

Global Trends in Mammalian Reproductive Health: A Cross-Species Study of Sperm Abnormalities

Nozomu Hirama^{1,2}, Marcia de A. M. M. Ferraz^{3,4}, Michael J. Noonan^{1,2,5}

¹Department of Biology, The University of British Columbia Okanagan, Kelowna, British Columbia, Canada;
²Okanagan Institute for Biodiversity, Resilience, and Ecosystem Services, The University of British Columbia Okanagan,
Kelowna, British Columbia, Canada;

³Clinic of Ruminants, Faculty of Veterinary Medicine, Ludwig-Maximilians University of Munich, Sonnenstr. 16,
Oberschleissheim, 85764, Germany;

⁴Gene Center, Ludwig-Maximilians University of Munich, Feodor-Lynen Str. 25, Munich, 81377, Germany;

⁵Department of Computer Science, Math, Physics, and Statistics, The University of British Columbia Okanagan, Kelowna,
British Columbia, Canada

Introduction

There are two main components to a sustainable population: survival and reproduction. Existing individuals must be able to survive long enough, as well as successfully reproduce to pass their genes onto future generations. Thus, in most cases, these two components are equally important in driving a population towards stability. However, with increasing interests in efforts to preserve pre-existing individuals, the reproductive component seems to be often overlooked in ecological research (Comizzoli et al., 2019). Yet, the truth is, if individuals are unable to successfully reproduce, populations may struggle to grow despite such conservation efforts. In fact, Manlik (2019) revealed that even in slow-growing species, where survival had been considered to have a larger impact on the population's dynamics, growth rates were significantly more sensitive to changes in reproductive features. As such, there is great potential for improvement in conservation planning with enhanced knowledge on animal reproduction (Comizzoli et al., 2018; Wildt et al., 2001).

While there are many ways to quantify reproductive health in animals, semen quality is a commonly used measure. Sperm cells are not only easy to sample, but also exhibit high morphological variation that can be used for easy assessment (Wysokińska, 2022). Specifically, morphological abnormalities in animal sperms have been recursively tied to a decrease in fertility across various taxa. In other words, high proportions of defects in sperm samples seem to indicate difficulties in successful reproduction, which could highly impact the viability of an animal population. This correlation between sperm defects and fertility has been documented in humans, mice, horses, and many other mammals, making sperm quality assessment an effective way to estimate the state of reproductive ability in a group of organisms (Guidobaldi et al., 2017; Love, 2011; Menkveld et al., 2011).

Fertility declines are a serious issue occurring across the globe that not only prevents successful production of offspring, but prevents the production of healthy, viable offspring as well. According to the World Health Organization (2023), it is estimated that approximately 17.5% of the adult human population experience or have experienced infertility during their lifetime, making this a major health concern in humans. While the direct cause of this increasing infertility rate cannot be identified, there seems to be indicators of sperm quality traits declining over the years. For instance, it has been reported that human sperm concentration and sperm count seems to be rapidly decreasing over the past few decades (Levine et al., 2017). Similarly, studies have revealed a potential decline in equine sperm motility over the years (Harris et al., 2023). These findings could hold major significance in conservation planning, as progressive drops in mammalian reproductive health would require extensive efforts to counteract that effect through survival rates, or prioritize species with greater declines, before it is impossible for a population to recover. Nonetheless, there is a great amount of information lacking on the exact extent at which these declines in reproductive health are taking place over a large timescale for a given species, let alone globally across a wide range of species. Here, we aim to characterize global trends in the reproductive health of terrestrial mammals through sperm morphology traits, as well as assess the current state of data-availability in peer-reviewed literature on mammalian reproduction. Additionally, we attempt to identify any phylogenetic differences in temporal trends of reproductive health, which can ultimately aid in reconsidering target areas for conservation research.

