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Integrating behavioral evidence and structured ethical assessment in conservation ARTs

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Article

Keywords: Animal Behavior, Northern White Rhinoceros, Ethical Evaluation, Assisted Reproductive Technologies, Wildlife Conservation

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11555567/v1

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Additional Declarations: No competing interests reported.

Abstract

Conservation efforts increasingly complement traditional strategies with novel biotechnological interventions where appropriate. Among these, assisted reproductive technologies (ARTs) have emerged as an important component of the conservation toolbox. Their application to wildlife conservation, however, extends beyond technical considerations, potentially exposing individual animals to pain, distress, physiological effects, and cumulative burdens that require careful ethical consideration. Conservation ARTs thus rais in this sense a significant ethical challenge, one that in fact cuts across much of conservation ethics: how to reconcile species conservation interests with individual animal welfare. This highlights the need for ethical frameworks capable of navigating these potentially competing values. The robustness of this process, however, depends not only on the ethical principles that guide it but also on the quality of the empirical evidence available to inform it. In this study, we illustrate how behavioral evidence can be integrated with structured ethical assessment to support robust and transparent ethical decision-making when using conservation ARTs. Using the Northern White Rhinoceros (*Ceratotherium simum cottoni*) as an illustrative example, we show how behavioral observations can provide ethically relevant evidence to strengthen the ethical evaluation of conservation interventions. Ultimately, this approach supports more transparent and evidence-informed decision-making in conservation programs relying on ARTs.

1. Introduction

The accelerating loss of biodiversity represents a major global challenge. Human-driven pressures, including habitat loss, agricultural expansion, overexploitation, pollution, invasive species, and climate change, continue to drive population declines and species extinctions worldwide [1–3]. Current extinction rates across several taxa are estimated to be between 100 and 1000 times higher than background levels, raising concerns that the Earth may be entering a sixth mass extinction [4–7]. In response, conservation efforts increasingly complement traditional strategies with novel biotechnological interventions where appropriate. Among these, assisted reproductive technologies (ARTs) have emerged as an increasingly important component of the conservation toolbox, offering new opportunities to preserve genetic diversity, facilitate reproduction, and support the recovery of critically endangered species [8–14].

The application of ARTs to wildlife conservation, however, extends beyond technical considerations. Their implementation often requires repeated handling and veterinary interventions, which may include restraint, anesthesia, and invasive procedures, all of which may affect the welfare of the animals involved. Although these interventions are typically undertaken to secure important conservation outcomes, they may expose individual animals to pain, distress, physiological effects, and cumulative burdens that require careful ethical consideration [15–21]. The application of conservation ARTs raises in this sense a significant ethical challenge, one that in fact cuts across much of conservation ethics: how to reconcile the interests of species conservation with the welfare of the individual animals on whom these interventions depend. While the primary goal of conservation has traditionally been the

survival of species and populations [22], ethical decision-making must also take due account of the welfare of the individual animals involved [23–26]. This highlights the need for decision-making frameworks capable of navigating these potentially competing values.

These frameworks should enable decision-makers to identify ethically relevant issues, evaluate alternative courses of action, and ultimately arrive at transparent and ethically justifiable decisions [27]. The robustness of this process, however, depends not only on the ethical principles that guide it but also on the quality of the empirical evidence available to inform it. Without reliable information on the consequences of conservation interventions for the animals involved, ethical evaluations risk being based on assumptions rather than evidence.

Among the different sources of empirical evidence available to inform ethical evaluations, behavioral observations are particularly valuable. Behavior can provide direct evidence of how animals respond to conservation interventions [28], allowing welfare impacts to be assessed from observed responses of the single individuals rather than inferred solely from the characteristics of the intervention. Empirical evidence therefore plays a critical role in ensuring that ethical deliberation remains responsive to the actual consequences of conservation interventions. In particular, behavioral monitoring can reveal both immediate and longer-term responses to repeated interventions [29], providing ethically relevant evidence for evaluating whether such interventions remain proportionate and justifiable over time.

Conservation efforts aimed at saving the northern white rhinoceros (*Ceratotherium simum cottoni*; hereafter NWR) provide a unique opportunity to apply this approach. Following decades of population decline driven primarily by poaching, only two females, Najin and Fatu, remain alive, neither of which is capable of reproducing naturally [30–34]. Consequently, the taxon is considered functionally extinct, and its survival depends on the application of ARTs, including repeated ovum pick-up (OPU) procedures, in vitro embryo production using cryopreserved sperm, and embryo transfer to southern white rhinoceros (*Ceratotherium simum simum*; hereafter SWR) surrogate females [11, 35]. The repeated nature of these interventions creates a particular context in which the recovery of the taxon depends on veterinary procedures performed on its last surviving individuals. This context offers a significant opportunity to examine how empirical behavioral evidence can inform ethical decision-making.

In this study, we illustrate how behavioral evidence can be integrated with structured ethical assessment to support robust and transparent ethical decision-making when applying conservation ARTs. For the integrated ethical analysis, the ETHical ASsessment (ETHAS) tool was used to identify procedural risks, welfare concerns, and ethical dilemmas at each stage of the procedure [17].

Using the NWR as an illustrative example, we show how behavioral observations can provide ethically relevant evidence to strengthen the ethical evaluation of conservation interventions. Ultimately, this approach supports more transparent and evidence-informed decision-making in conservation programs relying on ARTs.

2. Materials and methods

This study combined longitudinal behavioral monitoring of the last two NWR with the application of the ETHAS ethical self-assessment tool [17] in OPU procedures. The two sources of evidence were integrated to examine how behavioral evidence collected through repeated and prolonged observations can inform structured ethical assessment.

2.1 Ethical statement

The study was conducted in full compliance with the “Guidelines for the ethical treatment of nonhuman animals in behavioral research and teaching” [36], the “WAZA Code of Ethics” [37], and the “Italian Legislative Decree 26/2014” [38], which transposes European Directive 2010/63/EU [39] on the protection of animals used for scientific purposes.

Behavioral data collection was based on purely observational methods that did not interfere with the animals’ husbandry and management routines. Therefore, no additional ethical approval was required specifically for these non-invasive behavioral observations. The behavioral observations and video recordings were designed and conducted within the framework of the BioRescue conservation program, in coordination with Ol Pejeta Conservancy and the Kenya Wildlife Service (KWS). These activities were discussed and agreed upon during joint committee meetings involving representatives of the participating institutions, as documented in the corresponding meeting minutes.

All procedures related to OPU were performed by authorized veterinary personnel within the BioRescue project, in accordance with the principles of Good Veterinary Practice (GVP), relevant professional codes of conduct, and applicable national and international guidelines for veterinary interventions in wildlife conservation, including the OIE Guidelines on Veterinary Legislation [40] and the position statements of the World Veterinary Association (WVA; [41]). The anesthetic protocol was based on four drugs: butorphanol tartrate, detomidine hydrochloride, midazolam hydrochloride, and ketamine hydrochloride [32, 42]. The BioRescue project received ethical approval from the Internal Committee for Ethics and Animal Welfare of the Leibniz-IZW (approval number 2019-01-02).

Najin and Fatu, the two NWR females, were born and originally housed at Dvůr Králové Zoo, Czech Republic, and were transferred to Ol Pejeta Conservancy, Kenya, in December 2009 as part of an international conservation effort to save the taxon from extinction. Tauwa, the SWR female included in the study, was born in Lewa Wildlife Conservancy and was transferred at Ol Pejeta Conservancy in April 2010. Following the study, all three animals remained at Ol Pejeta Conservancy under their usual management and care. No animal was euthanized in connection with the study.

2.2 Subjects

The study was conducted on the last two living northern white rhinos, Najin, born in 1989, and her daughter Fatu, born in 2000. The two rhinos were housed at the Ol Pejeta Conservancy in Kenya (N 0° 4' 54.0048", E 37° 2' 20.8248") alongside Tauwo (born in 2006), a female SWR. They were housed in a large, enclosed space (10-acre boma), with a mud puddle, a vast herbaceous expanse that permitted free

grazing, and mature trees contributing to the creation of shaded areas. The facility was also equipped with accessible drinking troughs, providing adequate hydration for the animals. All the subjects could graze freely in the field, and they were provided with daily supplementation of carrots, hay and horse cubes. In the evening they also had access to four compartments 50 x 50 feet bomas with wooden fences and a small roof. During dry periods 400kg-bale of hay was provided every week in the night boma. When they spent the night in the small bomas, they would walk out in the larger enclosure at 7:00 a.m. where they stayed until 05:00 p.m. in the evening.

