological, economic, transformative, eudaimonic, and existential. Success would also provide for some of the involved females to expand their current range of accessible social behaviors. To reach this goal, the development of technologies and protocols, not yet available (at least for rhinoceros), is required. This means that it is not possible to establish with absolute certainty that the process is inevitably destined to succeed. However, some of the values conveyed by the project would still be fulfilled even in the event of a failure. Given its use of cutting-edge technologies, for instance, the scientific value of the project will still be high even in case of failure and the accumulated knowledge could be used to establish and improve similar procedures. Moreover, there are no opportunity costs falling on traditional conservation efforts, because the project draws from funds allocated for biotechnology and does not use the money raised for the purpose of funding conservation of other rhino taxa.

However, even in the case of success, some aspects must be taken into account when providing an overall evaluation of the project. One aspect concerns the welfare of the newborn calves. Although there is no reason to think that the calves will receive less attention than other white rhinos born in captivity or residing at Ol Pejeta Conservancy, it is not possible to know, in advance, if social interaction problems may arise due to rearing issues. A second aspect to be taken into consideration concerns the possible reintroduction of the NWR into the wild. In addition to all the welfare issues that can arise during a reintroduction (100), the chances of success for the operation lie on the possibility of removing the causes that led in the first instance to the decimation of this taxon, which have to be traced primarily in poaching.

Role of the Procedure in the Project

The OPU procedure is a key part of the project. In the SWR females, OPUs are performed to obtain the biomaterial needed for establishing new protocols for *in vitro* embryo production via ICSI and embryo transfer (ET). This is fundamental both for the "de-dooming" of the NWR as well as for establishing self-sustaining captive backup populations of SWR and helping with their future conservation. In the NWR females, OPUs are performed for producing embryos to be implanted as soon as the protocols for ET are ready. Presently, no alternative exists to this method of obtaining NWR oocytes, but, in the future, gametes could be obtained also from stem cell-associated techniques (5).

Value Beyond the Project

Beyond its immediate use in the project, carrying out the procedure conveys scientific, conservation, welfare, and social values. The refinement of techniques and protocols, the acquisition of new data, and the recurring veterinary screening of the animals can lead to technological and scientific improvement, which, in turn, may have positive repercussions on other conservation efforts. Moreover, the collection of biomaterials from the endangered taxa for cryopreservation has a scientific and conservation value independent from the project goals, due to its insurance value—meaning with this latter expression, the value inherent in the possibility that in the future the conserved biomaterial could be used for scientific or conservation purposes in ways unknown today or not yet developed. Given the international nature of the project, carrying out the procedure fosters knowledge transfer and the development and strengthening of links between people, groups, and institutions interested in conservation.

Risks and Costs

The main risks of the procedure are that it may harm the animals involved. This would be a problem from the point of view of each of the three value dimensions considered: the animal welfare dimension, for obvious reasons, but also the conservation dimension, since an accident could diminish the chances of saving the taxa, and the human dimension, since many people, for various reasons, care about the wellbeing and health of the two animals.

Specifically, there are three potentially critical factors in the procedure: ovarian stimulation involves a series of injections with a GnHR agonist which may accelerate pre-existing pathologies in certain individuals; the transrectal nature of the operation, which despite all caution may lead to enhanced infection risks; general anesthesia, which, while reducing the need of mechanically restraining the animal, can give rise to complications. In the event of a complication due to the procedure, the negative repercussions would be manifold. In addition to the harm caused to the animal involved, the possible economic damage to the owners should be considered. Other negative repercussion will be the suffering caused to people who had established relationships of some kind with the animal and the damage to the image of the project and for the entire community of conservationists.

Public View

The use of biotechnologies is particularly debated since, according to some, it distorts some fundamental aspects of the mission of conservation. Conservation ARTs could be accused in this sense to be a form of technofix, of creating a moral hazard, and of being a manifestation of hubris.

Evaluating the Conflicts and Addressing the Concerns

After building up the EM and mapping the factors involved in the assessment, the main goal of the ethical analysis is to evaluate the conflicts and to address the concerns. Conservation efforts raise inevitable conflicts, as their implementation usually affects different value dimensions and has to deal with complex sets of, often, irreconcilable demands. This is the case also with the OPU procedure that we have been analyzing, especially concerning two issues: the welfare of the involved animals, and the idea of conservation it may convey.

Concerns for the Welfare and Lives of the Animals Involved

Actions necessary for the conservation of the NWR taxon may be detrimental, in case of an accident or complication, to the welfare of the rhinoceros involved in the project, or even pose a threat to their life. However, refusing to intervene would mean failing the duty to conserve important elements of the biodiversity of Earth. A possible radical solution to this conflict would be to rely on an alternative biotechnology, such as the production of gametes from induced pluripotent stem cells. In this way, the same results could be obtained without the risks associated with the OPU procedure. The trouble with this solution, however, is that at the moment, this technology is not yet available for rhinoceros. Due to the age of the remaining NWR, waiting could mean losing the possibility of having both females alive when the first calf will be born, further limiting the generational transmission of skills and cultural traits. While behaviorally the NWR and SWR do not seem to differ decisively from each other, there are some unique elements in the repertoires of the two subspecies. In particular, eating habits seem to differ (46), as well as, to some extent, vocalizations (101). The role played by generational transmission in the expression of these behaviors is not clear, and it is also not clear whether they could be eventually recovered and passed to the future generations of NWR. Nonetheless, it would be unwise to miss this last opportunity, especially considering that the eldest of the two females, Najin, was able to carry out a pregnancy and rear an offspring.

The only viable solution, at present, is to reach an acceptable compromise among the different value dimensions involved. This means that no value demands can be disregarded, or on the contrary, assumed as the only important one to follow. For instance, however valuable we may consider the conservation effort for the survival of NWR, it cannot overrule the basic requirements of animal welfare. At the same time, it must be accepted that as veterinarian procedures, OPU interventions necessarily involve some level of risk concerning the life and the welfare of the animals.

Ovarian stimulation is the first potentially problematic issue of the procedure and should be avoided where there are concrete risks to promote tumor growth in the reproductive tract and induce malignancy.

A second issue is anesthesia, which can give rise to dangerous side effects or even results in the death of the animal. To cope with the matter, the OPU procedure on NWR makes use of an anesthesia protocol specifically devised (102). The main advantage of this protocol is that it is etorphine-free, preventing in this way all the possible side effects associated with this drug, which can be rather severe for the cardiovascular and respiratory systems (88–90), as well as risks of accidental exposure. The protocol is based on four different drugs (butorphanol tartrate, detomidine hydrochloride, midazolam hydrochloride, and ketamine hydrochloride), which interact synergistically with one another, enabling a reduction of their dosage and hence their possible side effects.

Moreover, each of these drugs—except for ketamine hydrochloride—has an antidote, and their effects can be reversed completely.

Butorphanol-based protocols are considered a valid alternative for immobilizing white rhinos (103) and have been shown to produce less respiratory depression and hypoxia (104). Currently, this protocol has been used on more than 500 rhinoceros of different species—both in captive, wild, or semi-wild conditions—and has shown no side effects even if repeatedly used in the same individuals. Consecutive repetition of the protocol makes it possible to better tailor it to the peculiarities of the specific animal. Moreover, the unnecessary use of anesthesia—something to be avoided especially in old animals—can be minimized by proceeding with a preliminary ultrasound screening when the animal is only lightly sedated (i.e., standing sedation), and then choosing whether to continue and proceed into full recumbent anesthesia or terminate the procedure. While frequencies of the procedure similar to those in use with cattle, buffalo, and horses are ruled out, these safer anesthesia protocols allow for the repetition of multiple OPUs on the same individual within a reasonable lapse of time (4).

Finally, a third issue comes from the transrectal puncture which is required to reach the ovaries. Even if restricted to a single penetration of the rectal wall, this puncture still poses a slight risk of infection due to the potential contamination of the puncture needle (47). To mitigate this risk, the rectum of the animal is thoroughly cleaned and disinfected before the procedure, following operative standards similar to those used in human medicine prior to colon resection (47).

In order to check each application of the procedure, an ethical self-assessment through a dedicated tool, ETHAS (105), is practiced before each intervention.

Table 6 recaps all the animal welfare issues and the minimization strategies adopted.

Table 6. Welfare issues and minimization strategies

Procedure	Animal welfare issues	Minimization strategy for the con
Ovarian stimulation	Ovarian stimulation increases the number of available follicles, helping in this way to maximize the collection of oocytes per anesthesia and reducing the number of interventions as much as possible. Con: Injections can be stressful for the animals. Ovarian stimulation may accelerate the progression of certain existing genital tract pathologies.	Exclusion of animals with severe genital tract pathologies from the OPU program.
Full anesthesia	Full anesthesia removes the necessity for mechanically restraining the animals during the procedure—with all the associated risks of injury. Con: May cause severe complications such as aspiration, respiratory depression, hypoxemia, hypertension, pulmonary shunting and ventilation/perfusion mismatch.	Specifically designed ethorphine-free protocol already tested on 500+ animals. The protocols employ four different drugs in order to lessen their individual dosages. For each drug with the exception of ketamine hydrochloridre a specific antidote is available to immediately reverse the effects. Preliminary ultrasound screening may remove possibility of unnecessary use of anesthesia. Ovarian stimulation, maximizing the number of oocyte recovery for each intervention.
Transrectal ultrasound-guided oocyte recovery	Con: Non sterility of the procedure, with the risk of infection.	Cleaning and disinfection of the rectum prior the procedure adopting operative standards from human medicine.

		Ovarian stimulation, maximizing oocyte recovery for every intervention.

Concerns About Conservation ARTs

Conservation ARTs push us far from a model of conservation where our main goal is to limit our interaction with the natural processes. Conservation ARTs, in fact, redefine one of the most paradigmatic of the natural processes, reproduction. In this regard, conservation ARTs may be accused to be hubristic, to be a technofix, or to create a moral hazard.

Without pretending to exhaust the complexity of these arguments, it can be nevertheless noted that they are often used to prove too much with too little. The hubris argument, for instance, is often grounded on the idea that some technologies— particularly those that, by breaking new ground, run the inevitable risk of producing unexpected consequences— may create more problems than they address, and eventually, may even lead to catastrophe. When this argument is used to urge caution, there is nothing suspicious in it, because, in applying a new technology, the risks are often real. However, if the argument is generalized to claim that every application of new technology, even when adopting the necessary measures and protocols, will produce uncontrollable negative consequences, then it is no more plausible.

Concerning the technofix argument, there could be few objections to the fact that conservation ARTs are an attempt to reverse the effects of an ongoing process, that is, human-caused extinction, through the use of technology. This remark, however, can be interpreted in two senses. In the first sense, it can be interpreted as an invitation to not lose sight of the causes that led to the current state of affairs regarding the NWR. This is important. Trying to reverse the decline of a population cannot be done without removing the original causes that led to this situation. Addressing the causes is, in this sense, a necessary condition for success. In a second sense, the previous remark can be interpreted as stating that there is something inherently wrong in working on the effects because this is not sufficient. This is misleading because something not sufficient might still be necessary. In the case of the NWR, for instance, the extinction clock cannot be brought back just by solving the issues that set it into motion, as reverting the population decline is also needed.

The moral hazard argument is based on the claim that having an easy way to revert extinction could make us even more reckless in our attitude toward biodiversity and the environment. To use an analogy, having a lifeboat at our disposal could make us more foolhardy in driving the boat. Again, if this argument is used to caution against the possible perils of new technology, it is sound. If it is used instead to convince us to abandon the technology, it is implausible. Lifeboats may make us more risk-prone, as much as car insurance is said to make drivers less prudent. However, people just do not stop using them because they might increase the risk of incidents. This is because their benefits, in case of an incident, are higher than the costs associated with the risks they may create. The same happens with conservation ARTs: their utility far surpasses the moral hazard they might pose by granting us with a certainly not easy, but nevertheless possible, way to reverse extinction.

Conclusion

Ethical analysis provides us with a way to reflect on a procedure or on a project and it is a necessary step in making its responsible implementation possible. This study presented a frame for the ethical analysis of conservation ART procedures based on the use of the EM to collect the ethically relevant factors to identify issues and value conflicts. The advantages offered by the use of the EM are manifold. In particular, the EM makes it possible to collect and organize the elements, starting from several principles and stakeholders, allowing for a more balanced approach in evaluating complex moral scenarios where different needs, interests, and ethical concerns may conflict.

The focus of the frame presented here is on procedures, and as such, it cannot replace a structured assessment of projects. Although it includes among its requirements the analysis of the general goals and of the context of the procedure, it should not be confused either with an overall evaluation of conservation ARTs or with a general scheme for evaluating complex projects. This does not undermine its utility. The acceptability of the procedures—with respect to the mission of conservation, the welfare of the animals, the people involved, and the public opinion—is an important aspect to discriminate between those projects that are conducted responsibly and those that are not. As applications of conservation ART to endangered taxa will become more and more common, the need to explore their ethical implications becomes increasingly important.

The case study we analyzed is exemplary in this sense. Although the analysis is specifically built around the OPU procedures carried out on white rhinoceros in the context of the conservation efforts to save the NWR, the EM can be used as a template for analyzing ART procedures performed on other rhino taxa and other endangered species. It is rather plausible that the standard scenario of ART procedures administered to rhinoceros or other species for conservation efforts will be simpler than this case. However, this would not reduce the need to carefully address the ethical issues involved.

Ethics statement

The animal study was reviewed and approved by Internal Committee for Ethics and Animal Welfare of the Leibniz-IZW—Approval No: 2019-01-02.

Author contributions

PB, TH, and BM: conceptualization. PB: methodology, original draft writing and preparation, and visualization. CG and SH: data curation. PB, BM, TH, SH, FG, RH, CG,

JS, IP, MS, GL, SC, SS, JZ, SN, SM, LK, IL, PO, and DN: editing and reviewing. BM: supervision and project administration. TH: fund acquisition. All authors contributed to the article and approved the submitted version.

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1.2. An ethical assessment tool (ETHAS) to evaluate the application of assisted reproductive technologies in mammals' conservation: the case of the northern white rhinoceros (*Ceratotherium simum cottoni*)

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Simple Summary

Applying assisted reproductive technologies (ARTs) to the conservation of endangered species may be the only way to save them from extinction. However, ART application can raise relevant ethical issues and could benefit from a comprehensive ethical assessment. Unfortunately, there is a lack of attention to the topic in the scientific literature and, to our knowledge, there is no tool for the ethical assessment of ARTs in the context of conservation that has been described. In the present paper, we show the effects of applying a dedicated ethical self-assessment tool, the Ethical Assessment Tool (ETHAS), to ovum pick-up and *in vitro* fertilization procedures performed within the BioRescue project. The BioRescue project is an international enterprise using ARTs to save the northern white rhinoceros from extinction. The situation of the northern white

rhinoceros is particularly critical as there are only two individuals of this subspecies still alive and they are both infertile females. The application of the ETHAS to the procedures contributed to the overall acceptability of the project and improved communication among the project's partners. In turn, the tool itself was also refined through an iterative consultation process between experts (both ethicists and scientists) and stakeholders.

Abstract

Assisted reproductive technologies (ARTs) can make a difference in biodiversity conservation. Their application, however, can create risks and raise ethical issues that need addressing. Unfortunately, there is a lack of attention to the topic in the scientific literature and, to our knowledge, there is no tool for the ethical assessment of ARTs in the context of conservation that has been described. This paper reports the first applications of the Ethical Assessment Tool (ETHAS) to trans-rectal ovum pick-up (OPU) and *in vitro* fertilization (IVF) procedures used in a northern white rhinoceros (*Ceratotherium simum cottoni*) conservation project. The ETHAS consists of two checklists, the Ethical Evaluation Sheet and the Ethical Risk Assessment, and is specifically customized for each ART procedure. It provides an integrated, multilevel and standardized self-assessment of the procedure under scrutiny, generating an ethical acceptability ranking (totally, partially, not acceptable) and a risk rank (low, medium, high), and, hence, allows for implementing measures to address or manage issues beforehand. The application of the ETHAS to the procedures performed on the northern white rhinoceros was effective in ensuring a high standard of procedures, contributing to the acceptability and improved communication among the project's partners. In turn, the tool itself was also refined through an iterative consultation process between experts and stakeholders.

Introduction

In the present global scenario, where an accelerated rate of extinction is paired with a severe decline in populations' abundance in surviving species [1,2], assisted reproductive technologies (ARTs) can make a difference in biodiversity conservation. ARTs can raise the chance of success of conservation breeding programs by both overcoming infertility issues and optimizing genetic management, avoiding inbreeding (or outbreeding) depression and risks of transmission of inherited diseases [3–5].

ARTs, in fact, may offer the only chance for survival of many endangered species with very fragmented populations or only few extant individuals. In this case, ARTs can be employed not only to boost the number of offspring, but also to enhance the genetic exchange between the fragmented populations (living both *in situ* and *ex situ*) without the need of actually translocating the animals [6], ARTs can also enhance the genetic exchange between living and dead generations by using gametes stored in cryobanks [7] or, in what could be a possible near-future development of this biotechnology, produced from stem cells [8].

While ARTs are a robust opportunity in the conservationist's toolbox—and one which promises to become increasingly important in the future—their application may raise several ethical issues. The use of ARTs can raise ethical concerns also in human medicine, some of which can be still valid when ARTs are applied to non-human animals, but many of the issues raised by the application of these technologies in conservation breeding projects are more specific [9–11]. These may range from issues also common in applications of ARTs to livestock [12–14] to more specific issues tied to the particular context of biodiversity conservation. For instance, ARTs need species-specific optimization in order to be successfully employed, and this, in turn, depends on detailed knowledge of the reproductive biology of the species involved [4]. Such knowledge may be difficult to obtain in already endangered species, due to the limited numbers of available individuals for research and the potential difficulties in accessing them [15]. In the end, its pursuit may pose several dilemmas to scientists and conservationists intentioned to both safeguard the remaining individuals of a species and obtain enough information for a last attempt to reverse its decline. It could also be claimed that important resources—in terms of time, space, people, competencies, and funding [16], which are needed to implement conservation projects involving ARTs, from the first step of species-specific optimization of the techniques to the breeding and reintroduction steps—could be perhaps better allocated to other more traditional forms of biodiversity conservation. Moreover, from a more theoretical perspective, applying ARTs could be seen as an exemplary case of "technofix" [11,17], that is, the short-sighted use of technology as a way to sort out the outcome of morally problematic activities instead of addressing their causes, or as an apparently "easy" solution to the decline in wildlife populations, with the risk of inducing complacency in the problem.

Above all, a crucial source of ethical concern regarding ARTs in biodiversity conservation is animal welfare. Many applications of ARTs require manipulation of live animals and, in some case, invasive procedures, with real risks for their welfare. This is of course also true for farm animals, where the issue has not received enough attention (for instance, [18,19]), but is further exacerbated in wildlife, where at least three factors intervene to complicate the matter. The first is the experimental characters of many ARTs applications to wildlife, with procedures less established than in livestock and which often stand in a gray area between research and veterinary practice. The second is our knowledge on animal welfare science, which, again, is scarcer in wildlife than in farm or laboratory animals. The third concerns manipulation of the animals. While livestock and, in general, domestic animals are more accustomed to being manipulated by people, operating on wildlife may be more stressful for the animals involved (and also for the staff performing the procedures) and may be more demanding in terms of restraint, sedation, or anesthesia. Moreover, this higher toll exacted in terms of animal welfare may be more difficult to mitigate, since excessive conditioning of the animals involved in the procedures could be undesirable due to the need for minimizing the effects of captivity [20].

In general, when an ethical assessment of a procedure involving individual animals has to be carried out, the golden standard would be a systematic project evaluation, requiring, among other things: (i) a risk assessment; (ii) an assessment of welfare conditions and pain, suffering, distress, and lasting harm imposed on the animals; (iii) a harm–benefit evaluation; and (iv) the application of the 3Rs (Replacement, Reduction and Refinement) [21]. This standard is, at least in theory, systematically applied when research projects involving laboratory animals are submitted to ethical committees for evaluation. With regard to wildlife, however, this standard evaluation is not performed systematically. Yet this evaluation is crucial, especially for projects involving ARTs.

Risk assessment, for instance, should be considered essential in these cases. Application of ARTs to wildlife and their biomaterial entails accepting a certain grade of uncertainty. This requires a prior definition of the ethically tolerable risk threshold for the procedures, which can be conducted only by performing a detailed risk analysis, based on traditional risk analysis [22], specific animal welfare [23] and ethical risk analysis [24], and application of the precautionary principle [25–28].

The assessment of potential pain, suffering, distress, and harm, alongside general welfare conditions of the individual animals involved in the procedure, should also be considered essential. However, pain, suffering, distress, harm, and, in general, the welfare of the individual animals have traditionally played a secondary role in biodiversity conservation. This is partly due to the fact that the goals of biodiversity conservation and of animal welfare are conceptually distinct and may sometimes diverge, since the former is mainly focused on species, whereas the latter is focused on individuals [29–31].

Nevertheless, excessive divergence may remove societal support for conservation projects [32,33]. Moreover, animal welfare is a crucial factor in the success of conservation breeding and reintroduction programs [20,34]. Yet, as already noted, the assessment of wildlife welfare may be harder to obtain. Knowledge on the issue is lacking if compared to laboratory animals. This is both due to fewer research works on the former subject than on the latter and to the larger number and diversity of wild vertebrate species compared to the few taxa employed in laboratory research [35]. These difficulties, however, do not remove the need to carefully assess the general welfare conditions and the specific potential pain, suffering, distress and lasting harm imposed on the animals during the application of the ART procedures.

The third important requirement is harm–benefit analysis. Again, while this is nowadays routine in the ethical assessment of laboratory projects involving animals [36], it is instead underrepresented in wildlife studies. In particular, harm–benefit analysis has been rarely applied to evaluate the impact on the health and welfare of wild animals involved in veterinary procedures aimed at safeguarding their species [37]. Nonetheless, it is progressively used to identify costs and benefits arising from conservation projects in relation to not only their economic impact [38], but also to their positive or negative consequences for the ecosystem and the local wildlife population.

The same can be said also for the fourth requirement, the application of the 3Rs, which has been widely satisfied in laboratory research but rarely in wildlife studies, where research conditions are more heterogeneous and it is harder to standardize a methodology for its implementation as has been done in laboratory research. However, as progressively stated [39,40], the 3Rs principle is crucial also for wildlife research. For instance, replacement can be obtained with non-invasive research techniques, reduction with optimized experimental design and refinement with better methods of capture, anesthesia and handling [39].

It may be countered that conservation interventions do not qualify—at least in a full sense—as research and, as such, should not be subjected to the same stringent standards involved in laboratory research. However, as already noted, the boundaries between research and veterinary practice are often blurred when applying ARTs to conservation breeding programs. Moreover, most applications of ARTs to wildlife may take place both in research and non-research scenarios. This raises a boundary problem, as the same activity may be subjected to different ethical standards of evaluation when performed in different contexts. To solve this inconsistency, it has been suggested that far from relaxing our ethical standards on research, we should instead extend them to all similar activities [41,42].

For all these reasons, conservation projects incorporating ARTs should be carefully scrutinized in order to evaluate their ethical acceptability, using the highest procedural standards and compliance with best practices and regulations as landmarks. Currently, despite the increasing interest in the use of ARTs in conservation, there is little attention to ethical assessment and, to our knowledge, there are no tools to evaluate the specific risks and ethical aspects involved. A simple search on Scopus (https://www.scopus.com/), with "ethical assessment" AND "reproduction" and "wild" and "animal" as keywords run in December 2020, gave no results. One of the reasons for this result could be that, with ARTs being applied to conservation breeding projects often in the gray area between clinical practice and research, their use in such context often does not require external ethical approval. It is therefore even more important that the practitioners and the researchers involved in these types of projects are able to evaluate the potential ethical relevant issues spanning from the procedures they use themselves. One way to enable practitioners and researchers to evaluate their procedures is to provide them with a comprehensive and customizable tool for the self-assessment of such procedures, which, once developed by experts with an ethics background (specifically, in applied ethics related to conservation and animal welfare), can be used also by people lacking such background. Self-assessment could also be an important step in preparation for an external overall evaluation of the ethical acceptability of a project and could help scientists to be proactive and to scrutinize the ethical issues surrounding their work [43]. In this paper, we present the self-ethical assessment of two ART procedures performed in the context of a conservation breeding program aimed at avoiding the extinction of the northern white rhinoceros (*Ceratotherium simum cottoni*—NWR). The procedures involved both southern white rhinoceros (SWR) females in European zoos and the last two surviving NWRs. The assessment was preformed using a self-assessment tool explicitly designed for conservation breeding programs, the Ethical Assessment Tool (ETHAS), as customized for the self-assessment of ovum pick-up (OPU) and *in vitro* fertilization (IVF) procedures. The aim of the study was to investigate both whether applying the tool could contribute to ensuring a high standard and improvement of procedures being assessed and, at the same time, how applying the first version of the tool in actual field conditions contributes to shape and improve the tool itself.

Materials and Methods

The Case

The NWR, a subspecies of the white rhinoceros (*Ceratotherium simum*), once ranged over much of the savannah of Central Africa [44–46]. However, between the 1970s and the 1980s, the wild population was reduced to only 15 individuals, and there have been no reported signs of their presence in the wild since 2007. Nowadays, it is declared as "possibly extinct in the wild" [47], as the only remaining individuals live in captivity. The last remaining individuals are two females, Najin and Fatu, who are under constant surveillance at Ol Pejeta Conservancy, in Kenya, and cannot have a viable pregnancy due to health and age-related issues. Najin is 31 years old and has a large ovarian tumor on her left adnexus. Moreover, she has very weak hind legs due to bilateral alterations of the Achilles tendons. Her 20-year-old daughter Fatu has developed untreatable degenerative endometriosis of unknown cause over her entire uterus [48]. Therefore, the only chance to save this iconic subspecies from the brink of extinction is to utilize ART procedures, using *in vitro* embryos gestated by recipient mothers of the sister subspecies—the southern white rhinoceros (*Ceratotherium simum simum*—SWR). In order to produce embryos, however, gametes have to be obtained first. During the last two decades, scientists have collected the semen from four NWR bulls and cryopreserved it in three different cryobanks [48]. No oocytes, instead, have ever been stored because of their low permeability to cryoprotectants and consequent susceptibility to chilling [49]. This means that ovum pick-up (OPU) has to be repeatedly performed on the two surviving females, in order to obtain viable oocytes, which are then sent to a specialized laboratory for incubating, maturing and performing *in vitro* fertilization (IVF), in order to obtain viable embryos. The embryos are then stored in liquid nitrogen, until transferred into an SWR recipient mother. OPU on Najin and Fatu was performed for the first time on August 22th, 2019, in collaboration with the Kenya Wildlife Service (KWS), and has been repeated three more times. Despite the difficulties caused by the COVID-19 pandemic, at present, there are five embryos ready for transfer.

When conducting an ethical assessment on ART procedures involving Najin and Fatu, the health-related issues of the two individuals are likely to be very relevant both because, as already said, they prevent the two animals from having a viable pregnancy and they impact on their welfare, mainly by modifying the risks that ART procedures create for the involved animals. In rhinoceroses, in general, OPU needs full anesthesia [50,51], with the animal lying down, and thus it may be a risky procedure even in healthy animals [48,52,53]. The scientific literature and best practices show that rhinoceroses quickly recover from ovum pick-up [54,55]—as fast as farm animals—making repeated anesthesia possible even within a short time period [51,56,57]. The health situation of the two NWRs may alter the risks posed by repeated anesthesia because their chronically ill status might affect their resilience to the procedure. However, the fact that they suffer from health issues increases the importance of being able to perform OPU with a higher frequency on them, in order to have more chances to succeed in saving the species from utter extinction, since their health issues might adversely affect their life expectancy and thus the time available for scheduling OPU.

Given the complexity of the ethically relevant issues involved, a sub-project dedicated to the development of a specific ethical self-assessment tool which could be used in mammalian conservation breeding programs was created within the BioRescue project—the international consortium led by the Leibniz Institute for Zoo and Wildlife Research of Berlin (Leibniz-IZW) and comprising the Czech Dvur Králové Zoo, Avantea laboratory, Max Delbrück Center for Molecular Medicine (MDC), Kyushu University and Padua University (and having the support of other international partners), which is in charge of the whole project that involves Najin and Fatu and aims at avoiding the final extinction of the northern white rhinoceros.

The Tool (ETHAS)

The Ethical Assessment Tool (ETHAS) is a flexible and customizable instrument for the ethical self-evaluation of specific ART procedures applied to mammals in biodiversity conservation projects. It includes and integrates with each other risk assessment (general, ethical and welfare), pain/distress/welfare evaluation, harm–benefit analysis and the 3Rs tenet application. As already stated, self-assessment tools help scientists to be proactive and to scrutinize the ethical issues surrounding their work and are preliminary for an external overall evaluation of the ethical acceptability of a project [43]. Their implementation fosters dialogue between all participants and may lead to the actual improvement of the procedures. Moreover, routinely performed ethical self-assessment helps scientists to comply with ethical principles, best practices with animals, relevant legislation and authorizations and ethical approval [35]. Self-assessment cannot replace ethical assessment by an external committee, but it contributes both to the final acceptance of the project, by anticipating its possible ethically critical issues (and hence allowing for timely and comprehensive design of mitigation strategies), and to the communication of its results to the general public.

ETHAS is based on checklists, a tool commonly used in medicine and other fields to identify errors, ameliorate operational standards and comply with best practices [58,59]. Checklists are a valuable tool for self-assessment. Their use improves research results and makes them easier to be communicated, contributing to the responsible conduct of research, thereby increasing its public acceptance [35,43]. Moreover, they can be used by both experienced and inexperienced personnel alike, and they are easily understandable and verifiable [59].

ETHAS's checklists aim to combine risk assessment with ethical acceptability assessment. Risk assessment is a crucial phase of risk analysis, and therefore it is very important for the overall ethical acceptability of wildlife conservation projects. As it is known, risk analysis is a three-step process: (i) risk evaluation/assessment, (ii) risk management and (iii) risk communication [60,61]. It allows a standardized, repeatable, transparent and documented evaluation of the risks posed by a course of action or a chain of decisions [62]. The use of ARTs on wild animals entails the acceptance of a certain level of risk, but this level must conform to the "as low as reasonably applicable principle" (ALARP) [63].

Therefore, the general frame of the ETHAS tool is based on two integrated checklists for self-assessment, the Ethical Evaluation Sheet (EES) and the Ethical Risk Assessment (ERA). Each ERA item is conceptually linked to a corresponding part of the EES checklist, which comprises, among others, all the relevant ethical aspects that are investigated in ERA. The link is reported in a column with an alphanumeric code.

There are customized EES and ERA versions for each ART procedure, but all share some common features. These constituent checklists of both EES and ERA have been developed on the basis of the current literature and best practices guidelines and refined through an iterative consultation process between experts (both ethicists and scientists) and stakeholders, which is still ongoing in the present stage of the project. They merge risk analysis, based on a combination of traditional, animal welfare and specific ethical risk assessments, with ethical analysis, based on pain/distress/welfare evaluation, harm–benefit analysis and the 3Rs tenet application, with the aim of defining the overall ethical acceptability of the procedure under assessment.

Ethical Evaluation Sheet (EES)

The Ethical Evaluation Sheet (EES) highlights potential ethical issues arising from the ART application. As with corresponding tools for the ethical assessment of research projects with laboratory animals [43,64–66], the general frame of EES consists of four main sections of investigation: (a) Documents; (b) Harm–benefit evaluation; (c) Procedure quality evaluation; and (d) Scientific team quality evaluation. For each specific ART procedure, it is necessary to detail a certain number of items within these main sections. In the first trial, the EES for the OPU procedure consisted of a total number of 83 items, whereas the IVF-lab EES consisted of 64 items. However, since some items are made up of sub-items, the total possible answers counted in the final score can be more. Regarding the OPU EES, the total number was 88, while in the IVF-lab EES, it was 81. After the revision of some items, detailed in Section 3.2, a second version of both the OPU and the IVF-lab EES was developed. The second version of the EES for the OPU procedure consisted of a total number of 86 items, with a total number of 91 items and sub-items, whereas the second version of the IVF-lab EES had 66 items, with a total number of 83 items and sub-items.

Table 1 shows the general structure of the EES checklists for OPU and IVF procedures in more detail and reports the scientific sources of information used in their development.

Table 1. Ethical Evaluation Sheet sections and bibliography.

EES Sections and Sub-Sections	Number of Items (Sub-items) OPU EES		Number of Items (Sub-items) IVF-Lab EES		Bibliography
	1st Trial	2nd Trial	1st Trial	2nd Trial	
A) Documents					[21,39,43,67–73]
	11 (13)	11 (13)	9 (10)	9 (10)	
B) Harm–benefit evaluation of the procedure					[36,64–66,69,74–81]
B1) Benefit evaluation	12 (14)	12 (14)	7 (7)	7 (7)	
B2) Harm evaluation	8 (9)	8 (9)	4 (8)	4 (8)	
C) Procedure Quality Evaluation					[21,36,39,40,54,64–66,75–82]
C1) Pre-screening consideration	6 (6)	6 (6)	6 (6)	6 (6)	
C2) Procedural steps evaluation	3 (3)	3 (3)	5 (5)	5 (5)	
C3) 3Rs evaluation (replacement, reduction, refinement)	23 (23)	23 (23)	14 (21)	14 (21)	
D) Scientific team quality evaluation					[62,64,76]
D1) Team and teamwork	13 (13)	14 (14)	12 (17)	12 (17)	
D2) Equipment	5 (5)	7 (7)	4 (4)	6 (6)	
D3) Laboratories and biobanks	2 (2)	2 (2)	3 (3)	3 (3)	
E) Final ethical evaluation of the procedure					[76]
	11 (11)	11 (11)	9 (9)	9 (9)	

The EES is designed to be filled in only once (unless the procedure's protocol is changed) before to start the procedures. In the case of the procedures performed during the present study, as it was a phase in the development of the final version of the tool, the EES was filled in by a member of the BioRescue team with an ethical background in applied ethics in conservation and animal welfare. However, as underlined in the Introduction, in the final version of the tool, any member of the team performing the procedures will be able to fill in the EES, without the need of a specific ethical background. During the EES compilation, it is asked to answer “yes” or “no” to all items, depending on whether the requirements are met or not. Moreover, for some EES items, it is required to add further information to explain the answer. The EES is evaluated using a semi-quantitative scoring model in which the answers “yes” or “no” assume the value of 0 and 1, respectively. The sum of the items’ outcome divided into three homogeneous ranges defines the rank of the ethical acceptability of the procedure: not acceptable, partially acceptable, acceptable. Therefore, the final score obtained from the EES compilation identifies one of the three acceptability ranks. Table 2 describes the EES final score for the OPU and IVF procedures performed in the present study.

Table 2. Acceptability ranking and scoring of the ovum pick-up (OPU) and *in vitro* fertilization (IVF-lab) Ethical Evaluation Sheets (EESs) applied in the present study.

Acceptability Ranking	**Score in OPU EES**		**Score in IVF-Lab EES**	
	1st Version	**2nd Version**	**1st Version**	**2nd Version**
Totally acceptable	0–29	0–30	0–27	0–27
Partially acceptable	30–58	31–60	28–54	28–55
Not acceptable	59–88	61–91	55–81	56–83

The identified acceptability level that represents the outcome of the EES assessment (defined as the first review level) defines the degree of the procedure acceptability. In case of a partial or not acceptable result in the ethical assessment, detected with the first review level, each section of the EES checklist is assessed individually. This second review level identifies at which section of the procedure corrective actions need to be planned. Finally, a third review level allows identifying the items whose requirement is not met and, therefore, the critical issues of the procedure to be reviewed before the procedure begins.

Ethical Risk Assessment (ERA)

The ERA checklist is specifically customized for each procedure under scrutiny by identifying the appropriate phases for risk assessment. The scientific literature on ARTs has been revised to analyze, in detail, each step of the OPU and IVF procedures and detect possible hazards and ethical risks whose occurrence could negatively impact on the animal welfare, staff safety and procedure outcome [83]. As shown in Table 3, the OPU ERA is composed of five different phases: A) Identification of the individual/s, welfare assessment and procedure planning; B) Ovarian stimulation protocol; C) Anesthetic procedure for oocyte recovery; D) Oocyte recovery by transrectal procedure; and E) Gametes packaging. The total number of items in the OPU ERA first version was 52, while in the second, it was 56. Since some items are made up of sub-items, the total number of the first version was 91, while that of the second one was 101. Table 3 shows the OPU ERA checklist in more detail and reports the scientific sources of information used in its development.

Table 3. OPU Ethical Risk Assessment (ERA) phases and bibliography.

	OPU Ethical Risk Assessment Phases	Number of Items (Sub-Items) 1° Version	Number of Items (Sub-Items) 2° Version	Bibliography
A)	Identification of the individual/s, welfare assessment and procedure planning	17 (34)	19 (36)	[19,67,84–88]
B)	Ovarian stimulation protocol	6 (8)	6 (8)	[50,54,89]
C)	Anesthetic procedure for oocyte recovery	10 (18)	13 (27)	[50,52–57,89–91]
D)	Oocyte recovery by transrectal procedure	12 (20)	11 (19)	[50,54,90,92–94]
E)	Gametes packaging	7 (11)	7 (11)	[95–97]

The IVF-lab ERA, instead, as shown in Table 4, is composed of nine phases: (A) Laboratory quality assessment and specimens processing; (B) Gametes shipping to the laboratory; (C) Gametes biobanking; (D) Gametes preparation for ICSI; (E) Intracytoplasmic sperm injection (ICSI); (F) Embryos culture; (G) Embryos cryopreservation and biobanking; (H) Embryos packaging; and (I) Embryos shipping. The total number of items in the IVF-lab ERA was 72. Since some items are made up of sub-items, the total number was 103.

Table 4. IVF-lab ERA phases and bibliography.

IVF-Lab ERA Phases	Number of Items (Sub-Items)	Bibliography
A) Laboratory quality assessment and specimens processing	17 (32)	[98–101]
B) Gametes shipping to laboratory	7 (8)	[7,54,102–104]
C) Gametes biobanking	7 (8)	[7,102,105,106]
D) Gametes preparation for ICSI	13 (16)	[54–107]
E) Intracytoplasmic sperm injection (ICSI)	6 (6)	[54,92,107,108]
F) Embryos culture	7 (7)	[54,109]
G) Embryos cryopreservation and biobanking	4 (11)	[54,102,110]
H) Embryos packaging	4 (7)	[109]
I) Embryos shipping	7 (8)	[109,111]

Each item and sub-item of the ERA checklists analyzes an element of the procedural step which could cause a hazard to the success of the phase under assessment. For each item, it is required to record a "yes" or "no" whether the requirement of the item is satisfied or not. Depending on the characteristics of the requirement and on the severity of the consequences associated with the hazard scenario, each item is scored differently (Table 5). For example, the consequences associated with a failure highlighted with items in phases A, B, C and D of the OPU ERA have different effects. Non-compliance with operational or animal management requirements has a more significant impact on animal welfare than non-compliance with operational instructions or documentary, structural, instrumental and environmental requirements (Table 5). The items of phase E of the OPU ERA have been evaluated with the risk categories of the IVF-lab ERA due to the consequences of the hazard impact on the gametes' safety. In the IVF-lab ERA, three scoring ranges were defined on the basis of the type and severity of the possible outcomes that the hazard scenarios could have on gametes and embryos.

Table 5. Description of risk categories and corresponding score used for phases A, B, C and D the OPU ERA and for phase E of the OPU ERA and all phases (A–I) of the IVF-lab ERA.

Phases	Categories	Characteristics of the Requirement	Score
OPU ERA (phases A–D)	Low	Documents, procedures, operating instructions, etc.	1
	Medium	Structural, instrumental and environmental requirements.	2
	High	Operational requirements.	3
OPU ERA (phase E) and IVF-lab ERA	Low	Factors affecting the process (documental and procedural support aspects).	1
	Medium	Factors related to the traceability and distribution of specimens, laboratory operator's safety, quality and availability of laboratory facilities.	2
	High	Factors related to the viability of gametes and embryos and to the instrumental requirements and the chemical reagents used.	3

The assessment uses a semi-quantitative scoring model where the risk is determined by a single value R that combines the probabilities (p) and consequences (x) associated with the occurrence of a hazard scenario [112]. The hazard scenario is identified with each ERA item. The probabilities are determined by the satisfaction or not of the item. The consequences depend on the characteristics of the requirement of the item and are classified into different levels of severity, in accordance with Table 5.

$$R = \sum_{i=1}^{n} p_i x_i$$

In the specific model, n corresponds to the number of scenarios chosen to describe the risk (number of items of the ERA checklist), pi can assume values of 0 or 1 depending on whether the requirement is met (yes) or not (no/no answer) and xi is from 1 to 3, as described in Table 5.

ERA checklists are designed to be filled in each time a procedure is performed. They have to be filled in by one to three different people, depending on the procedure under assessment, with two main aims: to have an overview of the procedure and to verify, in case of more persons involved in the assessment, if communication regarding ethically relevant issues among the participants is effective. Regarding the OPU procedure, for instance, if it is executed only by the veterinary staff of the zoo or facility hosting the animals, the ERA can be filled in just by the chief veterinarian. If the OPU procedure is executed by an external veterinary team, the ERA has to be filled in both by the external and internal veterinarians and the zoo or facility managing director. In the applications of the ETHAS described in the present paper, three different participants responded to the OPU ERA for both the procedures performed: the veterinarian responsible for the BioRescue project, the local veterinarian and the managing director of the facility where the procedure took place.