Materials and Methods

Literature review

Sperm morphology measures were taken from peer-reviewed articles selected through a semi-random search of all terrestrial mammal species. An initial list of easily accessible papers were obtained through a quick search of articles describing sperm morphology and/or reproductive health in order to understand where major interests in literature may lie. Many of these papers in the initial list included felid species such as the tiger (*Panthera tigris*) or the cheetah (*Acinonyx jubatus*). The dataset was then extended to include a wider range of taxa using a list of mammalian species in a randomized order. The list was taken from version 1.11 of the openly accessible Mammalian Diversity Database (2023). For the purpose of this study, any taxonomic order with more than 1000 species described were removed from the randomized list in order to ensure efficient search for literature; this resulted in the removal of the orders, Chiroptera and Rodentia. In addition to this, species with infraorder Cetacea was also removed from the search as these describe the majority of fully-aquatic mammals such as whales and dolphins. Fully-aquatic mammals generally exhibit physiological, behavioral, and habitat-related differences compared to fully-terrestrial ones, and therefore may impact the generalizability of global trends (Lanyon & Burgess, 2019). However, due to the difficulties in classifying semi-aquatic/terrestrial species such as polar bears, as polar bear reproduction takes place on land, no further modifications to the list of species were made.

The species were then fed into the Web of Science search engine in the randomized order, with no restriction on the timeframe. Our aim was to incorporate a wide range of publish date whenever available. Each search was conducted with the following search terms: (“scientific name” OR “common name” OR “alternative names”) AND (Sperm* OR Semen* OR Reproduct* OR Semin* OR Ejaculat*) AND (Normal OR Abnormal OR Motil*), where the

“scientific name”, “common name”, and “alternative names” were dependent on names provided in the Mammalian Diversity Database, while all other search terms were fixed across searches (Appendix A). The search results were then manually evaluated to select articles with numerical measure of normal or abnormal sperm morphology proportions and/or %motility. Where searches resulted in large numbers of papers (<100 papers), a random sample was taken to include papers from each of five equally divided segments of the publish date range. Additional papers were accessed from the citation lists when relevant studies had been referred to.

From the selected papers, the following six spermatic trait measurements were recorded where available: %morphologically normal sperm, %head abnormalities, %mid-piece abnormalities, %tail abnormalities, %motile sperm, and %intact acrosome. The three regional abnormalities (head, mid-piece, and tail) are based on commonly used and reported classification methods of abnormality types (Čeřovský et al., 2005; Perry, 2021). Additional information relevant to the paper or sampling methods were also recorded, as well as potential characteristics of the sampled specimens such as housing conditions as captive vs wild (Figure 1B).

Global trends in spermatic traits

We fit phylogenetically-controlled beta regression models to predict changes in the six spermatic trait measures over time from at most 1977 to 2024. This timescale spans from the earliest study included in our available dataset to the latest for the proportion of normal sperm. For other measures that did not have the full range of dates available, models were fit only where data was collected. Additionally, due to the lack of data available on exact timings of data collection for many of the studies, we used the publish date as a proxy of temporal standings of measurements in our dataset. Phylogenetic relationships between species in our dataset were based on 10000 samples of trees obtained from the VertLife repository (Upham et al., 2019). We estimated a consensus tree using the R package: phytools (version 2.1-1), which was then manually modified

based on divergent times and alternate names found in literature to account for missing species (Revell, 2024).

All percent measures were converted to proportions to have a range from 0-1. Thus, we fit six separate generalized linear regression model with a beta distribution and logit link to predict changes in these proportions. A beta distribution has the support of the interval [0,1], which ensures our predictions do not go beyond boundaries of spermatic trait proportions. Phylogenetic autocorrelation was taken into account by weighing the random effects of species by the phylogenetic covariance of species. This allows for the characterization of a global trend across time, generalizable to the body of mammalian species included in the study. Model behavior was assessed through estimated posterior distributions and chain convergence for each model. All analyses and data visualization were carried out in R (version 4.2.3, R Core Team) and all data and code used are openly accessible at

https://github.com/QuantitativeEcologyLab/Mammalian_Sperm_Abnormality.

Taxonomic trends in normal sperm proportions over time

In order to compare how different taxonomic groups are experiencing different trends in normal sperm proportion changes over time, we fit the same beta regression model used to model global trends, but instead without the phylogenetic covariance structure. This allows for the extraction of individual species-level slopes from the normal sperm regression. These slopes were then converted to describe estimated annual change in morphologically normal sperm proportions for each species. The slopes were visualized on a phylogenetic tree to identify any structure and localities in the slope intensities as well as signs (positive/negative slopes).