2.3 OPU procedures

In vivo oocyte collection in white rhinoceroses involves ovarian superstimulation, the administration of full anesthesia, and the transrectal, ultrasound-guided retrieval of oocytes [30,32,43,44]. During the study period, two OPU procedures were performed on Fatu following this standardized protocol on March 28 and July 9, 2021. During the same March session, Najin underwent a preliminary examination under standing sedation, and based on its outcome, it was decided to not proceed with the remaining part of the OPU procedure. Following a subsequent ethical evaluation in the following weeks, she was removed from the oocyte retrieval program before the July session [27].

2.4 Behavioral monitoring

Behavioral data were collected to generate empirical welfare evidence that could subsequently be integrated with the ethical assessment performed through ETHAS**.** Monitoring was conducted over six months, from February to July 2021. The subjects were video recorded under three conditions: (i) Control; (ii) Pre-OPU; (iii) Post-OPU. Each condition was assessed twice, across two cycles, resulting in a total of 59 days of data collection. The first cycle (C1) comprised the Control (February 16- 28), Pre-OPU (March 17- 26), and Post-OPU (March 29- April 06) periods, while the second cycle (C2) comprised the corresponding periods from May 7- 20, June 29- July 08, and July 10- 19, respectively.

The subjects were monitored using continuous focal animal sampling [45]. On each sampling day, their behavior was video recorded for 10 minutes at the beginning of every hour for each subject, from 09:00 a.m. to 04:00 p.m., as part of the daily husbandry routine. Supplementary Table S1 presents the hours of video recording, divided by subject and condition. The order of subjects was reversed each day to control potential order effects. Video footage was recorded using a SONY® HDR-CX240E, digital HD Video Camera Recorder operated by the field collaborators responsible for the care of Najin and Fatu.

2.4.1 Video analysis

Video recordings were analyzed using the Behavioral Observation Research Interactive Software—BORIS [46] by two observers. A species-specific ethogram was developed by integrating existing literature [47–54]. Behaviors were divided into “states”, which are behavioral patterns of relatively long duration, and “events”, which are behaviors that can be approximated as points in time [45]. These behaviors were then merged into categories. The final ethogram included 81 behaviors (78 state events and three-point

events) and 14 behavioral categories. Supplementary Table S2 contains the full ethogram, comprising the behavioral categories. Before beginning systematic video analysis, the two observers underwent training in which they analyzed the same set of videos (10% of the total recorded footage). They assessed inter-rater reliability using Cohen's Kappa coefficient. Training concluded once they reached a value greater than 0.90, indicating almost perfect agreement [55].

2.4.2 Statistical analysis

Data obtained from video analysis were used to calculate the proportion of time that subjects allocated to each behavioral category on each observation day (behavior duration/observation time). For this study, we focused on behaviors classified as states because vulva winking was never recorded and flehmen was recorded sporadically. The data were used to calculate the subjects' behavioral time budgets in each condition and analyze potential behavioral differences between subjects and across conditions.

We ran generalized linear mixed models (GLMMs), focusing on the most prevalent behavioral categories: affiliative, exploration, food-related, human-animal interaction, inactivity, locomotion, maintenance, and vigilance. We coded the proportion of time each subject allocated to behavioural categories per day as the dependent variable, the interaction between subject (factorial) and condition (factorial) as a fixed factor, and date (factorial) as a random factor. We ran GLMMs using the R-function glmmTB (family = beta) of the R-package glmmTMB with logit links [56]. The beta distribution is frequently used to model continuous data expressed as a proportion. Proportion data are constrained between zero and one, and we rescaled the data using the following equation: $p_\circ = (p(n - 1) + 1/2)/n$, where p is the original proportion, and n is the sample size [57]. We used a likelihood ratio test to determine if there was a statistically significant difference between the full and null models (ANOVA with the Chisq argument; [58]). When the fixed factor was a significant predictor, we performed pairwise comparisons using the Tukey test (R package: multcomp) and applied the Bonferroni correction to adjust the probability level [59]. All statistical tests were two-tailed, with the significance threshold set at $p < 0.05$.

2.5 ETHAS tool

The Ethical Assessment Tool (ETHAS) is a framework for ethical self-assessment originally developed to support ethical decision-making during the application of conservation ARTs [31]. Since its initial development, the framework has been further refined and extended to a broader range of conservation interventions involving different species and conservation contexts [17,20].

ETHAS is based on checklists and uses a multidimensional ethical framework structured around four key dimensions and the corresponding ethical goals evaluated during the assessment (Table 1).

Table 1. Ethical dimensions and goals at the basis of the ethical framework applied (from de Mori et al. 2024 [17])

Environmental Ethics	Animal Welfare Ethics	Research Ethics	Social Ethics
E1 - Conservation of the biomaterial and genetic information	A1 - Health and functioning	R1 - Scientific quality and innovation	S1 - Compliance with rules and regulations
E2 - Conservation of species	A2 - Allowance of positive affective states and avoidance of negative affective states	R2 - Transparent and effective data management	S2 - Health, safety and empowerment of the staff and other people involved
E3 - Conservation of biodiversity	A3 - Expressing natural behavior and living natural lives	R3 - Transparent and effective communication	S3 - Positive integration with the surrounding social environment

ETHAS comprises two separate checklists—Checklist A and Checklist B (formerly referred to as EES and ERA, respectively, [31]). Checklist A is used for project-level assessment and, in the present study, was completed by the project manager at the beginning of the project. Checklist B is used to evaluate individual procedures and was completed by the professionals involved, including veterinarians, before each OPU procedure. Because the present study focused on the ethical evaluation of individual OPU procedures, only Checklist B (then referred to as ERA) was included in the analysis.

2.6 Integration of behavioral monitoring and ethical assessment

Behavioral monitoring provided empirical information on the responses of the animals before and after each OPU procedure, whereas ETHAS Checklist B documented the ethical assessment performed before each intervention. Checklist B was completed on March 28 and July 8, 2021, before the respective OPU procedures. Behavioral findings were considered alongside the corresponding ETHAS Checklist B assessments for each OPU procedure, illustrating how empirical behavioral evidence can contribute to structured ethical evaluation.

The methodological approach proposed here is based on the complementary roles of these two components within a continuous process of ethical assessment. While ETHAS provides a structured framework for identifying and evaluating ethical concerns before an intervention, behavioral monitoring provides empirical evidence that can inform the interpretation of these concerns and contribute to the assessment of subsequent procedures. In this way, ethical assessment and behavioral monitoring can be connected through an iterative process in which the outcomes of previous interventions inform future ethical decisions.

3. Results

3.1 Behavioral monitoring

3.1.1 Behavioral time budget

In all conditions, the most prevalent behavioral categories in Najin's time budgets were inactivity (ranging from a minimum of 37.2% in C2 to a maximum of 63% in PS2) and food-related (min= 20.3% in C1; max= 50.4% in C2), followed by locomotion, human-animal interaction, exploration, vigilance, maintenance, affiliative, and play. No aggressive or abnormal repetitive behaviors were recorded. Time spent out of sight ranged from a maximum of 7.8% in C1 to 3.2% in PR1. The behavioral time budget for each condition is reported in Figure 1.

Similarly, Fatu dedicated most of the observation time to inactivity and food-related behaviors, followed by locomotion, exploration, human-animal interaction, vigilance, maintenance, affiliative, and play (Figure 1). In particular, the subject was inactive from a minimum of 40% of the time in PR2 to a maximum of 65.3% in PS2. The food-related category ranged from 41% in PR2 to 19.3% in PS2. Aggressive behaviors were recorded once in PR1, whereas abnormal repetitive behaviors were never observed.

3.1.2 Behavioral changes following OPU

First cycle. The full model for *exploration* ($GLMM_1$) was statistically different from the null model (likelihood ratio test: $\chi^2 = 13.137$, $df = 5$, $p = 0.02$). The subject was a significant predictor of the target variable (estimate = −0.69 ± 0.31, z value = −2.23, $p = 0.03$; Supplementary Table S3), with Najin allocating less time to checking the environment compared to Fatu in all conditions (Figure 2).

The full models for the behavioral categories *affiliative* ($GLMM_2$), *food-related* ($GLMM_3$), *human-animal interaction* ($GLMM_4$), *inactive* ($GLMM_5$), *locomotion* ($GLMM_6$), and *maintenance* ($GLMM_7$) did not differ from the null models (likelihood ratio test: *affiliative*, $\chi^2 = 2.776$, $df = 5$, $p = 0.73$; *food-related*, $\chi^2 = 7.250$, $df = 5$, $p = 0.20$; *human-animal interaction*, $\chi^2 = 1.876$, $df = 5$, $p = 0.87$; *inactive*, $\chi^2 = 1.089$, $df = 5$, $p = 0.95$; *locomotion*, $\chi^2 = 2.821$, $df = 5$, $p = 0.73$; *maintenance*, $\chi^2 = 3.135$, $df = 5$, $p = 0.68$).

The comparison between the full and the null models for *vigilance* ($GLMM_8$) showed a trend toward statistical significance (likelihood ratio test: $\chi^2 = 9.565$, $df = 5$, $p = 0.09$). The interaction between subject and condition significantly affected the proportion of time dedicated to the behavioral category. However, the Tukey test did not yield statistically significant differences.

Second cycle. No significant difference was found between the full and the null models for the behavioral categories *affiliative* ($GLMM_9$; likelihood ratio test: $\chi^2 = 3.977$, $df = 5$, $p = 0.55$) and *exploration* ($GLMM_{10}$; likelihood ratio test: $\chi^2 = 5.203$, $df = 5$, $p = 0.39$).

The full model for the category *food-related* ($GLMM_{11}$) was found to be significantly different from the null model (likelihood ratio test: $\chi^2 = 17.557$, $df = 5$, $p < 0.01$). Both subject and condition significantly influenced the target variable (subject: estimate = −0.63 ± 0.20, z value = 3.12, $p = p < 0.01$; condition: estimate = −1.12 ± 0.49, z value = -2.29, p =0.02; Supplementary Table S3). Najin spent more time feeding and foraging than Fatu. Both individuals engaged significantly more in food-related behaviors in

C2 compared to PS2; no significant variation was found between C2 and PR2 and between PR2 and PS2. The results of the Tukey test are reported in Figure 3 and Table 2.

Table 2. Full results of the Tukey test for *food related activities*, *human-animal interaction* and *inactive*. Note: p < 0.05 is boldface

Predictors	Estimates	SEM	χ^2	p
Food related activities				
C2 *vs* PR2	0.234	0.508	0.461	0.889
C2 *vs* PS2	1.327	0.462	2.872	**0.011**
PR2 *vs* PS2	1.092	0.520	2.101	0.089
Human-animal interaction				
C2 *vs* PR2	-0.715	0.231	-3.089	**0.006**
C2 *vs* PS2	0.757	0.260	2.913	**0.010**
PR2 *vs* PS2	1.471	0.268	5.483	**< 0.001**
Inactive				
C2 *vs* PR2	0.403	0.312	1.290	0.040
C2 *vs* PS2	-3.253	0.237	-13.747	**< 0.001**
PR2 *vs* PS2	-3.656	0.309	-11.840	**< 0.001**

The full and null models for *human-animal interaction* ($GLMM_{12}$) were significantly different (likelihood ratio test: $\chi^2 = 27.062$, $df = 5$, $p < 0.001$). The condition was found to be a significant predictor of the response variable (estimate = −1.03 ± 0.30, z value = 3.41, $p = p < 0.01$). The rhinoceros mainly spent time interacting with caregivers and visitors in PR2 compared to C2 and PS2. In PS2, both subjects dedicated the least amount of time to interactions compared to the other conditions (Figure 4 and Table 2).

The comparison between the full and the null model for the behavioral category *inactive* ($GLMM_{13}$) detected a significant difference (likelihood ratio test: $\chi^2 = 72.105$, $df = 5$, $p < 0.001$), with condition significantly affecting the selected behaviors (estimate = −3.22 ± 0.30, z value = 10.58, $p = 0.001$). In particular, Fatu and Najin engaged more in standing, resting, and sleeping in PS2 compared to C2 and PR2 (Figure 5 and Table 2).

The results of $GLMM_{14}$ (target variable: *locomotion*) and $GLMM_{15}$ (target variable: *maintenance*) did not show any significant variation between the full and null models (*locomotion*, likelihood ratio test: $\chi^2 = 9.458$, $df = 5$, $p = 0.09$; *maintenance*, likelihood ratio test: $\chi^2 = 7.843$, $df = 5$, $p = 0.16$).

Finally, results showed the full and the null model for *vigilance* ($GLMM_{16}$) were significantly different (likelihood ratio test: $\chi^2 = 14.084$, $df = 5$, $p = 0.01$). Even in this case, the condition was found to be a significant predictor of the dependent variable (estimate = 0.59 ± 0.27, z value = 2.21, $p = 0.03$). However, the Tukey test did not detect any significant variation among conditions.

3.2 ETHAS results

Three Checklist B assessments were completed by the professionals involved in the OPU procedures, including veterinarians, before each intervention: two before the March session (one for Fatu and one for Najin) and one before the July session (for Fatu only, as Najin had been excluded from the oocyte retrieval program). Percentages of responses for each section of the checklist B are shown in Figure 6.

For both OPU procedures involving Fatu, the majority of items received positive responses across all sections. The few unanswered items mainly concerned communication with stakeholders and verification of staff competencies. In the July assessment, one veterinarian did not complete the Gamete packaging section.

The assessment completed for Najin contained a substantially higher proportion of unanswered items across several sections. Besides staff competencies, unanswered items included questions on post-procedural behavioral and physiological monitoring, consideration of animal suffering, and the interruption of the procedure if predefined conditions were not met. Moreover, the Gamete packaging section was not completed.

Overall, the Checklist B assessments showed a consistent pattern across the two OPU procedures performed on Fatu, whereas the assessment completed for Najin differed reflecting the uncertainties associated with a procedure that was ultimately not carried forward.

4. Discussion

This study illustrates how longitudinal behavioral monitoring can be integrated with structured ethical assessment to support ethical decision-making during the application of conservation ARTs. We combined behavioral observations collected before and after OPU procedures performed on the last two NWR with the assessment provided by ETHAS. Overall, the behavioral findings did not provide evidence of marked welfare impairment following the procedures, although changes were detected in some behavioral categories during the second observation cycle. Importantly, the empirical behavioral findings were broadly consistent with the ethical evaluations documented through ETHAS, providing complementary perspectives on the welfare implications of the procedures. Considered together, these sources of evidence illustrate how empirical behavioral observations can contribute to a more transparent and evidence-informed ethical assessment of conservation interventions.

4.1 Behavioral observations

The behavioral observations suggest that the OPU procedures were not associated with major disruption of the behavioral repertoire of the animals. Across all experimental conditions, inactivity and food-related behaviors represented the predominant components of the behavioral time budget, consistent with previous descriptions of white rhinoceros activity patterns and the high energetic requirements of the species [50,60]. Moreover, aggressive behaviors were virtually absent and abnormal repetitive behaviors (ARBs) were never observed. Although the absence of ARBs cannot by itself exclude the presence of stress or negative experiences, ARBs are generally regarded as indicators of prolonged welfare challenges [61]. Together with the physiological findings previously reported for these animals [32], these observations suggest that OPU procedures did not result in marked impairment of animal welfare.

The behavioral changes detected during the second OPU cycle require careful interpretation. Increased inactivity and reduced food-related behavior could reflect the physiological effects of anesthesia and the recovery process, but they may also have been influenced by factors unrelated to the procedure itself, including seasonal variation, environmental conditions, or normal individual fluctuations in activity. In particular, examination of the daily behavioral data did not reveal a clear temporal trend in inactivity, which did not progressively decrease with increasing time since OPU. This observation suggests that factors other than post-procedural recovery may have contributed to the observed changes in activity levels. The reduction in human-animal interactions observed after the second procedure is also likely to reflect changes in husbandry routines associated with post-procedural management rather than a direct behavioral consequence of OPU. These findings highlight a broader challenge in behavioral welfare assessment: individual behavioral categories rarely provide unambiguous evidence of positive or negative welfare when considered in isolation. Rather, their interpretation requires consideration of the overall behavioral repertoire, the biological characteristics of the species, and the environmental context in which observations are collected.