Regarding the second aim—to verify if communication is effective—the three answers for each item are entered in an Excel spreadsheet, and the modal value that allows highlighting the most frequent responses per set of answers is calculated. The sum of the modal values is divided into three ranges, identifying the three categories of risk severity (low, medium, high). On the contrary, the modal value is not necessary at all for the IVF-lab ERA because it is compiled by only one person—the person responsible for the IVF laboratory. In this case, the sum of the values of each answer is divided into three ranges, corresponding to the three risk categories (Table 6).

Table 6. Risk ranks of the OPU and IVF-lab ERAs.

Risk Rank	Score in OPU		Score in IVF-Lab
	1st Version (October 2019)	2nd Version (December 2019)	Final Version (October 2019)
Low	0–63	0–73	0–61
Medium	64–126	74–146	62–123
High	127–190	147–220	124–184

Similarly to the EES, also for the ERA, three review levels can be applied: at an overall level (risk rank, first review level), at the phase level (second review level) and at the items level (third review level). The review levels allow revising the specific application of the procedure in case of the detection of a medium or high risk rank and applying risk management and risk communication strategies.

Final Overall Evaluation (EES + ERA)

The ETHAS generates a risk rank (low, medium, high) through the ERA and an ethical acceptability rank (totally, partially, not acceptable) with the EES. The overall final evaluation (ERA + EES) is calculated by combining the acceptability ranking obtained from the EES and the risk rank obtained the from ERA (Table 7). Therefore, ETHAS overall evaluation falls into three categories:

(1) Acceptable, when the ESS results in totally acceptable and the ERA detects low risks. The assessed procedure may be accepted without further actions.

(2) Acceptable with mitigation, when the EES results in partially acceptable and the ERA detects medium risks. The assessed procedure may be accepted only if critical issues are identified and addressed and the specific application of the procedure is revised.

(3) Not acceptable, when the EES detects a not acceptable result and the ERA detects high risks. The assessed procedure may be unacceptable until further improvements are enforced to eliminate the associated ethical concerns and procedural risks.

Table 7. Ethical Assessment Tool (ETHAS) overall final evaluation, obtained by combining results from the ESS and ERA checklists.

ERA / **ESS**	**Low Risk**	**Medium Risk**	**High Risk**
Totally acceptable	Acceptable	Acceptable with mitigation	Not acceptable
Partially acceptable	Acceptable with mitigation	Acceptable with mitigation	Not acceptable
Not acceptable	Not acceptable	Not acceptable	Not acceptable

Scoring of both checklists and the overall final evaluation have to be performed by the person completing the EES.

After the risk assessment, the ETHAS enables risk management of the possible highlighted hazards. Risk management (the second phase of a risk analysis process) allows raising awareness of the potential hazards and risks and enables the sharing and acceptance of the measures to be adopted to reduce the risks. Risk mitigation actions have to be chosen taking into account: (1) the characteristics of the requirements (in terms of scoring); and (2) what is reasonable and technically possible. Moreover, risk management allows an exchange of information and opinions between the staff involved in the ART procedures. Finally, the ETHAS enables also risk communication: through an iterative process among the staff directly involved in the procedures, information and opinions on hazards and their associated risks are exchanged, allowing a transparent and overarching discussion of results.

Application of the Tool

In a preliminary phase of ETHAS development, after consulting the relevant scientific literature and best practice guidelines on OPU and IVF procedures, a draft of the checklists was designed using a bottom-up approach, by witnessing several procedures and discussing with the teams performing them the main areas identified by the scientific literature and best practices. Relevant areas, not previously found in the literature search, but found to be relevant in the practical application of the ART procedures, were also added and discussed. The OPU procedures witnessed in the preliminary phase included both procedures performed on infertile SWRs in European zoos—who were involved in the BioRescue project both for approaching their infertility problems and for protocol optimization—and those (August 2019) performed on Najin and Fatu, in order to ensure suitable consideration of the relevant specific features of these individuals (e.g., their health status, as discussed in 1.1.) in the tool. The IVF procedures witnessed were all performed at the Avantea laboratory, which up to now is the only one that produced a viable rhinoceros embryo.

The preliminary phase led to the first version (beta1) of the ETHAS customization for OPU procedures (OPU EES + OPU ERA). The complete beta1 version can be found as Supplementary Material (File S1 and S2). The beta1 ETHAS version was then applied in October 2019 during an OPU procedure performed by the BioRescue team on three sub-fertile or infertile SWR females housed in a European zoo, in order to evaluate both the effects of conducting ethical self-assessment on the application of ART procedures and to improve the beta version of the tool itself.

The application of the beta1 version led to the revisions of some items, detailed in Section 3.2, resulting in the creation of an updated version (beta2) of the OPU EES and ERA. The beta2 version was applied in December 2019, during an OPU procedure performed by the BioRescue team on the last two NWRs in Kenya. Both procedures (October and

December) were performed following the BioRescue team's standardized protocols. Similarly, the first version (beta1) of the ETHAS customization for IVF procedures (IVF-lab EES + IVF-lab ERA) was first applied in August 2019 (Supplementary Material File S3 and S4), and the second one (beta2, after the changes detailed in Section 3.2) was applied in October 2019, at the Avantea laboratory.

Results

How Applying the Tool Contributed to the Refinement of The Procedures

EES

In both the first and second assessment trials, the ethical assessment of OPU and IVF-lab resulted in "Totally acceptable" in both EESs (Table 8). However, despite this result, the EESs were investigated at the second and third review levels to examine whether there were unmet requirements and, if so, in which sections and items they were found.

Table 8. EES results. Please note that the changes detailed in Section 3.2 were already included in the EES version used for the second OPU and IVF trials.

EES		OPU EES 1st Trial		OPU EES 2nd Trial		IVF-Lab EES 1st Trial		IVF-Lab EES 2nd Trial	
		Positive Answers	Negative Answers	Positive Answers	Negative Answers	Positive Answers	Negative Answers	Positive Answers	Negative Answers
A)	Documents	13 over 13	0 over 13	13 over 13	0 over 13	10 over 10	0 over 10	10 over 10	0 over 10
B)	Harm–benefit evaluation of the procedure	20 over 23	3 over 23	20 over 23	3 over 23	14 over 15	1 over 15	14 over 15	1 over 15
C)	Procedure quality Evaluation	32 over 32	0 over 32	32 over 32	0 over 32	32 over 32	0 over 32	32 over 32	0 over 32
D)	Scientific team quality evaluation	20 over 20	0 over 20	23 over 23	0 over 23	24 over 24	0 over 24	26 over 26	0 over 26
Total		85 over 88	3 over 88	88 over 91	3 over 91	80 over 81	1 over 81	82 over 83	1 over 83

The OPU EES in the first trial received a final score of 3 over 88, while in the second trial, it received a final score of 3 over 91. In both trials, the three negative answers were detected in the “Harm–benefit evaluation of the procedure” section. The first of the three unmet requirements was related to the fact that infertility is not widespread in the SWR wild population. For this reason, even if it is fundamental to optimize the procedure for this subspecies in zoos and facilities alike, there is no wilder population that can receive a direct benefit from this process. Nevertheless, the acquired knowledge on the rhinoceroses’ reproduction might turn out to be useful in the future, also for the other rhino species. The second concerns the possibility that the OPU procedure may have adverse side effects on the animal under it in case of a harmful event. Even if all the precautions are taken, the risk probability is never zero. Finally, the third one was related to the fact that any adverse event on the last two NWR females impacts this subspecies.

Regarding the IVF-lab EES first trial, the final score was 1 over 81, while the IVF-lab EES second trial obtained a final score of 1 over 83. Similarly to the OPU EES, the section that contained the not satisfied requirement in both trials was the "Harm–benefit evaluation of the procedure". The specific item was related to possible adverse side effects that can lead to biomaterial damage, even if all precautionary measures were taken.

ERA

The application of the OPU ERA first version, in a European zoo in October 2019, resulted in "low risk". Checklists filled in by the three respondents were analyzed for assessing both the procedure itself and the effectiveness of communication among the participants. In particular, the assessment of the procedure itself did not find any relevant nonconformity in the procedures. All potential issues were taken into account and suitable measures were enforced to minimize risks. The only negative score was concerning "previous experience of the local team" in OPU on rhinos, which was not a problem in itself because of the presence of the BioRescue veterinary staff, who coordinated and carried out the procedures.

When the answers of all three respondents were analyzed to assess communication, the obtained risk score was 57, over a total of 190. The "low risk" ranking notwithstanding, the second and third review levels were applied, and the ERA outcome was further investigated. Twenty items—distributed among the A and D phases—were identified. The characteristics of the requirements not met were related to "Documents, procedures, operating instructions" for 10 items and "Operational requirements" for the other 10 items. Apart from "experience of the local team", in all these cases, the problem was that the two local respondents did not answer to some items, although the BioRescue veterinarian had, so the modal value was 0. The same was true of the whole of phase E. Thanks to the third review level, it was possible to detect that the items that recorded "no" or “no answer” were mainly related to sub-optimal explicit communication of some issues between the three main people responsible for the procedure.

The highlighted communication issues in the first version were not detected in the second one. Consequently, the OPU ERA applied in December 2019 in Kenya resulted in “low risk” with a risk score of 0 over 220. Therefore, it was not necessary to proceed with the second and third review levels (Table 9).

Table 9. Results of the first and second assessment trials using the OPU ERA checklists. Please note that the changes detailed in Section 3.2. were already included in the ERA version used for the second OPU trial and that the results shown for OPU refer to the analysis of the answers of all three respondents.

OPU ERA Phases		1st Trial (October 2019)		2nd Trial (December 2019)	
		Positive Answers	**Negative Score**	**Positive Answers**	**Negative Score**
A)	Animal selection, procedure planning and welfare	27 over 34	10 over 75	36 over 36	0 over 79
B)	Ovarian stimulation protocol	8 over 8	0 over 21	8 over 8	0 over 21
C)	Anesthetic procedure	15 over 18	7 over 37	27 over 27	0 over 66
D)	Oocyte recovery by transrectal procedure	9 over 20	23 over 40	19 over 19	0 over 37
E)	Gametes packaging	0 over 11	17 over 17	11 over 11	0 over 17
Total		59 over 91	57 over 190	101 over 101	0 over 220

The application of the IVF-lab ERA, in October 2019, resulted in “low risk”, with a risk score of 0 over 184 (Table 10). All the requirements’ characteristics related to “Factors affecting the process (documental and procedural support aspects), “Factors related to the traceability and distribution of specimens, laboratory operator’s safety, quality and availability of laboratory facilities” and “Factors related to the viability of gametes and embryos and to the instrumental requirements and the chemical reagents used" were met for the rhinoceroses' biomaterial safety. It was not necessary to proceed with the second and third review levels. Therefore, there was no need to perform a second assessment trial after addressing problematic issues.

Table 10. Description of IVF-lab ERA standard checklist application and results.

IVF-Lab ERA Phases		October 2019	
		Positive Answers	Negative Score
A)	Laboratory quality assessment and specimens processing	32 over 32	0 over 54
B)	Gametes shipping to laboratory	8 over 8	0 over 14
C)	Gametes biobanking	8 over 8	0 over 17
D)	Gametes preparation for ICSI	16 over 16	0 over 28
E)	ICSI	6 over 6	0 over 16
F)	Embryos culture	7 over 7	0 over 11
G)	Embryos cryopreservation and Biobanking	11 over 11	0 over 19
H)	Embryos packaging	7 over 7	0 over 11
I)	Embryos shipping	8 over 8	0 over 14
	Total	103 over 103	0 over 184

Of course, also having established the inclusion of an ethical self-assessment in ART procedures as a routine protocol is to be considered in itself as an improvement of the procedures, as it ensures the high standards of the procedures themselves.

How Applying the Tool in Actual Field Conditions Improved the Tool Itself

As already explained, the tool is designed to be able to incorporate changes allowing it to be refined by means of consultation between ethicists, scientists and stakeholders following each application of it. After the application of the first version of the tool to the OPU procedure, some areas needing further addressing in the ERA and EES checklists were highlighted. The items added as a consequence of the process in the OPU EES and OPU ERA are shown in Table 11. The items added to the OPU EES were also added to the IVF-lab EES as they were also relevant to the IVF procedure.

Table 11. Items added to the first OPU EES, OPU ERA and IVF-lab EES standard versions to obtain the second ones.

New Added Items to OPU EES
Have the aspects related to the environmental impact of the staff travels been considered and have measures been taken to decrease it? (i.e., use train instead of airplane whenever possible, contributing to a certified carbon offset program for flights)
Have the aspects related to the environmental impact of the equipment and materials been considered and have measures to decrease it been taken?
Have the aspects related to the waste deriving from the procedure been considered and have measures to decrease it been taken?
New Added Items to OPU ERA
If the animal or animals have already undergone the OPU procedure, were the procedure and the recovery of the animal carried out without difficulties?
Does the facility have an ethical internal committee?
Have measures/actions to avoid or minimise possible animal's injuries due to its partial control of the awareness during a) and b) been planned? a) pre-anaesthesia b) post-anaesthesia recovery
Have measures/actions to avoid or minimise any animal distress or suffering, during a) and b), been planned? a) pre-anaesthesia b) post-anaesthesia
Are measures/actions to avoid or minimise the potential negative influence of a), b) and c) on the welfare of the animal/s involved in the procedure been planned? a) Visual/olfactory/auditory inputs from other individuals b) Visual/olfactory/auditory absence of inputs from individual/s of the same social group c) Absence of familiar keeper/s.
Are measures/actions to avoid or minimise the potential negative influence of a), b) and c) on the welfare of other animal/s not directly involved in the procedure been planned?

a) Visual/olfactory/auditory inputs from other individuals b) Visual/olfactory/auditory absence of inputs from individual/s of the same social group c) Absence of familiar keeper/s.
New Added Items to IVF-Lab EES
Have the aspects related to the environmental impact of the equipment and materials been considered and have measures to decrease it been taken?
Have the aspects related to the waste deriving from the procedure been considered and have measures to decrease it been taken?

Discussion

The application of the ETHAS to the procedures performed during the present study both contributed to the overall acceptability of the project and improved communication among the projects' partners while refining the tool itself, in view of its standardization and application to other contexts in which ARTs are used for mammalian conservation projects.

Regarding the procedures assessed in the present study, it is important to note how having applied a tool which integrated risk assessment (general, ethical, welfare), pain/distress/welfare evaluation, harm–benefit analysis and the 3Rs tenet more likely had the potential to make the assessment and, eventually, help in the detection of problematic issues than using only one of these approaches separately. If we analyze, in more depth, the results of the ETHAS assessment, the harm–benefit analysis part allowed highlighting both positive effects and harms that could be generated by the execution of the OPU and IVF-lab procedures on wild animals and their specimens. Among positive effects highlighted during the assessment were: routine health and welfare check-up of the animals involved; the possibility of propagation of the genetic material of the specimens involved; scientific knowledge and know-how improvements that might find positive applications in other fields; the development of new technologies and procedures to promote the health and welfare of wild animals; the development of protocols for the conservation of endangered wild species. It was also possible to check whether the BioRescue team was committed to sharing the outcoming benefits with local communities. The restoration of the NWR's wild populations can directly positively affect local communities' economies through tourism and indirectly improve the quality of local communities' lives, restoring the African ecosystem and landscape [113,114]. The ETHAS confirmed that the know-how deriving from the procedures' optimization was shared with local veterinarians.

The local staff was also directly involved in the compilation of the OPU ERA, since a general and comprehensive goal of the ETHAS is to facilitate discussion among participants. The testing of the ETHAS confirmed that the tool was effective in this respect. As the results of the OPU ERA checklists showed, after the first application, the issues with negative answers caused by a lack of communication were not detected in the second one. In general, better communication among participants helps to avoid, reduce or manage the risks of the procedures and to guarantee high standards. The application of the ETHAS to the laboratory procedures contributed to guarantee high standards also in the IVF procedure and to safeguard the biomaterial involved, as the three embryos created by NWRs are of exceptionally high conservation value.

Through the ETHAS, it was also possible to check for potential harms that may occur during the procedures and if everything possible was done to avoid their occurrence. The main potential harms highlighted by ETHAS application mostly concern the possible side effects of the veterinary procedures on the animals' health and welfare, correct preservation of the biomaterial and staff safety. However, since potential risks might occur during the veterinary procedures on wild animals, ETHAS application allowed highlighting the above-mentioned critical points, investigating whether action plans have been developed to deal with them and facilitating discussion around them between the staff members.

With regard to the animal welfare issues involved in the procedure, as highlighted by the positive results of the items specifically designed in the OPU ERA and EES, it was found that the team was committed to preserve and protect animal welfare, by monitoring the animals before, during and after the procedures, through physiological and behavioral analyses. Moreover, even if scientific evidence shows that the OPU procedure can be repeated on the same animal several times, the ETHAS allowed for checking if an adequate time-lapse between procedures was respected, as dictated by the best veterinary practices. Furthermore, specific items of the ERA checklist were included in order to analyze the welfare of other animals not directly involved in the procedures, such as herd mates sharing the same facilities or even enclosures.

Implementation of the 3Rs was another purpose of the ETHAS. Results showed that refinement, reduction and replacement were applied in the procedures whenever possible. For instance, refinement was applied by developing a new instrument for oocytes pick-up in rhinoceroses and by improving the procedures and techniques, with the aim of increasing the welfare of the animals involved, the efficacy of the procedures and the correct preservation of specimens. Another aspect related to refinement was the inclusion of items regarding environmentally friendly waste disposal in the EES, after the first trial. The replacement of laboratory media with synthetic ones, the replacement of materials with lower environmental impact and the replacement of procedures and equipment with a lower impact on animal welfare were considered and applied whenever possible. Finally, reduction was implemented by maximizing the number of sampling procedures under the same anesthesia to reduce the number of veterinary interventions as much as possible.

Furthermore, the applications of the ETHAS in different conditions (zoos and semi-captive management) have contributed to refine the accuracy and inclusiveness of the tool itself. OPU and IVF-lab ERAs underwent several applications that allowed improving the tool via a shared work between ethicists and experts. This process permitted reviewing and refining the checklists iteratively through a participative approach.

Last, but not least, a general and comprehensive goal of the ETHAS was to assist scientists to carry out a self-assessment in addressing ethical evaluation of ART application in conservation projects. The results of the present study show that the application of such an ongoing assessment was effective in ensuring the high standards of the procedures, including respect for animal welfare, and facilitating effective communication among participants. It is important to note that the application of a form of ethical self-assessment to procedures or projects constitutes in itself a contribution to their acceptability even if no problematic issue is detected. All this is a value in itself and can increase acceptance of this kind of project by the public.

Limitations and Future Developments

Self-assessment can also be seen as the main limit of ETHAS application, as the evaluation process can be interpreted as self-referential. Nevertheless, as already pointed out, the primary function of ethical self-assessment is to help scientists think, in detail and proactively, through ethical issues surrounding their research. Usually, ethical evaluation regarding conservation projects, when it is performed, is made by an external authority, which gives a general ethical approval to the overall project before it starts. On the contrary, ethical self-assessment offers the opportunity for an ongoing detailed scrutiny of all the main ethical aspects involved in the project, including the procedures that are carried out on animals, being proactive in detecting hazards for their welfare and taking measures to minimize them beforehand. In general, ethical self-assessment allows for a comprehensive and transparent evaluation process which can also be communicated to the public.

Another difficulty in applying such tool is the balancing between the need for standardization and that for customizing procedures and situations. Moreover, the fact that the tool is designed to evolve through iterative confrontation makes standardization more difficult. Notwithstanding, the ETHAS will continuously be tested in different contexts, species and procedures, in order to increase the comprehensiveness of the tool. However, it is important to note that the general frame and most of the tool are already adaptable to a more general use in different contexts, species and procedures, such as semen collection, embryo transfer, surrogate pregnancy and birth management, and to other innovative procedures regarding stem cell-associated techniques.

Conclusions

Ethical assessment of the application of ARTs in conservation is important for many reasons. In conservation breeding programs, for instance, animal welfare is a crucial element to be considered, alongside safety for the people involved and the quality of the procedures. Moreover, ethical assessment—especially when performed in the guise of self-assessment—allows anticipating the critical aspects that can compromise the ethical acceptability of a procedure and intervening before their eventual occurrence could damage the reputation of the whole conservation project and alienate societal support. As ARTs will become ever more important for conservation, the need to expand and deepen the ethical research on this topic will increase. An exemplary case, in this sense, is provided by the BioRescue project, which, alongside the development and testing of new approaches in the conservation of a "technically extinct" species, implemented a self-assessment tool designed for improving the procedures from an ethical standpoint. The application of such a tool within the project allowed for the mutual goals of improving some aspects of the communication among the projects' partners and improving the tool itself, to be applied in the near future to other contexts in which ARTs are applied for the conservation of other mammal species. Despite the obvious advantages of this kind of self-assessment, such an approach is almost underestimated in the literature dealing with ART in conservation, as shown by a simple Scopus search on the subject. Therefore, tools such as the ETHAS could raise the ethical standards of applications of ARTs to conservation and, in this way, contribute to their success.

Supplementary Materials

The following are available online at https://www.mdpi.com/2076-261 5/11/2/312/s1, File S1: OPU EES_1st trial, File S2: OPU ERA_october2019, File S3: IVF-lab EES_1st trial, File S4: IVF-lab ERA.

Author Contributions

Conceptualization, B.d.M.; methodology, B.d.M., D.F., M.M.S., I.P., F.A. and S.N. (Simona Normando); formal analysis, I.P., M.M.S. and F.A.; investigation, I.P., M.M.S., D.F. and F.A.; data curation, I.P. and M.M.S.; writing—original draft preparation, I.P. and M.M.S.; writing— review and editing, P.B., S.N. (Simona Normando), D.F., F.A., S.S., C.G., G.L., S.C., T.B.H., F.G., R.H., S.H., J.Z., J.S., R.V., S.N. (Stephen Ngulu), S.M., and D.N.; visualization, I.P.; supervision, B.d.M., T.B.H. and S.N. (Simona Normando); project administration, B.d.M.; funding acquisition, T.B.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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1.3. Application of decision tools to ethical analysis in biodiversity conservation

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Abstract

Achieving ethically responsible decisions is crucial for the success of biodiversity conservation projects. We adapted the ethical matrix, decision tree, and Bateson's cube to assist in the ethical analysis of complex conservation scenarios by structuring these tool so that they can implement the different value dimensions (environmental, social, and animal welfare) involved in conservation ethics. We then applied them to a case study relative to the decision-making process regarding whether or not to continue collecting biomaterial on the oldest of the 2 remaining northern white rhinoceroses (*Ceratotherium simum cottoni*), a functionally extinct subspecies of the white rhinoceros. We used the ethical matrix to gather ethical pros and cons and as a starting point for a participatory approach to ethical decision- making. We used decision trees to compare the different options at stake on the basis of a set of ethical desiderata. We used Bateson's cube to establish a threshold of ethical acceptability and model the results of a simple survey. The application of these tools proved to be pivotal in structuring the decision-making process

and in helping reach a shared, reasoned, and transparent decision on the best option from an ethical point of view among those available.

Introduction

Achieving ethically responsible decisions is crucial for the success of biodiversity conservation projects. At all levels of the decision-making process, hard choices and trade- offs need to be addressed (McShane et al. 2011) and conflicts must be mediated, lest they lead to failure (Redpath et al. 2013; Catalano et al. 2019). However, the path to ethically responsible decisions can be tortuous. The ethics of conservation is multidimensional and encompasses many value contexts (Minteer and Collins 2005a, 2005b; Biasetti and de Mori 2020). Besides the many facets of environmental and biodiversity values (Chan et al. 2016; Pearson 2016), conservation action needs to take into account the welfare of animals involved (Hampton et al. 2018; Johnson et al. 2019) and equity and justice for and well-being of people (Chan et al. 2007; Shoreman-Ouimet and Kopnina 2015). For these reasons, conservation decision-making is often ethically complex and requires careful analyses.

We considered 3 decision tools, the ethical matrix (EM) (Mepham 1996; Mepham et al. 2006), the decision tree (DT), and the Bateson's cube (BC) (Bateson 1986; Driscoll and Bateson 1988; Bateson 2005), and we adapted them for the ethical analysis of conservation decision-making. We then applied these tools to a case study to show how they can be used to reach reasoned, transparent, and shared ethical decisions. We sought to show how these tools can provide a systematic way to unpack ethically complex situations and identify the main nodes of the decision-making process; help structure the discussion; provide a guide for collecting information; offer a starting point for participatory processes; and, given their standardized form, be used to check consistency and compare cases.

All the tools we considered were either developed for ethical analysis (EM and BC) or have some established history of application to this end (DT), and their structure allows for taking into account different dimensions of value when analyzing a scenario. For these reasons, their application to conservation ethics seems particularly promising. While the EM has already been tailored to conservation (Biasetti and de Mori 2021), here we used it as a checklist for gathering ethical pros and cons and as a starting point for a participatory approach. Decision trees also have been used in conservation (starting with Maguire et al. [1987]), but to the best of our knowledge not in the ethical analyses of conservation efforts. Whereas the application of BC to conservation has been advocated (MacMahon et al. 2012), it is underused, and its potentialities are still not fully explored. In addition to their separate applications, we considered the tools' integrated use in a participatory decision-making process.

The tools

Ethical Matrix

The EM is a conceptual tool used to identify the value demands involved in a complex scenario. It was introduced by Mepham (1996) in the context of food ethics and it has since been applied to several fields, including conservation (Biasetti and de Mori 2021; Biasetti et al. 2021). It consists of a table listing general ethical principles in the first row and involved stakeholders in the first column. The remaining cells are filled with the value demands descending from the application of the principles to each stakeholder.

The general ethical principles in an EM are usually well-being, autonomy, and fairness and are derived from a simplified version of the ethical approach known as principlism (Beauchamp and Childress 1985; Beauchamp 2010), the purpose of which is to reflect the pluralism of common morality. Stakeholders in the EM for conservation (Table 1) can be ecological entities, individual animals, or people. A filled EM provides a detailed picture of the various demands related to the case coming from environmental ethics, animal ethics, and social ethics.

Table 1. General ethical matrix for conservation decision-making (from biasetti & de mori, 2021)

Stakeholders	**General ethical principle of well-being**	**General ethical principle of autonomy**	**General ethical principle of fairness**
Ecological entities	conservation	freedom from human intervention	equal treatment in relation to conservation
Animals	health and functioning absence of negative affective states and allowance of positive ones	living natural lives and opportunity to exert species-specific behaviors	equal treatment in relation to welfare
People	psychological and physiological welfare sustainable social, economic, and cultural welfare	freedom of choice capacity to exercise fundamental aspects of one's persona self-determination	equal and fair treatment

To fill an EM, it is necessary to identify the specific stakeholders and the category they belong to. Then, the general ethical principles are applied to obtain the value demands. The general EM in Table 1 can be used as a starting template to guide the process, which can be conducted top-down by experts, bottom-up in a participatory process, or with both methods. A completed EM can be used as a starting point for discussion in a decision-making process in conservation because it provides participants with a checklist of the ethically relevant interests which they can then analyze and determine whether they can be met. The compiled EM can also be used to gather the ethically relevant pros and cons of the options at stake by using the value demands as a yardstick. This makes it possible to anticipate the impact of different choices on stakeholders and to compare them.

Decision trees

Decision trees are flow-chart-like schemes employed, among other uses, to predict outcomes. A DT starts with a main decision node as the entry point, followed by as many branches as there are options to be discussed. Additional decision and chance nodes representing probabilistic events that may exert a relevant influence on a course of action form the crown of the tree, the branches of which culminate in end nodes that define a set of possible outcomes. When probabilities are assigned to chance nodes and payoffs to end nodes, it becomes possible to identify reasonable choices in face of uncertainty on the ground of the expected values of outcomes.

In ethical analysis of conservation, DTs are useful for comparing different options on the basis of a set of preestablished ethical desiderata. Chance nodes represent the probability of achieving or not achieving a specific desideratum. Payoffs assigned to end nodes reflect the importance of the desiderata achieved along that branch. The finished DT recapitulates the courses of action that can occur and identifies the available choice that, in face of uncertainty, should provide the expected ethical best outcome. To achieve this result, it is necessary to have previously established the ethical desiderata of the analyzed scenario. Generally speaking, in a DT the analysis becomes more realistic by incorporating more probabilistic events. However, the analysis also becomes more complicated and runs the risk of being obfuscated by trivial details. For this reason, ethical desiderata should be picked carefully to represent the most important values at stake.

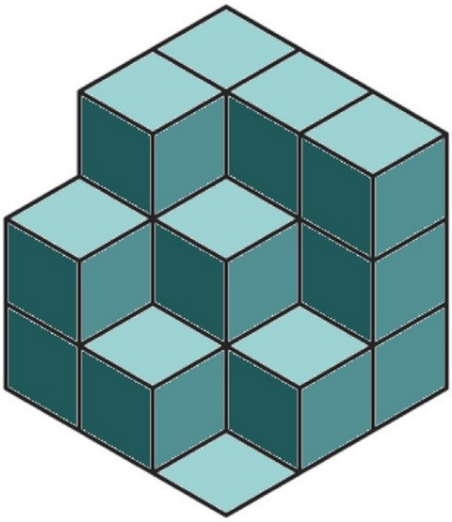
After identifying the ethical desiderata, the end nodes are ranked. In standard DTs, this is done by assigning payoffs measured in utility or other common metrics. This is not usually possible for a DT built for the ethical analysis of conservation scenarios because the values involved—such as protecting a species, guaranteeing animal welfare, respect fairness—do not share a common metric. However, incommensurability does not equate with incomparability, and ethical desiderata can be ordered lexically. In this way, it is possible to rank end nodes based on the expected realization of certain values.

Bateson's cube

The BC is a model for decision-making that displays the possible combinations between scores attributed along 3 dimensions. It was developed by Bateson (1986) to assess the ethical acceptability of scientific research involving animal experimentation (Driscoll and Bateson 1988). The idea behind BC is that the acceptability of research that can harm animals depends on its scientific quality and usefulness for people. In this way, the BC puts together 3 value dimensions related to a project: scientific value, social value, and welfare of the animals involved. In the original description of BC, these are denoted as scientific quality, medical benefit, and the likelihood of suffering (Bateson 1986; Driscoll and Bateson 1988).

When applying BC, a score from 1 to 3 is assigned to each dimension. The model then shows whether the combination is acceptable or not. The higher the chances of harming the animals, the higher the scientific quality and usefulness of the intervention has to be for it to be acceptable. Figure 1 shows a BC. Each small cube is 1 of the possible combinations of scores assigned along the 3 dimensions. The empty part of the BC represents the acceptable combinations, the solid part the unacceptable. Along a dimension (that of animal welfare in the original BC), more than the minimum score is required for the intervention to be considered acceptable.

Figure 1. The Bateson Cube displays the possibile combinations between 1 and 3 scores attributed along three dimensions. High scores represent high standards. Acceptable scenarios are represented by clear space. On one of the axes, at least a medium score is needed for the scenario to be acceptable.



In a decision-making process in which options are evaluated according to ethical criteria, the use of BC provides a way to assess each available option according to its acceptability. The BC can then be used to rank the options, based on the scores obtained. A more detailed ranking can be obtained by establishing a lexical ordering between dimensions.

When applying this tool to conservation, its dimensions must be adapted. The scientific value of the research, for instance, cannot be understood exclusively in terms of the quality of the research. In the original BC, this accounted for the fact that much research conducted on animals does not have a direct and visible benefit for people, but it can still have value in itself (that is, as scientific research trying to satisfy human curiosity) or for its eventual effects on more directly beneficial research. Both elements—scientific value and the possibility of eventual beneficial effects—are also part of conservation projects. However, they do not define the core of the mission of biodiversity conservation. This can be described as the maintenance and, eventually, the restoration of the natural diversity of life at all biological levels, from ecosystems to genes. When applying the BC to assess conservation, then, it is important to understand the dimension of scientific quality in terms of the capacity to fulfill this mission.

Similarly, social value cannot be understood merely as the estimated medical benefits, as in the original cube. Rather, this dimension should be conceived in its most general and literal sense, that is, as the set of all socially relevant consequences of the analyzed scenario.

Finally, animal welfare should be recognized as a multifactorial notion, and suffering, understood as the presence of negative affective states, as only 1 of its possible meanings (Fraser 2008). Allowance of positive affective states is another important meaning, as are health and functioning. Furthermore, given the context of conservation, where animals do not live in strictly controlled laboratory environments, the possibility of living natural lives and exercising species-specific behaviors (Bracke and Hopster 2006) needs also to be taken into account. Once adapted in this way, the BC can be used to analyze conservation efforts starting from the 3 ethically relevant dimensions usually involved: protection of biodiversity, animal welfare, and impact on people.

Case study

To show the potentialities of these tools, we applied them to a case study of the decision-making process regarding whether or not to continue collecting biomaterial on the oldest of the 2 last northern white rhinoceroses (NWR) (*Ceratotherium simum cottoni*), a functionally extinct subspecies of the white rhinoceros (*Ceratotherium simum*). We know of only 2 NWR in the world, both are female and neither can carry a pregnancy to term. Thus, the NWR is presently considered "functionally extinct" (Emslie, 2020), and the only chance to revert this state relies on the advancement of scientific techniques (Saragusty et al. 2016; Hildebrandt et al. 2021a). The strategy adopted by the Biorescue project—an international consortium led by the Leibniz Institute for Zoo and Wildlife Research— combines advanced assisted reproductive technologies and stem-cell associated techniques (Hildebrandt et al. 2018). Both approaches involve the use of biomaterial from living as well as from deceased individuals, in the form of cryopreserved gametes (Hermes et al. 2018) and fibroblast cultures derived from skin samples. The ultimate goal is to establish a self- sustaining and genetically healthy population to be reintroduced into the wild. Short-term goals include collecting additional biomaterial from the remaining individuals, creating viable embryos, and developing methods and protocols for successful embryo transfers in southern white rhinoceros (SWR) (*Ceratotherium simum simum*) recipient cows.

However, questions arose as to the suitability of Najin, the oldest of the 2 NWRs, as a donor of genetic material. At the time, Najin was 32 years old, had a number of health problems, and no embryos had ever been obtained from her oocytes. It was hence decided to start a decision-making process to discuss, both from a scientific and ethical standpoint, 3 options: continue performing ovum pickup (OPU) procedures on Najin while constantly monitoring her health conditions and reopening the decision-making process in case of a change in conditions; remove genetic material from Najin a final time by performing an ovariectomy; or stop using Najin as an oocyte donor.

Scientific information

Najin has weak hind legs and several documented reproductive tract pathologies described in white rhinoceroses (Hermes et al. 2006)—including a small leiomyoma in the cervix, multiple small leiomyomata in both horns of the uterus, a uterine adenoma in the right horn, and a septet-cystic neoplastic structure with a diameter of 25 cm in the left ovary.

An OPU in rhinoceroses is a relatively novel procedure that involves gonadotropin-releasing hormone (GnRH) stimulation, general anesthesia with an etorphine-free protocol, and transrectal ultrasound-guided oocyte recovery (Hermes et al. 2009, Hildebrandt et al. 2018). Oocyte harvesting from Najin and her daughter Fatu began on the 22 August 2019.

Interventions are planned with a minimum of 3 months pause between them to provide the animals with a safe interval to recover (de Mori et al. 2021; Biasetti et al. 2022). When the decision-making process was started after the fourth procedure, further OPU procedures on Najin were suspended. Before the suspension, 10 oocytes were collected from Najin from which no blastocyst-stage embryo was generated. In the same period, 34 oocytes were collected from Fatu in 4 procedures, and 5 embryos were generated (Hildebrandt 2021b). Najin was born in 1989. Ovarian response to GnRH stimulation before OPU in white rhinoceroses aged more than 30 years in the European OPU program has so far been very poor (n=5). This suggests that female rhinoceros after this age enter reproductive senescence in which GnRH stimulation becomes less effective. Moreover, further GnRH stimulations in Najin might promote uterine tumor growth, induce malignancy, and accelerate the growth of the cystic formation in the left ovary.

Abdominal surgery on rhinoceroses is extremely challenging; complete success of an ovariectomy is very uncertain and the procedure is risky for the animal. This intervention—to the best of our knowledge—has been attempted in white rhinoceroses 3 times, twice in an SWR (Bronx Zoo and San Diego Safari Park) and once in an NWR (San Diego Safari Park). Removal of ovarian tissue was done endoscopically. Only 1 animal survived the intervention and only for a short period. No oocytes were collected in a reported case of ovariectomy of a 32-year-old SWR (Pennington and Durant 2019).

Ovarian tissue harvested post mortem in senescent females has so far not yielded promising results in terms of oocyte retrieval or residual ovarian cortex. The fibrotic state of the ovaries of older, senescent females resulted in zero oocytes and limited amounts of germinative tissue harvested. However, this small but very limited germinative tissue removed during the procedure may become useful once *in vitro* follicle culture—a technique that has already been successful in some species (e.g., cats [Fassbender et al., 2007])—is developed for NWR (Hildebrandt et al., 2021a).

Ethical aspects

The relevant ethical aspects involved in the decision are the need to respect Najin's welfare, and, indirectly, that of Fatu; the need to preserve Najin's life for intrinsic and extrinsic reasons; and the need to foster the cause of biodiversity conservation. To respect Najin's welfare 3 complementary goals must be pursued: assure her physical health and functioning; minimize eventual unpleasant affective states while allowing for normal pleasures; allow development and performance of natural life functions according to her needs. It must be also taken into account that eventual harm to Najin's could also have a negative impact on Fatu, given their social bond.

Intrinsic reasons for respecting Najin's life stem from the need to respect life in itself. Extrinsic reasons stem instead from the importance Najin has acquired for people who have developed a feeling of connection and affective bonds (of varying degree) with her—from those who care for her daily to conservationists and the interested public.

There are several instrumental and noninstrumental reasons for conserving this subspecies from the standpoint of biodiversity conservation. The existence value of a taxon is already in itself an important reason for investing in its conservation. Furthermore, because great herbivores, such as rhinoceroses, are important ecosystem engineers (Owen-Smith 1988), their disappearance can cause further ecological impoverishment (Waldram et al. 2008; Cromsigt & te Beest 2014). Moreover, the success of the project could have a positive impact on the storytelling of biodiversity conservation, attracting new support, talents, and resources to its cause.

Ethical desiderata

We identified 3 primary ethical desiderata relative to the decision to be made: avoid major and minor accidents and obtain oocytes. Major accidents are defined as those that threaten the life of the animal. This desideratum follows from all 3 ethical aspects. Major accidents, besides being life-threatening, may be sources of welfare impairment and may compromise the chances of success of the conservation effort. Minor accidents are those that threaten the welfare of the animal but not her life. This desideratum follows mainly from the ethical aspect of respecting the welfare of the animal, but good animal welfare is also usually relevant to the success of conservation programs involving animal breeding (Greggor et al. 2018). Obtaining oocytes includes direct harvesting and *in vitro* methods (Hildebrandt et al. 2021a). This desideratum originates from the ethical aspect of conserving biodiversity.

Decision-making process

The participatory decision-making process occurred from March to October 2021. It started with semistructured interviews conducted by members of the Ethics Laboratory for Veterinary Medicine, Conservation and Animal Welfare of Padua University among the other members of the Biorescue consortium (n=20) to individuate options and collect the scientifically relevant information. A first draft document was prepared and distributed for collecting further observations and ideas. The tools were then applied to the case. Drafts of EM for each option were circulated among the members of the consortium for them to check and add items. Similarly, a sketch of the DT was discussed among all members. The task of estimating the probability of realizing the end node for each branch was assigned to the veterinarians in charge of performing the interventions. Finally, definitions were provided by members of the Ethics Laboratory for Veterinary Medicine, Conservation and Animal Welfare of Padua University for each dimension of the BC.

A second draft was prepared and presented during a meeting of the consortium in Ol Pejeta, Kenya. Further data were collected after the meeting through an online survey distributed among members of the consortium (n=20). Provided with all the relevant information through the draft document and the meeting, participants were asked to consider how likely was, in their opinion, the occurrence of the 3 desiderata (avoiding major accidents, avoiding minor accidents, obtaining oocytes) for each of the 3 options at stake. Response options consisted of a labeled 6-point Likert scale (1, extremely unlikely, to 6, extremely likely). The answer "I do not know" was also an option. The survey was based on an anonymous, computer-assisted self-completion questionnaire (CASI) (Hing et al. 2011) conducted with Google Forms. An email of invitation was used to distribute the link to the survey. Participants completed the self-administered questionnaire individually from 19 to 23 July 2021. The elaborated data were used to assess the 3 options through the BC. A final technical report was prepared and made available in October 2021 (Biorescue 2021).

Application of EM

Three kinds of stakeholders were included in the final EMs: biodiversity, Najin, and people involved, including staff performing the procedures, legal and economic managers of Najin, keepers, and all members of the consortium (Tables 2, 3, and 4). Pros and cons were obtained by using the general template in Table 1 as a checklist.