Results

Data distribution and availability of spermatic traits in mammals

Our search involved a total of 268 terrestrial mammalian species, with a maximum of 63 species having data available for spermatic traits ($n=63$ for normal sperm proportions). The timeframe of our data ranged from 1977 to 2024, with more studies in recent years. There was a fair spread of data collected from countries across the globe, although many samples were collected from repeated institutions and zoos (Figure 1A). The majority of our collected data were derived from captive-held specimen, which may impact the generalizability of our results to the entirety of terrestrial mammals. Additionally, while most semen samples were collected via electroejaculation, a commonly accepted method of semen extraction, there was no clear consensus in collection method, as plausibility depends on external conditions such as the body size of individuals (Figure 1B). Finally, we lack data regarding regional abnormalities compared to other spermatic traits. Not only were there fewer studies reporting specific types of morphological abnormalities, the studies that did in fact report regional proportions were also naturally prone to underestimation. Since researchers only report detailed breakdowns of types of morphological abnormalities that they were either interested in, or only those that are commonly acknowledged, we do not have any information on the abnormalities that were excluded from their reports. This may have a significant impact on the accuracy of regional abnormality estimates, leaving inferences based on the collected proportions difficult in subsequent analyses.

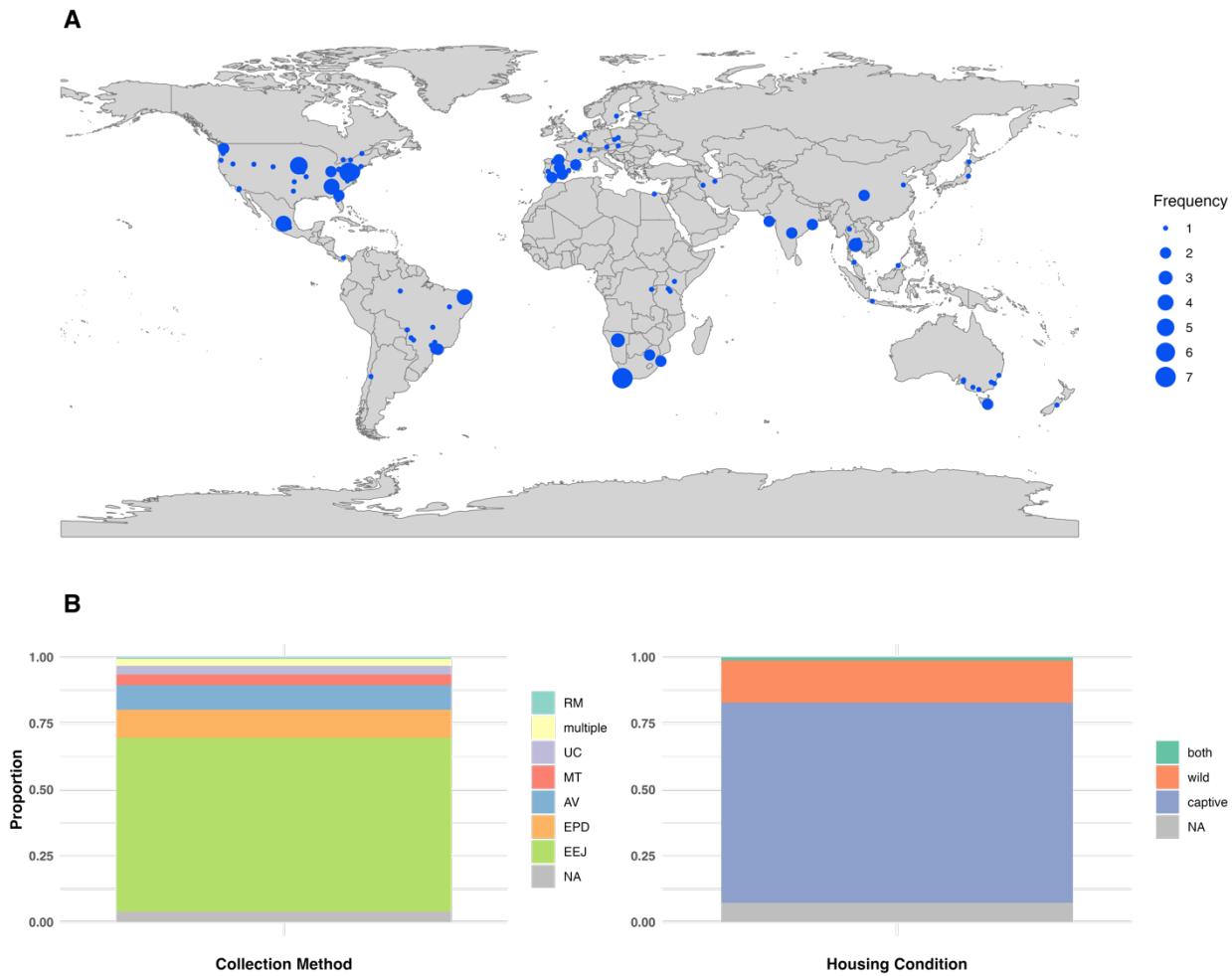


Figure 1. (A) Global distribution of sampled data collected from literature. The sizes of blue circles indicate the frequency of repetitive appearances for each coordinate location/institution. (B) Proportions of different collection methods and housing conditions reported, of only studies that had available data. Each of the abbreviations of collection methods stand for the following: EEJ (electroejaculation), EPD (epididymal sperm collection), AV (artificial vagina), MT (masturbation), UC (urethral catheter), and RM (rectal massage). Papers reporting more than one sperm collection method was marked as “multiple”. Housing conditions were only reported when clearly stated in writing. Studies that had a mixture was classified as “both” if clearly indicated a mixture, or “NA” if sufficient data were not provided to classify housing condition. Maximum proportions were seen for EEJ in collection method (0.66) and captive for housing condition (0.75).

Long-term trends in mammalian sperm quality

Our predictions of changes in spermatric trait proportions over the years indicated a mixture of inclines and declines. Based on the beta regression model fit, we observed that there was a general decrease in the proportion of sperm over the years, indicating that there may indeed be a health concern in terms of mammalian reproductive health (Figure 2A). Additionally, there

seems to be a slight decrease in the proportion of intact acrosomes as well (Figure 2B). This may have some effect on the viability of offspring produced, as documented by Guidobaldi et al. (2017), although further analysis may be required to identify whether or not there is a significant enough decline to apply to future research. In terms of regional abnormalities, we observed increases in head abnormalities while there seems to have been a drop in mid-piece abnormalities over the years (Figures 2C, 2D). As for tail abnormalities, there may be a slight increase in the proportion of these abnormalities, although the exact extent of this is unclear, especially due to the high variance observed in recent years (Figure 2E). Interestingly, our results suggest that the proportion of motile sperm, or %motility, did in fact exhibit little changes over the years (Figure 2F). This is contrary to previous beliefs and may indicate that sperm are still highly motile, yet the proportions of abnormalities have increased, which may ultimately lead to lower fertility regardless of the capabilities of sperm to reach the oocyte.