4.2 ETHAS

The assessment performed through ETHAS documented the ethical considerations underlying each OPU procedure. For the two procedures performed on Fatu, it showed a consistently positive evaluation across most sections, suggesting broad agreement among practitioners regarding the ethical acceptability of the intervention. In contrast, the assessment completed for Najin contained a higher proportion of unanswered items, reflecting the different ethical context of a procedure that was ultimately not carried forward. These findings illustrate the ability of structured ethical assessment to document procedure-specific ethical considerations while capturing differences between interventions and individual animals. Moreover, by explicitly documenting the ethical reasoning underlying each intervention before it takes place, ETHAS provided a transparent basis against which subsequent empirical evidence could be considered, allowing ethical deliberation to be revisited and further informed as new evidence becomes available.

4.3 Integrating behavioral monitoring in ethical decision-making

Taken together, the behavioral observations and the ETHAS assessments highlight the value of longitudinal behavioral monitoring not only for welfare assessment but also for ethical decision-making. ETHAS was originally developed to provide a structured framework for the prospective ethical evaluation of conservation ART procedures by encouraging practitioners to consider welfare, conservation, scientific, and social dimensions before an intervention is performed [17,31]. Here we suggest that longitudinal behavioral observations can complement this process by providing quantitative evidence on how animals actually respond to repeated interventions. The behavioral component of the ETHAS assessment relies primarily on unstructured observations, which, while providing valuable insights into individual animals, may be subject to observer-dependent variability. Structured behavioral monitoring can complement these observations by providing a systematic and standardized approach to detecting and quantifying behavioral changes associated with OPU, thereby contributing to a more comprehensive understanding of the animals' welfare. Rather than replacing structured ethical assessment, behavioral monitoring adds an animal-based source of evidence that can support the interpretation of ethical evaluations and contribute to a more informed assessment of subsequent procedures.

It is important to note that behavioral monitoring should not, however, be interpreted as a standalone measure of animal welfare. Behavioral responses are influenced by multiple biological and environmental factors, including seasonal climatic differences, which may affect temperature, food availability and food quality, and therefore require interpretation alongside physiological, veterinary, and other animal-based indicators. Within this broader evidential framework, however, repeated and prolonged behavioral observations can provide an additional source of empirical information significantly important for building a structured ethical assessment.

Although this study focuses on the last NWR, the proposed approach is not limited to this particular conservation context. Conservation ARTs are increasingly applied to endangered species under conditions of considerable uncertainty, where practitioners must balance expected conservation benefits against potential risks for individual animals. Integrating behavioral monitoring with structured ethical assessment offers a practical way to strengthen this process by complementing ethical deliberation with empirical evidence. Moreover, this approach may be applicable to a wider range of conservation procedures besides application of ARTs, including translocations, health assessments, and other management actions in which individual welfare and conservation goals must be carefully balanced.

More broadly, the approach proposed here suggests that ethical assessment should not be viewed as a single decision performed before an intervention, but as a dynamic process that evolves as empirical evidence accumulates. Decisions regarding conservation interventions are necessarily based on expectations about likely harms and benefits. However, the ethical justification of an intervention should remain open to revision in light of its actual consequences. When interventions are repeated, initial expectations can be progressively confronted with empirical observations, allowing subsequent ethical assessments to be informed by the outcomes of previous procedures. The methodological contribution of the approach proposed here lies precisely in establishing this connection between prospective ethical assessment and the empirical evaluation of intervention outcomes. Rather than representing

independent steps, ethical assessment and behavioral monitoring become components of a continuous process in which experience informs ethical deliberation and future decisions.

4.4 Limitations and future developments

The present study should be interpreted in light of several limitations. First, it is based on a unique case involving the last two NWR, and the behavioral responses observed cannot be directly generalized to other species or conservation contexts. Moreover, behavioral observations represent only one component of welfare assessment and should be interpreted alongside physiological, veterinary, and other animal-based indicators [62]. The longitudinal approach adopted here also requires repeated behavioral observations over extended periods, which may not always be practical in conservation programs operating under limited logistical or financial resources. A further limitation is that behavioral observations were restricted to daytime hours, potentially overlooking behavioral patterns occurring at night. The use of advanced monitoring technologies could help address this issue in future studies by extending behavioral monitoring beyond daytime observation periods.

Future research should evaluate the applicability of this approach across a broader range of conservation interventions, species, and ethical contexts, and explore how different sources of welfare evidence can be systematically incorporated into ethical decision-making frameworks. In particular, further studies should investigate how behavioral observations can be integrated with physiological, veterinary, and other animal-based indicators to support more comprehensive welfare assessments. Additional work is also needed to determine how empirical evidence collected over successive interventions can be most effectively incorporated into iterative ethical deliberation throughout long-term conservation programs.

5. Conclusion

Conservation ARTs increasingly require decisions that balance potential benefits for species conservation against possible costs for the welfare of individual animals. Supporting these decisions requires ethical assessment to be grounded in the best available empirical evidence. The present study illustrates one possible approach by integrating longitudinal behavioral monitoring with a structured ethical assessment framework.

More broadly, this study suggests that ethical assessment in conservation should evolve from a one-time prospective evaluation into an iterative process informed by empirical evidence. Integrating repeated and prolonged behavioral monitoring into structured ethical assessment allows the observed outcomes of previous interventions to inform the ethical evaluation of subsequent procedures, contributing to a more adaptive, transparent, and evidence-informed approach to conservation decision-making.

Declarations

Acknowledgements

The authors would like to thank all the keepers at Ol Pejeta Conservancy for their dedicated care of the northern white rhinoceroses and for their invaluable support throughout this study, and the late Richard McLellan and Rewind Rhino Extinction.

Additional Information

The author(s) declare no competing interests.

Funding

The authors acknowledge the following fundings:

ECCEAQUA project (MIUR; CUP: C26C18000030004)

Biorescue-2 (BMBF: 16LW0187K)

Biorescue-3 (BMFTR: 03LWU0016)

Nadace ČEZ PR20/1251 and PR21/46514

Data Availability

The data supporting the findings of this study are available from the corresponding author upon request.

Author Contributions Statement

BdM contributed to the conception and design of the work, the acquisition of data, and the drafting and substantive revision of the manuscript. EM and FB contributed equally to the acquisition, analysis, and interpretation of data, and to the drafting and substantive revision of the manuscript. IP contributed to the acquisition of data and the substantive revision of the manuscript. PB contributed to the conception and design of the work, the acquisition and interpretation of data, and the drafting and substantive revision of the manuscript. DM contributed to the acquisition of data and the substantive revision of the manuscript. FG, SH, JS, SS, IL, LK, DN, CK, SM, SN, PO, CG, MP, and AREI contributed to the substantive revision of the manuscript. EL and VB contributed to the acquisition and analysis of data, and to the drafting and substantive revision of the manuscript.

References

1. Saban, K. E. & Wiens, J. J. Unpacking the extinction crisis: rates, patterns and causes of recent extinctions in plants and animals. *Proc. R. Soc. B*292, 20251717 (2025). https://doi.org/10.1098/rspb.2025.1717