Table 2. Ethical matrix collecting ethical pros and cons concerning the first option discussed (continue performing ovum pick-up)

	Well-being	**Autonomy**	**Fairness**
Biodiversity	*conservation* pro: Not giving up on this option means at least leaving an open door for opportunistic harvesting after preliminary ultrasound screening. cons: The age, health conditions, and history of past procedures are	*freedom from human intervention* pro: No clear pro. con: To insist on this path without reasonable expectations of obtaining oocytes and embryos and with the possibility of damaging the animal would be a form of conservation	*equal treatment relative to conservation* pro: No clear pro. con: No clear con. The transrectal OPU protocol, which could be used in the future also for other taxa of rhinos or large mammals, can still be improved.

	not encouraging regarding the possibility of collecting new oocytes. Furthermore, no viable embryo has ever been obtained from oocytes collected from Najin in the past. A serious accident could damage the project.	obstinacy—in analogy with therapeutic obstinacy in human medicine.	
Najin	*health and functioning; Absence of negative affective states and allowance of positive ones* pro: No clear pro. con: The GnHR stimulation may worsen preexisting health conditions. Cyst growth may lead to a scenario where the life of the animal is seriously threatened by the possibility of a rupture. As prolonged standing sedation is not recommended due to the weak hind legs, preliminary screening for opportunistic OPU has to be done during a short time window.	*living natural lives and species-specific behaviors* pro: no clear pro. con: GnHR stimulation may worsen preexisting health conditions.	*equal treatment in relation to welfare* pro: No clear pro because the current levels of veterinarian screening could be maintained even if OPU procedures are suspended. con: No clear con.
People involved	*psychological and physiological welfare; sustainable social, economical, and cultural welfare* pro: No clear pro. con: A serious accident could cause stress and	*freedom of choice; capacity to exercise the various fundamental aspects of one's own persona, self-determination* pro: No clear pro, because stopping interventions on Najin does not mean	*equal and fair treatment* No clear pros or cons.

	grief in people close to the animal.	completely stopping this kind of interventions on other white rhinoceros (e.g. Fatu) , with all that can follow from the point of view of knowledge transfer and capacity building. con: a serious accident could damage the professional profile of the people involved.	

Table 3. Ethical matrix collecting ethical pros and cons concerning the second option discussed (ovariectomy)

	Well-being	**Autonomy**	**Fairness**
Biodiversity	*conservation* pro: There is the possibility of obtaining biomaterial from which to extract or produce gametes. con: Extraction of biomaterial can be done postmortem. Chances of obtaining oocytes depend on technology (*in vitro* follicle culture) not yet fully established for NWR. A serious accident could damage the project.	*freedom from human intervention* pros: No clear pros. Con: To insist on this path without reasonable expectations of obtaining oocytes and embryos and with the possibility of damaging the animal would be a form of conservation obstinacy—in analogy with therapeutic obstinacy in human medicine.	*equal treatment in relation to conservation* No clear pros or cons.
Najin	*health and functioning; absence of negative affective states and allowance of positive ones*	*living natural lives and species-specific behaviors* pro: Ovariectomy would solve some of the health conditions	*equal treatment in relation to welfare* pro: No clear pro. cons: Similar interventions in the past show that this is a

	pro: Ovariectomy would solve some of the health conditions relative to the genital apparatus. con: Invasive surgery is needed to perform the intervention. Similar interventions in the past show that this is a life-threatening procedure.	relative to the genital apparatus. cons: Recovery from the procedure could be long and hard. Removal of the ovaries may have adverse physiological effects.	life-threatening procedure. Even if it succeeds, it will require a long recovery process. It is not clear whether it would be fair, given this, to attempt the intervention on Najin, especially considering her age, because the risk of surgery and postsurgical complications is increased.
People involved	*psychological and physiological welfare; sustainable social, economical, and cultural welfare* pro: No clear pro. con: A serious accident could cause stress and grief in people close to the animal.	*freedom of choice; capacity to exercise the various fundamental aspects of one's own persona, self-determination* pro: No clear pro. con: A serious accident could damage the professional profile of the people involved.	*equal and fair treatment* No clear pros or cons.

Table 4. Ethical matrix collecting ethical pros and cons concerning the third option discussed (no further collection procedures)

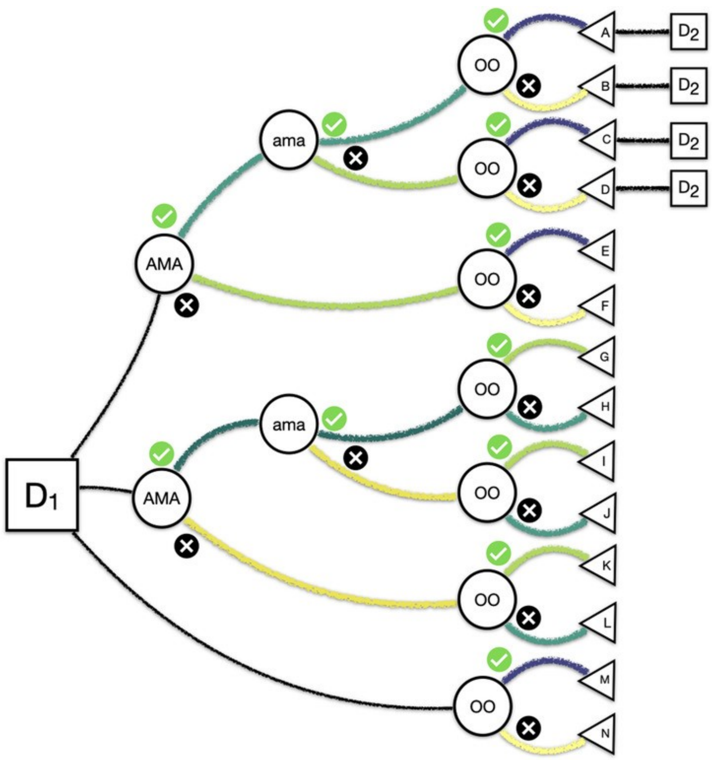
	Well-being	**Autonomy**	**Fairness**
Biodiversity	*conservation* pros: Najin's role in the project could still be crucial, thanks to her social competence, which would be vital to transmit to the next generation of NWR. Postmortem collection of ovarian tissue would still be possible, with some chance to obtain oocytes in the future	*freedom from human intervention* pro: Stopping the intervention if the estimated risks are higher than estimated chances of success would be a responsible choice and a demonstration of a nonhybristic attitude. con: No clear con.	*equal treatment in relation to conservation* pro: No clear pro. con: No clear con. The transrectal OPU protocol, which could be used in the future for other taxa of rhinoceros or large mammals, can still be improved.

	through *in vitro* follicle culture. con: There is no immediate chance of obtaining oocytes suitable for *in vitro* embryo production		
Najin	*health and functioning; absence of negative affective states and allowance of positive ones* pro: No specific welfare risks. This is the least risky choice. Con: No clear con. Tumor growth will likely still continue.	*living natural lives and species-specific behaviors* pro: Ending any type of intervention in the animal would mean allowing it to conclude the last arc of its existence in a more peaceful way—also considering how much Najin has already given to the cause of the survival of its taxon. Con: No clear con.	*equal treatment in relation to welfare* pro: The regular veterinary screening the animal has undergone in recent years can still continue. Con: No clear con.
People involved	*psychological and physiological welfare; sustainable social, economical, and cultural welfare* pro: This is the least risky choice. Con: No clear con.	*freedom of choice; capacity to exercise the fundamental aspects of one's own persona, self-determination* pro: No clear pro. Con: No clear con, because stopping procedures on Najin does not mean completely stopping this kind of interventions on other white rhinoceros (e.g. Fatu), with all that can follow from the point of view of knowledge transfer and capacity building.	*equal and fair treatment* No clear pros or cons.

Application of the DT

The starting decision node D1 branched into the 3 individuated options—continue to perform OPU (branch B1), perform ovariectomy (branch B2), and stop all procedures (branch B3) (Figure 2). The B1 and B2 branches were further extended by3 chance nodes representing the ethical desiderata: avoid major accidents (AMA), avoid minor accidents (ama), and obtain viable oocytes (OO). The B3 branch was extended with the OO chance node.

Figure 2. Final decision tree for Najin regarding whether to continue to perform OPU (branch B_1), perform ovariectomy (branch B_2), or stop all procedures (branch B_3). Abbreviations: AMA, avoid major accidents; ama, avoid minor accidents; OO, obtaining viable oocytes.



Probability	Scale	Color
Extremely unlikely	0,001	
Very unlikely	0,01	
Unlikely	0,1	
Likely	0,9	
Very likely	0,99	
Extremely likely	0,999	

The tree has 14 end nodes. Four of these lead to a new decision node identical to the one at the start. If the decision to carry out the OPU procedure on Najin gives rise to a course of action in which no major welfare accident occurs, then the starting question arises again. In all other cases, the decision-making process is concluded.

The end nodes were ranked to match their desirability (Figure 3). The ranking was based on each node's capacity to satisfy 4 desiderata: avoid major accidents, avoid minor accidents, possibility to repeat the procedure, and collect viable oocytes. The 4 desiderata are lexically ordered, meaning that higher-ordered desideratum trumps lower-ordered desiderata (i.e., avoiding major welfare accident trumps satisfaction of all other 3 desiderata). The desideratum possibility to repeat the procedure was added to reflect the option value in the decision to perform the OPU procedure.

Figure 3. Ranking and chances of realization of end nodes of the decision tree used to analyze from an ethical standpoint the three possibile conservation interventions on Najin. End nodes are listed from the ethical best (high) to worst (low) according to their capacity to satisfy the lexically ordered desiderata. Lexical ordering of desiderata goes from left (dark blue, higher lexical order) to right (light blue, lower lexical order). Chances of realization of each nodes is recapped in the rightmost column.

	Avoiding major accident	Avoiding minor accident	Possibility to repeat	Collecting viable oocytes	Expected realization
A					Less than extremely unlikely
B					Less than very unlikely
G					Less than extremely unlikely
M					Extremely unlikely
H					Less than extremely unlikely
N					Extremely likely
C					Less than extremely unlikely
D					Less than unlikely
I					Less than extremely unlikely
J					Less than extremely unlikely
E					Less than extremely unlikely
K					Less than likely
F					Less than likely
L					Less than unlikely

Desiderata were ordered lexically based on their capacity to comply with the ethical aspects described above). Avoiding a life threatening situation, for example, is important from the standpoint of respecting the life of the animal, but it also avoids possible suffering and ensures the ultimate success of the conservation project. Similarly, ensuring compliance with a good level of welfare, in addition to being a commendable objective in itself, is also important from the standpoint of conservation.

Probabilities for each outcome were determined by ranking the probability of occurrence of an event (unlikely, very unlikely, extremely unlikely, likely, very likely, extremely likely). At each bifurcation following an event node, the branches were classified according to this scale by the veterinarians in the consortium.

For branch B1 (continue OPU), chances of major accidents were estimated likely. If the cyst growth is further stimulated by hormones, there is a mechanical risk that the wall will rupture, an accident that occurred in another NWR, Nabire. The released content of the cyst may be life-threatening for the animal. Chances of minor accidents were similarly estimated likely because the growth of the cyst, even in a nonlife-threatening situation, is still a welfare impairment, as is the manipulation of the animal due to the condition of her legs. Chances of obtaining viable oocytes through OPU were estimated as extremely unlikely based on past results and data on performance of animals of the same age range as Najin.

For branch B2 (ovariectomy), possibilities of major accidents happening were estimated as very likely because of discouraging known precedents. Similarly, chances of minor accidents were estimated as very likely because of the invasiveness of the intervention and the need for prolonged recovery. Chances of obtaining oocytes were instead estimated as likely because the techniques required for obtaining oocytes from the germinative tissue eventually harvested from the ovaries, while not yet developed for rhinoceroses, have been developed for other mammals.

For branch B3 (do not perform OPU or ovariectomy), chances of obtaining oocytes were assessed as possible (thanks to the possibility of postmortem harvesting of ovarian tissue for future use *in vitro* follicle production) yet extremely unlikely.

Situation analyses

By cross-checking the chance of realization of an outcome with its ethical ranking (Fig. 3) it was possible to weight its effective value. The expected realization of the best ethical outcomes for branches B1 and B2 were rather low. In particular, the best outcome for B1 had the lowest chance of realization, and the best outcome for B2 had the second lowest chance of realization. For both branches, the end nodes with the highest chances of realization failed to satisfy most of the desiderata. For B1 the most probable outcome was the worst scenario of a major accident paired with no oocytes. For B2 this worst scenario had the second highest estimated chance of happening, and the most probable outcome was a major accident (Fig. 4).

Figure 4. End node analysis of the decision tree used to analyze from an ethical standpoint the three possibile conservation interventions on Najin.

End-node	Expected realization	Drawbacks
OPU		
F	Less than likely	Major accident, no oocytes. Worst scenario for B_1, highest estimated chance to happen if procedure is repeated
D	Less than unlikely	Minor accident, no oocytes. Possibility to repeat the procedure is nearly worthless as chances of obtaining oocytes remain low
B	Less than very unlikely	No oocytes. Possibility to repeat the procedure is nearly worthless as chances of obtaining oocytes remain low
E	Less than extremely unlikely	Major accident
C	Less than extremely unlikely	Minor accident
A	Less than extremely unlikely	No accidents, oocyte retrieved. Best scenario for B_1, lowest estimated chance to happen
OVARIECTOMY		
K	Less than likely	Major accident
L	Less than unlikely	Major accident, no oocytes. Worst scenario for B_2, second highest estimated chance to happen.
I	Less than extremely unlikely	Minor accident
J	Less than extremely unlikely	Minor accident, no oocytes
G	Less than extremely unlikely	No accidents, oocyte retrieved. Best scenario for B_2, second lowest estimated chance to happen
H	Less than extremely unlikely	No oocytes
NO FURTHER INTERVENTION		
N	Extremely likely	No oocytes
M	Extremely unlikely	

Fatu DT

A DT was also built for Fatu (Appendix 1) with the same procedures, and it was used to control consistency and coherence in the decision-making process.

Application of BC

The 3 dimensions of the cube were defined as avoiding a major accident, which in addition to damaging the welfare of the animal also puts her life at risk, with all that can follow for the people involved; avoiding a minor accident that damages the welfare of the animal; and obtaining oocytes.

High scores were used for high possibilities of satisfying the desiderata, low scores were used for low possibilities. On the axis avoiding major accidents at least a medium score was considered necessary (but not sufficient) for the result to be acceptable.

Scores were determined based on the data collected in the online survey. Sixteen participants completed the survey (response rate 80%). Descriptive statistics were used to examine the results (see Appendices 2 and 3), and for each scenario measures of central tendency were used to identify which scenario variants were considered the most likely by the respondents. The 6-point Likert results were converted in a 3-point scale and used to evaluate the acceptability of each option through the BC. Based on mode and mean scores, the options of continuing to attempt the OPU procedure on a regular basis or performing an ovariaectomy were deemed not ethically acceptable (with a worse result for ovariectomy). Ceasing to use Najin as a living donor of oocytes was instead considered acceptable.

Discussion

The final decision on Najin's future role in the project was to discontinue any further oocyte collection procedures (including the possibility of ovariectomy). The application of the tools was pivotal in reaching the decision. The EM highlighted numerous cons and a few pros for the options of continuing to perform OPU or attempting ovariectomy and a single substantial con (reducing opportunities for oocyte collection) with some partially counterbalancing pros for the choice of discontinuing all procedures. The DT showed that by choosing 1 of the first 2 options, the courses of events that most satisfied the ethical desiderata had a low probability of realization, as opposed to some of the courses of events that lead to ethically unsatisfactory outcomes. Analyses of the survey data modeled on BC showed that only the option to suspend interventions on Najin and to reshape her role in the project outside of oocyte donation was considered ethically acceptable.

The case study demonstrated the value these tools add to participatory decision-making in conservation. In particular, it showed the capacity of these tools to structure the processes and provide an organized framework for gathering relevant information and analyzing the available options.

Through the application of the tools, it was possible for participants to reflect on the ramifications of possible decisions and construct a shared, transparent, and reasoned justification for the chosen option. However, the tools did not determine the final choice. There are different and sometimes conflicting ethical approaches to conservation (Gamborg et al. 2012; Biasetti & de Mori 2020). Biodiversity conservation can intersect various dimensions of value, and which of these to prioritize depends on underlying assumptions.

The tools we applied here do not espouse a particular approach. They are not prescriptive, meaning that their structure does not reflect a specific value ordering. Instead, they are built to be compatible with different underlying value choices and to recognize the pluralism of views in conservation ethics.

Integrating the tools in the decision-making process

Combining the tools gave a robust structure to the decision-making process, allowing analysis of the available options from different angles, compilation of different points of view and articulation of a transparent decision-making process in which all elements that led to the final choice were organized and made explicit. The use of EM provided participants with a map of the value demands so they could easily compare the ethically relevant issues raised by each of the 3 options. The application of DT made it possible to identify the ramifications of the 3 options, classify them on the basis of their possibility of realization and ethical desirability, and combine data on the 2 latter aspects. Finally, the use of BC made it possible to model the data collected in the survey, establish a simple threshold for ethical acceptability, and adjudicate the 3 options accordingly.

By using the tools together, it was possible to integrate inputs from different participants and data collection methods. The pros and cons in the EM were collected in an unstructured manner, allowing participants to exchange information, opinions, and ideas. Estimation of the probability of realization of the chance nodes of the DT was carried out by the veterinarians in charge of the procedures. Scoring on the dimensions of the BC cube was provided again from all participants, this time in a structured manner, via a survey, to counteract the eventual influence of factors like personality and status.

Finally, the integrated use of tools gave a solid organization to the participatory decision-making process. Filling the EM was the starting point, building the DT was the central part, and modeling the BC was the conclusion. In addition to setting the pace, the tools were instrumental in collecting and framing the pieces of information and ideas that emerged during all phases in the decision-making process. In this way, it was made easier to build a technical report in which the scientific facts and ethical desiderata behind the choice were presented transparently and clearly.

Strengths and limitations

The usefulness of using an EM to support a participatory process is well known (Kaiser and Forsberg 2001; Kaiser et al. 2007). The structure of the EM encourages the participants to imagine themselves in the shoes of others, ensuring, as much as possible, a plural and comprehensive collection of the relevant value demands.

Here demands from the general template (Table 1) were used as a checklist to collect ethical pros and cons of each option. The advantage of using an EM to build a pros and cons list is 2- fold. First, the EM provides a structured frame for collecting and organizing the items in the list. Second, due to the pluralistic and comprehensive nature of the checklist provided by the EM, the resulting pros and cons list is compiled from a wide range of value perspectives. This kind of EM can be very useful when it comes to analyzing competing choices because it can be used to compare the different degrees of compliance of the analyzed options with the value demands on the template. This can be useful for understanding how the options differentially affect stakeholders. By comparing the EMs in our case study, for example, it was clear that the option that most respects the welfare of Najin was to cease all further intervention.

However, unless the different value demands and the degree of compliance are ranked or scored, the analysis that can be carried out remains qualitative.

The potential of DT in conservation was recognized early on (Maguire et al. 1987; Maguire 1991), although it remains an underused tool (Canessa et al. 2016), which, to the best of our knowledge, has never been employed before for the ethical analysis of biodiversity conservation decision-making. Application in the case study shows how the DT can be employed to analyze different options on the basis of a set of previously identified ethical desiderata. Building a DT, however, is never a neutral process because it is necessary to identify and select different pieces of information. More specifically, it is necessary to identify options, anticipate possible interfering events, establish their probability, and evaluate outcomes. Wrongful assumptions or estimations may lead to skewed representations of outcomes. For these reasons, DTs are vulnerable to bias and epistemic limitations and may fail to identify the best option (Regan et al. 2005). To obviate in part these limitations, a key point of the construction of the DT (estimating the possibility of realization of each chance node) was carried out by a restricted group of participants in charge of the procedures.

There are 3 limitations in the design of this specific DT. The first is that probabilities of realization of chance nodes are assigned through a simple scale. The second is that the DT does not take into account a possible course of events in which both major and minor accidents happen. In this case, a simplification was preferred, to reduce the number of possible outcomes, considering that a course of action in which a major accident occurs is already a really bad outcome per se. Finally, the tree is not complete. For example, the possibility that Najin might contribute to the conservation of the taxon by transmitting some of her social skills and competencies to the next generation of NWR was not included.

Including this element would have required making acceptable estimates of life expectancy of the animal and of the time still required to see the birth of an NWR calf. This would also have required the inclusion of an additional chance node at the end of several branches, multiplying in this way the number of outcomes.

In any case it is doubtful that by refining the DT to avoid the previous limitations it would be possible to obtain more optimistic evaluations regarding the OPU and ovariectomy options. In fact, by including in the analysis the capacity of Najin to contribute to the conservation efforts by transmitting her skills to the next generations, expected outcomes for the first 2 options would likely appear even worse.

To check the final results a DT was also built for Fatu (Appendix 1). In this DT, although the possibilities of realization of chance nodes concerning ovariectomies remain unvaried, the option of continuing performing OPU procedures fared better than the option of discontinuing every intervention. By comparing the 2 DTs it is possible to show that, given the same goals, desiderata, and set of choices, yet different circumstances, it is ethically acceptable to support different courses of action for the 2 females.

The use of the BC in conservation has been advocated (McMahon et al. 2012), but, based on the literature, it remains severely underutilized (but see Hickling [1994]). The BC, however, as shown by its application in the case study, is a very powerful tool thanks to its ease of use and the comprehensibility with which it represents the threshold of ethical acceptability in a 3-dimensional scenario. The 3 comparative dimensions are not commensurate (Bateson 2005), but this is not a negative aspect of the tool, the goal of which is to propose a practical rule to assess ethical acceptability based on a reasonable mediation between different points of view. Thus, the BC is useful in participatory decision-making processes because it is easy to apply and visualize, as long as what is represented by its dimensions has been established previously. Adapting the dimensions used in the original BC to the specific situation was an essential step toward exploiting the full potential of this tool. In this case, the 3 ethically relevant dimensions (scientific value, animal welfare, social value) were made to coincide with the desiderata identified in the participatory decision-making process. By adopting the ranking between desiderata already employed for the DT it was possible to order all the possible combination of scores in the BC to establish worst and best outcomes among acceptable and unacceptable results. Scores modeled on the BC for each option confirm the expectations derived from the application of the other 2 tools, showing the consistency of the decision-making process.

Although the use of surveys can be a valid alternative to other usual methodologies for obtaining scores for the BC—like evaluation by committees—the sample must be chosen to contain only experts and stakeholders. In this way, however, the sample is often destined to be numerically small, as in this study, where the number of respondents was 16 people.

Tools like the EM, DT, and the BC supply a flexible yet solid structure for ethical analyses in conservation and can assist in reaching balanced decisions, in which all the necessary factors are collected, considered, and scrutinized and the value choices are reasoned and made clear. In this way, these decision tools can contribute to the communication and responsible implementation of a project and hence to its success. As the case study showed, conservationists will increasingly need to address complex scenarios requiring ethical investigation. The need to refine the tools we employed for this task will increase over time.

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Supporting Information

Additional supporting information may be found in the online version of the article at the publisher's website.

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Section 2. Wildlife management and ethical evaluation of Animal-Visitor Interactions

2.1. Ethical reasoning and participatory approach towards achieving regulatory processes for animal-visitor interactions (AVIs) in South Africa

Adapted from:

Muzzo, A., Pollastri, I., Biasetti, P., Vogt, G., Manenti, R., & de Mori, B. (2023). Ethical reasoning and participatory approach towards achieving regulatory processes for animal-visitor interactions (AVIs) in South Africa. *Plos one*, *18*(3), e0282507.

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Abstract

South Africa's wide range of animal facilities offers many different types of Animal-Visitor Interactions, wild animal encounters where animals and visitors come closer than in normal circumstances. The aim of this study was to provide a map of the ethically relevant aspects involved in AVIs in South Africa as a first step towards regulating these activities. A participative approach based on the ethical matrix, a tool which organizes the ethical standings of the stakeholders by three bearing ethical principles (wellbeing, autonomy, fairness), was applied. The matrix was populated through a top-down approach and refined by engaging stakeholders in a workshop and two online self-administrated surveys. The outcome is a map of the value demands concerning Animal Visitor Interactions. This map shows how the ethical acceptability of AVIs is linked to different relevant issues like animal welfare, education, biodiversity conservation, sustainability, human competency, facility mission, impact on scientific research and socio-economic outcomes. In addition, results highlighted the importance of cooperation among stakeholders and suggested that attention for animal welfare can inform decision

making and inspire a multidisciplinary approach in implementing a regulatory frame for South African wildlife facilities.

Introduction

Wild animal encounters are increasingly popular activities offered to visitors by a variety of organizations and facilities: from zoos, aquaria, and sanctuaries proposing encounters with program or ambassador animals [1] to the nature-based tourism industry providing activities with free-ranging animals or in dedicated premises [2,3]. Some of these activities involve Animal-Visitor Interactions (AVIs)—that is, activities where visitors encounter the animals at a distance closer than allowed in usual circumstances [1,4]. These activities may include: low proximity AVIs where the experience, while still closer than usual, is mediated by a barrier of some sort (behind the scenes encounters, animal shows, etc.); medium proximity AVIs where visitors may experience close proximity without barriers, but with a relatively low expectation of direct contact (non-hand feeding, walkthrough or swim-through, etc.); and close proximity AVIs, where direct contact is an expected and essential part of the activity (touch-pools, direct animal feeding, tactile encounters, petting, animal riding, walk-with or swim-with activities, etc.). In any case, AVIs do not include behaviors that are not allowed but result in interaction (e.g., tank banging).

AVIs can impact human wellbeing (both of visitors and caregivers), animal wellbeing and welfare, and biodiversity conservation [5], with effects that may range from positive to neutral or negative [6-8]. Understanding the implications of AVIs on animal welfare, conservation, and people is a challenging and an emerging field of research [9,10]. This field of research is complicated by the many variables involved—the type of AVI, the species involved, the individual characteristics of the animals and their position on the wild-captive continuum, the type of facility, the management practices occurring, etc. For this reason, AVIs need to be investigated also from an ethical standpoint, taking into account the different value dimensions relative to respect for people, animals and biodiversity, and the way they relate with each other [9,11].

The need for a multidimensional ethical analysis of AVIs is made even more urgent by the proliferation of these activities, which goes along with the need of achieving a regulatory approach. Globally, wildlife tourism is a growing industry, and the possibility of interacting with animals provides great attractiveness. In this global context, South African wildlife tourism facilities may offer to their guests one of the greatest range of activities—giraffe-feeding, interactions with semi-captive elephants, lion and cheetah walks, snake demonstrations, meerkat interactions, carnivores-feeding shows, cub-petting, and so on. While AVIs require that complex trade-offs between profitability, animal welfare, and species conservation be made, if responsibly managed, they are conceived to be able to provide important opportunities for the local economy, biodiversity conservation, visitor education, and also for animal welfare [10]. At the same time, however, poor management can bring animal welfare, conservation, and economic sustainability into direct collision [10].

This study aimed to provide a map of the ethically relevant aspects involved in AVIs in South Africa as a possible first step towards regulating these activities. To consider a wide range of perspectives and include into the analysis the contextual variables from the South African scenario, a participative approach based on ethical reasoning was adopted. A workshop was organized and two online surveys were subsequently launched to build an Ethical Matrix (EM). The EM is a conceptual tool for conducting structured ethical analysis on existing or prospective technologies, situations, dynamics, and policy options, and to support decision-making [13,14]. It is not a prescriptive tool [15] but helps decision-makers in reaching responsible and defensible decisions [14] by summarizing the moral interests involved, pointing out the eventual conflicts, and anticipating the positive and negative impacts on the stakeholders of the issue under investigation. It was introduced in the literature by Ben Mepham in the context of food ethics [12] and it has since been applied to several fields including forestry [16], fishery technology [17,18], radiation restoration strategies [19,20], conservation practices and policies [21-23], as well as in the assessment of human-animal interactions [4, 24, 25]. The opportunities provided by structuring a participatory process through EM are well known [26, 27]. In particular, the use of the EM encourages the participants to take into consideration the others' perspectives, allowing in this way—as much as possible—for a plural and comprehensive collection of the ethically relevant aspects. In this study, a customized EM was created by collecting data through a participative process to be used as a first step toward regulating AVIs in South Africa.

Materials and methods

The study took place between November 2019 and December 2020 and consisted of building an EM by collecting the ethically relevant demands involved in AVIs in South Africa through a participatory process.

During the first phase of the study, stakeholders were defined following Mepham et al. (2006) [14]. Either interest groups (human or not human) "actively affecting" or "affected by" the issue were included. The proposals of the research group members were integrated into a brainstorming group, during which the final list was defined. The list included: a) animals involved in AVI; b) owners and managers; c) handlers; d) keepers and staff; e) veterinarians; f) government representatives; g) biodiversity; h) visitors participating in AVIs; i) animal rights groups. The EM was then sketched top-down by the members of the research group, using scientific and grey literature on the topic [4, 13-18, 21-29]. Subsequently, this first draft underwent a bottom-up process of refinement. During this second step of the study, data collected in a participatory process—a one-day facilitated workshop and two online surveys—were organized and analyzed, and were then used, along supplementary scientific and gray literature, to build the detailed Final

EM for AVIs. The outcome of the EM was then revised top-down, and multiple brainstorming sessions and revision phases allowed to define the concepts representing the stakeholders' interests, a draft report was prepared and distributed amongst participant stakeholders to obtain final feedbacks. Finally, a final report, including relevant data and the Final EM for AVIs was then completed and sent to the government representatives as a first step towards regulating AVIs in South Africa.

The study was performed in compliance with the relevant ethical and normative guidelines of South Africa. No approval of an ethics committee/institutional board was needed ate the time of the study. Workshop participants voluntarily joined the study and gave their oral consent for inclusion before participating. Participants were assured of anonymity unless specific requests for the contrary, and no personal information was collected. Survey respondents gave their informed consent for inclusion. A privacy notice was provided at the beginning of the survey to inform and assure that responses were anonymous and confidential and that information collected would be used for research purposes only. No personal information was collected, and only visitors over 18 years old could participate. Participation was voluntary and could be canceled at any time without any reason. No incentive or financial reimbursement was provided.

Step 1: The Participatory Process

After sketching an *interim* EM top-down based on the relevant literature (Table S1) and identifying the relevant stakeholders to be contacted, a participatory process involving a one-day workshop and two surveys was carried out to collect data. Data collected were then used to refine the EM top-down. The main goals of the participatory process was to ensure that stakeholders could personally advance and discuss their ethically relevant interests, and, at the same time, identify and discuss the interests of animals and biodiversity. Through the participatory process it was possible to collect data specifically to: (a) cross-check and confront the value-demands at stake; (b) assess the importance attributed by the stakeholders to the various value-demands identified.

The workshop

Workshops are part of the standard methodology of the bottom-up EM [14]. In this case, a one-day facilitated workshop, hosted by Shongweni Dam and Nature Reserve NPC (29°51’35’’S 30°43’20’’E) was organized on November 20th, 2019 in partnership with Conservation Guardians (www.conservationguardians.africa), who took care also of involving participants.

Relevant stakeholders (i.e., affected parties identified by the sketched EM) as potential participants were contacted. The invitation was sent by e-mail to 12 Facilities/Organizations/ Institutions, then followed up by phone call. Nine of Facilities/Organization/Institutions attended the workshop with one or more representatives, for a total of 18 invited participants. Their professions were: owners and managers of facilities (n=9); keepers (n=2); government representatives (n=2); wildlife veterinarians (n=1); and academic researchers (n=4). Owner and managers came from game farms, safari parks, zoos, aquaria, and facilities hosting elephants or lions. Their professional backgrounds included conservationists, animal welfare and behavior experts, field rangers, high-level keepers, and trainers.

The workshop was co-facilitated by University of Padova and Conservation Guardians members. Two researchers were tasked with taking minutes of the workshop (as suggested by [18]), preparing visual contents to support the process, and checking the logistical aspects. Audio recording of the workshop also took place, after written consent was given by all participants.

The workload was divided into four stages: opening, preliminary session, main session, and closure.

Opening of the workshop. The opening consisted of an introduction on the aims of the workshop, on ethics, and the EM. An operative definition and classification of AVIs was also discussed in this phase.

Preliminary session of the workshop. The preliminary session included a 1st round and a 2nd round. During the 1st round of the preliminary session, blocks of sticky notes were distributed among participants, who were asked to identify key animal welfare issues and key management issues concerning AVIs by writing them down [30]. Only one issue could be written on a single note, and no fixed limit to the number of notes that could be used was given. All sticky notes were then collected and displayed on a board; animal welfare issues on one side, management issues on the other. Each sticker was tagged with a pre-assigned numeric code, specifically assigned to each participant (as per [31]). This permitted researchers to identify the author of each note while assuring anonymity among participants, and minimizing the influence that they could have on each other.

Afterwards, researchers grouped the notes with similar themes, and assisted by the facilitators, assigned a temporary title to each cluster. Clusters and titles were then discussed with participants. Participants were invited to debate, agree or amend the composition of clusters and the temporary titles. During the discussion clusters were added to form larger grouping, others were instead split, and notes were moved from one cluster to another.

Once an agreement on clusters and title was reached, the 2nd round started. The goal of this round was to identify the perceived priorities of the participants amongst the clusters. Each participant voted three animal welfare clusters and three management issues clusters as her or his priority. Sticky notes were used for voting, and, after collection and counting, the six most voted clusters for each category were displayed on the board, animal welfare clusters on one side, management issues clusters on the other. The results provided a starting point for main session activities.

Main session of the workshop. During the main session, stakeholders were asked to advance their value demands. The basic structure of the EM was briefly recapped, and an empty matrix was displayed. Participants were asked to individually express, using sticky notes, their opinion on the necessary criteria for their wellbeing, autonomy, and fair treatment, also referring to the notes individuated in the previous step and still present on the board. During the entire exercise, facilitators were available to assist participants and give them further information. All participants then attached their stickers to the empty cells of the EM. An open discussion followed.

Closure of the workshop. During the closure phase, anonymous feedback from participants was collected using a questionnaire to support the SWOT analysis (Analysis of Strengths, Weaknesses, Opportunities, Threats) [32-34].

SWOT Analysis

As recommended [14], a SWOT analysis [32-34] was performed to evaluate strengths, weaknesses, opportunities, and threats of the workshop experience. The SWOT analysis focused on the methodology and aims of the workshop. The feedback form distributed was anonymous and participation voluntary. There was no time limit to complete the questionnaire and a researcher collected the filled forms one by one.

The surveys

The use of surveys introduced an element of novelty in the standard methodology of the EM. They were adopted to include the point of view of stakeholders that were difficult to involve in the workshop activities (i.e., visitors of facilities), and were necessary to be represented into the EM in order to follow criteria of inclusivity and completeness.

Two different surveys were specifically designed, one aimed at the staff of facilities and another at visitors. Both surveys aimed to investigate the value demands of the respondents, in order to identify their perceived criteria for their wellbeing, autonomy, and fair treatment. Moreover, the staff questionnaire also investigated the staff perspective on animal welfare and management issues related to AVIs, similarly to what was done during the preliminary session with stakeholders participants to the workshop.

Google Forms, a user-friendly web-based tool, was used to create and conduct the two online surveys, which were based on an anonymous self-administrated questionnaire. The surveys were set up using convenience sampling, also known as Haphazard Sampling or Accidental Sampling, a type of nonprobability sampling where members of the target population meet certain practical criteria [35]. In this study, such criteria were the accessibility and the willingness of the respondents to participate in the study. Due to privacy reasons, it was not possible to directly access visitors and staff emails. Therefore, facilities taking part in the study submitted the survey link to their past visitors—the ones who gave consent to the facility to use their email contacts—and to their Staff (keepers, educators, handlers, etc.).

The questionnaires were reviewed and pilot tested to identify confusing items, mistakes, and potential biases [36] by a small group of experts and not-experts, who were asked to complete the form and report what they found easy or difficult to understand, confusing and interesting. No data was analyzed in this phase, and the feedbacks were exclusively used to refine and finalize the questionnaires.

Data collection for the visitor survey began in April 2020 and continued until December 2020. The visitor questionnaire consisted of three sections: 1) "Demographical Section"; 2) "PV Section", for Participating Visitors (PV)—visitors who experienced AVI; 3) "NPV section" for Non-Participating Visitors (NPV)—visitors who did not experience any AVI (the visitor questionnaire is available in Table S2).

Demographical section. The demographical Section consisted of six items and included questions about the age, nationality, and gender of respondents, as well as on their self-perception and the period of the visit to the facility. At the end of the section, respondents were asked if they had experienced AVIs during their visit and were directed to the "PV Section" or the "NPV section" according to their answers.

PV section. The PV Section consisted of 15 items and included questions on the AVI experienced by the visitors (AVI description, questions n. 7-8), on their criteria for their well-being (questions n. 9-14), autonomy (questions n. 15-17), and fair treatment (questions n. 18-19), and general feedback and additional comments (questions n. 20-21).

NPV section. NPV Section included two questions, one asking why the respondent did not experience AVIs (question n. 22), the other collecting additional comments (question n. 23).

Data collection for the staff survey began in July 2020 and continued until December 2020. The staff questionnaire consisted of four sections: 1) "Preliminary Information Section", to allow redirection to either "AVI Section" or "No AVI Section"; 2) "AVI Section", dedicated to staff in care of animals involved in AVIs; 3) "No AVI Section", dedicate to staff in care of animals not involved in AVIs; and 4) "Staff Demographics Section" (the staff questionnaire is available in Table S3).

Preliminary information section. Questions in this section recorded the facility in which the respondent was working and sorted staff caring for animals involved in AVIs from staff not involved (questions n. 1-2).

AVI Section. Members of the staff caring for animals involved with AVIs were directed here from the preliminary information section. AVI section included six subsections, each with its specific goal: (a) to collect details about the AVIs and the animals under the responsibility and care of the respondent; (b) to collect their criteria for their wellbeing, autonomy and fair treatment, using five-points Likert scale (questions n. 4-16); (c) to identify three main animal welfare issues concerning AVIs and possible solutions or mitigation strategies (questions 17-18); (d) to identify three key management issues concerning AVIs and possible solutions and mitigation strategies (questions 19-20); (e) to investigate safety perception, by asking the respondents to indicate how often they feel unsafe during their work with animals, what are the main dangers concerning AVIs, and their suggestions on how to improve safety (questions n. 21-23); f) to get feedback, discover if, in the last year, the staff was involved in any meeting to promote animal welfare, conservation strategies, and educational activities for the visitors, and to collect suggestions on how to improve AVIs (questions n. 24-25).

No AVI section. Members of the staff not caring for animals involved with AVIs were directed here from the preliminary information section. No AVIs section included three subsections, each with its specific goal: (a) to collect details about the animals under the responsibility and care of the respondent (question n. 35); (b) to collect their criteria for their wellbeing, autonomy and fair treatment, using five-points Likert scale (questions n. 36-48); c) to get feedback, discover if, in the last year, the staff was involved in any meeting to promote animal welfare, conservation strategies, and educational activities for the visitors, and to collect suggestions on how to improve AVIs (questions n. 49-50).

Staff Demographics Section. This section grouped a wide range of demographical questions (questions n. 26-34 “AVI Section”; questions 51-59 “No AVI Section”.

Step 2: Final EM for AVIs

During the second step of the study, data collected in the participatory process (workshop and surveys) were organized and analyzed, and were then used, along supplementary scientific and gray literature, to build the detailed *Final EM* for AVIs. Stakeholders’ interests were defined during multiple brainstorming sessions and revision phases and reported in the Final EM for AVIs. Tables S2 and S3 summarize the link between the survey questions and the value demands of the respondents, and the staff perspective on animal welfare and management issues related to AVIs.

Data analysis—workshop

Materials from the workshop were checked, notes were associated with the correspondent participant codes, votes were screened, ranking of animal welfare and management clusters was performed. Issues and clusters were then analyzed according to authorship, to identify the preferences of each stakeholder.

Minutes, notes on the key discussion point, and audio recordings of the workshop were then used to craft a report. The draft report included the list of AVI animal welfare and management issues brought up by the participants; the thematic clusters into which they were collected; the ranking of the clusters; the notes of the discussion on the EM. After a first revision by the facilitators, the draft report was sent to all the workshop participants, inviting them to contribute, comment and revise. A final report was then prepared, including stakeholders' comments and revisions and the final EM built on the basis of data collected both from the workshop and the surveys, and sent to the government representatives.

Data analysis—surveys

Different statistical analyses were performed to understand the eventual impact of socio-cultural factors, time passed from the experience, and demographical factors (age, gender, etc.) on the perception and interests of visitors regarding AVIs. After descriptive analyses on data collected, a series of linear mixed effects models (LMMs) and generalized mixed effects models (GLMMs) were generated using as dependent variables: (a) the level of satisfaction with the experience of the respondents (question n. 9); (b) the level of safety perceived by the respondents (question n. 10); (c) the final profile of respondents (Amusement, Education, Emotion or Neutral—question n. 11-13; binomial error distribution); (d) the mindset of respondents (Animal-centric, Biodiversity-centric, Ethics-centric—question n. 14; binomial error distribution); and the economical affordability perceived by respondents (question n. 18). In each model generated, the following independent variables were included: (a) age; (b) gender; (c) self-definition of respondents; (d) and time passed from the visit. In the models in which final profiles and mindsets of respondents were not considered as dependent variables, they were added as independent factors. The facility in which the respondents performed their interactions was included as a random factor. After model building, the significance of the independent variables composing each model was assessed using Wald F and $\chi 2$ tests [37].

Questions n. 11, 12, 13 were analyzed both as separate questions and as a conjoint subset of questions to generate a "final profile" for each respondent. This final profile represents what the respondent prioritizes during the AVI experience between "need of amusement", "need of education" and "need to be emotionally close to animals". According to the answers to questions n. 11-13, each respondent was assigned to one of the four possible final profiles: "Amusement", "Education", "Emotion" or "Neutral". Respondents who showed prevalent interest in education, being emotionally close to animals or a prevalent attitude towards amusement in two or more of the answers were assigned to the final profile Education, Emotion, or Amusement, respectively. Respondents were classified Neutral if they choose one answer per type in the three questions ("need of amusement", "need of education", "need to be emotionally close to animals").

Question n. 14 aimed to identify which aspects of education were more important in the mindset of visitors among the proposed answers. Depending on what they prioritized between "learning about animals", "biodiversity and conservation", or "learning about the origin and welfare of the animals hosted in the facility, its mission, and the captive-related problems", respondents were classified to have an "animal-centric", "biodiversity centric" or "ethics-centric" mindset respectively.