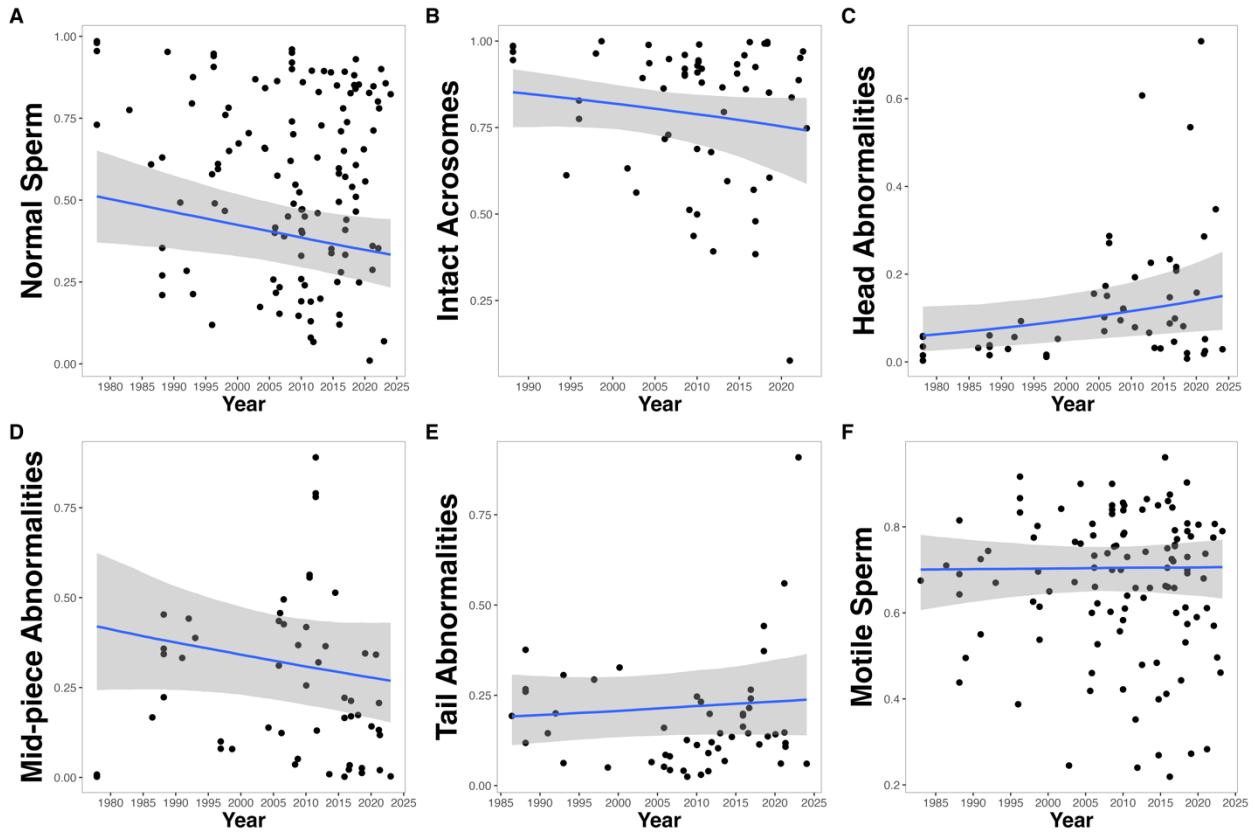


Figure 2. Estimated change in proportion of sperm morphology traits, computed from phylogenetically-controlled beta regression models (maximum $n=119$ for 63 species in normal sperm). The publish date of the papers were used as an estimate of data collection date. (A) Morphologically normal sperm proportion. (B) Proportion with intact acrosomes. (C) (D) (E) Proportion of head, mid-piece, and tail abnormalities, respectively. (F) Proportion of motile sperm obtained from %motile sperm measurement. % motile sperm was used instead of other motility measures such as progressive motility due to sample convenience and high accessibility to this measure.

Model behavior was assessed through posterior predictions and chain stability for all six models. The predictions for all parameters in the normal sperm proportion model returned stable, roughly bell-shaped, distributions with some variance around a mean. All chains also indicated convergent properties as the number of data points increases, stabilizing around the same means indicated in the prediction estimate distributions. Models for other traits resulted in similar behavior, although some instability was observed for head abnormalities estimates with respect to the dispersion parameter (Appendix B).

Taxonomic differences in effect sizes.

Using a non-phylogenetically-controlled model, assuming independence between species, we extracted species-level effect sizes as slopes of normal sperm proportion over time. Our results indicate that there does indeed seem to exist some variation among taxa in the extent to which normal sperm proportions have changed from 1977 (Figure 3). However, there is no apparent phylogenetic structure to these effect sizes and seems more of a random product of sample availability. Although there are certain groups of mammals such as the primates which seem to be experiencing more of a decline in normal sperm proportions over the years, with the lack of data available both within species and across species of the same taxa, broad generalizations cannot be made. Interestingly, however, some species revealed positive changes in the proportion of normal sperm per year such as the six-banded armadillo (*Euphractus sexcinctus*) with an estimated annual increase of 0.023 and the arctic fox (*Vulpes lagopus*) with an estimated increase of 0.025. Yet, once again there is limited data in both species, as the six-banded armadillo was only reported in papers after 2010, all coming from captive individuals, and the arctic fox only had a sample size n=1. Nonetheless, species with notable effect sizes, whether it be that they are experiencing harsh declines in reproductive health or gradual improvement in reproductive health over the years, may provide useful research opportunities, as they may possess some biological mechanism to either counteract this global decline in reproductive quality or be particularly vulnerable to this cross-species trend.