2. Torres-Romero, E. J. et al. Global scale assessment of the human-induced extinction crisis of terrestrial carnivores. *Sci. Adv.* **11**, eadq2853. https://doi.org/10.1126/sciadv.adq2853 (2025).
3. Keck, F. et al. The global human impact on biodiversity. *Nature* **641**, 395–400. https://doi.org/10.1038/s41586-025-08752-2 (2025).
4. McCallum, M. L. Vertebrate biodiversity losses point to a sixth mass extinction. *Biodivers. Conserv.* **24**, 2497–2519. https://doi.org/10.1007/s10531-015-0940-6 (2015).
5. Ceballos, G. et al. Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Sci. Adv.*1, e1400253 (2015). https://doi.org/10.1126/sciadv.1400253
6. Cowie, R. H., Bouchet, P. & Fontaine, B. The Sixth Mass Extinction: fact, fiction or speculation? *Biol. Rev.* **97**, 640–663. https://doi.org/10.1111/brv.12816 (2022).
7. Finn, C., Grattarola, F. & Pincheira-Donoso, D. More losers than winners: investigating Anthropocene defaunation through the diversity of population trends. *Biol. Rev.* **98**, 1732–1748. https://doi.org/10.1111/brv.12974 (2023).
8. Comizzoli, P. & Holt, W. V. Breakthroughs and new horizons in reproductive biology of rare and endangered animal species. *Biol. Reprod.* **101**, 514–525. https://doi.org/10.1093/biolre/ioz031 (2019).
9. Mastromonaco, G. F. & Songsasen, N. Reproductive technologies for the conservation of wildlife and endangered species. *Reproductive Technol. Anim. Acad. Press.* **7**, 99–117. https://doi.org/10.1016/B978-0-12-817107-3.00007-2 (2020).
10. Hildebrandt, T. B. et al. The ART of bringing extinction to a freeze – History and future of species conservation, exemplified by rhinos. *Theriogenology* **169**, 76–88. https://doi.org/10.1016/j.theriogenology.2021.04.006 (2021).
11. Hildebrandt, T. B. et al. De-extinction of the Northern white rhinoceros (*Ceratotherium simum cottoni*). *J. Reprod. Dev.* **72**, 88–95. https://doi.org/10.1262/jrd.2025-122 (2026).
12. Bolton, R. L. et al. Resurrecting biodiversity: advanced assisted reproductive technologies and biobanking. *Reprod. Fertil.* **3**, R121–R146. https://doi.org/10.1530/RAF-22-0005 (2022).
13. Hildebrandt, T. B. & Holtze, S. Advanced assisted reproduction technologies in endangered mammalian species. *Reprod. Domest. Anim.* **59**, e14700. https://doi.org/10.1111/rda.14700 (2024).
14. Comizzoli, P., Brown, J. & Holt, W. V. (eds) *Reproductive Sciences in Animal Conservation* (Springer Nature Switzerland, 2026). https://doi.org/10.1007/978-3-031-87707-0
15. de Mori, B. et al. Scientific and Ethical Issues in Exporting Welfare Findings to Different Animal Subpopulations: The Case of Semi-Captive Elephants Involved in Animal-Visitor Interactions (AVI) in South Africa. *Animals* **9**, 831. https://doi.org/10.3390/ani9100831 (2019).
16. Biasetti, P. et al. Ethical Analysis of the Application of Assisted Reproduction Technologies in Biodiversity Conservation and the Case of White Rhinoceros (*Ceratotherium simum*) Ovum Pick-Up Procedures. *Front. Vet. Sci.* **9**, 831675. https://doi.org/10.3389/fvets.2022.831675 (2022).

17. de Mori, B. et al. The ethical assessment of Assisted Reproductive Technologies (ART) in wildlife conservation. *Biol. Conserv.* **290**, 110423. https://doi.org/10.1016/j.biocon.2023.110423 (2024).
18. Palmer, C. & Sandler, R. Mapping the Ethics Landscape for the Use of Assisted Reproductive Technologies (ARTs) in Conservation. *J. Appl. Anim. Ethics Res.* **7**, 5–27. https://doi.org/10.1163/25889567-bja10058 (2025).
19. Faria, C. & Creating Life Creating Strife? Assisted Reproductive Technologies, Extinction, and Wild Animal Welfare. *J. Appl. Anim. Ethics Res.* **7**, 68–90. https://doi.org/10.1163/25889567-bja10060 (2025).
20. Biasetti, P. et al. Ethics at the Edge of Extinction: Assisted Reproductive Technologies (ART) in the Conservation of the Northern White Rhino. *J. Agric. Environ. Ethics*. **38**, 7. https://doi.org/10.1007/s10806-024-09943-z (2025).
21. Biasetti, P. & de Mori, B. Ethical and Regulation Aspects Relevant to the Study of Reproduction in Wild Species. In *Reproductive Sciences in Animal Conservation.*121–143 (2026). https://doi.org/10.1007/978-3-031-87707-0_4
22. Soulé, M. E. What is Conservation Biology? A new synthetic discipline addresses the dynamics and problems of perturbed species, communities, and ecosystems. *BioScience*35, 727–734 (1985). https://doi.org/10.2307/1310054
23. Fraser, D. Toward a Synthesis of Conservation and Animal Welfare Science. *Anim Welf.* **19** (2), 121–124. https://doi.org/10.1017/S0962728600001378 (2010).
24. Greggor, A. L. et al. Animal Welfare in Conservation Breeding: Applications and Challenges. *Front. Vet. Sci.* **5**, 323. https://doi.org/10.3389/fvets.2018.00323 (2018).
25. Johnson, P. J. et al. Consequences Matter: Compassion in Conservation Means Caring for Individuals, Populations and Species. *Animals* **9**, 1115. https://doi.org/10.3390/ani9121115 (2019).
26. Zemanova, M. Making room for the 3Rs principles of animal use in ecology: potential issues identified through a survey. *Eur. J. Ecol.* **7** https://doi.org/10.17161/eurojecol.v7i2.14683 (2021).
27. Biasetti, P. et al. Application of decision tools to ethical analysis in biodiversity conservation. *Conserv. Biol.* **37**, e14029. https://doi.org/10.1111/cobi.14029 (2023).
28. Berger-Tal, O. et al. A systematic survey of the integration of animal behavior into conservation. *Conserv. Biol.* **30**, 744–753. https://doi.org/10.1111/cobi.12654 (2016).
29. Greggor, A. L., Blumstein, D. T., Wong, B. B. M. & Berger-Tal, O. Using animal behavior in conservation management: a series of systematic reviews and maps. *Environ. Evid.* **8**, 23. https://doi.org/10.1186/s13750-019-0164-4 (2019).
30. Hildebrandt, T. B. et al. Embryos and embryonic stem cells from the white rhinoceros. *Nat. Commun.* **9**, 2589. https://doi.org/10.1038/s41467-018-04959-2 (2018).
31. de Mori, B. et al. 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*). *Animals* **11**, 312. https://doi.org/10.3390/ani11020312 (2021).

32. Hildebrandt, T. B. et al. In vitro fertilization program in white rhinoceros. *Reproduction* **166**, 383–399. https://doi.org/10.1530/REP-23-0087 (2023).
33. Roth, T. L. That was then, this is now – Over two decades of progress in rhinoceros reproductive science and technology. *Theriogenology Wild*. **4**, 100065. https://doi.org/10.1016/j.therwi.2023.100065 (2024).
34. Wang, G. et al. Genomic map of the functionally extinct northern white rhinoceros (*Ceratotherium simum cottoni*). *Proc. Natl Acad. Sci. USA*122, e2401207122 (2025). https://doi.org/10.1073/pnas.2401207122
35. Korody, M. L. & Hildebrandt, T. B. Progress Toward Genetic Rescue of the Northern White Rhinoceros (*Ceratotherium simum cottoni*). *Annu. Rev. Anim. Biosci.* **13**, 483–505. https://doi.org/10.1146/annurev-animal-111523-102158 (2025).
36. ASAB Ethical Committee/ABS Animal Care Committee. Guidelines for the ethical treatment of nonhuman animals in behavioural research and teaching. *Anim. Behav.* **195**, I–XI. https://doi.org/10.1016/j.anbehav.2022.09.006 (2023).
37. WAZA. WAZA Code of Ethics. *World Association of Zoos and Aquariums*. https://www.waza.org/priorities/waza-code-of-ethics/
38. Decreto legislativo 4 marzo. n. 26. *Normattiva*. (2014). https://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:decreto.legislativo:2014-03-04;26 (2014).
39. European Parliament and Council of the European Union. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off J. Eur. Union*. **L 276**, 33–79 (2010). http://data.europa.eu/eli/dir/2010/63/oj
40. World Organisation for Animal Health (WOAH). *OIE Guidelines on Veterinary Legislation*. (2021). https://www.woah.org/app/uploads/2021/03/a-guidelines-vetleg.pdf
41. World Veterinary Association (WVA). *Position Statements*. https://worldvet.org/policies/.
42. Göritz, F. et al. Etorphine free anesthesia protocols optimized for frequent reproductive interventions ranging from semen collection, artificial insemination to ovum-pick-up (OPU) in four rhino species. in Scientific Program, 15th International Elephant and Rhino Conservation and Research Symposium 50–51 (Singapore, 2016).
43. Hermes, R. et al. Ovarian superstimulation, transrectal ultrasound-guided oocyte recovery, and IVF in rhinoceros. *Theriogenology* **72**, 959–968. https://doi.org/10.1016/j.theriogenology.2009.06.014 (2009).
44. Hermes, R., Göritz, F., Streich, W. & Hildebrandt, T. Assisted Reproduction in Female Rhinoceros and Elephants – Current Status and Future Perspective. *Reprod. Domest. Anim.* **42**, 33–44. https://doi.org/10.1111/j.1439-0531.2007.00924.x (2007).
45. Bateson, M. & Martin, P. *Measuring Behaviour: An Introductory Guide* (Cambridge University Press, 2021). https://doi.org/10.1017/9781108776462
46. Friard, O. & Gamba, M. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325–1330. https://doi.org/10.1111/2041-

210X.12584 (2016).