Answers to open question n. 19 and n. 21 were studied and summarized in the results. To facilitate question n. 19 analysis, two different researchers independently assigned tags to each item in the answers (maximum six items per respondent) and then grouped them in broader categories. Afterwards, the work of the two researchers were compared, tags and grouping were reviewed and a final analysis was elaborated. This procedure was not meant to obtain quantitative data, but just to implement an effective summarizing process and improve its reliability.

The staff survey was analyzed with descriptive statistics.

All the analyses were performed in R 3.3 environment using the software packages car, LmerTest and glht (R Development Core 2018).

Results

Workshop results

In total, the 18 participants identified 76 animal welfare issues on the sticky notes (with a mean of 4.22 animal welfare issues per participant). After discussing the preliminary categorization proposed by the researchers, participants agreed to define 17 animal welfare clusters related to AVIs. During the 2^{nd} round, participants indicated (with three votes each) the animal welfare clusters, which, in their opinion, should be prioritized. The most voted cluster was *Human competency* (8 votes), followed by *Best practice, Compliance, Health* (5 votes each), and *Animal rights interference* and *Safety (Animal, Human*) (4 votes each). Table 1 presents the Animal Welfare clusters and votes, and Table S4 details identified issues, clusters, and votes.

Table 1. Animal Welfare Clusters and votes (OM = Owners and Manager; R = Researchers; HKS = Handlers/Keepers/Staff; GR= government Representatives; V= veterinarians). Full details in Table S4

Animal Welfare Clusters	Stakeholders who identified the issues	Stakeholders who voted for the cluster	Number of votes for cluster
Human competency	OM; V	GR; HKS; OM; R	8
Best practice	GR; R	OM; R; V	5
Compliance	GR; OM	HKS; OM; V	5
Health	OM; R; V	OM; R;	5
Animal rights interference	GR; OM	GR; OM; R	4
Safety (Animal, Human)	GR; OM; R	HKS; OM	4
Assessment (animal)	OM; R; V	OM; R	3
Implementing husbandry	HKS; OM; R	HKS; OM;	3
Regulating "rules"	GR	GR; HKS; OM	3
Training (animal)	HKS; OM; R	HKS; OM	3
Communication	OM; R	GR; OM	2
Population control (management)	HKS; R	GR; R	2
Regulating interactions	OM; R	OM	2
Zoonosis and diseases	OM; R	R	2
Five domains	HKS; OM	OM	1
Space	OM; R	V	1
Enrichment	R		0

In total, the 18 participants wrote 95 management issues on the sticky notes (mean 5.3 specific issues per participant). After discussing the preliminary categorization proposed by the researchers, participants agreed to define 14 management clusters related to AVI. During the 2nd round, participants indicated (with three votes each) the management clusters, which, in their opinion, should be prioritized. The most voted cluster was *Husbandry and care protocol* (7 votes), *Governance*, *Sustainability* (6 votes each), *Conflicting legislatory bodies*, *Legislation* (5 votes each), and *Communication*, *Conservation education*, *Training people* (4 votes each). *Communication* was considered both an animal welfare and a management issue. Table 2 reports the Management clusters and votes, and Table S5 details identified issues, clusters, and votes.

Table 2. Management Clusters and votes (OM = Owners and Manager; R = Researchers; HKS = Handlers/Keepers/Staff; GR = government Representatives; V= veterinarians). Full details in Table S5

Management cluster	Stakeholders who identified the issues	Stakeholders who voted for the cluster	Number of votes for cluster
Husbandry and care protocol	OM; R; GR;	OM; R; HKS; V	7
Governance	OM; GR; V	OM; R; GR; V	6
Sustainability	OM; HKS; GR	OM; R; HKS; GR	6
Conflicting legislatory bodies	OM;	OM; R; HKS; GR	5
Legislation	OM; R; GR;	OM; R; HKS;	5
Communication	OM; HKS;	OM; R	4
Conservation education	OM; R	OM; R	4
Training people	OM; R; HKS; GR	OM; R; GR	4
Human threats	OM; V	OM; R; HKS	3

Brand Reputation	OM;	OM;	2
Conflicting mandates	GR; OM; V	R; GR	2
Safety (Animal and humans)	OM; R	OM	2
Internal codes of conduct	OM; R; GR; V	V	1
Environmental threats	OM; R		0

All the original suggestions proposed by the participants during the discussion and reflecting their interests in terms of wellbeing, autonomy, and fairness during the *Main session of the workshop* are reported in Table S6. Table S6 was shared with the participants after the workshop, as well as the report of the day, summarizing the activities and the discussions. The workshop participants were invited to provide feedback and reviews, but no additional information were collected in this phase.

SWOT analysis results

Table 3 reports an evaluation of the Internal (strengths and weakness) and External (opportunities and threats) dimensions of the workshop done with a SWOT analysis. SWOT contents were obtained from the feedback questionnaire administered to the participants at the end of the workshop.

Table 3. Internal and External dimensions of the workshop

STRENGTHS	**WEAKNESSES**
Collecting different ideas, opinions, and perspectives on the topic. Having diverse stakeholders together at the same table for real-time confrontation and group discussion. Approaches and their novelty in this field appreciated by the participants (discovering and filling the Ethical Matrix, methods, materials); All the participants recognized the value and usefulness of the workshop in aiding the discussion on AVI.	Insufficient time for having a deep discussion in the main session and final synthesis. Not all stakeholders being represented at the workshop.
OPPORTUNITIES	**THREATS**
Interest in the Ethical Matrix as a new approach to problem-solving in the field of wildlife management. Possibility to integrate the participatory process with preliminary focus groups. Possibility to repeat the experience inviting other stakeholders and/or creating workshops dedicated to more specific topics. Possibility to organize other workshops, inspired by this experience, to aid the discussion on specific themes and develop possible guidelines or deliverables. Integrating the workshop process with the use of surveys.	Difficulty to have all necessary stakeholders at the table at the same time. Risk of «overdiscussing» issues and difficulty to produce an effective, synthetic deliverable. Difficulty in having stakeholders equally represented physically at the workshop (number of participants per stakeholder group). Stakeholders influencing other stakeholders (i.e., influence due to working relationships, influence that good communicators can have on others).

Visitor survey results

A total sample of 177 visitors answered the questionnaires, n=19 (11%) from facility A, n=150 (85%) from facility B, and n=8 (5%) from facility C. Table S7 summarizes demographic information and other independent variables collected from visitors who answered the questionnaires.

Only 4 out of 177 respondents did not experience AVI, for miscellaneous reasons, while 173 (98%) of the respondents experienced AVI with the elephants of facilities A, B, or C. The following results represent the subset of 173 respondents who experienced AVI.

The 94% (n=163) of respondents was “extremely happy” with the AVI experience, and the 6% (n=10) scored 4, so was “happy” with the experience (mean = 4.94, median = 5.00, mode = 5.00). When asked the safety perception during AVI, respondents declared to have a high safety perception: 94% (n=163) felt “extremely safe”, 5% (n=9) felt “safe” and only 1% (n=1) of the respondents felt “neither safe nor unsafe” (score 3) (mean = 4.93, median = 5.00, mode = 5.00).

Questions n. 11-13 investigated what respondents prioritized among three different needs. Answers were tagged according to the “Need of amusement”, “Need of education” and “Need to be emotionally close to animals”. When asked why they decided to participate in the activity (question n.11), most of the respondents fell into the “Need of education” category (76%, n=132). When asked what they were looking for when participating in the activity (question n.12) and what impressed them the most (question n.13), the majority fell into the “Need to be emotionally close to animals (61%, n=106 and 52% n=90 respectively). Table S8 presents the detailed results for each question.

According to the prevailing answers to questions n. 11, 12, 13, each respondent was assigned to an overall “final profile” among “Need of amusement”, “Need of education”, “Need to be emotionally close”, and “Neutral”. “Neutral” final profile was assigned to respondents who presented equally distributed answer types. Final profiles of respondents resulted distributed as follows: 53% (n=91) “Need of Education”, 42% (n=72) “Need to Be emotionally close”, 1% (n=2) “Need of amusement”, and 5% (n=8) “Neutral”.

Respondents who defined themselves as “Thrill seekers” in 100% of the cases showed a “Need to be emotionally close” profile. The “Need to be emotionally close” profile was also shown by 60% of “Curious tourists” respondents. The ones describing themselves as “Animal experts” or “Animal lover” more frequently had a “Need of Education” profile (82% and 51% respectively), and just 8% of the “Nature lovers” demonstrated a “Neutral” profile. The level of satisfaction with the experience was significantly lower in respondents with the “Need of amusement” final profile ($F= 7.51$, $p<0.01$; Table S9). From the analysis, it also emerges that respondents defining themselves as “Animal experts” or as professionals working with animals and the environment have a significantly higher probability to appreciate education (final profile “Need of Education”; $\chi^2= 5.44$, $p= 0.01$; Table S9).

Question n. 14 explored the specific interests of respondents concerning the learning opportunities offered by AVIs. Potentially, these experiences can stimulate the curiosity of visitors in these directions: (a) to learn about the animals involved (anatomy, physiology, ethology, captive animal welfare, husbandry, management, keeper-animal relationship, handler-animal relationship, training); (b) to learn about conservation of the animals involved (rehabilitation reintroduction, species survival plan, current challenges, poaching, conservation sustainability, understand impact); (c) to learn about habitats, biodiversity, and the interrelationship between wildlife and environments; (d) to practice ethical reasoning, investigating the mission statements of the facility, purpose of AVI, origin, history, and life of the animal housed in the facility (why these animals are here?). In this way, question n. 14 investigated whether the visitors approached the AVI experience with an “Animal-centric” (a), “Biodiversity-centric” (b and c), or “Ethics-centric” mindset (d). Respondents distributed as follows: 40% (n=70) “Animal-centric”, 32% (n=56) “Biodiversity-centric” and 27% (n=47) “Ethics-centric”.

The relation between mindset and demographic of respondents is reported in Table 4. A weak significant relationship occurs between older respondents and a Biodiversity-centric attitude (χ^2= 5.77, p= 0.01; Table S9), intended as a preferential interest in learning about nature and biodiversity (i.e., Habitat, interrelationship between wildlife and environment, interdependence, endangered species and relative survival plans, sustainable conservation programs, rehabilitation, and reintroduction programs, poaching, human impact on wildlife).

Table 4. Relation between mindset and demographic of respondents.

		Animal-centric	Biodiversity-centric	Ethics-centric
Age (range in years)	14-18	100% (1)	0	0
	19-25	44% (4)	22% (2)	33% (3)
	26-34	45% (10)	18% (4)	36% (8)
	35-54	40% (32)	35% (28)	26% (21)
	55-64	33% (14)	40% (17)	28% (12)
	over 64	53% (9)	29% (5)	18% (3)
Gender	Female	37% (41)	29% (32)	34% (38)
	Male	47% (29)	39% (24)	15% (9)
Nationality	Africa	36% (42)	38% (44)	26% (31)
	Australia	50% (1)	0	50% (1)
	Europe	53% (16)	27% (8)	20% (6)
	North America	50% (10)	15% (3)	35% (7)
	South America	25% (1)	25% (1)	50% (2)
Self-description	Animal expert	55% (6)	36% (4)	9% (1)
	Animal lover	39% (34)	26% (23)	34% (30)
	Curious tourist	20% (1)	60% (3)	20% (1)
	Nature lover	45% (28)	37% (23)	18% (11)
	Other	17% (1)	17% (1)	67% (4)
	Thrill seeker	0	100% (2)	0
Need for…	Amusement	50% (1)	50% (1)	0
	Education	40% (36)	38% (35)	22% (20)
	Emotion	39% (28)	25% (18)	36% (26)
	Neutral	62% (5)	25% (2)	13% (1)

Question n. 15 aimed to explore if visitors perceived to be provided with a sufficient level of information about the facility, specific information regarding the AVI, mandatory behavioral rules to be respected during the interaction, and information about the welfare of the interacting animals. In the 92% (n=159) of the cases, respondents believed to have received sufficient information about all the four topics. The 6% (n=10) of the sample confirmed to have received enough information for all the topics except for 'facility', as they declared that they did not look for information about this theme. The other respondents declared to not have received enough information about the behavioral rules (1%, n=2 of the respondents), or about the welfare of the animal/s they interacted with (1%, n=1). One respondent declared to have not looked for information on all the themes (1%, n=1).

Most respondents acquired information about the facility and its activities from friends/family (46%, n=79), internet (24%, n=41) or directly at the facility (20%, n=34). Other sources of information were hotels and holiday rentals (5%, n=8), travel agencies (3%, n=6), tourist centers (1%, n=2) and others (2%, n=3).

Understanding how visitors tend to choose an animal facility could give an insight into their needs and priorities. When asked about the reason why they chose the visited facility over other ones offering similar activities, 58% (n= 101) of the respondents reported that the reason had been the awareness around welfare standards offered in that specific facility. The 18% (n= 31) of the respondents said it was the closest facility on their travel route, for the 5% (n=9) of the respondents the visited facility was the only one they had heard of, 4% (n=7) chose the facility because of the possibility to do other interesting activities in the same facility, and 2% (n=4) because of the number of animals/species housed. The remaining 12% (n=21) provided miscellaneous reasons (recommended, for family/friends reasons, etc.).

The affordability of AVIs was investigated by asking the question "Do you think the price you paid is fair?" (question n. 18) and letting respondents express through five points Likert scale starting from "Extremely unfair" (1) to "Extremely fair" (5). The 77% (n=133) of respondents considered the price paid "extremely fair", 16% (n=28) considered it “fair” and 7% (n=12) “neither fair nor unfair” (score 3) (mean = 4.72, mode = 5.00).

Question n. 19 aimed to understand which factors visitors would consider important in a hypothetic rating system, that could rate the quality of the animal facilities offering AVIs by asking to indicate three criteria, from the most important to the least important. Respondents wrote 544 criteria, giving 0-6 criteria each. Considering the first three they wrote, a total of 519 criteria were grouped by theme for descriptive statistics. Overall, “Animal welfare and care” was the most cited criteria to evaluate a facility (34%, n=179), followed by “Education” (13%, n=65), “Staff” (intended as Staff competency, Animal-Staff Interaction and Relationship, and Staff welfare, 10%, n=54), and “Safety” (of animals and people, 10%, n=51). “Animal welfare and care” was indicated as the most important criteria to include in the hypothetical rating system by 70% (n=121) of respondents, followed by “Cleanliness and hygiene” of the facility (5%, n=8) and “safety” (5%, n=8). “Animal welfare and care” was indicated also as the second most important criteria to evaluate a facility by 20% (n=34) of respondents, followed by “education” (16%, n=27) and “safety” (14%, n=25). The most cited criteria respondents gave as their third option was “Education” (19%, n=33), “staff” (14%, n=25), “Animal welfare and care” (14%, n=24). The original list of criteria, the categorization process, and the answers’ details are available in Table S10.

When asked to indicate suggestions to improve their experience (question n.20), respondents equally distributed among the possible answers (Table 5).

Table 5. Distribution of answers to question n. 20

In your opinion, what should be done to improve the experience?	Frequency % (n)
Allowing longer interactions with the animals	10% (18)
Explaining if and how the facility cooperates with conservation programs	16% (28)
Illustrating if and how the facility also works as a rescue centre	13% (23)
Letting animals choose whether to interact or not with us	10% (18)
Offering more information about animal welfare issues	7% (12)
Offering more informative material and in general providing more educational content	5% (8)
Showing videos about the life of the animals in our facility when they are not interacting	24% (41)
Other	14% (25)

The last open-ended question asked the respondents about any additional feedback. 110 out of 173 (64%) respondents reported a comment, which, after a set of 20 questions, denotes a high degree of motivation and engagement.

No other significant results were obtained from the modeling and significance testing of the visitors' answers.

Staff survey results

A total sample of 14 staff members answered the questionnaires, n=4 (23.5%) from facility A and n =10 (58.8%) from facility B.

All the respondents had under their responsibility and cared for semi-captive African elephants involved in AVI. The majority were men (Male = 86%, n= 12; Female = 7%, n= 1; Prefer not to respond = 7%, n= 1), aged between 35 and 54 years old (79% of the respondents, n= 11; between 26-34 years old= 21%, n=3). The 64% (n= 9) came from Zimbabwe, 36% (n = 5) from South Africa. The majority of respondents have worked in the facility for six to ten years (72%, n= 10), only a few worked in the facility for two to five years (21%, n=3), and only one worked in the facility for more than ten years (7%, n= 1). Most of the respondents declared to have completed a high school degree (93%, n=13), while just one pursued further study getting a bachelor's degree in technology.

Most of the staff sample (64%, n= 9) selected two or more knowledge and know-how sources. The main sources to acquire knowledge and know-how were the education and training programs provided by the facility (86%, n= 12) and colleagues (71%, n= 10). Two respondents (14%) included their family as a source of know-how and knowledge, and two respondents declared that they acquired the knowledge thanks to their previous educational background (14%, n= 2), and eight said they also learned by doing (57%, n= 8).

Questions n. 4-16 explored to which degree the ethical demands of the stakeholder Staff were satisfied. Respondents were asked to express their level of agreement to various statements on a five-point scale. Most of the staff declared that the various demands expressed in the statement were fairly satisfied (mean = 4,18, median = 4, mode = 4). Table S11 indicates the "respect for" principle of the statements, the percentage of respondents choosing each score for each statement, the mean, median, and mode.

When asked to express the main animal welfare problems and/or important topics concerning AVIs (question n. 17) and possible solutions (question n. 18), the respondents indicated zoonotic diseases (four respondents over 14) or stated that there are no animal welfare issues (eight respondents over 14). Two respondents focused on the judgmental

attitude of some guests before doing the AVI and on the pressure exerted by animal rights organizations, without explicitly expressing welfare issues. The solutions indicated consisted in the use of preventive measures (e.g., hand sanitizers—four respondents over 14) or recommendations to guests and animal rights organizations to do more informative research to build an educated opinion (two respondents over 14). No other solutions were given. When asked to express management issues concerning AVI (question n. 19), nine respondents out of 14 reported no management issues. The other five respondents indicated as managing challenges the communication between staff members, the animal welfare assessment during the interactions, specific issues related to the management of young untrained calves, guest misinformation, and visitors not listening to and/or following instructions, so behaving inappropriately. To endorse communication between staff and visitors, respondents proposed team meetings and training sessions before the interactions, where effective communication can be practiced and learned, and efficient safety and animal welfare talk before the interaction begins. Moreover, ensuring that the staff is empowered to deal with potentially dangerous situations caused by unpredictable guests and a consistent presence of the manager during the interactions were also recommended. About the challenge of assessing animal welfare, it was remarked the importance of checking the animals before the interactions to ensure they are in good health, behave appropriately, and are not stressed or hurt. In the staff's opinion, the issues related to the management of calves may be addressed by additional training and by the employment of two dedicated staff members to engage, stimulate and follow the calves.
Staff safety perception was high (question n. 21). Respondents expressed how often they feel unsafe during their daily work with the animals through a five-point scale, ranging from "never "(1) to "always" (5). Nine respondents scored one (never feel unsafe), four respondents scored four, and one respondent reported that it always felt unsafe working with the animals.

The 43% (n= 6) of the respondents declared that in their opinion there were no safety issues (question n.22), one answering that they operate with a high staff to elephant ratio. Another argued that there are no significant risks when safety rules are respected. Non-compliant behaviors of the guests are highlighted as a safety issue from 14% of the respondents (n= 2): guests being where they are not supposed to be and doing what they are not supposed to do (i.e., going to an elephant by themselves, running, screaming shouting, etc.), while 14% of respondents (n= 2) indicated anything that can frighten the elephants (uncontrolled incidents like car crashes, explosions, fires, airplanes) as a source of safety problems. Three respondents declared that meeting a wild animal can be dangerous, or, to use their words, that elephants are "still animals", with "their own minds, hormones, and emotions, as such, if they are not respected they may injure you".

To address the listed issues, staff reported what is already done in their facilities, emphasizing some aspects of their safety procedures. Relevant safety procedures include ensuring that people stay in the assigned groups, following staff's indications, making sure handlers are in front of the animals before the encounter, and not allowing visitors to interact without the staff's supervision. To improve safety, they also highlight the importance of adequate training of the animals, avoiding performing interaction programs when environmental conditions are adverse (i.e., thunderstorm, heavy rain), and guests respecting the animals. More specifically, it was recommended to desensitize the elephant to as many variables as possible to make the animals more confident and less reactive.

Being part of management strategies to promote the wellbeing of the animals and contribute to conservation and education missions is in the interest of the staff as it promotes their autonomy and their fair treatment. All interviewed staff of facilities A and B declared to have been involved in a staff meeting to promote the wellbeing of the animals included in AVIs in the last year. 50% (n= 7) of the sample declared its engagement also in staff meetings to promote educational activities for visitors, and 43%

(n = 6) of the sample declared to have been engaged in meetings focused on animal wellbeing, educational activities, and conservation strategies (Table 6). The last question (question n. 25) asked respondents to write any suggestions on how to improve the AVI. Four respondents over 14 (29%) gave suggestions (reported in Table S12).

Table 6. Distribution of answers to question n. 24

In the last year have you been engaged in any staff meeting to promote any of the following?	**% Respondents (*n*)**
…animals' wellbeing	7% (1)
…animals' wellbeing AND educational activities	50% (7)
…animals' well-being AND educational activities AND conservation strategies	43% (6)

Final EM

The ethically relevant demands of the stakeholders (including animals and biodiversity) collected during the workshop were further organized following the frame of the EM. The inputs coming from the open discussions of the workshop, as well as the answers of the surveys, underwent a similar process. A report with the results were sent to the workshop respondents. No additional information, feedback, or review were collected from the participants after sending them the report of this first phase.

The research group carried out multiple phases of brainstorming and revision. Information, concepts, and ideas presented by relevant scientific literature were also evaluated during the analytical process and organized according to the EM framework. This process allowed to define the stakeholders' interests and value-demand and finalize the Final EM as shown in the synthetic version of the final EM presented in Table 7 and in Table S13.

Table 7. Customized Final EM

	WELL-BEING **Health & welfare** ***Maximizing the good, minimizing the harm***	**AUTONOMY** **Freedom & choice** ***Valuing differences and individual freedom***	**FAIRNESS** **Equity & justice** ***Avoiding discrimination***
Animals Involved in AVI	Animal Welfare Animals' Safety	Behavioural freedom Right to be captive and right to be rewilded	Avoid increasing objectification, animals = sentient beings Equity of treatment Respect their role of "ambassador animals" Animal welfare standards not being influenced by human dissents and conflicts Legal protection
Owners And Managers	Satisfactory working conditions Sustainability Well-being of animals, staff and visitors Property interest Having support and the approval of society and Institutions	Managerial freedom Professional development and support Recognition of the peculiar features of each facility	Fair legislation and regulations Equal possibility to communicate Fair assessment of the features of the facility Fair recognition of the actual/potential role of the facility in fulfilling Conservation and/or Education purposes, along with entertainment opportunities
Staff Involved in AVI	Safety Satisfactory working conditions Avoid cognitive dissonance *	Professional freedom Professional development Respect for caregivers' professional ethics Being able to be compliant with the law	Equal opportunities Fair staff recognition Respect for caregiver professional role

Veterinarians	Safety Satisfactory working conditions	Professional freedom Possibility to respect professional ethics Being able to be compliant with the law	Being respected as professionals Equitable standards of practice Fair price for their work
Government representatives	Development of the Country Personal fulfilment and self-realization Being supported in their work	Being educated and informed Possibility to respect their own institutional role Being provided with resources	Respect of regulations Respect for their institutional role Fair involvement of the different departments
Biodiversity	Conservation Mitigating human -animal conflict through education and poverty alleviation of the local rural communities	Autonomy from human intervention Availability of sufficient resources	Equal respect for each component of Nature
Visitors	Safety Satisfactory experience Possibility to be emotionally close to animals Avoid cognitive dissonance	Having the opportunity to see wild animals in a controlled environment Possibility to choose Education Informed consent	Affordability Accessibility Equal opportunities
Animal Rights Groups	Sustainability of their business Personal fulfilment and self-realization of their members	Freedom to propose their long-term vision on SA tourism industry with regard to AVI Freedom to communicate their ideas regarding AVI Education and access to information, avoiding miseducation	Being recognised as a group of people advocating their own perspective on AVI Equal access to communication

Discussion

Workshop

Participants attending the workshop had the opportunity to reflect and discuss several topics related to AVIs, including animal welfare and management issues, their interests and value-demands, and those of the other stakeholders. The workshop activities emphasized the cooperative and constructive attitude of the participants, fostering the exchange of ideas and perspectives. This provided stakeholders with an opportunity to step back from their own starting assumptions, relate with other standpoints, and participate in finding a common synthesis.

Reconsidering one's starting assumption in the light of others' standpoints is indeed a crucial requirement for the success of a participatory process. The different evaluation of animal welfare and management issues between the 1st and the 2nd round of the preliminary session proves that this result was accomplished. In particular, the most voted clusters at the end of the preliminary session were not the most cited at the start, with some participants choosing to prioritize issues originally proposed by others. In this sense, the workshop successfully created a space for "compromise" between different and often diverging value demands.

The results of the process of identifying and prioritizing animal welfare issues concerning AVIs share common points with WAZA (World Association of Zoos and Aquaria) animal welfare strategy [38] and with the Five Domains welfare model [39,40]. This consonance gives evidence of the awareness and knowledge of animal welfare of the participants. This shows that the categories involved in the participatory process are key actors to act in the interests of the animals, invest in their welfare, and improve South African tourism facilities. Going back to the animal welfare themes highlighted, it should be noted that: (a) some of them only indirectly affect animal welfare, like communication and animal rights interference; (b) human competency was universally recognized of critical importance; (c) data collected do not provide species-specific insights—not surprising, given the impossibility to define a ‘one size fits all’ welfare strategy [6]; (d) participants showed awareness for zoonosis-related risks, before the COVID-19 pandemic. The fact that participants highlighted themes like communication and animal rights interference as potential causes of concern, not only evidences the analytic attitude of the participants, but also drives attention towards these human dynamics, their potential indirect effects on animal welfare, and the urge to integrate them in the ethical debate.

Human competency was highlighted as a key animal welfare issue and received the highest number of votes. This should raise attention in decision-makers: regulating human competency aspects may contribute to improving animal welfare in South African facilities, and their benefit to the national tourism brand. Moreover, having identified human competency as an important factor for animal welfare, participants *de facto* anticipated some aspects of the 2020 Five Domains Model, which includes the human dimension of animal welfare in its framework [41].

Concerning management, the participants focused on the need for clear legislation and regulation (see themes as legislation, conflicting legislative bodies, conflicting mandates), aside from a series of other issues (i.e. human threats, environmental threats, brand reputation) which may have detrimental effects on the challenge to optimize animal management and care (husbandry and care protocol).

Visitor survey

Visitors participating in the study were highly satisfied with their overall AVI experience, which could be considered an encouraging starting point in terms of respecting their well-being, autonomy, and fair treatment. Interestingly, 58% of the visitor respondents stated that they chose the facility because they were aware of its animal welfare standards. This may sound surprising considering that even experts struggle in assessing animal welfare. Moreover, a gold standard protocol for the welfare assessment of semi-captive elephants (or, in general, of semi-captive wild animals) is lacking, as well as recognized criteria to inform tourists [42]. Even though it would be interesting to understand what respondents intended for animal welfare, this data shows that visitors care for it, and acceptable levels of animal welfare guide visitors in their choices. One possible conservative explanation of this outcome could be that it is mainly the result of a social desirability response bias, a form of motivated misreporting in which people falsely report the socially desirable answer [43]. However, even if based on a social desirability response bias, the outcome remains the same: animal welfare guides visitors in their choice.

This result is in accordance with Miller's findings that zoo visitors are less likely to support animal facilities when they perceive animal welfare as being poor [44]. It is also supported by another finding of this study. When asked to list criteria for a hypothetic rating system to rate the quality of animal facilities offering AVI, the respondents indicated Animal Welfare or Animal Care as the most important criteria in 70% of the cases.

Staff survey

Handlers are the ones that spend more time with the animals, building unique relationships with them. To perform a detailed ethical analysis of AVIs, it is fundamental to collect their perspective, as they are at the frontline in providing animal care and in ensuring the safety of visitors and animals. Among the insights provided by survey results, it is interesting to discuss their perception of animal welfare and of their wellbeing, autonomy, and fair treatment.

When asked to indicate animal welfare issues, two main trends can be observed: (a) denying the presence of any animal welfare issues; and (b) indicating zoonosis transmission as a major concern. While a focus on zoonosis diseases by handlers seems to reflect common concerns during the COVID 19 pandemic, the fact that most of them did not identify any welfare issue can be explained in different ways. One hypothesis consists in handlers sincerely not perceiving any animal welfare problem. This hypothesis, in turn, would lead the way to another interesting research question: to which extent is this perception linked to high standards of animal welfare offered by the facilities, and to which extent is it instead influenced by the socio-cultural background of handlers? Another hypothesis could be that handlers were reluctant to provide information, given that facilities are currently subject to pressure from activists.

The handlers of facilities A and B seemed satisfied concerning their well-being, autonomy, and fair treatment, indicating that they did not perceive their interests as threatened. This finding is encouraging, also considering the positive outcomes in terms of animal welfare correlated with the satisfaction of caregivers [45,46]. Handlers seemed particularly satisfied in terms of professional freedom (the majority strongly agreed with the statement "I am able to fully apply my knowledge and skills to my job"). On the other hand, data collected suggest that, according to staff perception, there is room for improvement in terms of economical reward and in terms of feeling appreciated and respected.

EM Discussion

The EM developed during this study should provide decision-makers with a framework of the value-demands and ethically relevant aspects involved in AVIs in South Africa to be used as a starting point for the discussion around their regulation.

The Final EM highlights that stakeholders share a common interest: animal welfare. Whether directly or indirectly, respect for animal welfare may provide benefits to all the parties involved. For animals, it is important for obvious reasons tied both to their well-being and autonomy. For veterinarians is important because: a) it is a requirement coming from their professional ethics; b) it evokes positive feelings associated with the accomplishment of duties and vocations, and reduce the risks of compassion fatigue. Similarly, for the staff respecting animal welfare means following professional duties and benefitting from a positive relationship with the animal both on an emotional and on a safety level. By respecting animal welfare, owners and managers may benefit from an increase in the long-term sustainability of the enterprise and brand reputation. Indeed, this study supports the claim that visitors' perception of animal welfare may be very important from a business perspective [44]. Moreover, data collected during the workshop suggest that certain owners and managers perceive their well-being as linked to those of animals and staff. By advocating animal welfare, government representatives may contribute to their mission of protecting the animals and promoting the South African brand reputation. Likewise, animal rights groups should be interested in animal welfare as well. Finally, respect for animal welfare can also positively affect biodiversity when an AVI is paired with conservation education [21, 25, 47].

As shown by the data from the survey, visitors are also interested in animal welfare, and could especially benefit from having reliable information on the standards adopted by the facility. More specifically, being informed allows them a) to express their freedom to choose whichever facility represents better their expectations; b) to fulfill their right of informed consent; and c) to avoid cognitive dissonance, that is, the unpleasant psychological stress resulting from having an experience with animals and enjoying it while, at the same time, being concerned about their animal welfare.

The methodology followed to develop an EM for AVI allowed to disentangle complex value-issues and helped each stakeholder to put itself in the shoes of every other interest group [21]. The results showed that all the stakeholders involved identified the welfare of the animals involved in AVI as priority. Once animal welfare is recognized as a priority to define the degree of acceptability of AVI practices, decision-makers can evaluate how to incorporate this result in future policies [21]. This result is in line with [31]. The EM developed during this study could therefore helps decision-makers in take decisions and anticipate value conflicts [48] and the focus can be moved on how to assess animal welfare before, during, and after AVIs, and how to communicate animal welfare standards to the tourists.

Strengths, limitations and future developments

During the participatory process, some stakeholder groups, such as animal rights groups and veterinarians, although they had been contacted, were not sufficiently represented, and therefore their requests should be further investigated. Moreover, the staff and visitors interviewed with the questionnaires came almost all from elephant facilities, and, due to the COVID-19 pandemic and associated facility closures, it took more time than planned to collect data and it was not possible to collect surveys from other facilities. Therefore, along with the need to redefine priorities and activities concerning AVIs due to COVID-19 long-term implications, it would be important to collect more data also from facilities offering different AVIs.

An additional point deserving attention is specifically linked to the workshop activities. During the main session, in which participants were asked to advance and discuss their value demands, some stakeholders identified their interests with those of other stakeholders, as can be seen from Table S6. More specifically, some managers, veterinarians, and staff linked their wellbeing to those of the animals. From one perspective, this could be due to legal or professional reasons. An owner must keep her or his animals healthy to avoid legal repercussions and have more visitors. A caregiver must provide the animals with their needs to respect his or her professional ethics. A veterinarian is compelled by his or her responsibilities to act in the animal’s interests.

And so on. Besides these motivations, however, two more hypotheses could explain why some participants identified their interests with those of other stakeholders: (a) it could reflect sincere emphatic feelings; (b) the ethical reasoning task was not fully understood or the stakeholders were not used to this kind of introspective tasks (some stakeholders reported in the feedback form some difficulties on this regard). To improve future similar workshops, these last two hypotheses should be considered. To verify whether and how emphatic feelings towards other stakeholders play a role in the interests of participants, a brief questionnaire could be included at the beginning of the workshop experience. To overcome problems linked to an insufficient comprehension of the task by the participants it would be useful to provide in advance the participants with briefing documents containing information about the EM and the role and goals of ethical analysis. Moreover, during the workshop, it would be useful to allocate more time to dispel doubts and to complete unusual and cognitively demanding tasks.

The results of the participative processes could have been affected by selection bias, so they need to be interpreted cautiously. While selection bias could have affected the results of the workshop and the surveys, this is not negatively affecting the overall results of this study, the final EM of AVI. In fact, both the workshop and the surveys were designed to collect as much inputs as possible to define the final EM presented. All the inputs have been considered for defining the stakeholders' interests by an inclusive approach.

Conclusion

The EM showed to be a useful tool to perform a structured ethical analysis on AVIs in South Africa as a first step towards their regulation. In particular, the integrated approach—combining workshop and surveys—adopted in this study assured the direct or indirect engagement of a great part of the affected stakeholders and improved the quality of the representation of their ethical standings. The result is a detailed map of the value demands involved which should facilitate decision-making.

The EM highlights animal welfare as a crucial and transversal issue. In this way, the conclusions of this study fully support the need to develop scientific assessment tools capable to evaluate the welfare of wild animals involved in AVIs considering the peculiar semi-captive and free contact management conditions. Moreover, despite different perspectives on whether and how animals in the "wild-captive continuum" should be managed [42], the EM reflects the overriding importance of compromises and collaboration between the stakeholders to ensure the best possible outcomes for the animals under human responsibility and care.

In addition to the central issue of animal welfare, the study highlights several other relevant issues related to AVIs, like education, biodiversity conservation, sustainability, human competency, facility mission, impact on scientific research, and socio-economic outcomes. In this way, the study shows the importance of an interdisciplinary approach to the issue, and the need to integrate several different criteria to build an official accreditation system dedicated to South African wildlife facilities.

Implementing workshop activities and providing the stakeholders with more opportunities to share their perspectives is of crucial importance to find sustainable solutions and set long-term goals for wildlife tourism evolution in South Africa. As advocated by D'Cruze et al., suitable goals for AVIs should be both biodiversity conservation, education, scientific research, animal welfare, and entertainment [1].

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Supporting Information

S1 Table. Interim EM. - https://doi.org/10.1371/journal.pone.0282507.s001

S2 Table. Questions included in the visitor survey, question type, and question purpose. - https://doi.org/10.1371/journal.pone.0282507.s002

S3 Table. Questions included in the staff survey, question type, and question purpose. - https://doi.org/10.1371/journal.pone.0282507.s003

S4 Table. Animal welfare issues. - https://doi.org/10.1371/journal.pone.0282507.s004

S5 Table. Animal management issues. - https://doi.org/10.1371/journal.pone.0282507.s005

S6 Table. Full list of participants' notes, from Workshop 3rd round, grouped by Stakeholder and by ethical principle. - https://doi.org/10.1371/journal.pone.0282507.s006

S7 Table. Demographic information and other independent variables collected from respondents of facilities A, and B. - https://doi.org/10.1371/journal.pone.0282507.s007

S8 Table. Answers to questions 11–13 accordingly to the need embodied by the respondents. - https://doi.org/10.1371/journal.pone.0282507.s008

S9 Table. 1. Results of LMMs analysis performed using the level of satisfaction of respondents performing the experience as dependent variable. 2. Results of GLMMs analysis performed using the “need of education” of respondents performing the experience as dependent variable. 3. Results of GLMMs analysis performed using the “biodiversity centric attitude” of respondents performing the experience as dependent variable. - https://doi.org/10.1371/journal.pone.0282507.s009

S10 Table. Distribution of categories identified in visitors answers to question n.19 “Imagine an official five star rating system that rates the quality of the facilities in which animal-visitor interactions take place. In your opinion, on which things should the rating be based? Please list the three most important things on which to evaluate the quality of these animal facilities (from most to least important)”. - https://doi.org/10.1371/journal.pone.0282507.s010

S11 Table. Answers to questions 4–16, “respect for” principle of the statements, the percentage of respondents choosing each score for each statement, the mean, median, and mode. - https://doi.org/10.1371/journal.pone.0282507.s011

S12 Table. Suggestions given by respondents in question n. 25. - https://doi.org/10.1371/journal.pone.0282507.s012

S13 Table. Final detailed EM. - https://doi.org/10.1371/journal.pone.0282507.s013

S1 File. -https://doi.org/10.1371/journal.pone.0282507.s014

S1 Data. - https://doi.org/10.1371/journal.pone.0282507.s015

2.2. Emotional States of African Elephants (*Loxodonta africana*) Kept for Animal–Visitor Interactions, as Perceived by People Differing in Age and Knowledge of the Species

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Simple Summary

We investigated the use of the qualitative behavior assessment (QBA) to evaluate the emotional state of African elephants managed in captive and semi-captive environments by three groups of people with differing ages and levels of knowledge of the species. We also examined whether their assessments correlated with behaviors exhibited by the animals. Fifteen video-clips of a total of 18 African elephants recorded in three different situations (release from the night boma; interactions with visitors; return to the night boma) were used. The result of the performed analysis supported that the consensus found was not due to chance. This notwithstanding, all the adjectives used by the three observer groups were not strong descriptors of the consensus variables resulting from statistical analysis. All three groups showed a degree of separation between captive and semi-captive management, with semi-captive animals rated as being in a more positive emotional state. For all three groups of observers, stereotypic “trunk swirling” behavior

correlated with negative emotional descriptors. Although definitive evaluation of animal welfare requires the services of experts, more studies are needed to investigate the perception of elephants' emotional states amongst visitors of different ages and background.

Abstract

This study aimed to investigate how three groups of people of differing ages, and with differing knowledge of the species, perceived the emotional state of African elephants (*Loxodonta africana*) managed in captive and semi-captive environments. Fifteen video-clips of 18 elephants, observed during three different daily routines (release from and return to the night boma; interactions with visitors), were used for a free choice profiling assessment (FCP) and then analyzed with quantitative methods. A general Procrustes analysis identified two main descriptive dimensions of elephant behavioral expression explaining 27% and 19% of the variability in the children group, 19% and 23.7% in adults, and 21.8% and 17% in the expert group. All the descriptors the observers came up with showed a low level of correlation on the identified dimensions. All three observers' groups showed a degree of separation between captive and semi-captive management. Spearman analyses showed that stereotypic "trunk swirling" behavior correlated negatively with first dimension (free/friendly versus sad/bored) in the children's group; second dimension (agitated/confident versus angry/bored) amongst the adults; and first dimension (active/excited versus agitated/bored) amongst the experts. More studies are needed to investigate other potential differences in assessing elephants' emotional states by visitors of different ages and backgrounds.

Introduction

African elephants have complex cognitive abilities, a sophisticated social structure, and a vast behavioral repertoire, and they generally attract a large number of visitors in zoos and other animal facilities [1,2]. Concerns have recently been raised regarding the effects on welfare of conditions under which elephants are held and managed in European and American zoos [3,4]. For instance, a high prevalence of factors which might be indicators that the welfare of the animals is not optimal have been reported. These include stereotypic behaviors [5] and health issues, such as ovarian acyclicity [6,7], obesity, foot problems [8], infectious diseases [9,10], and compromised survivorship [11]. Therefore, ensuring the welfare of elephants in zoos can be challenging [12,13]. For these reasons, there is growing scientific interest in developing, validating, and publishing methodologies for assessing the welfare of elephants in zoos, whose results, include indices to assess behavioral and/or psychological changes and their consequences [12,14–16]. However, zoo elephants are not the only elephant populations living in controlled conditions, and the external validity of protocols validated for elephants in zoos has never been investigated [17], leaving open to debate their suitability to be applied to other sub-populations of elephants under human control (e.g., semi-captive elephants in South African and Asian facilities).

In Africa, for instance, the management of elephants housed in game reserves, or in privately-owned facilities usually differs from the management of elephants held in zoos [18]. While elephants in zoos are held in fenced enclosures, elephants held in game reserves and other privately-owned facilities are generally afforded the opportunity to spend part of the day moving freely in the bush. Despite remaining under the supervision of their handlers, the latter are left free to express their natural behavioral repertoire without being subject to the imposition of specific activities, except for safety reasons [19], or in cases where they disperse excessively. Therefore, visitors to most of these facilities are able to observe the animals in their semi-natural environment. Additionally, most South African facilities offer visitors some form of organized animal–visitor interaction. These activities can vary greatly in their style and content including, for example, hearing talks on characteristics of the species, observing elephants in their night areas, or witnessing ad hoc training sessions. In some cases, visitors are afforded opportunities to approach the elephants and thus become involved in close-up animal–visitor interactions (AVIs) such as feeding or elephant-back safaris.