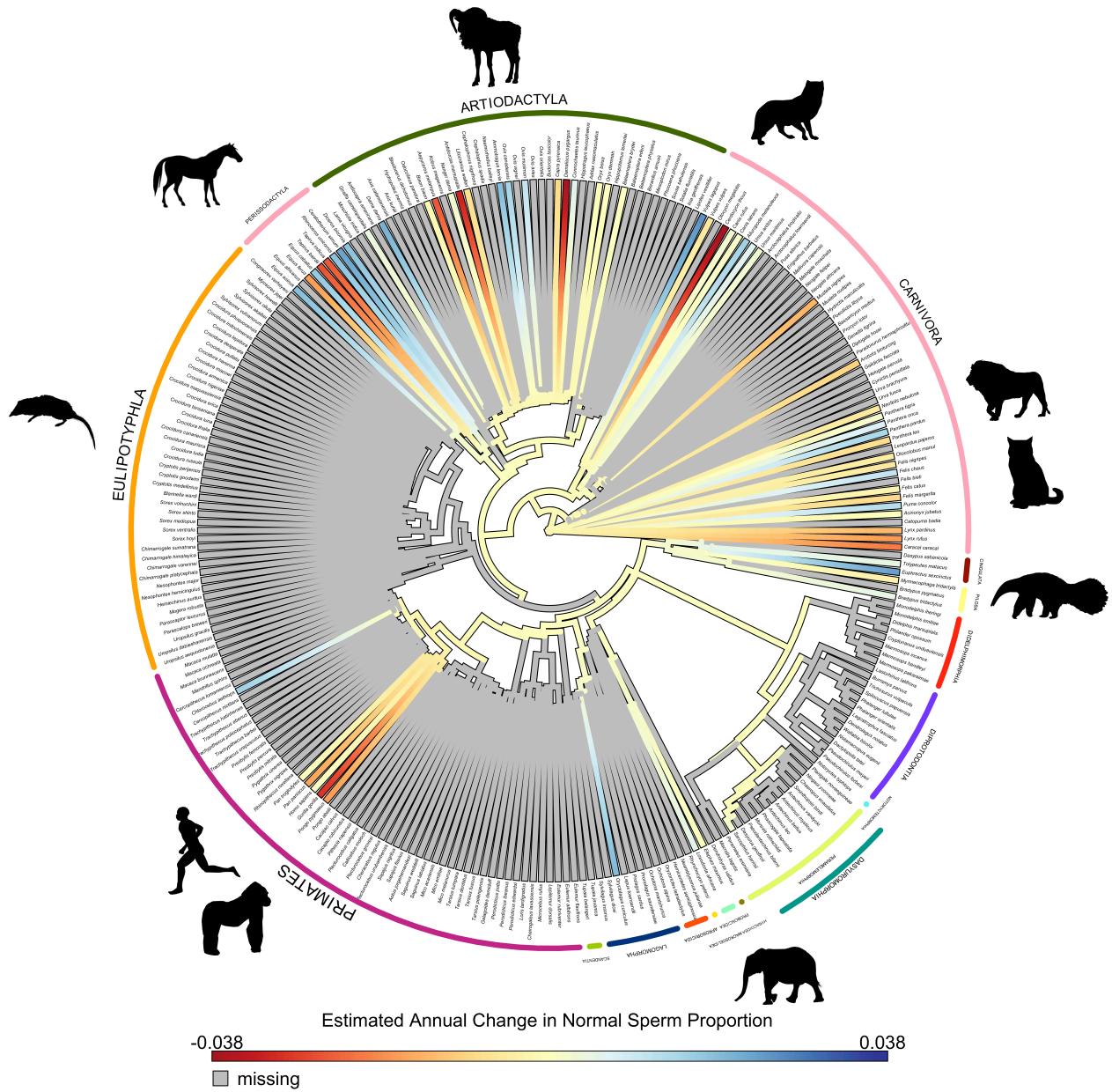


Figure 3. Phylogenetic tree of all 268 species involved in the present study. Colored branches indicate the estimated annual change in normal sperm proportion. Grey branches indicate species with missing data. Exterior arcs indicate positions of phylogenetic orders. Different branch lengths indicate varying divergent times across taxa.