47. Owen-Smith, R. N. The Social Ethology of the White Rhinoceros *Ceratotherium simum* (Burchell 1817). *Z. Tierpsychol*. **38**, 337–384. https://doi.org/10.1111/j.1439-0310.1975.tb02010.x (1975).
48. Owen-Smith, R. N. *The Behavioural Ecology of the White Rhinoceros*. PhD dissertation, University of Wisconsin (1973). https://rhinoresourcecenter.com/library/references/the-behavioural-ecology-of-the-white-rhinoceros/
49. Kunes, M. & Bičík, V. *Social and Sexual Behaviour in Captive Breeding Groups of White Rhinoceros*. Biologica 39–40 (2002). https://www.semanticscholar.org/paper/FACULTAS-RERUM-NATURALIUM-%28-2001-%E2%80%93-2002-%29-BIOLOGICA-Kunes-Bi%C4%8D%C3%ADk/d9d37cf5a4a1c2858bb1a42c412539b8b359d3d9.
50. Hutchins, M. & Kreger, M. D. Rhinoceros behaviour: implications for captive management and conservation. *Int. Zoo Yearb*. **40**, 150–173. https://doi.org/10.1111/j.1748-1090.2006.00150.x (2006).
51. Metrione, L. Relationships of social behavior and the captive environment to reproduction in female Southern White rhinoceros (*Ceratotherium simum simum*). Dissertation, 1-232 (2010). https://rhinoresourcecenter.com/library/references/relationships-of-social-behavior-and-the-captive-environment-to-reproduction-in-female-southern-white-rhinoceros-ceratotherium-simum-simum/
52. Cinková, I. & Bičík, V. Social and reproductive behaviour of critically endangered northern white rhinoceros in a zoological garden. *Mamm. Biol.* **78**, 50–54. https://doi.org/10.1016/j.mambio.2012.09.007 (2013).
53. Metrione, L. & Eyres, A. *Rhino Husbandry Manual* (2014).
54. Jenikejew, J., Chaignon, B., Linn, S. & Scheumann, M. Proximity-based vocal networks reveal social relationships in the Southern white rhinoceros. *Sci. Rep.* **10**, 15104. https://doi.org/10.1038/s41598-020-72052-0 (2020).
55. Kaufman, A. B. & Rosenthal, R. Can you believe my eyes? The importance of interobserver reliability statistics in observations of animal behaviour. *Anim. Behav.* **78**, 1487–1491. https://doi.org/10.1016/j.anbehav.2009.09.014 (2009).
56. Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A. & Smith, G. M. *Mixed Effects Models and Extensions in Ecology with R* (Springer, 2009).
57. Smithson, M. & Verkuilen, J. A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. *Psychol. Methods*. **11**, 54–71. https://doi.org/10.1037/1082-989X.11.1.54 (2006).
58. Dobson, A. J. *An Introduction to Generalized Linear Models* (CRC, 2002).
59. Bretz, F., Hothorn, T., Westfall, P. & Hall/CRC. *Multiple Comparisons Using R*(Chapman and, (2016). https://doi.org/10.1201/9781420010909
60. Field, C. R. Zoological Society of London,. A comparative study of the food habits of some wild ungulates in the Queen Elizabeth Park, Uganda. Preliminary report. In *Symposium of the Zoological Society of London*, No. 21, 135–151 (1968).

61. Mason, G. J. Stereotypies: a critical review. *Anim. Behav.* **41**, 1015–1037. https://doi.org/10.1016/S0003-3472(05)80640-2 (1991).
62. Mellor, D. J. Updating Animal Welfare Thinking: Moving beyond the Five Freedoms towards A Life Worth Living. *Animals* **6**, 21. https://doi.org/10.3390/ani6030021 (2016).

Figures

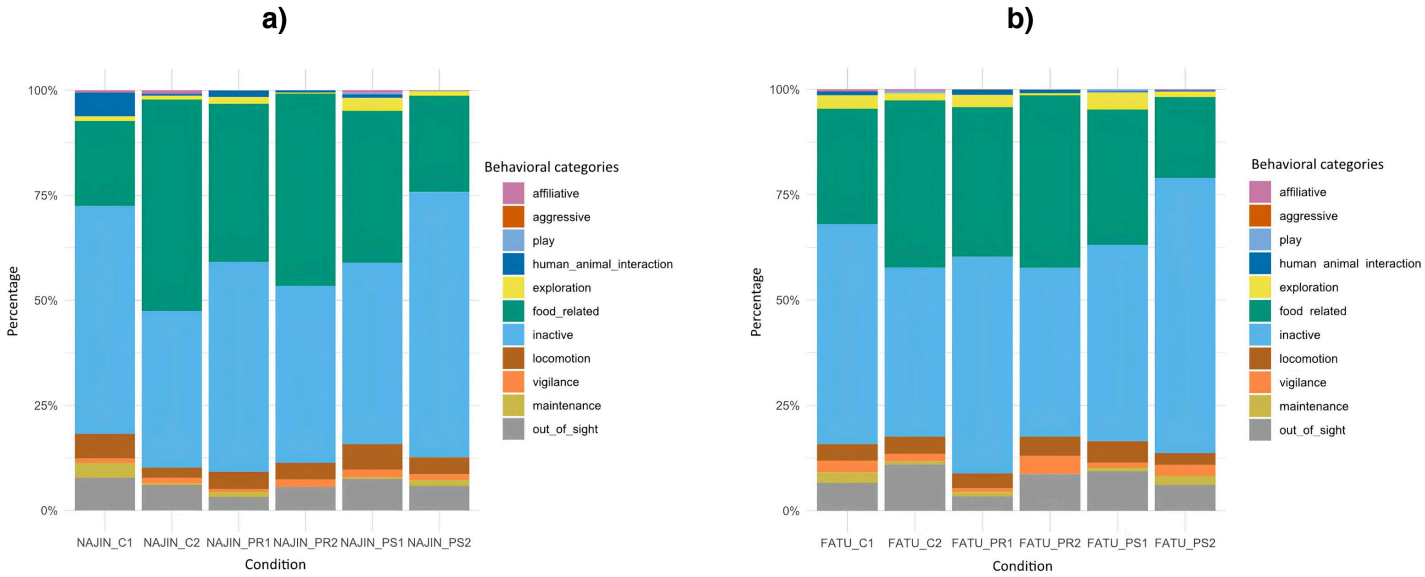


Figure 1

Proportion of time dedicated to each behavioral category by Najin (a) and Fatu (b)