In 2013, a group of researchers from the University of Padua began a project aimed at developing a protocol for assessing the welfare of African elephants kept in semi-captive environments and involved in AVIs in South Africa [17]. This assessment protocol involves a merger between scientific and ethical approaches. In this project, qualitative behavioral assessment (QBA), carried out by experts, was included as a method to validate the emotional valences (negative or positive) both of possible behavioral welfare indicators and of management procedures that could possibly affect welfare (see [17] for details).

Developed by Wemelsfelder and colleagues [20,21], the QBA is a reliable method, and has been cross validated against quantitative behavioral or physiological measures (for example [22,23]). QBA is empirically based on observation of behavioral signs that reflect an animal's emotional state and does not consist of unfounded projections of human emotions [20,21,24]. Moreover, as stated by Wemeslfelder and colleagues [20] (p.208), "[...] its being based on human perception does not make it a study of human perception. Human observers and their perceptive powers are used as an assessment tool [...]". Therefore, it has been shown to represent an integrative evaluation tool for use in animal welfare studies, and is hence included in several protocols for welfare assessment, such as Welfare Quality® and the European Animal Welfare Indicators Project (AWIN) [25,26]. It determines the animal's physical and physiological state, and additionally represents a valid measure of animals' demeanor [22,27,28]. The 'holistic' approach recognizes animals as sentient beings with different personalities and capable of experiencing positive and negative emotions [29]. QBA uses the 'whole-animal' approach, which measures how animals respond to the environment and how they deal with it, rather than measuring only the animal's physical behavior [30]. Specifically, it focuses on the dynamic expressivity of the behavioral demeanor, characterizing and quantifying it, through the use of lists of descriptors. Such list can either be supplied predefined (e.g., [31–33]), or developed through a methodology known as free-choice profiling (FCP), in which each observer generates their descriptors [20,21,28,34].

The QBA has been shown to be able to assess an animal's affective state quickly, reliably, and non-invasively [32], both under semi-captive and on-farm conditions [31]. Therefore, in the few last years, the QBA has been applied to a range of different species and different contexts [20,27,28,30,32,35–38]. Recently, it has also been used to address human–animal relationships (HAR) in zoos [34].

The main aim of this study was to investigate how three distinct groups of people differing in age and knowledge of the species (i.e., children, non-expert adults, and experts) perceived the behavior of African elephants in its emotional connotations, using free choice profiling assessment (FCP). Additionally, it aimed at investigating whether a difference exists between the descriptors generated by experts observing elephants held in captive management and elephants held in semi-captive management, and whether similar differences could be found amongst other observers. It is important to note that QBA adjectives proposed for elephants were developed in zoo conditions [14], and the findings demonstrate a fair degree of separation in the experts' scatterplots between videos recorded in captive and semi-captive management systems. This study, therefore, cautions against using adjectives generated exclusively in a zoological context when developing a QBA for elephants in semi-captive conditions. As a sideline of investigation, we also evaluated whether correlations existed between quantitative behavioral assessment and the results obtained from FCP carried out by experts on the same videos, and whether similar results could be found also when non expert observers' FCP were concerned. Besides, in the context of the project, the FCP procedures running with children and adults provide an idea of the emotional impact on visitors of the "elephants' experience", and the results are therefore potentially useful in ethical evaluations when considering the interests of visitors [17].

Animals, Materials, and Methods

The research was conducted in Italy and South Africa. Five institutions were chosen, and each facility gave permission to film the animals and collect data. The study was observational in nature and was made in accordance with both the ethical requirements of the participating facilities, and relevant national and international regulations. The husbandry routines of the animals involved were not changed or affected by the study. All the observers gave their consent for inclusion before they participated, and the informed consent of parents of each child was given. When the study was performed, no approval of ethical committees was needed in the country leading the project.

Places, Animals, and Their Management

Five institutions and an overall of 18 African elephants were included in the study:(1) Zoo Safari Ravenna, Italy (44◦19′36.4″ N 12◦16′29.8″ E) a zoological garden housing two female elephants; (2) Indalu Game Reserve, Mossel Bay, Garden Route, Western Cape Province, South Africa (34◦10′56.9″ S 21◦48′22.4″ E), where at the time of the study, six elephants were housed, three males and three females; (3) Garden Route Game Lodge, Garden Route, Western Cape Province, South Africa (34◦12′31.2″ S 21◦38′00.1″ E), which held two male elephants; (4) Adventures with Elephants, Bela Bela, Limpopo Province (24◦46′54.1″ S 27◦57′05.3″ E) which held seven elephants in the reserve, although only five adult animals were included in the study; (5) National Zoological Garden (NZG), Pretoria, Gauteng Province (25◦44′05.1″ S 28◦11′24.9″ E) which owned three elephants, a male and two females. The five institutions were chosen for their different management typologies. We considered "captive" facilities to be those in which animals were kept in fenced enclosures for the whole day, and in which food supply was completely provided by human intervention. On the other hand, we defined "semi-captivity" as the condition in which animals were allowed to roam in the so-called "free choice activity" for at least part of the day, thus experiencing a varied environment, rich in vegetation and stimuli, while foraging and socially interacting with their conspecifics [19]. In most of these facilities, during "free choice activity", the elephants are herded to an area where they are free to choose what to do and to roam around in the bush, without any imposed activity and under the supervision of the handlers. Handlers refrain from interacting with elephants unless for safety and security reasons or to avoid excessive dispersion which could hamper herding at the end of the free choice activity time. All the male elephants included in the study were treated with GnRH (gonadotropin releasing hormone), a non-surgical method of managing testosterone and musth in bull elephants [39] that have reached their sexual maturity. Table 1 provides a summary of the elephants included in the study, the age range of the individuals, whether the animals were trained, whether they were involved in interactions with visitors, and management's typology.

Table 1. Summary of each study location, including the elephants' name, their age at the study time, the presence or absence of a training program, whether the animals were involved in interactions with visitors, and whether held in the captive or semi-captive management typology.

Institution	Elephants included in the study	Age range (years old)	Training	Interaction with visitors	Management typology
Zoo Safari Ravenna	Dumbo (female), Robin (female)	35 - 45	No	Yes	Captive
Indalu Game Reserve	Mooketsi (male), Bakari (male), Teboco (male), Amari (female), Madiwa (female), Shanti (female)	11 - 23	Yes	Yes	Semi-captive
Garden Route Game Lodge	Salati (male), Moya (male)	17-35	No	No	Semi-captive
Adventures with Elephants	Chishuro (male), Chova (male), Nuanhedi (female), Shan (female), Mussina (female)	15 - 20	Yes	Yes	Semi-captive
National Zoological Garden	Charlie (male), Thandi (female), Landa (female)	33 - 36	No	No	Captive

Videos Gathering and Processing

Videos were collected in Italy and South Africa during a period ranging from October to December 2016. Sessions from Italy were video recorded with a Samsung NX1000 mirrorless camera or a SONY A7R II mirrorless camera. Videos recorded in South Africa were shot with a JVC Everio full HD water-resistant camera. Except in the National Zoological Gardens, video recording took place at different times of day, usually beginning with a first recording session when the animals were released in the morning (usually at 7:00 a.m.) and ending with a final session when the animals went back into their bomas or indoor areas for the night (usually at 5:00 p.m.). In the National Zoological Garden, video- recording sessions began around 8:30 a.m., and ended around 7 p.m. The order in which individual animals were filmed was scheduled to change every day. However, because of contingent events (operator safety, environmental and weather conditions, hidden animals, etc.), it was not always possible to comply with the order as planned.

For the present study, fifteen videos were chosen from 1200 collected. In the chosen 15 videos, a total of 18 elephants are visible, for an overall duration of 20:51 min. Each video was cut in post-production.

Of the fifteen videos chosen for this study:

- Five videos show "re-entry": the elephants in the process of returning to the night boma or indoor area Four of these videos show the entire group of animals at their respective facilities, while one video, shot at the National Zoological Gardens, shows only Thandi (the dominant female in her group) since the animals at the NZG were returned separately to the night area. These "re-entry" videos range in length from 1:04 to 2:02 min, for a total duration of 7:29 min.
- Five videos show "release": the elephants being released from their night areas. These videos are divided as above, and have a duration ranging from 0:51 to 2:01 min, for an overall duration of 6:37 min.
- Five videos show "interaction with humans". In these videos, the dominant elephant of each group is shown interacting with humans. These videos have a duration ranging from 1:00 to 1:45 min, for an overall duration of 6:45 min.

The decision to film at given times (during entry and release from the night bomas, and during animal-visitor interactions) was made for three related reasons: (a) because they were the most similar moments in the elephants' daily routines in all facilities involved in the study; (b) because it was logistically feasible to video record the animals during these times; and (c) because the re-entry and release times presented opportunities during which it was most likely that (almost) all the elephants in each different facility would be visible in the video recordings.

Qualitative Behavioural Assessment

Observer Groups

Three mixed-sex groups of Italian observers were involved in the study: one group comprising fourteen children between 8 and 13 years old, one group comprising thirteen adults ranging from 20 to 50 years old, and one group of ten experts in African elephants whose members ranged from 25 to 53 years old. None of the observers in the first two groups had had previous experience of animal observation, qualitative behavioral assessment, free choice profiling methodology, or African elephants. In contrast, the expert group included Italian zookeepers with knowledge of the management of African elephants, veterinarians who work in zoos where African elephants are kept, and more generally, Italian researchers who work with African elephants and are involved in handling elephants, welfare assessment, and animal behavior.

The number of observers in each group, and the number of videos analyzed both fall within the limits of what has already been done in scientific studies using FCP [22,24,28,31–34,38,40,41].

Free Choice Profiling

In this study, the FCP methodology, as described by Wemelsfelder and colleagues [21], is used to evaluate the behavioral expression of the elephant.

Consistent with normal practice for FCP studies, the FCP methodology employed consisted of two separate phases held on separate dates [21]. In the first phase, observers watched each video. At the end of each video, observers had three minutes to write down terms that in their opinion best described the emotions they thought the animals were feeling. All participants generated terms in their native language (i.e., Italian). In the second phase (on the second date), the same observers were asked to watch the same videos again, in the same sequence as before. Each of the observer's terms was printed in a list, with each observer receiving a list of their own individual terms. Each term was paired with a visual analogue scale (VAS). Observers were now asked to mark a line on the scale at the point considered appropriate between "minimum" (0 mm) and "maximum" (125 mm), thus determining a quantitative value for each term. If an adjective was deemed irrelevant for a certain video or if the observer considered that the video did not provide adequate information to decide how to rate that adjective, the observer was instructed to tick the space marked "non-pertinent" (NP) that appeared alongside the VAS. To avoid every possible impact, the VAS was left entirely blank with no marks or measurement indicators.

Observers were asked to come up with descriptors completely spontaneously in the first phase, and were also free either to choose new descriptors for each video, or to use terms they had already used for other videos. They were also asked to concentrate on choosing the best possible descriptors, and to refrain from communicating with each other in order to minimize external influences.

Each observer's scores were thus obtained by measuring the distance in millimeters between the "minimum" point of the VAS, and the line the observer had drawn on the scale. The scores were entered into 37 data matrices (one for each observer), providing scores for the 18 animals on the basis of the observers' personal vocabularies. Zeros were added to individual matrices every time the observers left a blank so that all observer configurations acquired equal dimensionality.

General Method of Analysis

Data were analyzed separately for the three observer groups using generalized Procrustes analysis (GPA) through a GenStat software edition written by Françoise Wemelsfelder (Genstat 2016, VSN International, Hemel Hempstead, Hertfordshire, UK). GPA is a multivariate technique that identifies patterns in data that do not consist of fixed variables. It detects the level of consensus between observer scoring patterns, giving the percentage of variation between observer configurations explained by the consensus profile. For more detailed explanation of GPA procedures, see [20]. The statistical significance of this consensus is determined through a permutation or randomization test [42], that allows discriminating whether the consensus is a significant feature of the data set or an artefact of the Procrustean calculation procedures. A one-way Student's t-test ($n = 100$) is used to determine whether the true observer consensus profile falls significantly outside the distribution of randomized profiles ($p < 0.001$).

GPA provides a Procrustes statistic for each pair of transformed observer configurations, which quantifies the percentage of the total variance between observer configurations explained by the consensus profile. The relative distance between transformed observer configurations and the "best of fit" can be projected visually in a so-called "observer plot". Principal coordinate analysis (PCO) estimates the center of distributions of the relative distance between the observer and a standard deviation and draws a 95% confidence region for the consensus profile.

Observers lying outside this region are potentially outliers who, in some sense, may differ from other observers in their assessment of samples [20]. Once these outliers are excluded, GPA can be repeated to assess whether and how their data influenced the consent profile. At this stage, observer 12 of the children group was an outlier. Since there was a valid reason (e.g., problems with vocabulary), he was excluded from further analysis. Moreover, observers 4, 5, and 13 of the adult group and observers 1 and 6 of the expert group were outliers and were therefore excluded. The consensus profile of the three groups improved after the first exclusion of outliers. Although other outlier observers were identified in the three groups, it was decided to carry out the outlier exclusion procedure only once because further applications of this process would have reduced the sample size of the three groups too much.

GPA thus transformed the now 13 different video-scoring (representing elephants scoring) configurations of the children's group, the 10 different video-scoring configurations of the adult's group, and the 8 different video-scoring configurations of the experts' group into a three multidimensional consensus profile, entirely independent from any interpretation by the experimenter.

Through the principal component analysis (PCA), the number of dimensions of the consensus profile is reduced, identifying the principal axes and determining how much variation between the videos these dimensions explain. Each video was attributed a score on each of these dimensions. Scores were then reflected in several two-dimensional 'elephant-plots' showing the distribution of the videos along the principal axes of the consensus profile. A standard error ellipse indicates the reliability for each video's position on the axes.

These dimensions are then interpreted by correlating them to the original individual observer data matrices. This step of the analysis produces two-dimensional individual observer interpretative 'word charts', showing the association between all terms of a particular observer and the two or more principal axes of the consensus profile. The higher a term correlates with an axis (or dimension), the more weight it has as a descriptor for that axis. The extent to which individual observers concur in their judgment of elephants' expressions is indicated by the degree of semantic convergence between charts. If observer assessments show significant convergence, then the consensus profile can be used to appraise qualitative differences between individual animals, defined by the position of individual animals on the plot.

Given that, in the majority of the studies using FCP, the respondents were native English speakers or generated terms in English, or the terms given in a language other than English were translated into English for statistical analysis (e.g., [40]), the process described above was followed a second time using the English translations of the Italian terms given by respondents instead of using the Italian terms themselves. The original Italian terms were translated into English using a consensus among three native English speakers (see Appendix A for details). This was done in order to verify that using two different languages did not alter the GPA results, as the consensus profile calculation is supposed to be done independently from the semantic information provided by the terminologies chosen by the observers.

Quantitative Behavioral Assessment

The behaviors shown by the 18 elephants in the 15 video clips were analyzed quantitatively, by a single observer using the continuous focal animal sampling technique, as defined by Martin and Bateson [43], and using a dedicated Behavioral Observation Research Interactive Software, BORIS [44]. The working ethogram used in this study is based on previous research on the behavior mainly of African elephants [45–50], and adapted according to the behaviors observed in the videos (Table 2). Trunk movements were considered a category on their own, even if some of them have been categorized as stereotypies in Asian elephants [51].

For videos in which multiple elephants were registered, each animal was observed individually, recording each behavior performed. When animals showed events behaviors simultaneously with states (e.g., elephant walks and “ear flap”), both were recorded. States behaviors were set as mutually exclusive because (the start of) a new state was considered most likely to reflect an intervening variation in the animal’s motivation and emotional state. Then, the total time in which each animal was visible in the video was calculated, excluding the time in which they were out of sight. The relative duration of each behavior was calculated over the total visible time of that video. The frequencies have been expressed as the number of occurrences of the behavior on the total time of the video.

Table 2. Working ethogram. Behaviors marked with “§” represent *events.*

Category	Behavior	Definition
Locomotion	Walk	Animal takes more than 2 steps forward, but not in a stereotypic pattern.
	Walk backwards	Animal takes more than 2 steps backwards, but not in a stereotypic pattern.
	Change direction §	Animal makes a change in its direction while walking.
Standing	Stand	Animal stands in a still position with its eyes open or closed, possibly exhibiting other behaviors at the same time but without changing place.
Feeding	Eat	Animal is in the process of putting food in its mouth with its trunk.
	Foraging	Animal is actively searching for food with its trunk.
Eliminatory	Urinate	Animal discharges urine out of the body.
	Defecate	Animal discharges feces out of the body.
Ear Movements	Ear open	Animal keeps one or both ears separate from the body with an angle ≤90°.
	Ear flap §	Animal moves one or both ears from 0° to ≤90° and from ≤90° back to 0°.
Trunk movements	Trunk swirling §	Animal swirls downwards its trunk’s first half, second half or both on its circular axis [51].
	Trunk up §	Animal swirls its trunk as before but upwards.
	Trunk swing §	Animal moves its trunk from one side to the other (left <-> right; up <-> down) once or in a repetitive manner.
Self-directed movements of the trunk	Trunk—body part §	Animal touches its own body in specified part with its trunk.

Investigative movements of the trunk	Trunk forward §	Animal stretches its trunk toward something it wants to reach.
	Trunk manipulation	Animal picks up something in its trunk and physically interacts with it by pulling, pressing, rotating, or moving it in general.
Head movements	Head shake §	Animal heavily agitates its head.
Stereotypies	Pacing	Animal takes steps forward or backwards in an unvarying, repetitive manner. In this study, we are consider pacing after the repetitive fifth step forward/backwards.
	Rocking	Side-to-side and/or back-and-forth repetitive swaying of the body.
Out of sight	Out of sight	Animal is not visible, partially, or completely.
Commands	Commanded trunk up §	Animal performs a "trunk up" after being commanded to do so.
	Commanded change direction §	Animal changes direction after being commanded to do so.
	Commanded yes/no §	Animal moves its head in a nodding manner or from side to side after being commanded to do so.
Interactions with tourists	Being fed by tourists	Animal receives food in its mouth or in its trunk by a tourist.

Relationship between Quantitative and Qualitative Data

Spearman rank correlation analyses were performed to correlate scores on the first and second PCA dimension axis, separately calculated for children, adults, and experts' groups with the elephants' different behavioral patterns. The behaviors shown overall one or two times in the 15 videos were not included in the analysis, while others were grouped. Therefore, the behaviors included in the statistical analysis were: walk, walk backward, stand, feeding (expressed as the sum of eating behavior and feeding by tourists), ear open, ear flap, trunk swirling, trunk movements, trunk manipulation, and rocking.

Statistical analyses were carried out using IBM SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA) and Excel for Windows 2007 (Microsoft Inc., Redmond, WA, USA).

Results

Observer Plot and Their Statistical Significance (Consensus Profile)

Observers generated a total of 641 terms to describe the elephants they were shown, with an average of 17.32 terms (range 5 to 39) per observer. In particular, the children group came up with an average of 11.57 terms (min: 5, max: 17); the adult group an average of 23.07 terms (min: 12, max: 39); and the expert group an average of 17.9 terms (min: 9, max: 28).

The Procrustes statistic values of the three consensus profiles (adults, children, and experts) are presented in Table 3, both as resulting from the Italian and from the English version of the terms. Overall, as expected, the results concerning the two languages were quite similar. Therefore, as use of English terms appears to be the usual procedure in the literature (e.g., [40]), only the results regarding the English will be presented and discussed further in the present paper.

Table 3. Procrustes statistics for the observer groups ($p < 0.001$).

Observer Group	Consensus Profile (%)		Randomized Procrustes Statistics (Mean ± SD)		Student *t*-test (df = 99)	
	Eng.	**Ita.**	**Eng.**	**Ita.**	**Eng.**	**Ita.**
Children	57.20	60.37	53.29 ± 0.35	55.55 ± 0.42	55.3	57.3
Adults	70.37	70.37	66.24 ± 0.32	66.24 ± 0.32	67.9	67.9
Experts	68.50	68.50	65.10 ± 0.35	65.10 ± 0.35	66.8	66.8

The GPA showed that the consensus profile explained a significantly higher percentage of the variation between observer matrices than the mean of 100 randomized profiles, meaning that none of the consensus profiles was an artefact of GPA procedures.

The observer plots, after the outlier observer reduction, are shown in Figure 1. These plots reflect the relative distance between individual observers as a measure of the level of consensus between individual observer assessment. Numbers represent individual observers, while the dotted circles enclose a 95% confidence region for what may be considered the normal population of observers.

Interpretation of the Consensus Profile

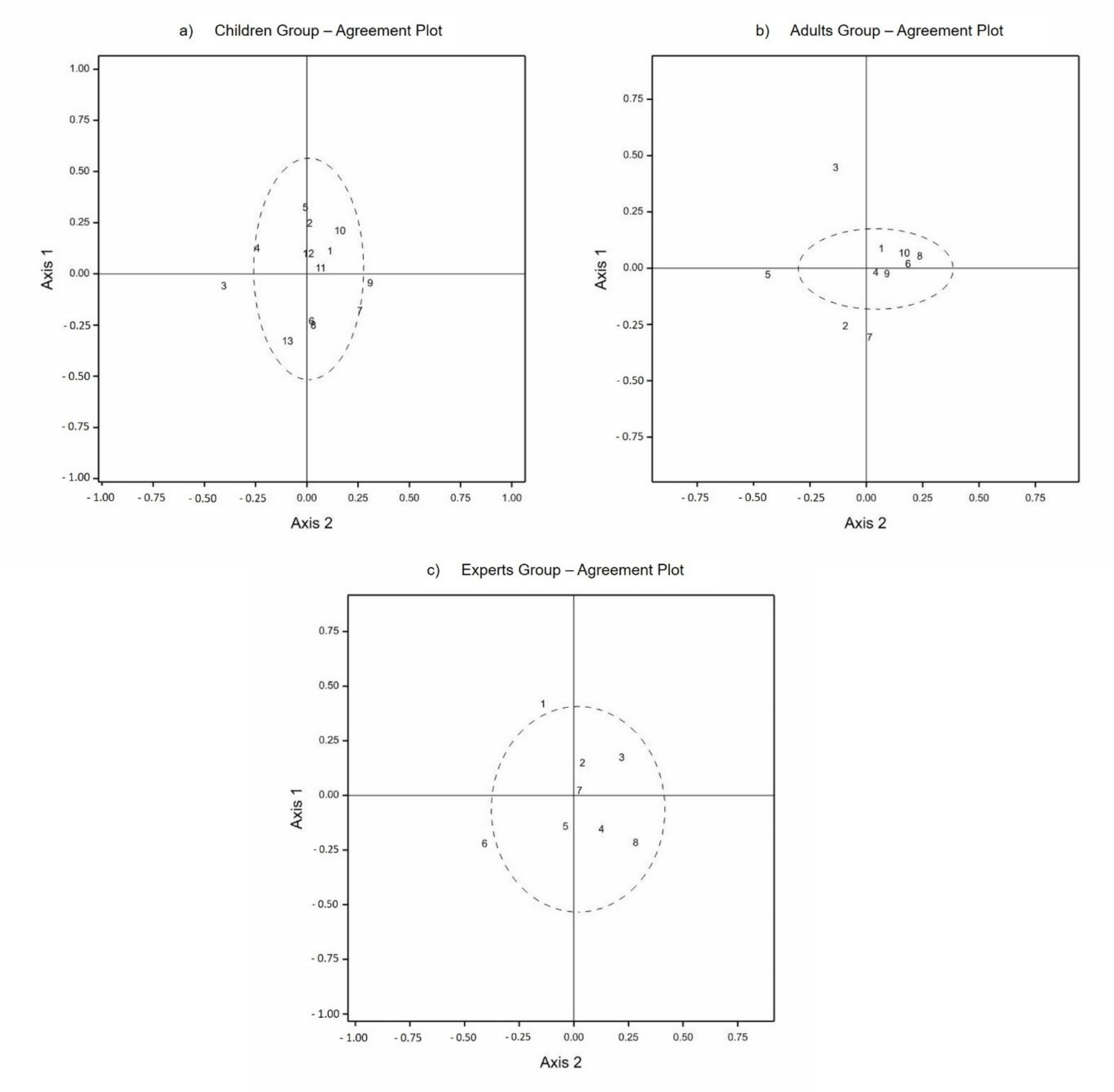
The first dimension of the children's consensus profile explains 27.1% of the variation between the elephants' emotional state in the 15 videos, while the second dimension explains 19.4% of this variation. The first dimension of the adult consensus profile explains 23.7% of the variation, while the second dimension explains 15.8%. Finally, the first dimension of the expert consensus profile explains 21.8% of the variation, while the second one explains 17% of this variation.

To give a more general overview of the observer interpretations, Table 4 lists the two terms which held the highest positive and negative correlations with dimensions 1 and 2 for each observer, divided by groups.

In particular, the terms used most frequently by the 13 children to characterize the first dimension of the consensus profile were 'free', 'friendly', 'hungry', and 'playful' versus 'sad', 'bored', 'apathetic', and 'stressed'. The terms used most frequently to characterize the second dimension of the consensus profile were 'bored', 'sleepy', 'tired', and 'apathetic' versus 'friendly', 'hungry', 'sad', and 'curious'.

Moreover, the terms used most frequently by the 10 adults to characterize the first dimension of the consensus profile were 'united' and 'gregarious' versus 'annoyed' and 'apathetic', while the terms used most frequently to characterize the second dimension of the consensus profile were 'agitated', and 'confident' versus 'angry' and 'bored'. However, even if adult observers mostly used different descriptors, their meaning was similar (e.g., ‘united’ and ‘gregarious’) or, in any case, coherent with different nuances of the same behavior and thus provided a comprehensive characterization of it (e.g., ‘hurried’, ‘restless’).

Figure 1. Observer plots of (a) children, (b) adults, and (c) experts.



Finally, the terms used most frequently by the eight experts to characterize the first dimension of the consensus profile were ‘active’ and ‘excited’ versus ‘agitated’ and ‘bored’, while the terms used most frequently to characterize the second dimension of the consensus profile were ‘agitated’ and ‘annoyed’ versus ‘calm’ and ‘relaxed’. Nevertheless, in general, where observers used different terms, the meanings of these terms tended to be either similar in mood/tone (e.g., ‘agitated/jumpy/wary’ and ‘bored/boredom’) or complement each other in mood/tone (e.g., ‘in alert/on the defensive, ‘group cohesion/integrated between them’). In some cases, however, terms appear to contradict each other in tone (e.g., ‘excited’ and ‘quiet’, or ‘safe’ and ‘wary’ and ‘tension’).

Table 4. Terms used by the observer groups showing the highest correlations with the first and second dimensions of the consensus profile (axis 1 and axis 2). The loading values near 0 indicate no correlation, whereas if near 1 indicates highly and positively correlated values. The closer the values are to −1, the more inverse the correlation is. The values in brackets indicate the number of observers using the specific terms, unless used by 1 observer.

Group	Axis	Positive Correlation	Negative Correlation
Children			
	Axis 1	Free (3), Friendly (2), Hungry (3), Playful (2), Amused, Himself/itself, Joyful Loadings varying from 0.35 to 0.77	Sad (6), Bored (2), Apathetic, Stressed, Impatient, Sleepy, Wants to go out Loadings varying from −0.19 to −0.89
	Axis 2	Bored (3), Sleepy (2), Tired (2), Apathetic, Compact, Nervous, Patient, Scared, Stressed Loadings varying from 0.18 to 0.87	Friendly (3), Hungry (2), Sad (2), Curious (2), Calm, Feels bad, Free, Stuck Loadings varying from −0.28 to −0.88
Adults			
	Axis 1	United (2), Gregarious, Hurried, Impatient, Jumpy, Ordered, Relieved, Restless, Supportive Loadings varying from 0.42 to 0.88	Annoyed, Apathetic, Confident, Greedy, Happy, Hungry, Repetitive, Skeptical, Sense of disappointment, Uninterested Loadings varying from −0.38 to −0.85

	Axis 2	Agitated, Confident, Content, Curious, Free, Keeps distance, Lost, Methodical, Not friendly, Skeptical Loadings varying from 0.39 to 0.77	Angry, Bored, Frustrated, Happy, Impatient, Jumpy, Not happy to return, Stereotyped, Stressed, Waiting Loadings varying from −0.28 to −0.83
Experts			
	Axis 1	Active, Excited, Group cohesion, Integrated between them, Naturalness, On the defensive, Quiet, Safety Loadings varying from 0.47 to 0.77	Agitated, Bored, Boredom, Friendly, Frustrated, Seeking attention, Stereotyped, Waiting Loading varying from −0.43 to −0.83
	Axis 2	Agitated, Annoyed, Expectation, In alert, Jumpy, Safe, Tension, Wary Loadings varying from 0.43 to 0.70	Calm (2), Relaxed (2), Complicity, Curious, Obedient, Satisfied Loadings varying from −0.07 to −0.78

Figure 2 indicates the strength of correlation (r-values) for all descriptors of dimension 1 and dimension 2. All the descriptors used by the three observer groups show a weak level of correlation on both dimensions.

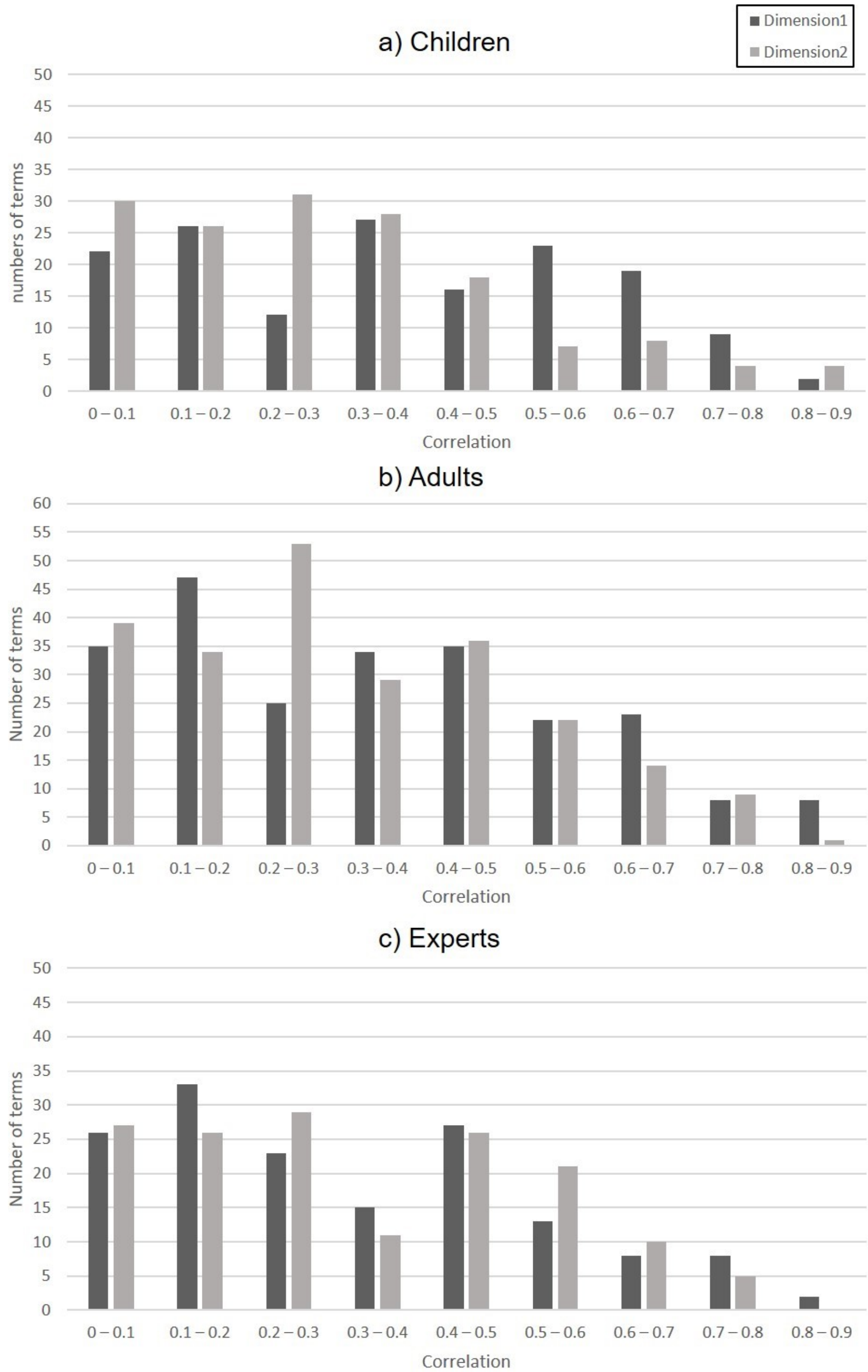
Scatter Plots

The videos were subsequently classified according to their management typology (captive or semi-captive—see Materials and Methods for definition). Videos representing animals in captivity were assigned the number 1, while videos representing animals in semi-captivity were assigned the number 2, accordingly with Table 1. Therefore, three elephant scatter plots (Figure 3) were created, one for each observer group, based on the consensus score of each observer. The plots describe how the animals shown in the 15 videos distribute along the two main dimensions according to observer’s perception.

The animals, represented by the videos, are evenly distributed over the two dimensions in children, adults, and experts' plots, which suggests that these dimensions adequately characterize observed variances in behavioral expression.

The scatter plot obtained from the children' consensus score (Figure 3a) shows a clear separation between the captive and semi-captive elephant group. The captive elephants represented by the red triangles are shifted towards the negative side of the first dimension (sad/bored as opposed to free/friendly) and equally distributed between the positive and negative side of the second dimension (bored/sleepy and friendly/hungry). The elephants in semi-captivity, represented by the purple squares, are shifted towards the positive side of the first dimension (free/friendly as opposed to sad/bored) and positive side of the second dimension (bored/sleepy as opposed to friendly/hungry).

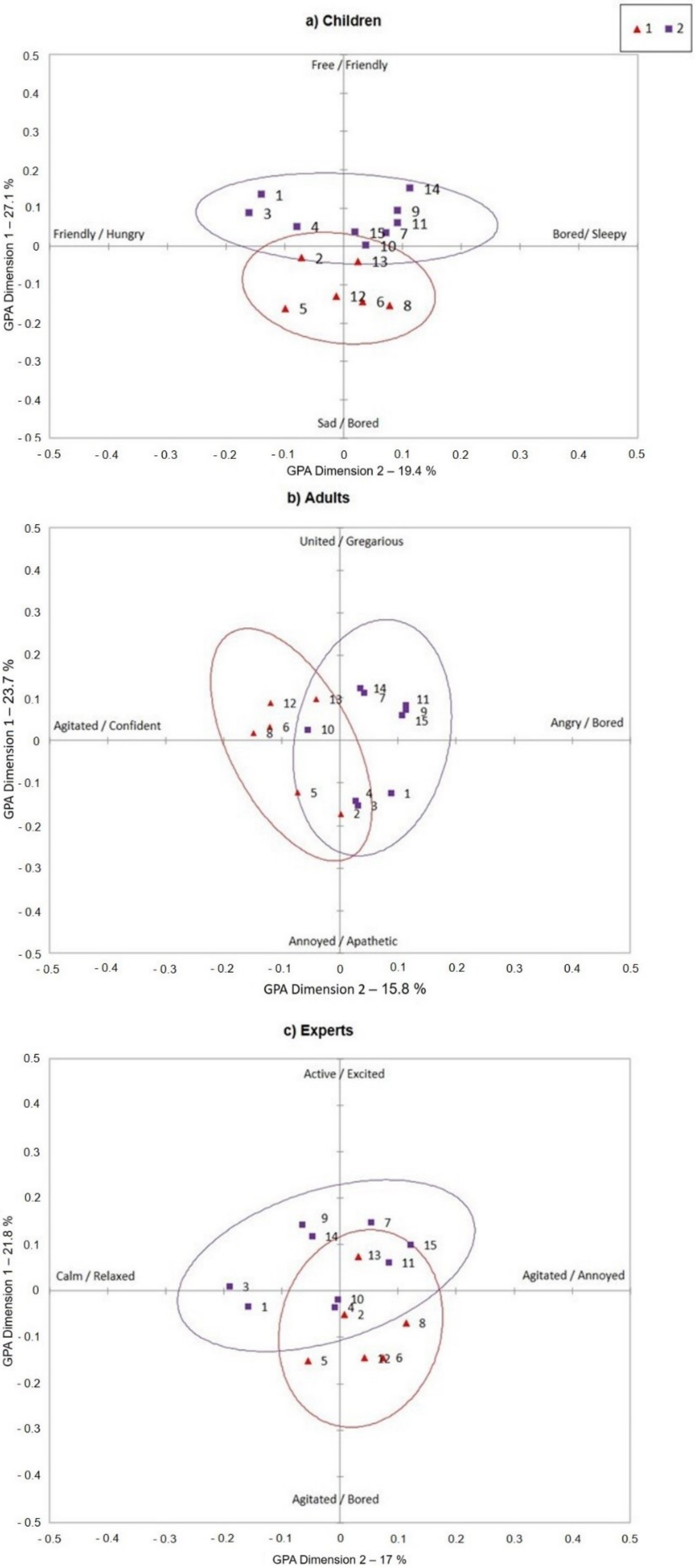
Figure 2. Correlation of (a) children, (b) adults, and (c) expert observer terms with dimension 1 and dimension 2.



The scatter plot obtained from the adults' consensus score (Figure 3b) also shows a clear separation between the captive and semi-captive elephant group. The captive elephants are shifted towards the positive side of the first dimension (united/gregarious as opposed to annoyed/apathetic) and the negative side of the second dimension (angry/bored as opposed to agitated/confident). The elephants in semi-captivity are shifted towards the positive side of the first dimension (united/gregarious as opposed to annoyed/apathetic) and positive side of the second dimension (agitated/confident as opposed to angry/bored). Finally, the scatter plot obtained from the experts' consensus score (Figure 3c) shows that the captive elephants are shifted towards the negative side of the first dimension (agitated/bored as opposed to active/excited) and the positive side of the second dimension (agitated/annoyed as opposed to calm/relaxed), while the elephants in semi-captivity are shifted towards the positive side of the first dimension (active/excited as opposed to agitated/bored) and negative side of the second dimension (calm/relaxed as opposed to agitated/annoyed).

Interestingly, videos 2 and 13, showing captive elephants and video 10, showing semi-captive elephants are included in the overlap area in all the three observers' groups.

Figure 3. Generalized Procrustes Analysis (GPA) consensus score—elephant video scatter plots. The scatter plots represent the distribution of the videos (represented by the 1–15 numbers) on dimensions 1 and 2 of the consensus profile for (a) children, (b) adults, and (c) experts and the separation between videos representing captive (red triangles) and semi-captive (purple squares).


a) Children
1
2
Free / Friendly
Friendly / Hungry
Bored/ Sleepy
Sad / Bored
GPA Dimension 1 – 27.1 %
GPA Dimension 2 – 19.4 %
b) Adults
United / Gregarious
Agitated / Confident
Angry / Bored
Annoyed / Apathetic
GPA Dimension 1 – 23.7 %
GPA Dimension 2 – 15.8 %
c) Experts
Active / Excited
Calm / Relaxed
Agitated / Annoyed
Agitated / Bored
GPA Dimension 1 – 21.8 %
GPA Dimension 2 – 17 %

The Correlation between Quantitative and Qualitative Data

Table 5 presents the results of the Spearman correlation, used to investigate the relationship between the behaviors recorded on the videos through the quantitative evaluation and the projection of each video on the first and second dimensions obtained with the GPA. The table reports the moderate and weak correlation ($r_s > \pm 0.5$) and a statistical level of $\alpha \leq 0.01$.

Table 5. Spearman's rho correlations between generalized Procrustes analysis (GPA) scores on dimensions 1 and 2 of the three observer groups separately, and the relative duration/frequency § of the behavioral patterns shown by the 18 elephants in the 15 videos.

Group	Dimension	Spearman's Rho (r_s)	*p*-Value	Sample Size	Behavior
Children	First Dimension	−0.641	0.010	15	Stand
		−0.704	0.003	15	Trunk swirling §
	Second Dimension	0.686	0.005	15	Walk
		−0.753	0.001	15	Feeding
Adults	First Dimension	0.829	0.000	15	Walk
	Second Dimension	−0.777	0.001	15	Trunk swirling §
Experts	First Dimension	0.742	0.002	15	Walk
		−0.733	0.002	15	Trunk swirling §

The scores of the first dimension of the children group negatively correlate with elephants standing still ($r_s = 0.64$, $n = 15$, $p = 0.01$), and showing "trunk swirling" behavior ($r_s = 0.70$, $n = 15$, $p < 0.01$). Those results indicate that the elephants described as being more free/friendly on GPA dimension 1 (as opposed to sadder/more bored) spent a smaller proportion of time standing still and had fewer trunk swirling events. The scores of the second dimension negatively correlate with the "feeding" behavior ($r_s = 0.75$, $n = 15$, $p = 0.01$) and positively with the proportion of time spent walking ($r_s = 0.68$, $n = 15$, $p < 0.01$), indicating that elephants described as more friendly/hungry on GPA dimension 2 (as opposed to more bored/sleepy) spent a higher proportion of time eating and spent a smaller proportion of time walking.

The scores of the first dimension of the adults group positively correlate with elephants walking ($r_s = 0.82$, $n = 15$, $p < 0.01$), indicating that elephants described as being more united/gregarious on GPA dimension 1 (as opposed to more annoyed/apathetic) spent a larger proportion of time walking. The scores of the second dimension correlated negatively with "trunk swirling" ($r_s = 0.77$, $n = 15$, $p < 0.01$), indicating that elephants described as being angrier/more bored on GPA dimension 2 (as opposed to more agitated/confident) had more trunk swirling events.

Finally, the scores of the first dimension of the experts group positively correlate with elephants walking ($r_s = 0.74$, $n = 15$, $p < 0.01$) and negatively with "trunk swirling" ($r_s = 0.73$, $n = 15$, $p < 0.01$). Therefore, elephants described as being more active/excited on GPA dimension 1 (as opposed to more agitated/bored) had fewer trunk swirling events and spent a larger proportion of time walking.

Discussion

As a part of a larger project aimed at developing a protocol to specifically assess the welfare of elephants kept in semi-captive environments and involved in close-up AVIs [17], to be coupled with an overall ethical evaluation of the AVI themselves [52], the present study aimed to investigate how three groups of people, differing in age and knowledge of the species (i.e., children, non-expert adults, and experts), perceived the behavior of African elephants (*Loxodonta africana*) managed in captive and semi-captive environments, and whether the descriptors they generated were different for the elephants living under the two different management systems. Moreover, it also aimed to investigate the possible correlations between behavioral patterns observed and quantified using a traditional quantitative method applied in ethology and the results of the FCP. It is important to note that, in the context of the University of Padua's project, the result obtained in the present study from the experts will be included in the protocol section regarding welfare assessment. Instead, results obtained by adults and children will be linked to the protocol section in which the AVI effect on the stakeholder "visitor" is assessed during the overall ethical assessment of AVIs. This notwithstanding, it is worth describing, in the context of the present paper, to what extent the results of the naïve observers' group were similar to those of experts.

To our knowledge, the FCP and QBA methodologies were previously applied to African elephants just once, by Wemelsfelder and colleagues [53]. Twelve observers, of whom four were elephant experts and eight farm animal experts, assessed 28 clips taken at the Amboseli National Park, and 8 clips recorded in a UK Zoo/Safari park using a FCP methodology. Results showed good agreement between observers, who managed to come up with convergent terminologies, and a meaningful dimension relevant to health and welfare. From the word chart of two elephant expert observers presented in [53], it can be noted that the terms used by the elephant experts are similar to the terms used by the experts engaged in this study, although the language originally used by the respondents of the present study was not the same as that of respondents in the Wemelsfelder and colleagues study [53].

In the present study, all three observer groups, regardless of age and knowledge of the species, achieved good agreement in their qualitative assessment of the emotional expression of the African elephants. The consensus profiles of the children, adults, and expert groups explained 60%, 70%, and 68% of the variation, respectively. The statistical software is supposed to work independently from the semantic information provided by the terminologies chosen by the observers. However, it was created for terms generated in the English language. In this study, the descriptors gave by observers were in Italian, and then translated into English. By repeating the same analyses with both Italian and English terms, no differences in results were found. Therefore, it is unlikely that the different language originally used is the explanation of such variability. This notwithstanding, it would be interesting to assess whether different results could be obtained by involving English children, adults, and experts in the same study or asking Italian respondents to generate terms directly in English. Moreover, it would be interesting to perform the same study with native African observers living in close proximity to elephants, as familiarity with the animal species can affect QBA results [54]. Language notwithstanding, the terms used by the experts in the present study were similar to the list of terms included in protocols already developed to assess the welfare of zoo elephants [14,16]. From the 12 terms included in those protocols, we can note that terms such as "content", "relaxed", "agitated", "tense", "frustrated", "wary", "playful" appear in the results, defining positively and negatively the first two dimensions. Although this finding could be due to experts being aware of such protocols, it is interesting to note that all the three groups in the present study used a somewhat similar vocabulary to describe the elephant's emotional state. For example, the negative end of the first axis was characterized by words such as "stressed", "apathetic", "bored" for children, "annoyed", "sense of disappointment", "apathetic", "repetitive" for adults and "frustrated", "bored/boredom", "stereotyped" for experts. The aforementioned finding suggests that children and naïve adults, in the recreational moments of close-up experiences with the animals, can have a rough perception of the general emotional state of the animals with which they are interacting, and thus, indirectly of their welfare.

In agreement with previous studies, this methodology discriminated between animals held in two different types of management: captive and semi-captive. For example, Temple and colleagues [55], assessing the Iberian pig welfare through the QBA, found that this methodology was useful to discriminate farms (intensive or extensive rearing conditions) on the basis of the expression of behavior. From the distribution of the elephant videos on the two main dimensions of the GPA, it can be seen that, although there is a slight overlap, there is a separation between the elephants held in the captive or semi-captive management typology for all three groups of observers, with the animals in semi-activity moved towards the free/friendly or active/excited end. In contrast, the animals in captivity moved towards angry/bored.

This finding suggests that using adjectives generated exclusively in a zoological context when developing a QBA for elephants in semi-captive conditions may increase the risk of missing some welfare relevant points specific to the semi-captive context. The inclusion in the present study of distinct facilities, with varying typologies of management, allowed to create a "baseline" list of adjectives as vast as possible that could include most of the emotions expressed by the animals both in the zoo context and in contexts that could somewhat differ from the zoo one. It is interesting to note that, in the present study, naive observers were also able to discriminate between the two conditions, creating scatterplots in which there was an even greater degree of separation than amongst experts.

This finding agrees with the findings of Duijvesteijn and colleagues [33] and Wemelsfelder and colleagues [41], highlighting the importance of involving participants from different backgrounds and with a varying degree of familiarity with the studied animal species in order to obtain a balanced assessment of animal welfare from a QBA study [28,33,41]. Breeders or people who work with animals on a daily basis, were found to assess animal welfare more positively, focusing more on health than other stakeholder groups, while urban citizens or animal scientists were found to perceive natural behavior as the most important feature [33,41,56,57]. However, this should not lead to an underestimation of the importance of knowledge of the species, and of expertise in animal welfare assessment, as pitfalls could arise when people with little or no experience have to evaluate welfare without expert guidance.

Since zoos and facilities that offer “elephant experiences” to visitors usually rely on the sale of tickets to sustain themselves, they should both manage their animals according to objectively assessed good welfare levels and understand the factors that can affect the visitors’ perception of the welfare of the animals. This is significant since visitors are likely to choose facilities according to their perceptions of the emotional states of the animals in those facilities. Based on findings from the present study, elephants housed in less restrictive management environments were scored by naïve adults and children (i.e., average potential tourists) as tending to more positive emotional states.

Moreover, since education is one reason for involving animals in AVIs, it is important to promote both their welfare and the expression of their species-specific natural behavior. It has been shown that seeing animals express their natural behavior enhances the emotional value of observing them and this, in turn, increase visitors’ conservation-mindedness [58]. The present study also highlighted the potential benefits of involving children when assessing the perception of the emotional state of animals in controlled environments (e.g., high ability to discriminate between management conditions), as well as the limits of such an approach (e.g., low agreement in their use and scoring of terms). Children represent not only the main users of animal facilities but also the new generation on whose sensitivity conservation of biodiversity is likely to depend in the future. As far as we know, this study represents the first time that a group of children is included in a qualitative behavioural assessment, potentially opening a new path in exploring children’s perception of emotions in non-human species.

The present study found correspondence between FCP results and quantitative assessment of behavior in the same way found by Rousing and Wemelsfelder [23], and Rutherford and colleagues [24].“Trunk swirling” was associated with the negative end of the first axis ("bored") for experts, supporting the idea of this behavior as a possible correlate for mainly negative emotional states in elephants. In the scientific literature, "trunk swirling" has been described as a stereotypy for Asian elephants by de Mel and colleagues [23], and the present finding further supports its possible association with mainly negative mental states, at least in the perception of observers. However, the link between stereotypies and compromised welfare is a complex one, as, for example, stereotypies can emancipate from their causal situation, although, where data exist, in the 68% of cases, the situations which cause/increase them also decrease welfare [59]. Moreover, as a general rule, a single behavior is better evaluated in the context of the other behaviors and postures contextually shown by the animal expressing it. Of course, the correlation goes both ways and it may be possible that experts generated more negative adjectives because they saw more stereotypic behavior and recognized it for what it was. However, there were some similarities among the three groups in this respect, too. "Trunk swirling" was associated to the negative end of the first axis ("sad", "stressed") for children, and the negative end of the second axis for adults ("frustrated"). Such findings suggest that animals performing such behavior are perceived even by naïve observers as being in a more negative emotional state than those not doing so. Alternatively, performing such behavior could be associated with other aspects of the elephants' demeanor that are interpreted as being associated with negative emotional states in the perception of people, irrespectively of their expertise with the species. Other correlations can be found for "walking" and "feeding" behavior. The results showed that adults described elephants that spent a higher proportion of time walking as more united/gregarious and the experts as more active/excited. However, in contrast, the children described the elephants as more bored/sleepy when the elephants spent a higher proportion of time walking. The children group also described the elephants that spent a higher proportion of time eating as more friendly/hungry on the second axis. Using a FCP methodology, three groups of people (i.e., children, non-expert adults, and experts) assessed video-recordings of African elephants (*Loxodonta africana*) managed in captive and semi-captive environments, coming up with a similar terminology to that used by experts in other studies, both in zoos elephants [14,16], and in elephants living in more natural conditions [53]. Moreover, they were also able to differentiate, to some extent, between videos recordings of elephants in captive environments, and those in semicaptive environments, generally associating more emotionally positive descriptors to the latter. Although the present study did not aim at ranking different forms of management in terms of welfare, it is important to note that videos from semi-captive management projected somewhat differently from those in captive management in the experts' consensus, suggesting that the terms generated in the two conditions either differed in themselves or their scores did (or possibly both). This suggests that the validity of using methods and protocols designed for captive conditions should not be taken for granted when assessing the welfare of semi captive individuals.

It is suggested that the animals' housing conditions could influence observer perception regarding the welfare of the animal, and consequently the QBA rating [60]. Depictions of the natural environment in videos of elephants under semi-captive management might create contextual bias among observers, leading them to score a more positive affective state in these situations. However, since Wemelsfelder et al. [61] did not detect serious distortions in observer assessment of pigs' expressions in different contexts, the difference in scoring found in the present study is likely to be due more to the behavior of the elephants themselves, than to different visual contexts. Furthermore, an interesting correlation was found between a behavior (i.e., trunk swirling) described in the scientific literature as a stereotypy, and negative emotional descriptors in all the groups of observers. The finding that people can perceive an animal performing a stereotypy as "stressed", "frustrated", and/or "sad", further highlights the importance of ensuring conditions conducive to good welfare and natural behavior in animals exposed to the public, not only because they are of outmost importance to the animals' quality of life, but also for the impact they can have on members of the public and their conservation mindness. Although we cannot rule this out completely, it is unlikely that the differences found were due to the different periods in which the videos were filmed (video collection ranged from October to December). On one hand, there is evidence of seasonal changes in movement [62] and social structure amongst wild elephants, depending on rainfall patterns and the availability of resources. In fact, in cases of abundance of resources, elephant groups tend to stay together. In contrast, groups tend to split when competition for resources is high. Such behavior is typical of their fission–fusion social structure [63]. Moreover, different seasons might present a significant change of vegetation in certain areas, influencing the food intake behavior (grazing vs. browsing) [64]. On the other hand, to our knowledge, changes in behavior between seasons has not been described under captive conditions in African elephants, as resources are controlled and thus unlikely to affect behavior. In this regard, it is important to note that the only facility included in this study that was not in South Africa was a zoo where the elephants' diet is controlled thorough the year.

Conclusions

During the last decade, qualitative behavior assessment has been progressively validated and applied to a wide range of domestic species and some wild species. Recently, QBA has also been included in protocols to assess elephants' welfare in zoos. In this study, three groups of observers were asked to evaluate their perception of the elephant's demeanor using the FCP method. The African elephants included in the assessment were managed not only in captive but also semi-captive environments and, to our knowledge this is the first application of FCP to assess "elephants' experiences" in semi-captive facilities. Results suggest that developing a QBA for elephants in semi-captive conditions by the use of adjectives generated exclusively in a zoological context may be a sub-optimal approach. In this regard, the descriptors generated in the present study could be a more suitable option in designing a QBA for welfare assessment of elephants both in zoos and in “elephants’ experiences” facilities. Given that zoos and interactions between zoo visitors and animals play a role in conservation education, naive observers’ perceptions of the emotional state of the animals could be a useful tool to assess their welfare. In this study, naïve observers, particularly children, deemed to be representative of zoo visitors and “elephants’ experiences” participants, for the first time were included in the QBA assessment. Due to the link between the emotional value of seeing wild animals in a controlled environment and conservation-mindedness [58], further research is required to investigate which factors influence the naïve people’s perception of animals’ demeanor. Finally, this study suggests that the captive and semi-captive environment in which the animals are kept may influence African elephant emotional expression. However, further studies are needed to investigate the impact of the environment (captive and semi-captive) on African elephants’ demeanor.

Author Contributions

Conceptualization, S.N. and V.S.; methodology, S.N., B.C., E.S., V.S., I.P.; software, B.C.; formal analysis, B.C.; investigation, V.S. and I.P.; resources, B.d.M., G.V., D.G., S.N., S.H.; data curation, I.P., B.C., V.S.; writing—original draft preparation, I.P., E.M., S.N.; writing— review and editing, I.P., S.N., B.C., G.V., D.G., V.S., E.S., E.M., S.H., B.d.M.; supervision, B.d.M., S.N.; project administration, B.d.M., G.V.; funding acquisition, B.d.M., G.V. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

Ethical review and approval were waived since, when the study was performed, no approval of ethical committees was needed in the country leading the project. Additionally, the study was observational in nature and was made in accordance with both the ethical regulations of the participating facilities and relevant national and international regulations and the husbandry routines of the animals involved were not changed or affected by the study. All the observers gave their consent for inclusion before they participated, and the informed consent of parents of each child was given.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Appendix A

The observers gave their terms in Italian, which were then translated into English to verify that using two different languages did not alter the GPA results. The consensus profile calculation, in fact, is supposed to be done independently from the semantic information provided by the terminologies chosen by the observers.

Therefore, three different people were involved in the translation process (one native American speaker and two bilingual English-Italian speakers). The translators were given all the words used by the observers and asked to translate them independently.

Subsequently, the English translations were compared and checked for concordance between the three. If a concordance was found between two or three of the three translators, the word given by the majority was chosen. If the three people gave different translations of the terms, online translators were used (DeepL—Wordreference—Google Translate) to identify the best interpretation of the Italian word.

In some cases, different terms in Italian, which have similar meaning, were translated into the same term in English as a result of the previously described process (e.g., "appagato" and "soddisatto" were both translated into satisfied). In this case, if the two original Italian adjectives were used only by different observers, no action was taken, and both remained translated into the same English term, whereas if the same observer had used the two (or more) Italian terms that resulted in having been translated into the same English adjective, other correspondences were found so that each of the different Italian adjectives was translated into a different English one. In the example above, as one observer had used both adjectives to describe elephants, “appagato” was translated into fulfilled, while “soddisfatto” remained satisfied.

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2.3. The Animal-Visitor Interaction Protocol (AVIP) for the assessment of *Lemur catta* walk-in enclosure in zoos.

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Abstract

Animal–Visitor Interactions (AVI) are activities offered by zoos and other tourism facilities, in which visitors come into close contact with animals. These activities can promote conservational and educational content, raise conservation mindedness and responsibility for the environment and animal welfare, but if not properly managed can jeopardize visitors' and animals' well-being and conservation efforts. The Animal-Visitor Interaction assessment Protocol (AVIP) has been designed to perform an integrated and multidisciplinary assessment of these activities, encompassing the "One Health, One Welfare" approach. AVIP throughout six different steps allows to assess the effects of AVIs both on animals, visitors, and the staff involved. Results can assist zoos to improve management decisions, ensure a transparent evaluation of their activities and promote conservation education goals. Lemurs walk-in enclosures have become increasingly popular among zoos, nevertheless studies focused on their assessment are still scarce. To validate AVIP to this particular AVI, we applied it to assess a walk-in enclosure hosting five *Lemur catta* in an Italian zoo. Results of behavioural and physiological analyses suggested no changes in animal welfare level and the Animal Welfare Risk Assessment showed low animal welfare risks. Two Visitor Experience Surveys were used to interview 291 visitors, showing that the assessed AVI could help promote the zoo's conservation objectives and visitor education. Risk Assessment found low and medium risks to the health and safety of visitors. Results were then combined to perform a final ethical assessment. Some potential ethical concerns were detected, but the outcomes indicated that these conflicts were well managed. In the context of recent findings AVIP demonstrated its potential for application also in assessing AVIs involving primates. Our findings confirmed the usefulness of AVIP in assessing and monitoring AVIs, allowing to gain key information in a single process on multiple welfare-related parameters, educational impact, safety of the main stakeholders involved, and ethical concerns.

Introduction

Animal-Visitor Interactions (AVIs) are very common in modern zoos and aquaria worldwide [1]. Recent studies show that AVIs can be a powerful way of maximizing both education and memorable experiences [2,3], increasing positive attitudes of zoo visitors towards natural and conservation issues, and facilitating the multiple goals of modern zoos regarding conservation, education, research, and animal welfare [1]. AVIs include animal presentations, behind the scenes encounters, close-up encounters, and walk-in enclosures with free-ranging animals [4,5].

Despite the requirements provided by the Guidelines of the World Association of Zoos and Aquaria (WAZA) [6,7], the overall impact that AVIs can have on animal welfare, health, and safety, as well as on visitors' experience, conservation educational outcomes, and safety, are still not assessed on a regular basis [8]. Although some studies exist (as reviewed by [9–12], scientific research on these impacts is still needed due also to the wide range of species involved and the considerable variability of AVIs [1,8]. To date, studies have shown that involving live animals in educational and entertainment activities in zoos, such as AVIs, not only can increase educational opportunities, but can be useful in changing visitor attitudes regarding wildlife, as well as rising conservation mindedness and responsibility for the environment [9– 14]. Also the effects that zoo visitors can have on the welfare of captive animals have been the focus of recent scientific interest [15–17], showing that presence and density of visitors, as well as their behaviour, may have an impact on animal welfare. This impact can be either negative (undesirable), positive (enriching), or merely a changing variable that has no effect [17–22].

However, studies have also shown that AVIs not properly managed can lead to welfare problems for the animals involved [2,23], transmit erroneous conservational and educational messages (e.g., perceive wild animals to be suitable pets), have detrimental impacts on the conservation of the species involved (e.g., wildlife illegal trade), thus jeopardizing the zoo's mission [2]. Therefore, due to the increasing number of AVIs provided in zoos, it has become especially important to assess their impact both on animals, visitors and staff involved [16].

Following WAZA recommendations [6,7] and due to the different kinds of AVIs proposed in zoos worldwide [1], an Animal-Visitor Interaction assessment Protocol (AVIP) has been designed to perform an integrated and multidisciplinary assessment of these activities [24].

Thanks to its multi-disciplinary approach, AVIP encompasses the 'One Health, One Welfare' concept, which recognizes that many aspects of animal well-being are intrinsically linked to those of humans [25]. Throughout six different steps, AVIP allows to assess the effects of AVIs both on animals, visitors and the staff involved (Fig 1).

In zoos, walk-in enclosures AVIs have become increasingly common [26,27] as they allow a particular kind of AVI where visitors experience close proximity to wild captive animals without physical barriers. There is evidence that the closeness generated by this kind of setting is preferred by visitors, as they can see animals in a more naturalistic way

[28]. Studies found this kind of encounters exert positive effects on visitor attitude, like increasing interest towards the animals and developing a connection with the wild ones [29]. For example, Price et al. (1994) highlighted that visitors' reactions to free-ranging cotton-top tamarins (*Saguinus oedipus*) were more favorable compared to the reactions to caged tamarins. Indeed, visitors spent more time watching them, made more comments about them, and perceived them to have improved welfare and a higher educational value than their caged counterparts [30]. Nonetheless, research recently showed that negative impact on animals could occur in some scenarios. This has been observed particularly in walk-in enclosures with shy species or individuals, if they are unable to avoid unfamiliar visitors, sights, and sounds [1]. For example, Learmont et al. (2018) found that quokkas (*Setonix brachyurus*) were less visible in their preferred areas when visitors were present, indicating that visitors were at least moderately fear-provoking for that group of animals [31]. Similarly, Larsen et al. (2014) discovered that koalas (*Phascolarctos cinereus*) spent more time being vigilant as the number of visitors and noise increased [32]. Sherwen et al. (2015) noted that western grey kangaroos and red kangaroos (*Macropus fuliginosus fuliginosus,* and *Macropus rufus*) showed not only an increase of time spent in vigilant behaviour and locomotion, but also a decrease in time spent resting [26]. Moreover, the kangaroos increased the time spent in retreat areas when visitors were present [26]. This notwithstanding, research on the effects of these walk-in enclosures on animal welfare is still limited [26].

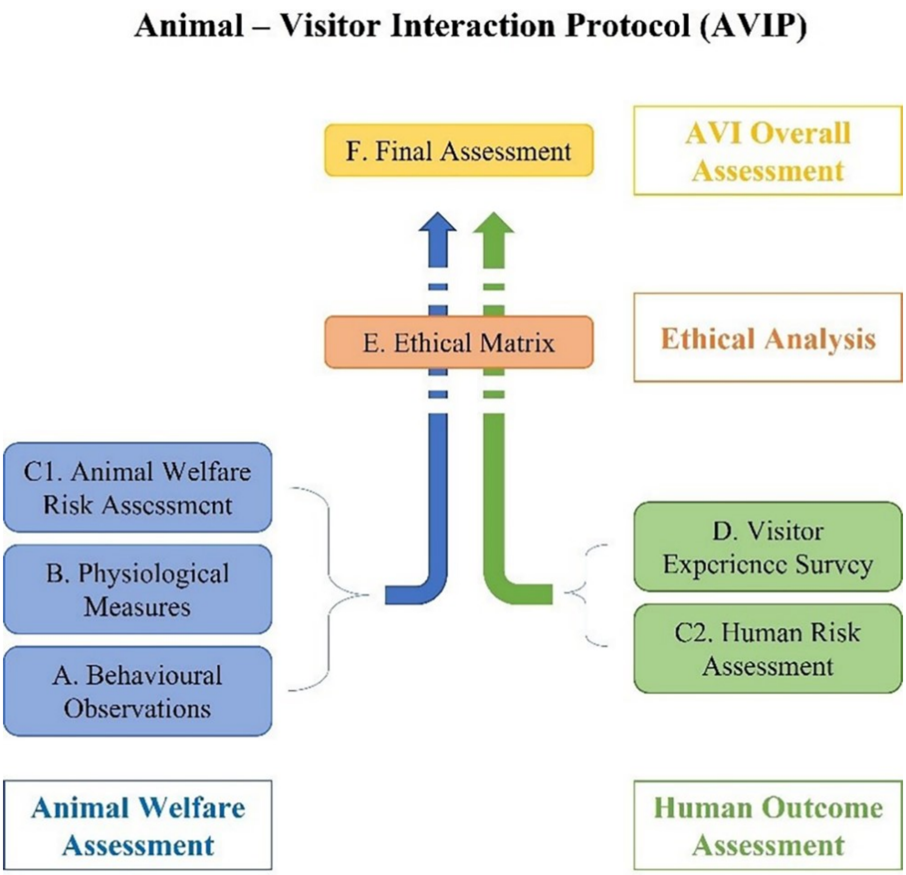

Fig 1. Description of the Animal-Visitor Interaction Protocol (AVIP). The behavioural (step A) and physiological (step B) assessments, together with the Animal Welfare Risk Assessment (step C1), enable to evaluate the effects and consequences of AVIs on animal welfare and health. Steps from C2 to F allow to assess the impact of AVIs on humans involved and to make a final evaluation: Step C2 allows evaluating the impact of AVIs on the safety and welfare of visitors, while step D investigates changes in visitors' attitudes towards animals and conservation issues, as well as their education and experience in terms of motivation and expectations. The ethical assessment of AVIP (step E) enables to interpret and discuss results obtained by comparing them with an Ethical Matrix, representing the ideal situation for all stakeholders. Finally, through the final checklist (step F), it is possible to provide an explicit result of the evaluation process, by which strengths and weaknesses of an AVI can be identified, managed and communicated. Since both the variation in zoo animal responses to visitors and visitor

experience may be the result of species and situation-specific differences, individual animal characteristics, enclosure design, or AVIs nature, the AVIP protocol should be adapted every time to the specific context of the AVI to be evaluated. As a result, AVIP can help to improve management decisions and to ensure a transparent evaluation of AVI activities.

Non-human primates are commonly housed in walk-in enclosures. In particular, lemur walk-in enclosures have become increasingly popular in zoos, as viewing lemurs has been found to be attractive to visitors [28,33–35]. However, even if research on non-human primate behaviour, welfare, and visitor effects is vast, scientific evidence of the impact of walk-in enclosures on their overall welfare is limited to few species. For example, Jones et al. (2016) found that allowing visitors into the exhibit of crowned lemurs (*Eulemur coronatus*) had a positive effect by decreasing lemur aggression [36]. Studies on lemurs, in particular *Lemur catta*, have shown the suitability of this species to this type of enclosure. Manna et al. (2007) found that the ring-tailed lemurs (*Lemur catta*) housed in a walk-in exhibit were more active and spent less time resting when visitors were present within the enclosure [37]. Collins and colleagues reported that behaviour and behavioural diversity of ring-tailed lemurs at Fota Wildlife Park (Ireland) were little affected by visitors, highlighting their habituation to them [38]. According to Goodenough et al. (2019), the time of day and weather (which vary with visitor numbers) have a negative impact on the behaviour of a group of 19 ring-tailed lemurs housed in a walk- in enclosure at West Midlands Safari Park (Worcestershire, UK) [28]. However, they showed that this impact had a greater influence on the animals' behaviour than the presence of visitors in the enclosure. Furthermore, the advantages of walk-in enclosures in terms of visitor experience and education outweigh the negative effects of visitors on animal behavior. Similarly, Farhall et al. (2010) found that the behaviour of nine lemurs housed in a walk-in enclosure at Cotswold Wildlife Park (CWP) was significantly influenced by the weather but not by time or visitor presence, whereas they showed that enclosure use was affected by weather, time, and visitor presence [39]. Conversly, Snipp (2004) found that visitor presence within a walk-through lemur exhibit had little influence on the behaviour and exhibit use of the animals [40]. Also Hosey et al. (2016) reported that the visitor presence in a lemur walk-in enclosure did not affect the animals' behaviour, and that the levels of lemur–lemur wounding did not correlate with the number of people in the zoo [41]. However, as animals' reactions to visitors varied among the species and zoos, in AVI assessment, it is important to consider the temperament, adaptation to the surroundings, and characteristics of the species and the individuals involved. For example, in some cases, visitors' access may be restricted (so that they cannot touch, feed or approach the animals too closely) or may be without control, representing a variable that could affect the animals.

The present study is focused on AVIP application on a specific AVI involving a group of ring-tailed lemurs (*Lemur catta*) housed in a walk-in enclosure at the Giardino Zoologico di Pistoia (Pistoia, Italy). During the interaction, Giardino Zoologico di Pistoia's visitors are allowed to enter the lemur enclosure and have a close encounter with the animals while receiving information about them. Therefore, each step of the AVIP protocol [24] was customized to focus on the specific features of the AVI under evaluation. AVIP is different from other evaluation methodologies of AVIs, in that it allows at the same time to gain key information on multiple welfare-related parameters, analyse the educational impact, the safety of the main stakeholders involved, and ethical concerns in one protocol, following WAZA guidelines and a "One Health, One Welfare" approach. Although the obtained results refer to a specific situation, they are discussed in the context of recent findings regarding visitor presence, zoo animal welfare, and behavioural research on primates in zoos. Our findings confirmed the usefulness of AVIP in assessing and monitoring AVIs, also when involving primates.

Materials and methods

Ethical approval

The study was performed in full compliance with the Guidelines for the Treatment of Animals in Behavioural Research and Teaching (2006) and with the WAZA Code of Ethics and Animal Welfare (2003), and with authorization from the Giardino Zoologico di Pistoia (www. zoodipistoia.it), Pistoia, Italy. This zoological institution is accredited by the EAZA (European Association of Zoos and Aquaria) and UIZA (Unione Italiana Giardini Zoologici e Acquari) and it has rigorous standards for animal welfare. Zoological gardens in Italy are expected to perform behavioral observations of the individuals in their care (D. Lgs.73/2005). Since all recording procedures were non-invasive and the husbandry routines of the animals involved were not changed or affected by the study, this study does not fall in any of the categories for which approval of an ethic committee is required by Italian laws (D.Lgs. 26/14, implementation of the Directive 2010/63/EU). Informed consent was obtained from all the surveys participants. A privacy notice was provided in the survey to inform and assure that responses were anonymous, confidential, and that information collected would be used for research purposes only. No personal data were collected. Participation was voluntary and could be cancelled at any time without any reason. No financial or gift compensation was proposed for completing the survey. The participation in the surveys did not interfere with the day at the zoo or the daily life of participants.

Animals, housing, and husbandry

The animals involved in the present study were a group consisting of five ring-tailed lemurs (*Lemur catta*), all born at Giardino Zoologico di Pistoia: Sakalava born in 2006, Mandrare born in 2001, Bekili and Andribe born in 2000 and the only male housed, Ankarana, born in 2005. All five individuals were related. Lemurs were housed together in an enclosure ("Voliera dei lemuri") consisting of an indoor area, not visible to visitors, that opens to an external area. The internal area, of 15 m2, 3 m high, consisted of two rooms equipped with branch platforms, a nest box, and heating. The external area was 200 m2, 5 m high, and delimited by a nylon net. It can be divided into two different parts: a lower artificial area, consisting of a cemented path used for the transit of the keepers and visitors inside the enclosure, and a 1 m raised area consisting of soil with natural vegetation (trees, shrubs, and grass). In this second part of the external enclosure, natural and artificial supports were placed at different heights. In addition, on two sides of the outdoor area, there were doors used for the entrance and exit of the public.

From the outside of the enclosure, visitors could see inside through two glass-walls, one placed in the middle of the enclosure and the other one at the end (S2 Fig).

Animals were free to access the indoor area all day. Moreover, in wintertime and until the weather temperatures become milder, the internal area was heated.

Feeding provisions took place twice a day, in the morning and evening. Fruits and seasonal vegetables were given to the lemurs into feeding bowls located in the internal area. Water was provided ad libitum in both areas of the enclosure. Moreover, leaves, flowers, and berries were naturally present in the enclosure and always available to the animals. Enclosures were cleaned daily in the morning.

The animal-visitor interaction: “A tu per tu con i lemuri”

At the Giardino Zoologico di Pistoia, visitors have the possibility to enter the outside area of the lemurs’ enclosure for a closely encounter with the animals. This interactive activity, “A tu per tu con i lemuri,” differs depending on whether it is a weekday or weekend day. Over the weekend (Saturday, Sunday, and public holidays), the interaction is scheduled from two to four times a day. An educator opens the enclosure to the public, and small groups of on average six people are guided in the external area of the enclosure, where the activity takes place. During the activity, the educator presents some information about lemurs (geographical distribution, behaviour, social system, conservation status, the zoo’s conservation projects, etc.).

When the talk finishes, and visitors have no more questions, the interaction ends, and the educator leads the visitors to the exit. Each session lasts around 5/10 minutes.

During the weekday, the activity occurs differently. In those days, the lemur’s enclosure is opened by an animal keeper only once a day for 20 minutes in the afternoon. No information about the animals is given to visitors, and on average six visitors can access the enclosure at the same time. Once inside, they can stay there as long as they wish or depending on the number of people waiting outside.

In both situations, the opening and closing of the gates are always regulated by the zoo staff, and only happens in the presence of them. In their absence, public can only observe the lemurs from the outside, through glass—walls. Furthermore, before entering the enclosure, visitors receive information on behavioural rules to be respected, and posters indicating the timing, regulation, obligations, and prohibitions are shown (e.g., to stay in the artificial part of the enclosure, not to touch the animals, not to smoke, etc.). Moreover, visitors are asked to leave strollers and food out of the enclosure, to disinfect shoe outsoles, and to cleanse their hands with disinfectant gel provided by the keepers both at enclosure entrance and exit. The zoo staff checks that the rules are respected, and controls that visitors maintain a minimum distance of one meter from the lemurs. During the interaction, the animals are observed without requiring them in any way to stay close to the visitors.

AVIP procedures and data collection

The aforementioned AVI "A tu per tu con i lemuri" was assessed in August and September 2018 at the Giardino Zoologico di Pistoia (Pistoia, Italy—43°55’46.6"N 10°51’59.7"E). To carry out the evaluation of “A tu per tu con i lemuri” AVI, some details of the AVIP methods previously described for a giraffe feeding AVI [24,42] were adapted to the new context. Therefore, S1 Table describes the adaptations applied for the first five steps (A, B, C, D, E), while step F (i.e., the final checklist) was instead not modified from [24].

Animals were recorded in four different situations: during weekday afternoon activity (EEK), during weekend morning activity (EE1) and weekend afternoon activity (EE2). Moreover, on weekday morning, a control session (CON) was carried out, following the same recording timing of EE1 (no longer than 20 minutes). In these different situations, the animals were recorded before, during and after the AVI (S1 Fig). Animals were recorded using continuous focal animal sampling [43,44] and behaviours were analyzed using a working ethogram (S2 Table). The ethogram was adapted from previous studies [45–54] and finalized during a one-week period of preliminary observations using ad lib observations, as defined by [43].

Moreover, during the one-week preliminary period, the observer practiced the recognition of individual animals, thanks to specific signs made with temporary hair dye (BioKap1 Spray Ritocco, Bios Line S.p.a. (PD)).

During observations, the researcher was supposed to record any abnormal behavior, any attempt of the animals to avoid contact/close proximity with interacting visitors, and any escape attempt. Also, the researcher was instructed to record any other behavioral pattern not highlighted by the preliminary observations but deemed of potential interest.

The lemurs' enclosure was virtually divided into several areas (S2 Fig): areas 2, 4, and 6 correspond to the cemented path used for people transit inside the enclosure; areas 1, 3, and 5 correspond to the natural part of the enclosure, not accessible to visitors, in which natural and artificial supports allow the lemurs to use the space vertically; RI correspond to the night enclosure. During each recording session, the position of the animals in each area was recorded.

The lemurs' individual faecal samples were collected in the enclosure, stored at -20°C, and then analysed to detect any changes in levels of faecal glucocorticoid metabolites (FGM) between periods of low density of visitors (during the weekday) and periods of high density of visitors (during the weekend). To measure the cortisol level matched to the recorded day, the samples were collected the following day, as there is a time interval between the release of cortisol and FGM excretion in the faeces [55,56].

The information gathered by the two previous steps was used to perform an Animal Welfare Risk Assessment. Following the methodology provided by the EFSA guidelines [57], through a series of phases it is possible to identify any risk for animal welfare and to calculate a Welfare Score, as described in S1 Table.

Moreover, for each recorded interaction day, a Visitor Experience Survey was administered after the AVI to the participants. On the same days, a second Visitor Experience Survey was delivered near the zoo exit to visitors who either attended or did not to the AVI programs.

Visitor Safety Risk Assessment was performed following the Department for Environment, Food, and Rural Affairs (DEFRA) [58] and EAZA [59] documents. This step aims to highlight possible risks for visitors' safety and health, identify measures to eliminate or reduce hazard exposure, and examine the existing prevention and protection measures. It also allows to find preventive and protective actions that can be implemented to ensure the protection of the visitors during AVIs. Therefore, through several phases, it is possible to calculate the risk, defined as the probability that a hazard occurs multiplied by the magnitude of the damage that can derive from the hazard if it happens.

To highlight possible ethical concerns, results obtained from the previous A-D Steps were analyzed using an Ethical Matrix (EM) [60]. The Ethical Matrix is a conceptual tool that frames the ethically relevant demands in a complex situation where several moral conflicts are difficult to address. The EM's purpose is to summarize all the ethical demands, in terms of needs and interests involved in a given situation, of the relevant stakeholders allowing decision-makers to analyze the different points of view and map potential ethical conflicts. The EM consists of a table where the rows list the three main ethical principles of common morality (well-being, autonomy, fairness), and the first column lists the various stakeholders involved. All the cells of the EM are filled with specific moral demands. In AVIP, the EM is tailored to represent the ideal situation of the AVI under scrutiny for all stakeholders (Step E).

Finally, through the Step F final checklist, strengths and weaknesses of the AVI are identified, allowing be sharing and communicating.

Faecal sample analyses

As recommended, to extract steroids from nonliquid matrices (such as dried solids), faeces were subjected to an organic phase extraction using ethanol [61]. Extraction and determination of FGM were carried out as previously reported by [62]. Following the protocol, faecal samples were kiln dried at 55˚C for 24 hours, thoroughly crushed, and five aliquots of pulverized feces (0.20 g each) were put into extraction tubes, which were then sealed with a Teflon cap (Pechiney Plastic Packaging Inc., Menasha, WI). Next, 1 mL of ethanol (Sigma-Aldrich, St. Louis, MO) for every 0.1 g of solid was added to each tube, and the mixture was shaken vigorously for 30 minutes. Samples were centrifuged at 3,300g for 15 minutes, and the supernatant was recovered in a clean tube for evaporation to dryness in a SpeedVac (Thermo Fisher Scientific, Waltham, MA). Extracts were stored at -80˚C. Extracted samples were dissolved in 100 mL of ethanol followed by at least 400 mL of kit Assay Buffer (Arbor Assays, Ann Arbor, MI); then, they were vortexed and rested for 5 minutes twice, to ensure complete steroid solubility. FGM was determined using a multi-species cortisol enzyme immunoassay kit (K003; Arbor Assays, Ann Arbor, MI) validated for dried faecal extracts. All analyses were repeated twice. It is uncertain to which extent native molecules and immunoreactive metabolites of cortisol were quantified in the kit used. As a consequence, the terminology faecal glucocorticoid metabolites were used. Inter-assay and intra-assay coefficients of variation were less than 10%.

The test's sensitivity was determined by measuring the least amount of hormone standard consistently distinguishable from the zero-concentration standard and was calculated to be 24.5 pg/mL.

According to the manufacturer, the cortisol kit presents the following cross-reactivity: 100% with cortisol, 18.8% with dexamethasone, 7.8% with prednisolone, 1.2% with corticosterone, and 1.2% with cortisone. Serial dilutions (1:4, 1:8, 1:16, and 1:32) of faecal samples were assayed to test for parallelism against the standard curve ($P < 0.05$ for all assays). The mean recovery rate of cortisol added to dried feces was 97.2%.

Statistical analysis

Behavioural observations (Step A).

A first analysis was performed on the quantity of "Not visible". Since the length of the "Not visible" periods (the time the animals were not visible) was not negligible relative to the length of the observed behavioral patterns to be recorded, the time the animals were not visible was deleted from the sample, reducing the sample period accordingly, following Lehner 1996, p. 193 [63] for subjects disappearing from view. As a consequence, data for states were represented as the ratio between the duration of each behavior in a given session and the time in which the animals were visible in the same session. Similarly, event's frequency was calculated as the number of occurrences of the behavioural pattern in a given session divided by the number of minutes the animal was visible in the same session.

Then statistical analyses were run, both on the five lemurs as a group, and at the individual level (as advised by [15,64]), by comparing the observations made in analogous sessions (e.g., "pre-" vs. "pre-") (as in [42,65]). Therefore, behaviours performed in the ten control sessions (CON) were compared with the ten weekend morning interactions (EE1), and the behaviours observed in the eight weekday afternoon interactions (EEK) were compared with the eight weekend afternoon interactions (EE2). The Shannon-Weiner diversity index H [66], also referred to as Entropy [67] was calculated to investigate the behavioural diversity of the lemur group on the visible time and the enclosure use on the total time. The decision to calculate the latter on total time and the former on visible time was taken because, during observations, it had been always possible to know where an animal was, but not to always discriminate what behaviour the animal was performing (e.g., only a part of the animal was seen; the animal was not outside, so he/she was inside). The index has been calculated using the following formula:

$$H = -\sum (p_i \log p_i)$$

where p is the proportion of time engaged in the behaviour of the ith. Higher values indicate greater behavioural diversity or even enclosure use. The H index of analogous sessions (e.g., "pre-" vs. "pre-") were compared following the previous comparisons (CON vs EE1 and EEK vs EE2).

Finally, the ambient temperatures recorded were compared between control and interaction episodes to verify that they did not differ, as this could be a source of bias for the results.

All the comparisons were made using the Mann-Whitney U-test. Even if no data set was used twice in the comparisons, Bonferroni correction for multiple comparisons was applied in order to be more rigorous. Therefore, behaviours are considered to significantly differ if the *p*- value was less than 0.025 (0.05/2 because there are two comparisons). Differences with *p*-value between 0.05 and 0.025 are considered as tendencies. All statistical analyses were performed using the software IBM SPSS Statistics 21.0 (IBM Corp, Armonk, NY).

Physiological measures (Step B).

The samples of each individual were analyzed to detect differences in FGM concentration between periods of low density of visitors (during the weekday–"LOW") and periods of high visitor density (during the weekend–"HIGH"). Student t-test was performed with alpha set at 0.05. The statistical analyses were performed using the software IBM SPSS Statistics 21.0 (IBM Corp, Armonk, NY).

Visitor experience survey (Step D).