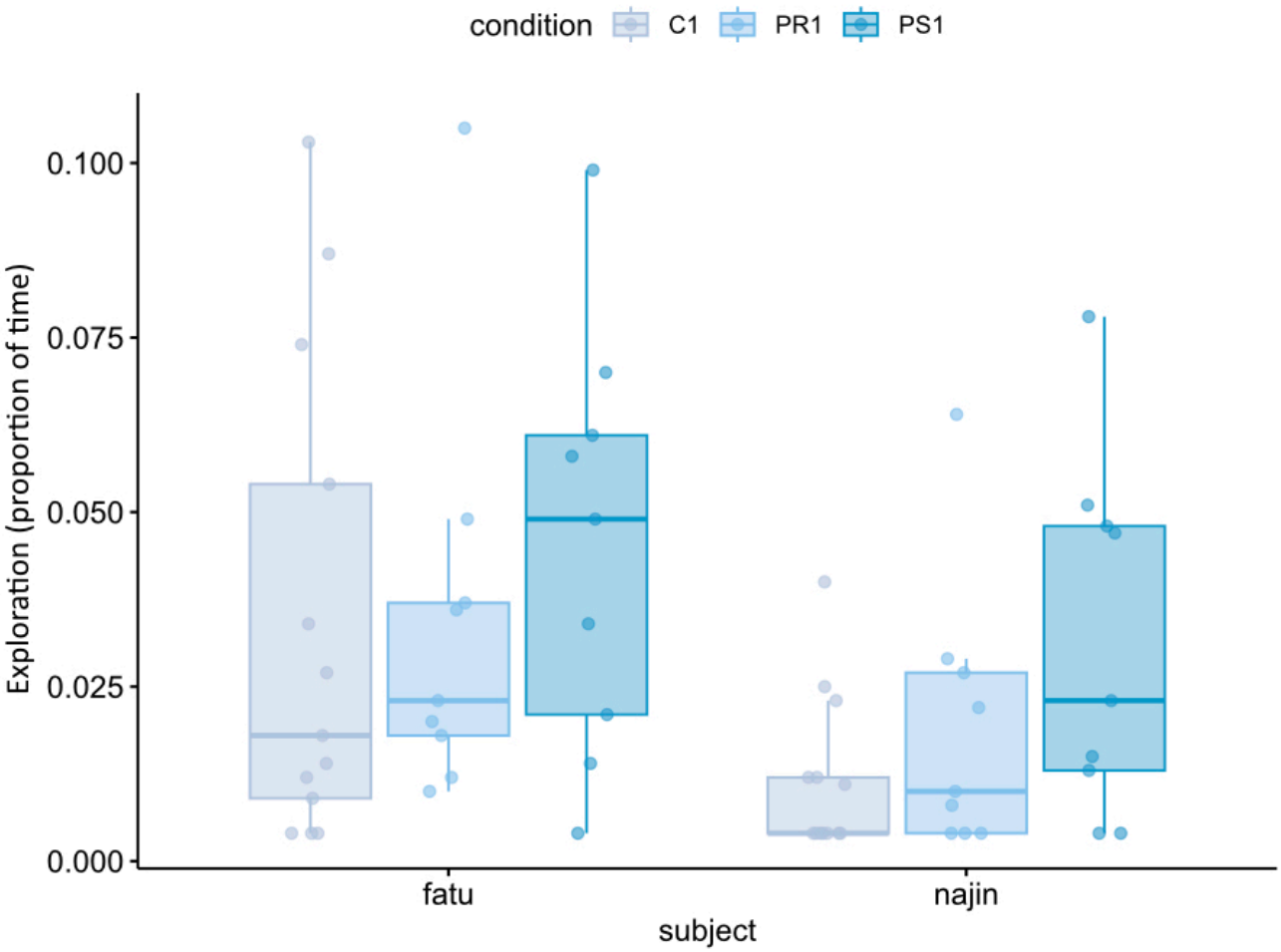


Figure 2

Proportion of time allocated to *exploration*by the subjects in the selected conditions. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR. C1= control condition 1; PR1= pre-OPU 1; PS1= post-OPU 1

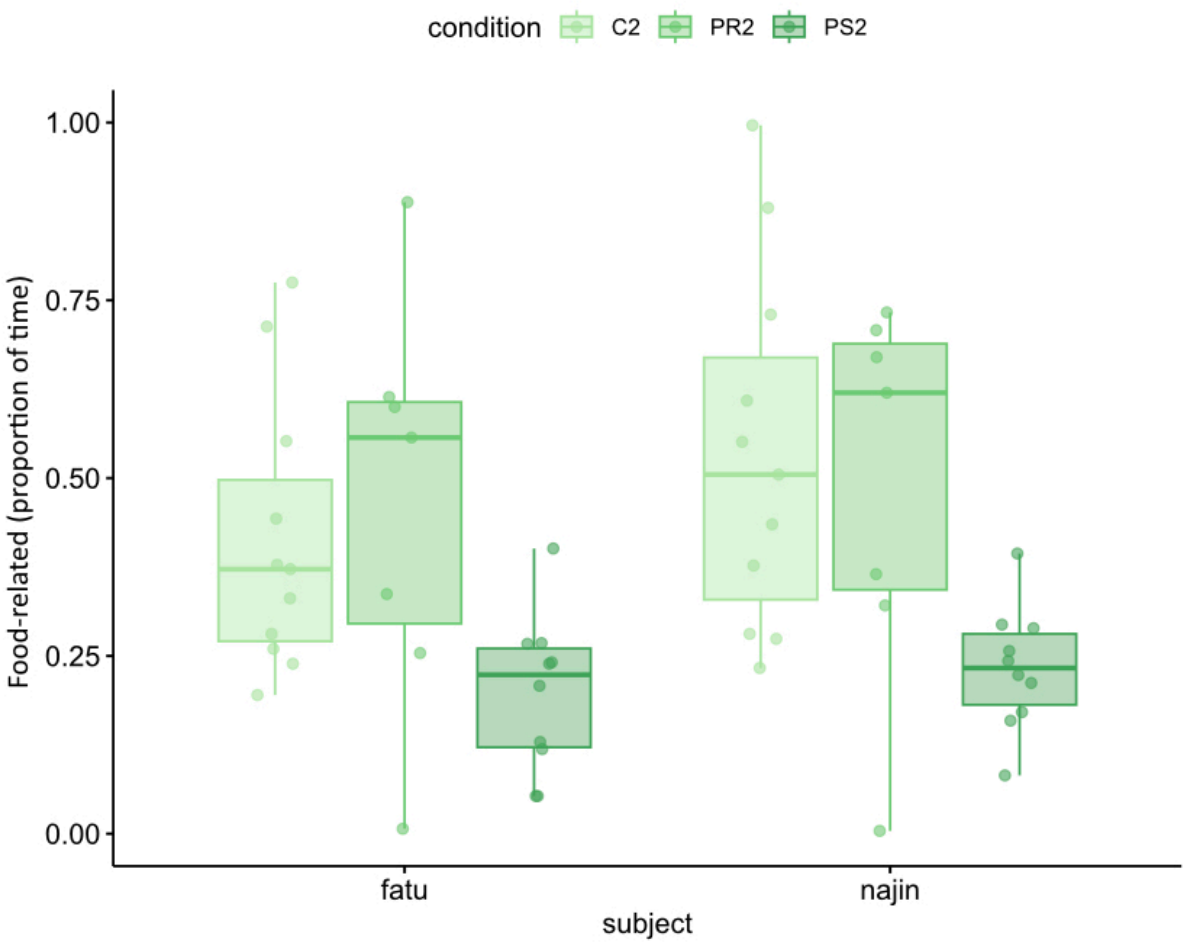


Figure 3

Proportion of time allocated to *food-related*activities by the subjects in the selected conditions. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR. C2= control condition 2; PR2= pre-OPU 2; PS2= post-OPU 2.