A Chi-squared contingency test was done to investigate whether groups (PostQ vs. GenQ) or respondents who joined an interaction or who did not join an interaction have distinctive characteristics (sex, educational level, pet ownership, natural childhood, first visit, age). Independent samples t-test was used to investigate the Likert scale statements. The analysis of visitor satisfaction was investigated with the Kano Model: the answers of each respondent to the functional and dysfunctional questions of each attribute were classified by the combination of the answers. For each attribute, the combination chosen by the majority of the respondents defined the classification of the attribute: Exciter, Indifferent, Questionable response, Reverse, Must-have, Linear. The frequency of respondents choosing Linear, Exciter, Must-have and Indifferent were used to calculate the customer satisfaction and dissatisfaction coefficient (CS) (see [24,68,69] for further details about the procedure).

All statistical analyses were performed using the software IBM SPSS Statistics 21.0 (IBM Corp, Armonk, NY).

Results

Animal welfare assessment (Step A to C1)

Behavioural observations (Step A).

The statistically significant differences detected during the group comparisons CON vs EE1 and EEK vs EE2 of analogous sessions (e.g., "pre-" vs. "pre-") carried out when the animals were visible are reported in Table 1.

Table 1. Medians, interquartile range of the behaviours which resulted significantly different among sessions and Mann-Whitney U-test results. Behaviours in *italics* indicate events. Only behaviours which significantly differed or showed a tendency to differ are reported. *P*-values not reported in bold are those which after Bonferroni correction are not significant but represent a tendency.

Behaviour	Session	Median	IQR	Mann-Whitney U-test	*P*-value
Self-grooming	CON – pre	0	0.05	U = 924	**p = 0.012**
	EE1 – pre	0.02	0.21		
Eating natural food	CON – pre	0	0	U = 1071	**p = 0.023**
	EE1 – pre	0	0		

Huddling	CON – dur	0	0.7	U = 883	**p = 0.004**
	EE1 – dur	0.27	1		
Mutual licking	CON – dur	0	0	U = 1067	p = 0.031
	EE1 – dur	0	0		
Sunning	CON – post	0	0	U = 1150	p = 0.042
	EE1 – post	0	0		
Urinate	CON – post	0	0	U = 1125	**p = 0.023**
	EE1 – post	0	0		
Eating natural food	EEK – dur	0	0	U = 680	**p = 0.011**
	EE2 – dur	0	0		
Huddling	EEK – post	0	0	U = 635.5	p = 0.047
	EE2 – post	0	0.68		
Grooming by conspecific	EEK – post	0	0	U = 720	p = 0.042
	EE2 – post	0	0		
Sniffing	EEK – post	0.18	0.32	U = 573.5	**p = 0.012**
	EE2 – post	0	0		
Yawn	EEK – post	0	0	U = 720	p = 0.042
	EE2 – post	0	0		

No differences were detected in ambient temperatures in the comparison between control and interaction episodes. Results of the analyses performed on behaviours at the individual level (as advised by [64]) are reported as supplementary material (S3 Table). The Mann-Whitney U test detected a statistically significant difference in the behavioural diversity index, showing that during the pre-session of the control the animals performed more state behaviours (or more animals performed different behaviours) than during the pre- session of the morning weekend interaction (CON-pre: H index ranging from 0 to 0.75; EE1-pre: H index ranging 0 to 0.71; U = 845; p = 0.005).

Overall enclosure use was higher on the dur-session of the control compared to the dur-session of the weekend morning interaction (CON-dur Entropy index ranging from 0 to 0.47; EE1-dur Entropy index ranging from 0 to 0.40; $U = 927.5$, $p = 0.01$). No other differences were detected. Analyzing the proportion of time the animals spent in the different areas in the comparison CON-dur vs EE1-dur, the Mann-Whitney U test detected that the animals spent more time in the internal area of the enclosure during the during session of the control that the during session of the weekend morning interaction (RI-CON-dur median = 0; IQR = 0.42; RI-EE1_dur median = 0; IQR = 0; $U = 929$, $p = 0.002$).

Physiological measures (Step B).

We analyzed a total of 41 faecal samples, 19 collected in “LOW” sampling days, and 22 in “HIGH” sampling days. Measured FGM ranged from 16.88 to 173.95 ng/g feces. The mean FGM concentration values were lower in the "LOW" sampling days (mean ± SE: 69.320 ± 6.621 ng/g) than in the "HIGH" periods (mean ± SE: 96.532 ± 5.952 ng/g) for all the subjects. Analysing the mean FGM concentration, the statistical test did not detect any significant difference between “LOW” sampling days and “HIGH” sampling days for any of the five lemurs (Fig 2).

Animal risk assessment (Step C1).

Results obtained by the previous steps were integrated in the Animal Welfare Risk Assessment.

Problem formulation phase—Identification of factors of animal welfare concern.

The “Management Checklist: Staff action and Procedures” (Table A in S1 Appendix) did not record negative answers, showing that the management and enclosure were adequate to maintain a high standard of welfare during the interactions.

Welfare risk assessment—Exposure assessment.

Scenario 1 and scenario 2 have different consequences (behavioural changes that indicate that the animals were at risk of perceiving negative subjective experiences and injuries) while recognizing the same exposure factors (improper approach of the visitor). Based on the analysis of the video recordings of the during-sessions (dur-EE1, dur-EE2 and dur-EEK), the frequency of exposure was identified as negligible. For scenario 3, a low probability of effective contacts was defined. Even when visitors wash and disinfect their hands before the interaction activity, the spreading of some pathogens by droplets or aerosol may be possible in the absence of preventive measures. No clinical signs attributable to infectious or diffusive disease were observed during the study.

Moreover, all periodical clinical examinations performed by the zoo veterinarian, which were supported by laboratory tests for specific zoonotic agents, had negative outcomes. Nonetheless, the probability of disease spreading was considered low and not negligible as a precautionary measure.

Welfare risk assessment–Consequence characterization.

In our study, no consequences on animal welfare were observed, but as a precautionary measure, the probability has been defined as low and not as negligible. We cannot exclude, due to the short period of observation, the possibility that there could be negative long-term consequences for welfare.

Welfare risk assessment–Risk characterization.

The WS value was calculated for each exposure scenario. The results are presented in the following table (Table 2).

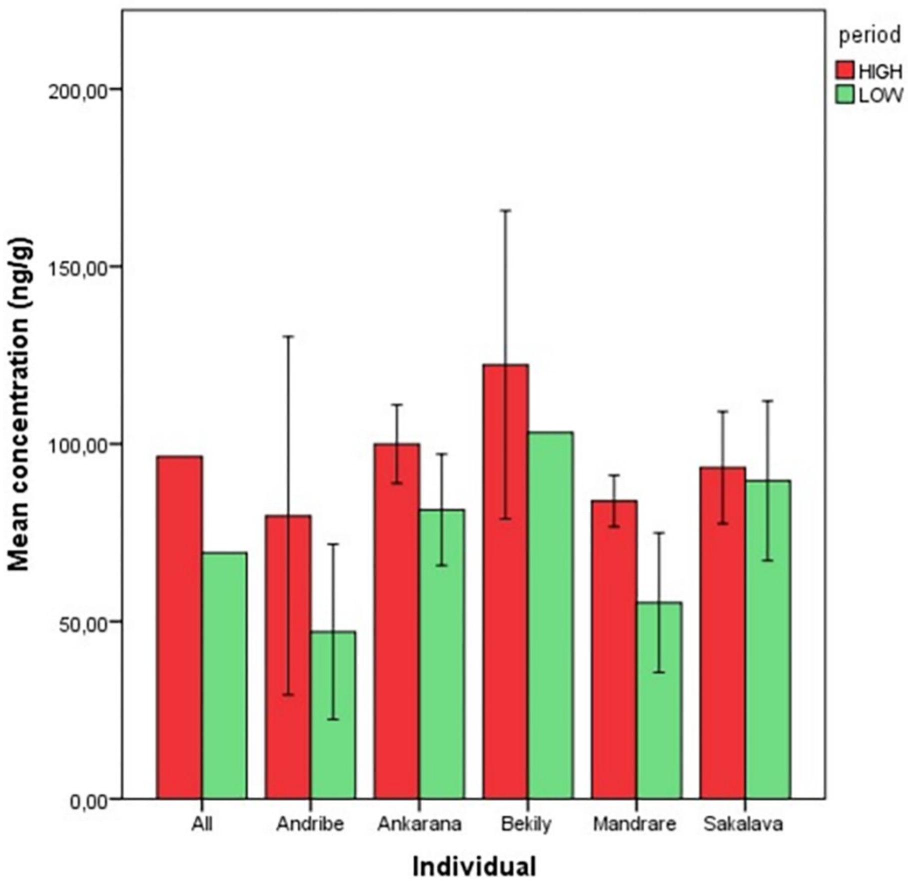

Fig 2. Mean concentrations of faecal cortisol metabolites (FCM) of the five lemurs during the "LOW" and "HIGH" sampling. Mean value ± Standard error are reported.

Human outcome assessment (Steps C2 and D)

Human risk assessment (Step C2).

The "Management Checklist: Preventive and Protective Measures" (Table A in S2 Appendix) applied for the analysis of the preventive and protective measures needed to guarantee adequate safety standard of people during interactions recorded eight negative answers. The items concerned:

Table 2. Welfare score values (FE = frequency of exposure; FC = frequency of consequences; MA = magnitude of consequences; WS = Welfare Score). Adapted from [42].

Exposure Assessment		Consequences Characterization				Risk Characterization
Hazard Description	**FE**	**Animal-Based Indicators**	**Severity**	**Duration**	**FC**	**WS = FE * MA * FC**
Improper approach (scenario 1)	1	Behavioural observation	1	1	2	2
Improper approach (scenario 2)	1	Skin Lesion	1	1	2	2
Effective contact with a zoonotic agent (scenario 3)	2	Zooanthroponosis	1	1	2	4

- The existence of a safe area, and thus the possibility to store personal item in the safe area (5.5., 1.2)
- The existence of a service access point differentiating from visitors' entrance or exit, nor the transition zones (5.1; 5.4)
- The existence of an appropriate number of hand-washing stations accessible to all visitors regardless of age or height (5.11)
- The absence of automatic (or foot-operated) washing stations (6.6)
- The use of Personal Protective Equipment (PPE) (7.1; 7.2)

The human risk assessment procedure was carried out in five different phases.

Phase 1: Hazard identification: (1) Anthopozoonoses: in Table B in S2 Appendix is reported a list of the main zoonotic agents (both fungi and bacteria). (2) injuries: the visitor and staff involved in the AVI can suffer injuries caused by scratches and bites.

Phase 2: Hazard characterization (P): the results of this second phase are detailed in Table 3 and expressed in terms of probability (P).

Table 3. List of relevant zoonosis in lemurs and other animals reported for animal-visitor interactions (detailed in Table B in S2 Appendix). Values of risk characterization for existing control measure and additional control measure to be implemented (phase 2–5). (P = probability; D = damage; R = risk score; RR = risk rating; OR = Oral route; DC = Direct contact; A = Aerosol; CM = Contact with infected material and ingestion; CF = Contact with body fluids; L = Low; M = Medium; H = High).

Hazard characterization – Exposure assessment			**Risk characterization Phase 4 (Existing control measure)**				**Risk characterization Phase 5 (Additional control measure to be implemented)**			
Exposure Condition/ Scenario	**Hazard**	**Consequences**	**P**	**D**	**R**	**RR**	**P**	**D**	**R**	**RR**
A / CF	*Mycobacterium tubercolosis*e *M. bovis*	Pulmonary tuberculosis/ extrapulmonary tuberculosis	1	3	3	L	1	3	3	L
OR	*Klebsiella pneumoniae*	Septicemia, abscess	2	2	4	M	1	2	2	L
OR	*Escherichia coli*	Mild/severe diarrhea; Haemolyticuraemic syndrome	2	3	6	M	1	3	3	L
OR	*Salmonella enterica*	Mild/severe diarrhea	2	2	4	M	1	2	2	L
OR	*Shigella* spp	Mild/severe diarrhea	2	2	4	M	1	2	2	L
OR	*Vibrio* cholera	Mild/severe diarrhea	2	2	4	M	1	2	2	L
OR	*Yersinia pseudotuberculosis, Y. enterocolitica*	Acute enterocolitis, diarrhea, septicemia	2	2	4	M	1	2	2	L
OR	*Campylobacter fetus* subsp *jejuni*	Bacteriaemia, Guillain-Barre syndrome	2	3	6	M	1	3	3	L
DC	*Leptospira* spp.	Kidney damage, liver failure meningitis, death	2	3	6	M	1	3	3	L
OR / A / DC	*Francisella tularensis*	High fever, chills, headache, focal ulcers, swollen lymph nodes	2	3	6	M	1	3	3	L

A / CM /CF	Methicillin resistant *S. aureus* (MRSA), Extended spectrum beta-lactamase (ESBL)	Skin infections, urinary tract infections (UTIs), intra-abdominal and respiratory infections	2	3	6	M	1	3	3	L
DC	Rabies lyssavirus	Cerebral dysfunction, death	1	3	3	L	1	3	3	L
DC / A	*Lymphocryptovirus*	Lymphadenopathy	1	2	2	L	1	2	2	L
OR	*Cryptosporidium* spp.	Mild/severe diarrhea	2	2	4	M	1	2	2	L
OR	*Giardia duodenalis*	Mild/severe diarrhea	2	2	4	M	1	2	2	L
OR	*Entamoeba* sp.	Stomach cramping, dysentery	2	1	2	L	1	1	1	L
OR	*Encephalitozoon cunicoli, E.intestinalis; E. bieneusi*	Diarrhea, Disseminated infection	2	2	4	M	1	2	2	L
DC	*Trichophyton mentagrophytes*	Reddish ring-shaped rash, that may be itchy/ eventually itchy	1	2	2	L	1	2	2	L
DC	Injuries	Bites and scratches	1	2	2	L	1	2	2	L

Phase 3: Exposure Assessment: the results of the exposure assessment are detailed in Table 3 and expressed in terms of damage (D).

Phase 4: Risk characterization: the results of the risk characterization are detailed in Table 3 and expresses in terms of risk score (R) and risk categories (risk rating—RR) obtained with the existing control measures (Tables C-E in S2 Appendix).

Phase 5: Risk characterization: predicted with the additional control measure that could be implemented.

Visitor experience survey (Step D).

A total sample of 291 visitors answered questionnaires, N = 153 (53%) from the PostQ and N = 138 (47%) from the GenQ. Table 4 below summarizes demographic information and other independent variables collected from visitors who participated in “A tu per tu con i lemuri” activity with the PostQ, and from visitors interviewed with the GenQ nearby the zoo exit.

Table 4. Demographical information for PostQ and GenQ respondents.

Demographic	Category	PostQ respondents		GenQ respondents		X²	df	*p*-value
		Percentage	*n*	Percentage	*n*			
Sex	Female	52%	79	50%	69	0.072	1	.789
	Male	47%	72	49%	67			
	No Answer	1%	2	1%	2			
Age	14-18	7%	10	1%	2	9.412	5	.094
	19-25	12%	18	7%	10			
	26-34	17%	26	25%	35			
	35-54	55%	84	54%	75			
	55-64	5%	7	7%	10			
	65+	3%	4	3%	4			
	No Answer	3%	4	1%	2			
Education	Elementary school graduate	2%	3	1%	2	0.829	4	.935
	Middle school graduate	12%	19	10%	14			
	High school graduate	49%	75	49%	67			
	University degree	27%	41	30%	42			
	Higher degree / PhD	7%	11	8%	11			
	No Answer	3%	4	1%	2			
Education on nature/animals	Yes	12%	19	14%	20	0.252	1	.616
	No	86%	131	84%	116			
	No Answer	2%	3	1%	2			
Number of past visits	1	63%	97	64%	88	2.144	3	.543
	2-3 times	29%	45	30%	41			
	from 4 to 10	3%	4	4%	6			
	more than 10	3%	4	1%	1			
	No answer	2%	3	1%	2			
Annual ticket / Membership	Yes	4%	6	3%	4	0.227	1	.634
	No	95%	145	96%	132			
	No Answer	1%	2	1%	2			
Pet ownership	Have a pet	64%	98	66%	91	0.041	1	.839
	Not have a pet	33%	51	33%	45			
	No Answer	3%	4	1%	2			
Member of an environmental association	Yes	14%	21	22%	30	3.254	1	.071
	No	85%	130	77%	106			
	No Answer	1%	2	1%	2			

Among respondents of the GenQ ($n = 138$), only 20% ($n = 27$) of them joined the "A tu per tu con i lemuri" on the same day of the survey and the 46% ($n = 64$) of the interviewed participated at least one of any activity on that day. The most followed activities by GenQ respondents, among those proposed, were the bear talk ($n = 28$, 20%), the walk-in with lemurs ($n = 27$, 20%) and the penguin talk ($n = 23$, 17%). Similarly, among the PostQ respondents ($n = 153$), the most followed activities after the walk-in with lemurs were the bear talk ($n = 38$, 25%), and the penguin talk ($n = 33$, 22%). Therefore, it has been tested if any variables differed between visitors who joined the lemurs AVI and visitors who did not. The test did not find any statistical differences between the two groups for any variable tested.

No statistically significant difference was identified for any of the five statements investigated with the 5-point Likert Scale (S1 Table) between PostQ($n = 149$) and GenQ ($n = 135$) visitors, nor between visitors who did the activity with lemurs ($n = 176$) and those who did not participate ($n = 108$). However, an independent-samples t-test indicated that statement B, "I don't think I will take the time to learn more about animals", scores were higher for weekend visitors ($n = 173$, mean = 2.08; SD = 1.17) than for weekday visitors ($n = 111$, mean = 1.84, SD = 0.89, $t(282) = -1.787$, $p = .05$).

There was no significant difference in the scoring given by the weekend visitors ($n = 96$, mean = 4.48; SD = 0.725) respect to the weekday visitors ($n = 52$, mean = 4.38; SD = 0.745) when asking them if they would suggest to friends to participate in the lemurs AVI ($t = -.832$, $p = .407$). The great majority of the PostQ answered that they absolutely would suggest to friends to participate ("absolutely probable"; $n = 87$, 59%). According with the Net Promoter Score (NPS), 59% ($n = 87$) of visitors could be considered as promoters, 28% ($n = 42$) as passive and 13% ($n = 19$) as detractors. According to the formula, the NPS resulted in 46%. However, the NPS calculated for the weekend visitors was higher than for the weekday visitors, resulting in 49% for the firsts and 40% for the second ones.

The pre-information experience was said to be satisfactory by 93% of the sample (n = 140), with a higher satisfaction level in the weekend visitors (99%, n = 96) compared to the weekday visitors (83%, n = 44). When asked why the pre-information was not satisfactory, the most frequent answer was that visitors had not received any information. As previously explained, during the weekday, the enclosure was opened by a keeper, and there was no provision of a guide to give information to visitors.

The majority of the attributes investigated with the Kano Model were a "Linear" requirement (Table 5), but "Direct contact with animals" and "Information about animals" were "Exciter" for 36% (n = 53) and 35% (n = 52) of the respondents, respectively. This means that for them the direct contact and information about the animals were unexpected attractive features, which provided high satisfaction. In Table 6 the CS coefficients for respondents' satisfaction or dissatisfaction are presented.

Table 5. Attributes investigated with the Kano Model and their distribution within the categories.

Attribute	N (%)					
	Must have	Linear	Exciter	Indifferent	Reverse	Questionable
1. Direct contact	7 (5%)	21 (14%)	53 (36%)	34 (23%)	23 (16%)	10 (7%)
2. Information about animals	9 (6%)	44 (30%)	52 (35%)	36 (24%)	2 (1%)	6 (4%)
3. Information about conservation issues	18 (12%)	71 (48%)	27 (18%)	20 (13%)	4 (3%)	9 (6%)
4. Information about animal welfare	28 (19%)	87 (58%)	16 (11%)	12 (8%)	0 (0%)	6 (4%)
5. Presence of a guide	16 (11%)	83 (56%)	37 (25%)	8 (5%)	0 (0%)	3 (2%)

Table 6. Customer satisfaction and dissatisfaction coefficient (CS). The closer the satisfaction coefficient is to +1, the more the presence of the attribute influences respondents' satisfaction. On the contrary, the closer the dissatisfaction coefficient is to -1, the more the absence of the attribute influences respondents' dissatisfaction. If the CS is adjacent to 0, it means that the attributes have a low influence on visitor satisfaction or dissatisfaction.

Attribute	Satisfaction	Dissatisfaction
1. Direct contact	0.643478	-0.24348
2. Information about animals	0.680851	-0.37589
3. Information about conservation issues	0.720588	-0.65441
4. Information about animal welfare	0.72028	-0.8042
5. Presence of a guide	0.833333	-0.6875

Overall ethical assessment (Steps E and F)

Ethical analysis (Step E).

Cells of the Ethical Matrix (EM, Tables 7 and S4) were populated to represent the ideal situation in which all the stakeholder's moral demands, corresponding to the three prima facie ethical principles (well-being, autonomy, fairness), are respected during the AVI. From the comparison between the content of the EM cells and results obtained with the other steps, few non-conformities and potential conflicts were detected.

The lemur walk-in enclosure at Giardino Zoologico di Pistoia was opened in 2009, but only since 2013 the individuals involved in this study have moved in and started participating in the specific AVI. Veterinary records did not show any differences in the frequency of veterinary interventions between the five years before 2013 and the five years after (until this study was conducted). Results of this study did not indicate any adverse stressful effects on lemurs' welfare, neither throughout the behavioural and physiological analysis, nor throughout the animal welfare risk assessment, therefore, it is possible to state that their well-being was respected (LW). Results indicate also that the enclosure allows a good degree of control on the environment also during the AVI. Visitor passage area within the enclosure represents only a third of the horizontal space of the lemur enclosure, and animals are not forced to be always visible, and they have free access to the internal area, which is never accessible to visitors (LA).

Table 7. Outline of the customized ethical matrix. Adapted from [24].

	WELL-BEING	AUTONOMY	FAIRNESS
ZOO ANIMALS PARTICIPATING IN THE AVI	Physiological and psychological welfare (LW)	Behavioral freedom (LA)	Intrinsic value (LF)
WILD ANIMALS AND THE ENVIRONMENT	Species and biodiversity conservation (WW)	Freedom from human intervention (WA)	Respect for the worth of every individual (WF)
VISITORS PARTICIPATING IN THE AVI	Physiological and psychological welfare (AW)	Self-determination (AA)	Fair treatment (AF)
VISITORS NOT PARTICIPATING IN THE AVI	Safety and psychological welfare (VW)	Self-determination (VA)	Fair treatment (VF)
KEEPERS INVOLVED IN THE AVI	Satisfactory and safety working conditions; professional realization (KW)	Professional freedom (KA)	Fair treatment (KF)
EDUCATORS INVOLVED IN THE AVI	Satisfactory and safety working conditions; professional realization (EW)	Professional freedom (EA)	Fair treatment (EF)
MANAGEMENT STAFF	Satisfactory working conditions; professional realization (MW)	Management freedom (MA)	Fair treatment (MF)

VETERINARY STAFF	Satisfactory working conditions; professional realization (VSW)	Professional freedom (VSA)	Fair treatment (VSF)
ZOO	Economic sustainability, support from society (ZW)	Mission fulfilment (ZA)	Adequate legislation and access to resources (ZF)

Therefore, animals were shown to express most species-specific behaviours and use all the space available to them, respecting their well-being, autonomy and fairness (LW, LA, LF). The other statistically significant differences did not appear to be relevant in diminishing the welfare state of the animals. Moreover, since part of the zoo ticket is devoted to lemur's *in-situ* conservation project, the five ring tailed lemurs at Giardino Zoologico di Pistoia appear to contribute to the conservation of their species (LF and WF).

The zoo entrance ticket includes every talk and activities offered within the park, including the AVI discussed here. Visitors are therefore respected in their freedom to participate in the activities and receive information about the animals without any additional cost and are free to choose between several alternative free talks and activities (AA, VA and VF). As results have shown, there is great participation (AA and VA, AF, and VF). Besides, the absence of an educator during the weekdays causes some concern about visitors' well-being, autonomy and fairness, as they are more likely to be satisfied when receiving information about animals, animal welfare, and conservation issues during the AVI. Results of risk assessment showed low and medium risks for visitors' health and safety. This means that, although there are signs and behavioural norms for visitors to follow within the enclosure, and staff monitors visitors' compliance, there is some concern related to their well-being (AW). The implementation of additional control measures (e.g., periodic veterinary checks, as is routinely done at the Giardino Zoologico di Pistoia) reduces these physiological risks. On the other side, their psychological well-being, according to results of the surveys, was respected. The NPSwas 49%, and 59% of visitors answered that they absolutely would suggest to friends to participate. The CS coefficient confirmed, moreover, that direct contact and information about the animals had a high influence on respondents, which provided higher satisfaction to them (AW).

In this AVI, no concerns were identified regarding the management staff. The efforts of the management staff guarantee both animal welfare and the quality of the experience for visitors. It is therefore recognized that management staff has the necessary resources to train educators and keepers to meet the educational and entertainment needs of visitors, while ensuring the welfare of both visitors and animals involved (MW and MF). Both keepers and educators involved in AVI were satisfied with the working environment and their roles, thus respecting their wellbeing (KW and EW). They have adequate training in working with both animals and visitors during AVIs, and they are always available and ready to deal with any problems.

Finally, as the results show, they contribute to fulfill the mission statement of the zoo, in terms of welfare, conservation and education (EF, KF and MF).

Veterinarians interact with all animals, including those involved in AVIs, and with most of the other stakeholders included in the EM. Veterinarians are essential with their work, as their activity have a crucial role in safeguarding the welfare of the animals and the safety of visitors. Therefore, their well-being and safety must be monitored. As in the case of the other human stakeholders, the risk assessment results showed low and medium risks of contamination (VSW). Moreover, veterinarians have discretionary power on how to monitor and protect animals, especially the ones involved in AVIs, have access to fair working conditions, and contribute to the quality of education and the implementation of biosecurity activities (VSA, and VSF). Therefore, in this AVI, no concern was identified with regard to veterinary staff.

Results also showed that Giardino Zoologico di Pistoia fulfills the goals concerning animal welfare and conservation education by offering the "A tu per tu con i lemuri" AVI (ZW, ZA and ZF). The zoo offers a wide range of educational and entertaining activities, where visitors are provided with multiple information. The participation in any of these activities, including the AVI and talks is free of charge. They are indeed included in the entrance ticket price, and part of it is donated to *in-situ* conservation projects (ZF). Even if it was not possible to count the number of visitors participating in the interaction, the participation in the assessed AVI was high, both in the weekend and in the weekdays. Finally, results of the questionnaires showed that visitors were satisfied and willing to recommend the experience, thus contributing to the good reputation of the facility (ZW and ZA).

Final assessment (Step F).

The eleven entries checklist developed for the overall assessment recorded the following (Table 8):

Table 8. Final assessment checklist. Adapted from [24].

N.	Entry	YES	NO
1	The behavioural analysis did not identify any behavioural sign suggesting welfare issues (Step A).	x	
2	The analysis of physiological parameters (endocrine or others) did not identify any physiological sign suggestive of welfare problems (Step B).	x	
3	Only a negligible or low risk of welfare health was detected in the risk assessment analysis of physiological parameters (Step C1)	x	
4	No critical issues were detected when conducting an accurate 'management and enclosure analysis' within the welfare risk assessment (Step C1)	x	

5	A negligible or low risk was detected for the health/welfare of the people (visitors and staff) in the risk assessment analysis (Step C2)	x	
6	During the AVI, indications are given to increase awareness about wildlife conservation and animal welfare, and to promote sustainable behaviours among visitors (Step D)	x	
7	The visitor experience analysis detected a positive emotional impact (Step D).	x	
8	The visitor experience analysis detected a positive educational impact (Step D)	x	
9	The visitor experience survey detected a positive impact on the conservation mindedness and/or animal welfare awareness of the visitor (Step D)	x	
10	An ethical evaluation was done to highlight possible conflicts (Step E)	x	
11	If any ethical concern was identified with the AVI, the zoo staff is working toward a solution (Step E)	x	

Discussion

Despite walk-in exhibits offering close animal encounters are common in modern zoos [1], their impact on animal welfare, health, and safety, as well as on visitors' experience, education, and safety has been poorly researched. Bringing visitors into close proximity with animals is a form of interaction which needs to be carefully evaluated, even though it does not involve direct animal-visitor contact. Indeed, according to WAZA guidelines [6,7], it is of primary concern that the well-being of animals and the safety of visitors, as well as the educational and conservation impact of the proposed AVI are adequately monitored. AVIP stands as a key framework to fulfill these aims since it provides a holistic and interdisciplinary approach based on the integration of different assessment processes. In fact, it aims to evaluate animal physiological and behavioural welfare-related parameters, animal and human (both visitors and staff) health and safety risks, and educational and conservation outcomes. Results are then combined and compared with the content of an ethical matrix to carry out an ethical assessment which highlights key ethical concerns and informs whether the interaction is justifiable while suggesting potential practical solutions.

Currently, lemur walk-in enclosures are commonly present in zoos [26], and rigorous research to regularly assess this kind of AVIs is needed. The scientific studies assessing this kind of AVIs usually focus on few welfare parameters, or alternatively on visitors' effects and exhibit design, or on educational contents, without fully exploring the undesirable outcomes [8]. AVIP allows an integrated assessment of lemur walk-in enclosure AVIs, evaluating the impact of these activities on both animals and visitors. The AVIP application described in this study allowed to assess for the first time different interconnected aspects during lemur-visitors interaction: animal welfare and safety, human welfare, safety and experience. Moreover, the ethical analysis, through the EM, allowed to analyze and reconstruct the framework of morally relevant interests considering the different stakeholders involved and the Overall Assessment provided a detailed result of the evaluation process suggesting improvements. Behavioural results, both at the group and individual level, indicated that the five ring-tailed lemurs were probably used to the presence of visitors during the AVI at the time of this study. The variations in grooming (self-grooming and groomed by conspecific) detected in relation to the AVI (pre and post) both at the group and individual levels do not suggest a stereotypical overgrooming as no bald skin patches have been detected in any individual [49] and the percentage of time spent in them is low. Moreover, no differences have been found in the aggression levels. Thus, the variations in grooming do not indicate conciliatory contact [70]. This result agrees with Perry et al., (2011), and Hosey et al., (2016) that found that visitors do not affect lemurs' behaviour or welfare, and visitors do not increase wounding rates [41,71]. Moreover, no other stereotypical behaviours were detected, although pacing has been evaluated as the common stereotypic behaviour in prosimians [54].While the H index did not detect any relevant significant difference associated with the AVI, it is interesting to note that the lemurs spent more time outside (in areas where also the visitors could stay) during the weekend morning interaction than during control sessions, suggesting that the animals did not feel threatened by the presence of visitors (which is corroborated also by the absence of an increase in scanning behaviour) [72], and they might also be interested in being outside where they could see the people inside the enclosure.

The physiological analysis further supported these results. Although the mean values of FGM concentration for each individual were lower in the weekdays, when there were fewer visitors in the zoo, no statistically significant difference has been found when more groups of visitors entered the enclosure. Overall, as the EM is suggesting, there were no significant differences suggesting an adverse visitor effect during the AVI (LW). However, due to logistical issues, it was possible to have control sessions only on weekday mornings. Still, it could have been interesting also to perform control sessions for each activity (morning/afternoon, weekend/weekday).

Most of the differences in behaviours are found between the two types of interactions and not between AVI and control sessions. However, differences that emerge between the two interactions (EEK and EE2) are not relevant to suggest a negative effect on the welfare of lemurs.The assessed AVI does not involve direct animal-visitor contact, although it allows visitors to closely observe the animals. Nonetheless, it also provides animals with the opportunity to express species-specific behaviours, to escape and avoid unpleasant stimuli. The enclosure design allows animals to have an always accessible retreat space and to avoid close proximity to humans, that might represent a source of stress, not only during AVIs but also for zoo animals in general [73]. Moreover, the resources present in the enclosure allow lemurs to express a diverse repertoire of behaviours, including grooming and affiliative behaviour, and facilitates the absence of intra-group aggression, satisfying their autonomy and wellbeing (LA and LW). These results suggest that at Giardino Zoologico di Pistoia lemurs are not negatively affected by the presence of the visitors inside the enclosure during the AVI (LW, LA). However, respect for visitors fairness (AF) can conflict with well-being and autonomy of the lemurs participating in AVI, if more people are interested in joining the activity (LW, LA).

Visitors were never observed performing behaviours that did not comply with the park's rules. In rare situations where visitors showed negative behaviour towards lemurs (e.g., quick approach), the most common action of the animals was not responding or retreating. Furthermore, lemurs were never observed receiving food or voluntarily approaching visitors, even during the afternoon session on weekdays, when the supervision of keepers was not as close as during the weekend, and the number of visitors inside the enclosure at the same time was higher. Besides, even though all zoo staff was participative, it was not possible to have a count of visitors entering/exiting the enclosure for each activity and the time they spent watching the animals from outside the enclosure.

AVIP protocol foresees that the behavioural and physiological results obtained with the dedicated steps are the basis of the animal welfare risk assessment (phase C1). This phase allows for the calculation of the lemurs' welfare risk, assesses the adequacy of the enclosure to maintain, during AVI activities, a high level of welfare for the animals involved, and examines the suitability of management procedures to prevent welfare risks to the animals. Therefore, the risks of spreading zoonoses and other health and safety problems of lemurs and visitors that may occur as a result of AVI were considered, analysed, and compared with the EM. In "A tu per tu con i lemuri" AVI, no animal welfare relevant consequences were detected. However, as a precautionary measure, the probability of disease spreading was defined as low and not negligible. In fact, possible future negative consequences on welfare cannot be excluded a priori, although periodical clinical examinations supported by laboratory tests for specific zoonotic agents are conducted. Through the development of three different scenarios, it was possible to classify the animal welfare risk as ‘low.’ However, in the third scenario, the WS reached 4, a threshold level of attention. This scenario considers the risks associated with zooanthroponosis. Therefore, an epidemiological investigation involving specific clinical observations and laboratory tests should accompany passive surveillance (permanent periodical veterinary control), leading to the detection of any new-onset zoonotic disease. However, the overall results of the Animal Welfare Assessment (steps A–C1) allow to conclude that the ideal situation of the well-being, autonomy and fairness regarding the interaction corresponded with the actual situation, with no need to discontinue the AVI.

AVIs must convey educational and conservation messages, since combining visitor-animal interactions with an educational experience may enhance visitor learning [2,3,29]. The presence of a guide and the information about conservation issues and animal welfare have been shown to strongly influence visitors’ satisfaction following results from the surveys, respecting their wellbeing, autonomy and fairness (AW, AA, AF). From the comparison of the EM and the Human Outcome Assessment results appears that visitors were mainly satisfied with the information given before the beginning of the activity. In general, people visiting the Giardino Zoologico di Pistoia chose to attend these activities, although many of them took place late in the afternoon. As questionnaires were administered in the afternoon (around 5 p.m.), some talks were not performed yet, and visitors’ participation to some of them was not recorded.

However, despite general satisfaction of visitors, the absence of a guide and the relative information provided during the weekdays, influenced respondents’ dissatisfaction, as also shown by the CS coefficients. The absence of information given to visitors did not affect animal welfare but created a concern for visitors' well-being, autonomy and fairness (AW, AA, and AF). Due to the educational role these activities offer to visitors, it is important to give visitors information not only about how to behave during the AVI but also about the individual animals, their welfare and conservation.

Direct contact with the animals during the interaction is a critical issue in all AVIs as it could be linked to the risk of contamination. Therefore, the AVIP Human Outcome Assessment includes a specific risk assessment to carefully evaluate the risks related to human health during the AVI. In the assessed AVI, low and medium risks for visitor health were detected, creating a risk for their well-being (AW). The additional measures that would bring the risk from medium to low are mainly related to items 5.11 and 6.6 (Table A in S2 Appendix). Consequently, a service access point differentiating visitor entrance and exit, or transition zones, and the use of PPE are measures that should be implemented to enhance visitor safety.

However, implementing the use of PPE could have a negative psychological impact on the animals and on visitor experience because the animal could be perceived as dangerous. Moreover, the presence of a safe area and the possibility of storing personal items near the enclosure should be considered not only for the visitors' safety, but also for their experience, and for respecting their well-being (AW). In this analysis, SARS-CoV-2 was not considered as this study was conducted before the emergency of the CoViD-19 epidemic. At present, however, it is difficult to adequately perform the hazard characterization phase as scientific studies have not yet provided robust scientific evidence regarding the susceptibility of these animals to SARS-CoV-2.

AVIP must always be customized to the specific AVI. The specific application of AVIP to the lemur walk-in at Giardino Zoologico di Pistoia, compared to past AVIP applications (e.g., giraffe feeding [24,42], turtle experience [not published], etc.), had some limitations. First, due to the design of the AVI proposed to the Giardino Zoologico di Pistoia's visitors, it was not possible to perform a pre-questionnaire before the beginning of AVI, and a matched post-questionnaire to the same visitors, to test the educational impact of the activity on visitors (as in [24,74]), nor to perform a long-term follow-up data collection (as in [75,76]). The inclusion of this assessment is important as AVIs must convey educational contents. A second factor was the assessment of the visitor effect. The control situation recorded in this study is not sufficient to extrapolate the visitors effect from the behavior of the animals, also because visitors, although not present within the enclosure, could see lemurs from different positions from outside the enclosure and consequently, lemurs could constantly see visitors [16]. Therefore, it is not possible to exclude that there have been reciprocal influences between the behavior of animals and that of visitors. As recommended by Goodenough et al., (2019) the weather was considered, as they demonstrated that it is a factor exerting a strong effect on the behavior of lemurs [28].

As a result of the overall assessment process, AVIP highlights strengths and weaknesses of the AVI under evaluation and proposes management decisions to address concerns depending on the outcome. Regarding the specific "A tu per tu con i lemuri" AVI, the main suggestions for improvement arising from the AVIP assessment were i) to implement the presence of a guide also during weekdays to assist visitors in obtaining information, ii) the placement of lockers to store personal items before AVI, and iii) periodical meetings for the staff involved in AVI (keepers and educators) to recall them the correct rules to be followed in order to continue to safeguard the health and welfare of animals and visitors. However, for an exhaustive assessment it would be also advisable to assess the specific educational impact of the walk-in enclosure on both weekend and weekday visitors. Anyway, from an ethical point of view, and from the comparison between the ideal situation described in the EM and the results obtained with the A-D steps, it is possible to conclude that the zoo addressed the highlighted concerns notwithstanding the complex framework of the AVIs. In the final checklist no negative answers were recorded.

Although it would be interesting to do a multicenter study to examine the different AVIs involving lemurs in different zoos, the protocol is not meant to compare different interactions, as differences in animals' age, group composition, characteristic of AVI, etc., as well as subjective variability of individuals, would not allow relevant comparisons between different facilities. Nonetheless, even if a more detailed evaluation of every single aspect involved in the AVI (e.g., behavioural assessment, physiological assessment, educational impact, etc.,) could eventually offer more in-depth and comprehensive inputs, AVIP promotes a holistic framework which satisfies WAZA recommendations and the One Health–One Welfare approach. Its outcome could help zoos willing to perform an AVI self-assessment through AVIP, as the Giardino Zoologico di Pistoia did, to develop targeted interventions to optimize both animal welfare,health and visitors experience, and safety during the specific AVI under assessment.

Conclusions

Given the increasing demand for AVI activities, and their supposed educational and recreational value, a multidisciplinary tool is crucial to evaluate their impact both on animals, visitors and the staff involved. AVIP is designed to support each facility in the self—assessment of its interaction activities. The aim of AVIP is to monitor all key aspects of a specific interaction, and to ensure that welfare of the animals, their safety and safety of visitors are carefully considered. AVIP results can support the work and commitment of zoos to both animal welfare and conservation education, while assisting in improving their management decisions and ensuring a transparent evaluation of their activities.

Result of AVIP application which are presented here are related to a specific AVI involving five lemurs at Giardino Zoologico di Pistoia and the specific circumstances at the time in which the study was performed. The results highlighted that there is no need to discontinue the assessed AVI as no concerns for lemurs, visitors, and staff were found, and ethical concerns were well addressed. This application shows that AVIP can be useful also in assessing walk-in enclosures with *Lemur catta* in zoos, confirming its potential to address WAZA recommendations for an overall evaluation of animal-visitor interactions.

Supporting information

S1 Fig. Diagram of the observational schedule. https://doi.org/10.1371/journal.pone.0271409.s001

S2 Fig. Diagram of the ring-tailed lemur enclosure ("Voliera dei lemuri") at Giardino Zoologico di Pistoia. https://doi.org/10.1371/journal.pone.0271409.s002

S1 Table. Relevant adaptations in the materials and methods applied for the AVIP A-E steps. https://doi.org/10.1371/journal.pone.0271409.s003

S2 Table. Ring-tailed lemurs ethogram. Working ethogram used in the study (adapted from [45–54]. Behaviors marked with "§" represent events. https://doi.org/10.1371/journal.pone.0271409.s004

S3 Table. Medians, interquartile range of the behaviours which resulted significantly different among sessions and Mann-Whitney U-test results performed at the individual level. Only behaviours which significantly differed or showed a tendency to differ are reported. https://doi.org/10.1371/journal.pone.0271409.s005

S4 Table. Customized ethical matrix. Adapted from [24]. https://doi.org/10.1371/journal.pone.0271409.s006

S1 Appendix. Animal welfare risk assessment. Tables (Tables A–E) used to perform the animal welfare risk assessment. https://doi.org/10.1371/journal.pone.0271409.s007

S2 Appendix. Human risk assessment. Tables (Tables A–F) used to perform the human risk assessment. https://doi.org/10.1371/journal.pone.0271409.s008

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Section 3. Wildlife management and ethical evaluation during COVID-19 emergency

3.1. The impact of Covid-19 pandemic on zoos and aquariums: assessing staff perception in Italian zoological facilities.

Adapted from:

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Abstract

At the end of 2019, the advent of COVID-19 drastically changed the working dynamics of millions of realities. Facilities operating in the animal care sector, such as zoos and aquariums, have been affected by the pandemic in many ways. This exploratory study tackled the perceived impact of the COVID-19 pandemic on Italian zoological facilities from the perspective of members of the staff. From June to November 2021, we administered a survey consisting of 14 questions and collected 107 answers, 61 from keepers and 46 from other zoological staff (e.g., directors, educators and guides). We analyzed data using the relative importance index (RII) to detect the most relevant issues that affected zoos and aquariums during the COVID-19 pandemic according to the two subgroups. We calculated the Spearman's rank correlation to assess the agreement order between keepers and other zoological staff. Results highlighted a shared concern regarding the economic damage caused by COVID-19 and its persistence in the future. Educational activities were also affected by the periods of lockdown and the difficulties caused by the emergency measures adopted to avoid the spread of the virus. We detected

some differences between the answers of keepers and other zoological staff. Still, despite their different roles, results showed that all the zoological staff agreed that the pandemic significantly impacted zoological facilities in all of their sectors. Building upon this exploratory study, a follow up survey may deepen the critical issues and gauge if they are going to persist even after the most critical pandemic period.

Introduction

After the onset of the COVID-19 pandemic in March 2020, zoos and aquariums (hereafter referred as zoological facilities) worldwide had to deal with prolonged closure periods due to lockdowns and restrictive measures adopted to reduce the spread of SARS-CoV-2. Italian zoological facilities (that at the time of the study were 35, Mite, 2021) firstly closed to the public from March to May 2020 (DPCM 2020, 8 March). After this first closure, they were granted the permission to reopen to visitors following targeted procedures, such as the introduction of a visitor cap and the adoption of specific health protocols (EAZA 2020; Gili, Vasconi & Gagliardi, 2021; Bandoli & Cavicchio, 2021). From November 2020 to April 2021, Italian zoological facilities faced multiple closure events according to local SARS-CoV-2 infection rates (DPCM 2020, 3 November). Only in late Spring 2021 they could reopen their gates but had to continue applying anti-COVID-19 measures according to National directives.

Notwithstanding, during the whole pandemic period zoological facilities could not suspend routine husbandry and care practices (e.g., feeding, enrichment provision, animal training, and veterinary surveillance) and continued to sustain daily expenditures to properly care for their animals (Pepper & Voigt, 2021). In Italy, with few exceptions, zoological facilities are private institutions, and they must comply with public companies budget, while retaining a role more similar to a museum than to an entertainment venue (Paknazar, 2021). Therefore, the care and maintenance of animals, the costs of the management and staff, and all the activities carried out are only based on ticket revenues without the support of any public funding (Gili et al., 2021). Therefore, with no financial income for several months, Italian zoological facilities sustained huge economic losses. Furthermore, the main activities regularly carried out by these institutions (i.e., research, conservation and educational activities) were forced to undergo remarkable changes to be in line with the pandemic situation.

As a consequence of the lockdown, the numerous closures imposed, and the restrictions on the number of visitors, zoological facilities faced several managerial and financial issues. In addition, the stressful working conditions experienced by the staff of these facilities and their psychological consequences also need to be considered. Although zoological facilities have been listed among the non-essential business, animal keepers have undoubtedly distinguished themselves as essential workers (Bandoli & Cavicchio, 2021). All the staff, from the directors to the keepers, continuously worked during the lockdown, taking care of the animals, trying to maintain the animals' well-being, and carrying out - as far as possible - conservation and education projects, despite the limits imposed by the pandemic. Most zoological facilities had to change their management procedures. The need to reduce staff members, create separate animal care teams, along with safety training courses, were some of the main changes put in place to prevent the risks of infection among staff members and ensure animal well-being. Moreover, after the first cases of animal infections in zoos (McAloose et al., 2020), new guidelines and protocols for routine husbandry practices have been introduced, making staff's daily tasks more stressful in some situations. We developed and administered an online questionnaire targeted at Italian zoo and aquarium staff personnel to investigate the general impact of the pandemic on the operation and activities of these facilities with a focus on animal management and staff profession.

Material and Methods

The survey was managed by the Ethics Laboratory for Veterinary Medicine, Conservation and Animal Welfare of Padua University in collaboration with the Unione Italiana dei Giardini Zoologici ed Acquari (Italian Union of Zoos and Acquariums, UIZA). The survey did not obtain any specific funding, and no personal information was collected. The data controller was the University of Padua, that has guaranteed that the data collected has been treated in compliance with EU Reg. No. 679/2016. No ethical approval was requested in the Country at the time of the study. The questionnaire was created with the collaboration of a team that included ethicists, veterinarians, zoologists, a sociologist, and a psychologist, and it was intended to be filled by staff members of zoos and aquariums. A first questionnaire was initially developed in November 2020 and sent to 38 participants to perform a pilot test until April 2021. However, after the pilot test, the emergency scenario in Italy changed, allowing zoological facilities to admit tourists. At the beginning of June 2021, the Ethics Laboratory for Veterinary Medicine, Conservation, and Animal Welfare organized and coordinated a meeting with the staff of Italian zoological facilities to discuss and share the difficulties encountered during the months of closure and the strategies put in place to deal with them. Therefore, the questionnaire was updated to reflect the new COVID-19 circumstances, and to include the issues that emerged during the national meeting. The final questionnaire was uploaded on Lime Survey, an open-source online statistical web program (https://www.limesurvey.org/). The Lime Survey link for the survey, active from June 2021 to November 2021, was sent via e-mail to every Italian zoo and aquarium, with the request of sharing it with all the staff through e-mail or chat. It was completely anonymous, and participants were free to leave it at any moment. The questionnaire consisted of 14 questions (four four-point Likert scale, nine closed-ended single-choice, and one open-ended question) divided into three parts. The first part aimed to identify the respondents' role within the facility (e.g., keeper, director, guide, etc.). The second part of the survey aimed to investigate the impacts of the COVID-19 pandemic on: a) zoos and aquariums in general, b) the staff's professional activities, and c) animal management. Finally, the last part of the survey included questions about the demographic characteristics of respondents (gender, age, educational background, pet ownership, and environmental association fellowship) and an open-ended question to allow them to leave their e-mail address or indications to better circumstantiate their answers.

Statistical Analysis

Data was exported from Lime Survey and analyzed using Excel and the software IBM SPSS Statistics 21.0 (IBM Corp, Armonk, NY). Descriptive statistics were presented as counts and percentages to summarize the collected data. To measure the impacts of COVID-19 pandemic on zoos and aquariums, the 4-Point Likert Scale answers were converted into numeric values: strongly agree = 4 points; agree = 3 points; disagree = 2 points; strongly disagree = 1 point. To detect agreements in the answers depending on the respondent's role, the dataset was divided into two subgroups, keepers (K) and other respondents (OW - All respondents excluding keepers). To compare the mean values of the variables, data was analyzed using the Relative Importance Index (RII). The Relative Importance Index (RII) specifies the ranked degree of importance of the issues examined. It is especially useful for questionnaires that use a Likert scale (Tholibon et al., 2021). The RII formula is showed in Eq. 1.

$$RII = \frac{\sum W}{A * N}$$

Where W is the respondent's weighting of each factor, which can range from 1 to 4. A represents the highest weight (in this case, 4), and the total number of people is labeled as N. The Relative Importance Index ranges from 0 to 1. The Higher the value of RII, the more important is that factor.

Finally, to assess the order of agreement between the two subgroups the Spearman's rank correlation was calculated.

Results

From the 194 responses obtained, we excluded questionnaires with incomplete answers (n=54) and questionnaires filled in by zoo and aquarium staff who do not directly deal with animals or who were not working at zoological facilities during the pandemic period due to the imposed closures (e.g., front office, waiters, etc.; n=33) for a final number of 107 responses.

Of the 107 participants, 53 (49.5%) were females, 52 (48.6%) males, and 2 (1.9%) preferred not to say. The participants' age ranged from 23 to 66 years (mean ± SD = 38.45 ± 10.34 years). 35.5% (n=38) of the participants were aged 23–32 years, 31.8% (n=34) were aged 33–42 years, 17.8% (n=19) were aged 43–52 years, 11.2% (n=12) were aged 53–62 years, 1.9% (n=2) were aged over 62, and 1.9% (n=2) preferred not to say. When asked to report their educational degree, 69.2% (n=74) of the participants had a university degree or post-university degree, 26.2% (n=28) had a high school degree and 4.7% (n=5) a secondary school degree. The majority of respondents (84.1%, n=90) stated to own a pet at home, while 15.9% (n=17) did not. Most of respondents (77.6%, n=83) where not associated with any environmental association.

The 107 respondents were mainly keepers (57%, n=61), and staff from the educational department (guides and educators, 15%, n=16) and from the research and conservation department (e.g., scientific managers, curators, etc., 11%, n=12). Table 1 reports the role of the respondents and the classification into the two subgroups, "Keepers" (K) and "Other Workers" (OW).

Table 1. Role and subgroups of the respondents within the facilities. In the right column, percentage and number of respondents are reported.

Role (Subgroup)	% (n)
Director (OW†)	6 (6)
Research / Conservation Department (OW†)	11 (12)
Educational Department (OW†)	15 (16)
Keeper (K‡)	57 (61)
Veterinarian (OW†)	9 (10)
University trainees (OW†)	2 (2)

†OW = Other Workers; ‡K = Keepers

The general impact of the COVID-19 pandemic on zoos and aquariums

The issue that was perceived to be the most relevant in affecting zoos and aquariums during the COVID-19 pandemic was the one related to environmental education activities (RII_{ALL}=0,818; RII_{K}=0,779; RII_{OW}=0,870). Keepers also identified the issue related to negative economic effects as the most relevant issue affecting zoological facilities and their workers, with an RII value of 0,799. The statement that obtained the lower RII value was "The government bodies have rescued and supported the animal heritage in its maintenance costs as it is a collective asset, and the economic support of visitors has ceased because of the pandemic" (RII_{ALL}=0,291; RII_{K}=0,291; RII_{OW}=0,310). The two subgroups showed less agreement in responses to the statement "The pandemic was an opportunity to rethink the different activities for visitors" with an RII difference of 0,248 (RII_{OW}=0,690; RII_{K}=0,443) (Table 2). There was a strong positive correlation on answers between K and OW (Spearman's rank correlation, $r=0.916$, $p<0.001$).

Table 2: Table 2 reports the frequencies of responses to each statement (where strongly agree = 4; agree = 3; disagree = 2; strongly disagree = 1), RII, and the item mean with Standard Deviation (SD) for all the respondents (ALL), keepers (K) and other workers (OW) to the question "How much do you agree with the following statements regarding the effects of the recent COVID-19 pandemic on zoos and aquariums?".

Statement	All respondents (ALL, n=107)							Keepers (K, n=61)						Other workers (OW, n=46)					
	Frequency of responses (n)				I don't know	RII	Item Mean (SD)	Frequency of responses (n)				RII	Item Mean (SD)	Frequency of responses (n)				RII	Item Mean (SD)
	1	2	3	4				1	2	3	4			1	2	3	4		
To face the emergency, Italian zoological facilities did not cooperate to highlight the issues caused by the pandemic	1	17	35	24	28%	0,551	2,206 (2,230)	0	8	19	13	0,512	2,049 (2,165)	1	9	16	11	0,603	2,413 (2,313)
The pandemic has strengthened the connection between zoological facilities and local communities.	17	33	21	4	30%	0,379	1,514 (1,498)	8	22	9	1	0,340	1,361 (1,343)	9	11	12	3	0,429	1,717 (1,681)
The pandemic, the related restrictive measures, and the economic issues have damaged conservation projects supported by zoos and aquariums.	3	5	63	26	9%	0,715	2,860 (2,558)	3	1	35	12	0,648	2,590 (2,416)	0	4	28	14	0,804	3,217 (2,735)
During the pandemic, the educational activities were able to continue in alternative forms.	24	43	27	9	4%	0,530	2,121 (1,824)	12	28	12	5	0,508	2,033 (1,756)	12	15	15	4	0,560	2,239 (1,911)

The government bodies have rescued and supported the animal heritage in its maintenance costs as it is a collective asset, and the economic support of visitors has ceased because of the pandemic	56	33	2	0	15%	0,299	1,196 (0,854)	31	17	2	0	0,291	1,164 (0,868)	25	16	0	0	0,310	1,239 (0,834)
Local administrations have supported zoos and aquariums of their territory	44	34	7	2	19%	0,329	1,318 (1,119)	23	20	4	1	0,324	1,295 (1,116)	21	14	3	1	0,337	1,348 (1,123)
Restrictions adopted due to the pandemic decreased the educational effectiveness of zoo visits	8	14	39	37	8%	0,703	2,813 (2,569)	7	9	19	18	0,631	2,525 (2,388)	1	5	20	19	0,799	3,196 (2,790)
The pandemic was an opportunity to rethink the different activities for visitors	7	21	46	12	20%	0,549	2,196 (2,078)	6	10	22	4	0,443	1,770 (1,811)	1	11	24	8	0,690	2,761 (2,387)
The pandemic was an opportunity to implement digital communication tools for zoos and aquariums to stay connected to visitors	8	7	48	29	14%	0,659	2,636 (2,465)	5	4	30	11	0,602	2,410 (2,290)	3	3	18	18	0,734	2,935 (2,678)
The pandemic has compromised the social role of zoos and aquariums	9	24	33	8	31%	0,439	1,757 (1,788)	5	13	18	3	0,398	1,590 (1,669)	4	11	15	5	0,495	1,978 (1,934)

The closures imposed by the pandemic have affected the educational role of zoos and aquariums	2	12	48	38	7%	0,752	3,009 (2,679)	0	5	33	16	0,709	2,836 (2,561)	2	7	15	22	0,810	3,239 (2,828)
Zoos and aquariums, and the people who work there, have been very negatively affected by the COVID-19 pandemic from an economic point of view.	0	7	41	53	6%	0,815	3,262 (2,894)	0	5	28	24	0,779	3,115 (2,764)	0	2	13	29	0,864	3,457 (3,057)
The dissemination of scientific knowledge of zoos and aquariums' host species has been negatively affected by the pandemic	1	8	46	43	8%	0,764	3,056 (2,748)	1	4	28	22	0,742	2,967 (2,686)	0	4	18	21	0,793	3,174 (2,828)
The pandemic has had a medium / long-term adverse effect on nature conservation interventions.	0	19	51	30	7%	0,727	2,907 (2,565)	0	14	28	16	0,721	2,885 (2,522)	0	5	23	14	0,734	2,935 (2,621)
The pandemic and the related restrictive measure affected environmental education activities.	0	5	56	43	3%	0,818	3,271 (2,838)	0	5	32	21	0,779	3,115 (2,728)	0	0	24	22	0,870	3,478 (2,978)
During the pandemic, zoos and aquariums lacked public support.	0	12	49	32	13%	0,699	2,794 (2,561)	0	5	36	16	0,746	2,984 (2,618)	0	7	13	16	0,636	2,543 (2,485)
The pandemic has increased fear of contracting diseases (COVID-19 and/or other) through animal contagion.	7	37	37	11	14%	0,551	2,206 (2,000)	6	25	15	5	0,496	1,984 (1,811)	1	12	22	6	0,625	2,500 (2,226)

The pandemic has increased the public consideration of the social role of zoos and aquariums.	11	41	20	5	28%	0,404	1,617 (1,565)	7	22	12	1	0,373	1,492 (1,449)	4	19	8	4	0,446	1,783 (1,707)
In issuing provisions to deal with the pandemic, the government bodies considered the peculiarities of zoos and aquariums.	59	34	6	0	7%	0,339	1,355 (0,986)	31	19	3	0	0,320	1,279 (0,958)	28	15	3	0	0,364	1,457 (1,022)

2

The impact of the COVID-19 pandemic on Zoological facilities staff's professional activities

Most respondents did not convert their work into smart working (71%, n=76). Of those who converted their daily work to smart working (29%, n=31), they converted it from 10% to 100% (mean ± SD = 47,58% ± 26,54). When asked how smart working affected them on a scale from 0 to 5 (where 0 = at all and 5 = a lot), results showed that the average evaluation is 2,89 (where 7% of respondents (n=2) reported 0, 14% (n=4) 1, 10% (n=3) 2, 34% (n=10) 3, 21% (n=6) 4, and 14% (n=4) reported that they were significantly affected by selecting 5).

The issue that was perceived to be most relevant in affecting the zoos and aquariums staff's profession during the COVID-19 pandemic, and that will continue to be significant in the future, was the economic damage (RII_{ALL}=0,755; RII_{K}=0,758; RII_{OW}=0,750). All respondents and OW identified the risk of infection due to handling potentially infected animals as the least relevant issue (RII_{ALL}=0,371; RII_{OW}=0,391). Whereas keepers identified the statement "From the very first moments of emergency management, I felt supported by the network of zoos and aquariums" as the least relevant factor in affecting the zoos and aquariums staff's profession during the COVID-19 (RII_{K}=0,352). The two subgroups showed less agreement in responses to the statement regarding the possibility of contributing to the decisions made by the facility during the emergency, with an RII difference of 0,174 (RII_{OW}=0,641; RII_{K}=0,467). There was a positive correlation between the items between keepers and OW (Spearman's rank correlation, r=0.795, p=0.010).

Table 3: Tables 3 reports the frequencies of responses to each statement (where strongly agree = 4; agree = 3; disagree = 2; strongly disagree = 1), RII, and the item mean with Standard Deviation (SD) for all the respondents (ALL), keepers (K) and other workers (OW) to the question "How much do you agree with the following statements regarding the effects of the COVID-19 pandemic on your professional activity?".

Statement	All respondents (ALL, n=107)							Keepers (K, n=61)						Other workers (OW, n=46)					
	Frequency of responses (n)				I don't know	RII	Item Mean (SD)	Frequency of responses (n)				RII	Item Mean (SD)	Frequency of responses (n)				RII	Item Mean (SD)
	1	2	3	4				1	2	3	4			1	2	3	4		
Even after the return to the normal situation pre-covid, there will continue to be significant economic damage to the sector in which I work	0	4	93	9	1%	0,755	3,019 (2,510)	0	2	55	4	0,758	3,033 (2,502)	0	2	38	5	0,750	3,000 (2,519)
It made me uncomfortable to manage people's fear of the possibility that the animals I care for could infect humans	30	52	17	2	6%	0,451	1,804 (1,466)	26	24	9	2	0,447	1,787 (1,437)	4	28	8	0	0,457	1,826 (1,504)
I felt at risk of interpersonal contagion due to the way in which my profession was exercised	21	51	26	6	3%	0,526	2,103 (1,756)	13	23	21	2	0,533	2,131 (1,793)	8	28	5	4	0,516	2,065 (1,707)
During the emergency situation, I have been able to give my contribution to the decisions implemented by the facility in which I work	21	20	37	15	13%	0,542	2,168 (2,032)	15	14	17	5	0,467	1,869 (1,765)	6	6	20	10	0,641	2,565 (2,341)

Due to the pandemic, I had to start wearing special protective equipment to take care of specific exhibits, and this made me uncomfortable	14	48	26	15	4%	0,579	2,318 (2,009)	8	26	17	10	0,619	2,475 (2,119)	6	22	9	5	0,527	2,109 (1,853)
The consequences of the pandemic, in general, are more negative for my work with wild animals than for other work fields with animals.	9	38	24	11	23%	0,470	1,879 (1,814)	4	26	15	3	0,463	1,852 (1,708)	5	12	9	8	0,478	1,913 (1,945)
I felt at risk of contagion from handling potentially infected animals	46	52	3	0	6%	0,371	1,486 (1,068)	33	24	2	0	0,357	1,426 (0,992)	13	28	1	0	0,391	1,565 (1,161)
The extraordinary work organization measures taken during the emergency had a negative influence on my work	14	27	50	14	2%	0,640	2,561 (2,209)	8	22	24	5	0,590	2,361 (2,016)	6	5	26	9	0,707	2,826 (2,441)
From the very first moments of emergency management, I felt supported by the network of zoos and aquariums	16	36	25	2	26%	0,400	1,598 (1,516)	11	23	7	2	0,352	1,410 (1,355)	5	13	18	0	0,462	1,848 (1,707)

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The impact of the COVID-19 pandemic on animal management

The issue that was perceived to be most relevant in affecting the management of the animals during the COVID-19 pandemic was the financial loss that forced the postponement of structural and organizational investments aimed at promoting animal welfare (RII_{ALL}=0,759; RII_{K}=0,717; RII_{OW}=0,815). This statement also showed less agreement between the two subgroups, with an RII value difference of 0,174 between keepers and OW. The statement "In issuing provisions to combat the pandemic, the governing bodies have taken into account the fact that most of the animals present in the zoos and aquariums are owned by the facilities themselves", was identified as the least relevant issue (RII_{ALL}=0,278; RII_{K}=0,283; RII_{OW}=0,272). There was a positive correlation between the items between Keepers and Other Workers (Spearman's rank correlation, $r=0.969$, $p<0.001$).

Table 4: Tables 4 reports the frequencies of responses to each statement (where strongly agree = 4; agree = 3; disagree = 2; strongly disagree = 1), RII, and the item mean with Standard Deviation (SD) for all the respondents (ALL), keepers (K) and other workers (OW) at the question "How much do you agree with the following statements regarding the effects of the COVID-19 pandemic on animal management?".

Statement (management)	All respondents (ALL, n=107)							Keepers (K, n=61)						Other workers (OW, n=46)					
	Frequency of responses (n)				I don't know	RII	Item Mean	Frequency of responses (n)				RII	Item Mean	Frequency of responses (n)				RII	Item Mean
	1	2	3	4				1	2	3	4			1	2	3	4		
It was a source of discomfort to take extraordinary measures to minimize the risk of contagion for the animals	10	48	31	9	8%	0,549	2,196 (1,909)	7	31	16	4	0,545	2,180 (1,838)	3	17	15	5	0,554	2,217 (2,000)
The animal food supply was a cause for concern	7	23	42	28	7%	0,680	2,720 (2,434)	6	15	24	13	0,656	2,623 (2,326)	1	8	18	15	0,712	2,848 (2,571)
The increased use of protective devices and disinfectants has generally had a negative impact on animals	13	64	8	4	17%	0,423	1,692 (1,447)	9	40	6	0	0,439	1,754 (1,379)	4	24	2	4	0,402	1,609 (1,532)
In issuing provisions to combat the pandemic, the governing bodies have taken into account the fact that most of the animals present in the zoos and aquariums are owned by the facilities themselves	31	37	2	2	33%	0,278	1,112 (1,014)	13	23	2	1	0,283	1,131 (1,071)	18	14	0	1	0,272	1,087 (0,933)
Restrictive measures have caused an increased risk of behavioral problems for the animals	19	36	27	6	18%	0,458	1,832 (1,691)	16	23	14	1	0,443	1,770 (1,526)	3	13	13	5	0,478	1,913 (1,888)

The pandemic and related restrictive measures have forced changes in animal training activities	3	15	46	31	11%	0,689	2,757 (2,517)	2	8	26	19	0,705	2,820 (2,561)	1	7	20	12	0,668	2,674 (2,458)
The restrictive measures have caused a decrease in the risk of behavioral problems for the animals kept	9	51	16	1	28%	0,381	1,523 (1,401)	5	30	10	0	0,389	1,557 (1,403)	4	21	6	1	0,370	1,478 (1,399)
The economic consequences of the pandemic forced the postponement of structural and organizational investments aimed at promoting animal welfare	0	6	43	46	11%	0,759	3,037 (2,772)	0	4	25	23	0,717	2,869 (2,667)	0	2	18	23	0,815	3,261 (2,904)
Animal welfare was positively influenced by the increase in the use of protective equipment and disinfectants	12	45	14	3	31%	0,364	1,458 (1,401)	5	26	10	3	0,406	1,623 (1,558)	7	19	4	0	0,310	1,239 (1,161)
Due to the pandemic, animal movement/exchange between facilities caused concern	0	8	47	34	17%	0,685	2,738 (2,569)	0	3	29	17	0,660	2,639 (2,509)	0	5	18	17	0,717	2,870 (2,646)

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Discussion

Zoological facilities host and manage thousands of animal species, even endangered ones, with the aim of promoting conservation, education, and research activities. SARS-CoV-2 pandemic has caused an unexpected and deep global crisis affecting zoos and aquariums worldwide at different levels. During the pandemic, zoological facilities were forced to close their gates for several months. However, the constant care of the animals could not be stopped. Consequently, Italian zoological facilities, which mostly rely on tickets revenue, faced operational and ethical challenges (Bandoli & Cavicchio, 2021). Although each institution has its own peculiar characteristics, and each of them faced unique challenges (Paknazar, 2021), the questionnaire aimed to deepen the main issues faced at the Italian level through a nationwide survey.

According to Fine et al., 2021, Bandoli & Cavicchio, 2021 and Gili et al., 2021, one of the most important impacts the pandemic caused on zoological facilities was the financial one. Even without visitors, zoological facilities sustained costs to manage the staff and the animal heritage (Bandoli & Cavicchio, 2021, Gili et al., 2021). This impact was even more substantial on Italian zoological facilities, which are mainly private and have not received financial support from national and local authorities. Our results further highlighted how the pandemic negatively impacted zoos and aquariums in Italy from an economic point of view. According to respondents, these impacts will have consequences even after the pandemic, resulting as one of the most relevant issues from the survey answers. Therefore, the negative economic impact has forced Italian zoological facilities to postpone investments aimed at promoting conservation and animal welfare. However, this last aspect affected the two subgroups differently, with keepers being more concerned than the other staff members.

Most respondents indicated that the authorities, which play a regulated role in the Nature Conservation Efforts, did not support zoos and aquariums of their territory in issuing provisions to deal with the pandemic and did not take into account that most of the animals are owned by the facilities themselves, or that they host wild animals seized from illegal trade.

In Europe, the European Association of Zoos and Aquaria (EAZA), during the pandemic period, started a program of cooperation and mutual assistance to avoid Member Facilities going bankrupt and thus-guaranteeing the welfare of all hosted animals. EAZA encouraged its members to cooperate as much as possible by creating a community initiative that included a “COVID-19 subgroup" of the EEP (EAZA Ex situ Programme) Committee to respond to emergencies in a tailor-made way (EAZA, 2020b). However, when asked if they felt enough supported by the network of zoos and aquariums to face the emergency in Italy, respondents agreed that the support was not enough. Moreover, they felt that no common front between the zoological facilities took place to highlight the difficulties. Consequently, also the connection that zoological facilities had with the territory and local communities they belong to was compromised.

Furthermore, considering the social role of zoological facilities in the education of people and nature conservation, respondents agreed that the pandemic had a negative impact on the social role of zoos and aquariums. Moreover, they considered that not only the support of associations was lacking, but also the support of the public throughout the pandemic period.

This result contrasts with the findings reported by Fine et al., 2021, where the staff indicated that public engagement had changed because of the COVID-19 pandemic and felt that the pandemic had brought conservation issues to the forefront of public consciousness. Moreover, this result also contrasts with Bandoli & Cavicchio, 2021 and Gili et al., 2021, in which the authors describe that to face the lost revenues, many Italian zoological facilities launched successful public fundraising activities and species adoption programs, obtaining support from the public.

As mentioned above, one of the main goals of the zoo and aquarium community is educating visitors and general public, giving them opportunities for leaning, understanding and connecting to nature (EAZA, 2016). During the pandemic period, zoological facilities have developed new educational activities in order to maintain their social role of education. And, even during the reopening of the zoological facilities, most of the pre-pandemic educational activities were modified to ensure the safety of visitors and educators. However, although respondents agreed that the pandemic was an opportunity to implement digital communication tools to stay connected with visitors, they did not agree that educational activities were able to continue. Therefore, respondents indicated that the promotion of scientific knowledge and environmental education activities were negatively affected by the pandemic and the related closures, and that the new modalities of the visits due to the restrictions decreased the educational effectiveness of the visit itself. Interestingly, the other workers mainly agreed that the pandemic was an opportunity to rethink the different activities for visitors, whereas keepers mainly disagreed, showing an important difference between their RII values.

Even if the assessment of COVID-19 pandemic effects on nature conservation is beyond the scope of this analysis, also in our study respondents agreed that the animal transfer between facilities for conservation purposes caused concern as already highlighted by other researchers (Fine et al., 2021). Based on the results of the survey, and according to Fine et al. (2021), also the provisioning of food for the animals was a cause of concern.

Notwithstanding, keepers took constantly care of the animals housed in the facilities, maintaining high standards of animal welfare during the whole pandemic. Moreover, during the periods of closures and reopening, researchers were able to study the visitor effect on several animal species, monitoring the welfare of the animals in the absence of visitors for prolonged periods (for example, Williams, Carter, Rendle & Ward, 2021; Riley et al., 2021). When asked Italian zoological facilities staff if in their opinion the restrictive measures caused a risk for an increase of behavioral problems for the animals, Italian respondents showed two different and conflicting opinions. They disagreed that the restrictive measures caused an increased risk of behavioral problems. However, they also agreed that the restrictive measures caused a decrease in the risk of behavioral problems for the animals. These conflicting answers could reflect differences at the facility or species/individual levels.

After the first cases of SARS-CoV-2 transmission from keepers to zoo animals, animal care staff had to implement measures (e.g., protective equipment and disinfectants) to reduce the risk of infecting the animals under their care (Mathavarajah, Stoddart, Gagnon & Dellaire, 2021; EAZA, 2020c). Moreover, several studies showed how the COVID-19 pandemic negatively affected mental health both worldwide and in the Italian population (Rossi et al., 2020). Stress, anxiety, depression, insecurities, and the risk of contagion could even be exacerbated after the discovery of the keeper-to-animal transmission risk. In line with these studies, respondents of our survey agreed that the extraordinary measures taken during the emergency negatively influenced their daily work, indicating it as one of the three most critical issue during the pandemic.

Therefore, checking for the staff working condition and the related stress, respondents mainly disagreed that they felt at risk of interpersonal contagion or - in particular keepers - at risk of contagion when handling potentially infected animals. Keepers also disagreed that they felt uncomfortable in wearing special protective equipment to take care of specific exhibits or that it was a source of discomfort to take extraordinary measures to minimize the risk of contagion.

Strenghts, limitatons and future developments

Although exploratory, our study allowed to detect key issues which affected Italian zoological facilities during and immediately after the COVID-19 pandemic and, despite the differences in their roles, keepers, directors, educator and researchers mainly detected the same issues, showing that the pandemic deeply affected zoological facilities. However, this study had limitations. Firstly, the questionnaire was directed to Italian zoological facilities in general and the study did not explore individual differences between the facilities or differences between zoos and aquariums. It would be important to explore the critical issues that were detected in the light of these differences. Secondly, despite the significant effort to communicate this research initiative to the target audience, the percentage of respondents were low. This could be explained by the emergency in itself, not easily allowing zoos' staff to dedicate time and energy to this type of investigation. Notwithstanding, despite the limited number of surveys collected there are few cases in which respondents did not express their opinion selecting the response "I do not know". This can be explained by the fact that the staff wanted to contribute by giving their opinion on the challenges faced and that have affected their daily work. Finally, yet importantly, the survey was just focusing on a specific period. It would be important to propose a similar survey to deepen the critical issues that were detected and understand if they are closely linked to the pandemic period or if they will continue to affect zoos and aquariums even after the crisis abated.

Conclusions

In this study we investigated the effects of the COVID-19 pandemic on Italian zoos and aquariums focusing on the impact on animal management and on the professional activities of the staff. The study was performed using a questionnaire, specifically developed for the staff working at zoological facilities (director, keepers, educational and research department). We detected some differences between the answers of keepers and other zoological staff. Still, despite the differences in their roles, results showed that all the zoological staff agreed that the pandemic deeply affected zoological facilities. Building upon this exploratory study, a follow up survey may deepen the critical issues and gauge if they are going to persist even after the most critical pandemic period.

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1

General Discussion

This Ph.D. thesis presents a framework for the ethical analysis of biodiversity conservation actions and wildlife management under different conditions: the application of Conservation ART, Animal-Visitor Interaction activities, and wildlife management during emergencies. Due to the pluralistic nature of biodiversity conservation, the need to consider the pluralism of points of view when dealing with wildlife conservation and management is urgently needed, as the value conflicts can be morally problematic (Cortes-Capano et al., 2022). However, there are different and sometimes conflicting ethical approaches to conservation (Gamborg et al., 2012; Biasetti & de Mori, 2021). Conservation ethics, for instance, allows to consider various dimensions of value, the safety of the people involved, the quality of procedures, animal welfare, and so on, to anticipate the critical aspects that can compromise the ethical acceptability of a conservation project. Therefore, evaluating the ethical acceptability of conservation projects—concerning the conservation mission, the animals' welfare, the people involved, and public opinion—could help discriminate between those projects conducted responsibly and those not. Consequently, intervening in the possible critical factors before their eventual occurrence could help maintain the reputation of the whole conservation project and its social importance and support. In this thesis, different ethical and multidisciplinary frameworks have been used to collect the ethical and all the other relevant factors to identify issues and value conflicts. The tools presented provide a way to reflect on wildlife management procedures or wildlife conservation projects to make responsible and balanced decisions, taking into consideration the pluralisms of point of views.

As Conservation ART for endangered taxa becomes increasingly common, the need to explore their ethical implications becomes even more crucial. Ethical evaluation of Conservation ART can contribute to ensuring the success of conservation projects and protecting the welfare of the concerned animals. Moreover, it contributes to raising the quality of procedures on and research with wildlife, fostering consistency, transparency, and communication among the stakeholders. The BioRescue project analyzed in the first section of this Ph.D. thesis is exemplary in this sense. This project is developing and testing new approaches in the conservation of a "technically extinct" species, the

Northern White Rhinoceros (*Ceratotherium simum cottoni*). The application and combination of different ethical and self-assessment tools have made it possible to evaluate the procedures involved and complex scenarios showing the ability of these tools to structure decision-making processes, provide an organized framework for collecting relevant information, and anticipate potential risks associated with the application of Conservation ARTs. By recognizing the pluralism of opinions and values, and by the collection and organization of different points of view throughout different ethical tools, it was, therefore, possible to reflect on the ramifications of potential decisions and build a shared, transparent, and reasoned justification of the chosen option, making the decision-making process explicit. The use of the Ethical Matrix offers several advantages in conservation (Biasetti & de Mori, 2019, 2021). As also evidenced by the various EM applications in this thesis, thanks to the EM it is possible to gather and organize principles and points of view of the different stakeholders involved, allowing for a more thought-out approach for assessing complex moral scenarios where different needs, interests, and ethical concerns may conflict.

In the first study presented in Section 1 (Biasetti et al., 2022a), the EM was used to collect the ethically relevant factors to identify issues and value conflicts in projects involving endangered species. Therefore, to provide a framework for evaluating ART procedures, OPU procedures performed on the northern white rhinoceros have been used as an example of its application. The EM has been used also with other tools (i.e., Decision trees and Bateson Cube) to integrate inputs from different data collection methods and stakeholders and reach a final decision on Najin's future role in the project (Biasetti et al., 2022b). Therefore, the EM provided a map of the value demands so that it was possible to compare the ethically relevant issues raised by each viable option, highlighting the pros and cons. The DT made it possible to identify the ramifications of the choices and to classify them based on their possibility of realization and ethical desirability. The use of BC made it possible to model the data collected in the survey, establish a simple threshold for ethical acceptability, and adjudicate the three options accordingly. Finally, the combined use of the three ethical tools showed that the only option considered ethically acceptable was to suspend interventions on Najin and to reshape her role in the project outside of oocyte donation.

Finally, the results of the applications of ETHAS (de Mori et al., 2021) to assess the implementations of Conservation ART procedures (OPU, embryo transfer, etc.) showed that the new tool, developed iteratively with different stakeholders, contributed to raising the quality of procedures, and to foster consistency, transparency, and communication among the involved project partners. Moreover, it has been shown that the implementation of ETHAS before each veterinary procedure allowed for the mitigation of potential procedure-associated risks for the animals, the staff, and the biomaterials and ensured– by taking into consideration potential risk factors – the animal welfare. Importantly, ETHAS also allowed taking into consideration even factors such as ARTs procedure overall sustainability, possible positive social consequences, scientific and technological contributions, and research ethics issues. ETHAS, thanks to the self-assessment it foresees, for each implementation of the procedures provides the necessary means to assess acceptability in research and veterinary requirements and in relation to the identification, evaluation, and mitigation of potential procedure-associated risks and animal welfare issues.

The assessment of various types of Animal-Visitor Interactions in zoological facilities was another area taken into account by this Ph.D. As required by WAZA (WAZA, 2016), these activities need to be regularly assessed in order not to jeopardize the zoological facilities' educational, conservation, and research aims. Moreover, ethical questions have been raised around AVIs due to welfare concerns they can pose (Learmonth et al., 2021; Spooner et al., 2021). AVIs can be considered justifiable if there are educational benefits and no negative impacts on welfare (Spooner et al., 2021). In this sense, Section 2 presents ethical and multidisciplinary approaches for the assessment of AVIs in South Africa and Italian zoological facilities.

The usefulness of using an EM to support a participatory process is well known (Kaiser & Forsberg, 2001; Kaiser et al., 2007), even in analyzing other wildlife management procedures, such as AVIs (Biasetti et al., 2020; de Mori et al., 2019). As the structure of the EM encourages the participants to imagine themselves in the shoes of others, ensuring, as much as possible, a plural and comprehensive collection of the relevant value demands, in Section 2, the EM has been combined with a participatory approach - a workshop and two different surveys - to map the ethical issues of AVIs in South Africa. The integration of the participatory process results in the first sketch of the EM, allowed to develop a final EM that considered most of the stakeholders point of view. Interestingly, the final EM highlighted that animal welfare is crucial for every stakeholder. Many other relevant issues related to AVIs were highlighted, such as visitors' education, biodiversity conservation, sustainability, and impact on scientific research. This study, therefore, showed both the importance of an interdisciplinary approach to assess AVIs, the need to integrate several different points of view in such assessments, and of developing scientific assessment tools capable of evaluating the welfare of wild animals involved in AVIs considering the peculiar semi-captive and free contact management conditions (Muzzo et al., 2023). The latter result was further confirmed by the second study presented in Section 2 (Pollastri et al., 2021). The QBA adjectives proposed to describe the elephants' demeanour developed in zoo conditions differed from those used to describe it in semi-captive, and the experts' scatterplots demonstrated a degree of separation between the two management systems. Moreover, this second study of section 2 suggests that even non-experts in animal behavior and welfare (i.e., children and naive adults) can have a rough perception of the general emotional state of the animals and, thus, of their welfare. This result agrees with other studies on QBA that highlighted the importance of involving participants from different backgrounds and familiarity with the studied animal species to obtain a balanced animal welfare assessment (Wemelsfelder et al., 2012; Napolitano et al., 2012; Duijevesteijn et al., 2014). Moreover, this result further emphasizes the need to integrate different stakeholders in ethical decision-making regarding wildlife management.

Following WAZA recommendations and the "One Health, One Welfare" approach, it has been applied AVIP (Normando et al., 2018; de Mori et al., 2019) to assess another kind of AVI. AVIP considers a multidisciplinary and holistic approach to measure both animal and human risk assessments, physiological and behavioral animal welfare, and human well-being outcomes. The ethical assessment, by comparing the obtained results with the content of an Ethical Matrix, informs whether the interaction is ethically justifiable. Therefore, Section 2 presents the first application of AVIP to assess a walk-in enclosure involving non-human primates (Pollastri et al., 2022). This application, in addition to the previous ones (Normando et al., 2018; de Mori et al., 2019), showed that a multidisciplinary approach, combined with an ethical assessment, could help zoological facilities in addressing issues related to AVIs and to take preventive and precautionary measures to preserve and protect the welfare, health and safety of the animals, the staff and the visitors. Thus, applying AVIP protocols help zoological facilities to follows the WAZA guidelines and perform a transparent evaluation of these activities, to give them the opportunity of preserve the health and welfare of animals and visitors, and to consider stakeholders point of view in the final ethical evaluation.

The results highlighted that there was no need to discontinue the assessed AVI as lemurs, visitors, and staff needs were respected. From an ethical point of view, and from the comparison between the ideal situation described in the EM and the results obtained with the A-D steps, it was possible to conclude that the zoo addressed the few highlighted concerns notwithstanding the complex framework of the AVIs.

The results of the last study presented may be useful in understanding the impact of the COVID-19 pandemic on Italian zoological institutions. Given the research, educational, and conservational role of zoological facilities, this study highlighted the considerable impact the pandemic had on them. The lack of fees paid by the visitors impacted the conservation to some extent, as well as the changing in the educational modalities modified visitor education. Given the results of the study and its limitations, it would be very interesting to perform a similar study to understand if the obtained results are directly linked to the pandemic period or if they reflect a more general situation of zoological facilities in Italy.

Conclusion

This doctoral thesis aims to demonstrate how an ethical evaluation is crucial in wildlife management and conservation. Implementing ethical frameworks and tools in conservation allows an understanding of the decision-making processes and constraints related to solutions, and consequences. Therefore, the ethical analysis makes it possible to face wildlife management and conservation challenges by taking into account all the interested parties and correctly planning the conservation efforts. Due to the pluralistic nature of conservation, the ethical evaluation – as shown throughout this thesis – allows to include in decision-making process several point of view and values. The use of multidisciplinary, holistic and ethical tools allows for the sharing of expertise between scientists, conservationists, ethicists, and other stakeholders, and take ethically sound and transparent decisions in wildlife management and conservation, raising the ethical standards and setting the bar of wildlife conservation projects and management as high as possible.

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