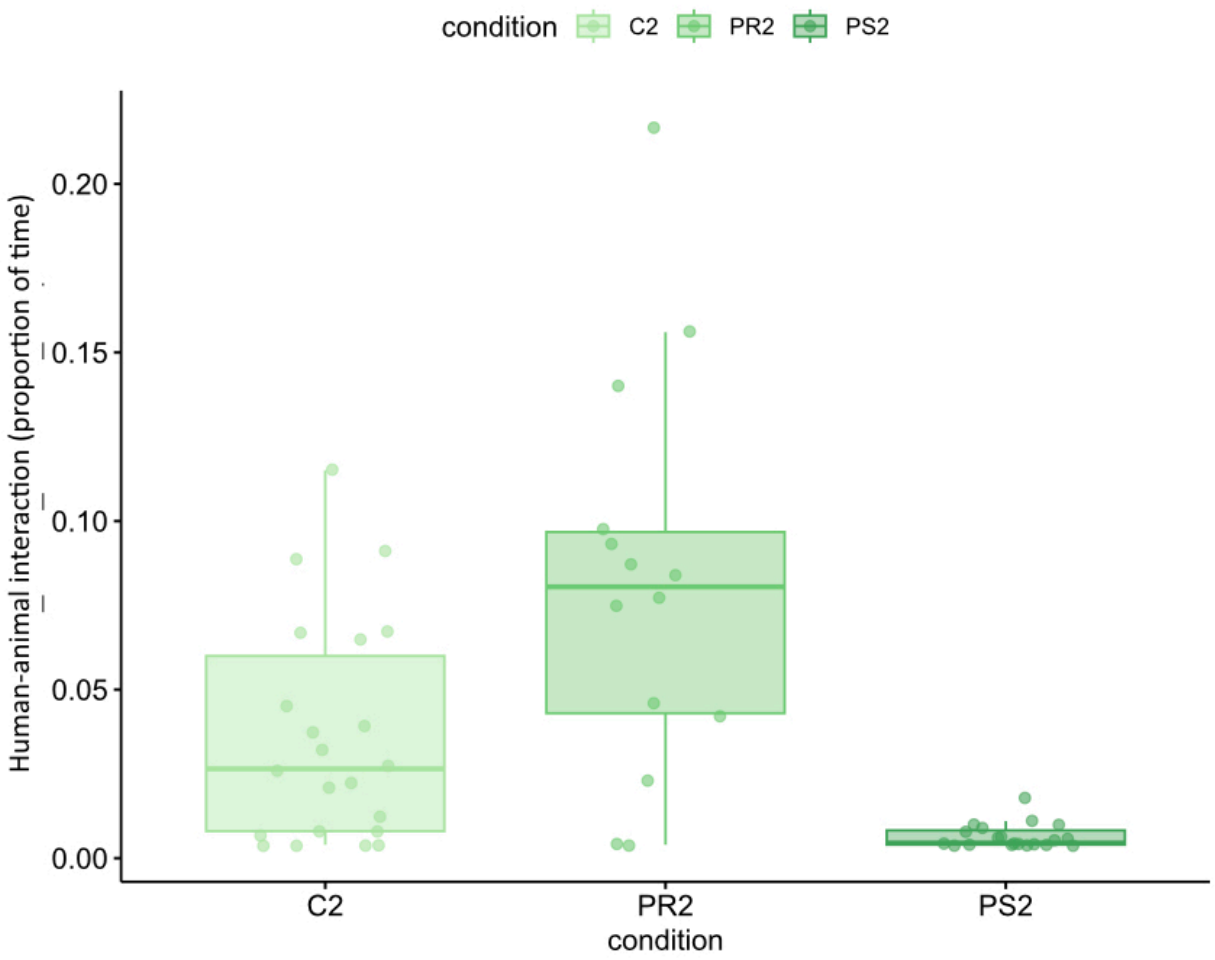


Figure 4

Proportion of time allocated to *human-animal interaction* by the subjects in the selected conditions. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR. C2= control condition 2; PR2= pre-OPU 2; PS2= post-OPU 2

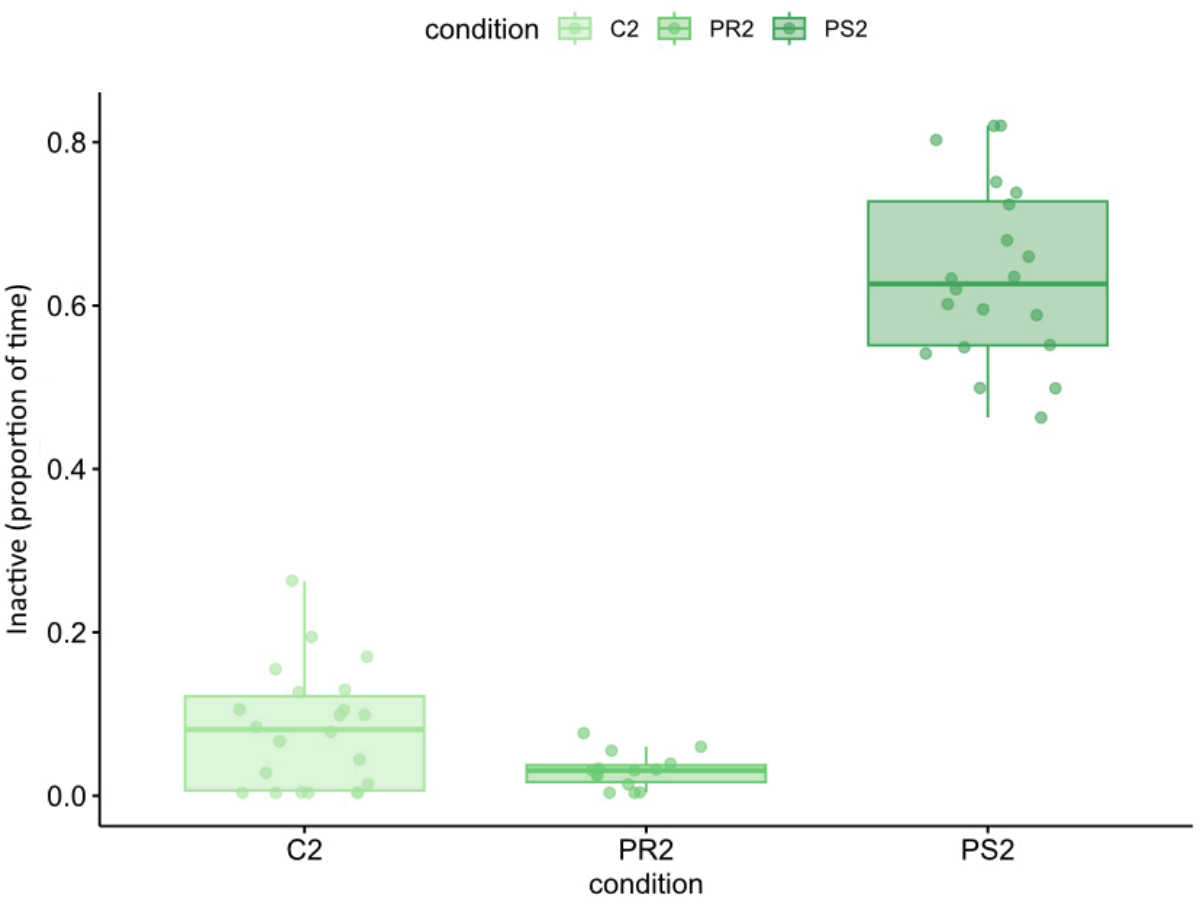
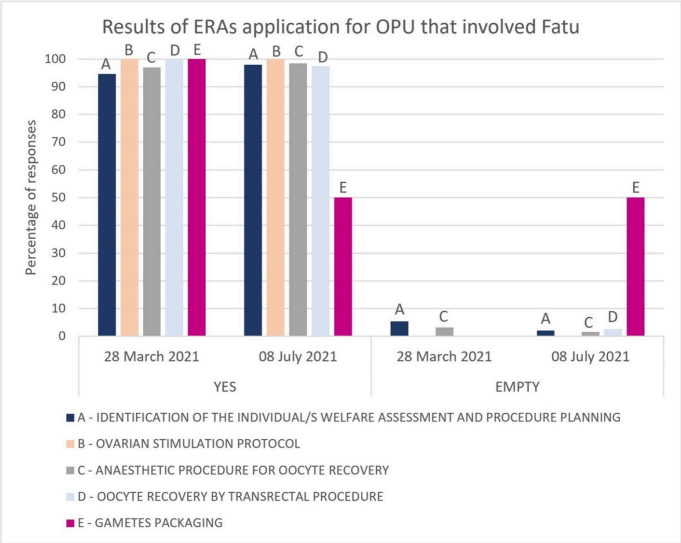


Figure 5

Proportion of time allocated to *inactive*by the subjects in the selected conditions. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the inter-quartile range, IQR. C2= control condition 2; PR2= pre-OPU 2; PS2= post-OPU 2.

a)



b)

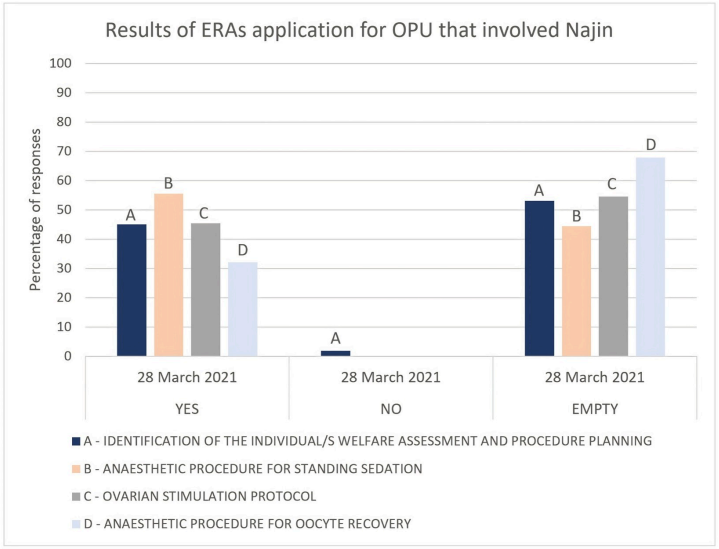


Figure 6

Percentage of positive and empty responses for the Checklist B application for OPU that involved Fatu (a) and Najin (b), divided by checklist section. Percentages are calculated on the total number of items per section.

Supplementary Files

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