Discussion

As our literature review suggests, there is a great bias in data availability across taxa in the field of reproductive biology, as well as inconsistencies in data collection methods of sperm in mammals. Yet, at the same time, there are numerous studies that report findings regarding

mammalian reproductive health in one way or another. Since the current study was only able to examine data availability in a total of 268 terrestrial mammal species, it can be speculated that further literature searches can greatly expand upon the current dataset. Although the measurements necessary lies in the literature, we still lack reliable information on how reproductive health has changed over the years in these animals. Our findings regarding the significant decrease in normal sperm proportions while motility remained fairly constant, may provide alternate explanations to what was discussed by Harris et al., (2023) and their findings of motility declines in equines over the years. Further investigation will therefore be necessary in order to truly determine if there is a different trend between normal sperm morphology and sperm motility. Additionally, we observed large variation in the distribution of spermatoc trait proportions over the years across the board. With this, it is difficult to conclude whether our observed trends are truly occurring in mammals, or is simply reflective of the sparsity of data, and particularly the lack of measurements in early years around the 1980s. Yet, there still remains great significance in the literature-review portion of the current study, as it identifies gaps in our knowledge on mammalian reproduction. Not only this but, as the dataset and work are openly accessible, it provides easy opportunities for anyone to expand on this study in the future. Future expansion of the dataset should focus on two main points: the incorporation of a wider range of species and the accumulation of a larger number of samples within each species. The former can be achieved by continuing the literature search with the list of species that has already been created. The latter is not a major challenge either, as extending to other search engines such as Google Scholar or extensively checking the citations list for all papers involved in the study are some of the many ways we can obtain more information.

Aside from the sample size and data availability, however, there is a significant underlying issue with the representability of mammalian species within our dataset. Since the majority of specimens involved in the assessment of reproductive health come from captive individuals, it may be debated that our results cannot actually be applied to wildlife conservation as whole. Captive individuals are more prone to inbreeding as zoos often participate in controlled breeding, making the breeding population much smaller than the entire population of a given species. If time and sample sizes permit, comparison of temporal trends in sperm quality traits across captive and wild individuals may lead to important findings. However, with the imbalance in efforts regarding semen collection in captive settings and wild settings, a much larger dataset may be required for significant findings down this path. Additionally, there are challenges associated with distinguishing gene pools of captive populations from wild populations as often times papers reported the existence of wild-born individuals raised in captivity. There may be external factors such as environmental toxin accumulation that could be impacting these two populations differently as well in terms of reproductive capabilities. Tying in global predictors that were not considered in this study such as the wide-spreading distributions of microplastics could enhance our predictions, allowing for us to identify potential causes behind some of the trends characterized in the present study.

Connecting the observed global trends is expected to have great application in the conservation field. Particularly, differences in effect sizes of changes in normal sperm proportions could help shape efforts to species preservation in a more efficient manner. It is truly the comparison of reproductive health statuses that can help us achieve a more targeted approach to population growth while giving animal reproduction the attention it deserves. As of now, there were no significant differences in effect sizes across species grouped by the IUCN status of

extinction risks (Appendix C). Therefore, it is difficult to identify if there are any areas of conservation priority in terms of declining fertility. However, once again, with more data collected in the future, we suggest that there will be useful applications provided by this study.

Conclusion

Overall, the present study reports significant findings in how mammalian reproductive may be tending towards a decline over the past years. We present a holistic approach to this topic, describing a wide range of taxa over a long temporal scale. Future efforts in expanding the dataset as well as incorporating a wider range of predictors in our statistical models will most likely improve upon our findings. With the world expressing greater interests in infertility in humans over the past few years, it is not too late to apply these concepts to the entire body of terrestrial mammals (World Health Organization, 2023). By ensuring we have reliable knowledge in the field of animal reproduction, we can also ensure that our conservation efforts are not going to waste.

Acknowledgements

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Appendix A

The screenshot shows the search interface of the Web of Science database. It features four search rows, each with a dropdown menu and a search bar. The first row has 'All Fields' selected. The second row has 'And' selected and 'Abstract' chosen from the dropdown. The third row has 'And' selected and 'Abstract' chosen from the dropdown. The fourth row has 'And' selected and 'Abstract' chosen from the dropdown. Below these rows are buttons for '+ Add row', '+ Add date range', and 'Advanced search'. At the bottom right are 'Clear' and 'Search' buttons.

All Fields

Example: liver disease india singh
Sperm* OR Semen* OR Reproduct* OR Semin*

And ▾ All Fields

Example: liver disease india singh
"Lion" OR "Panthera leo"

And ▾ Abstract

Example: marine protected areas
Normal OR Abnormal OR Motil*

And ▾ Abstract

Example: marine protected areas
Sperm* OR Semen* OR Ejaculat* OR Semin*

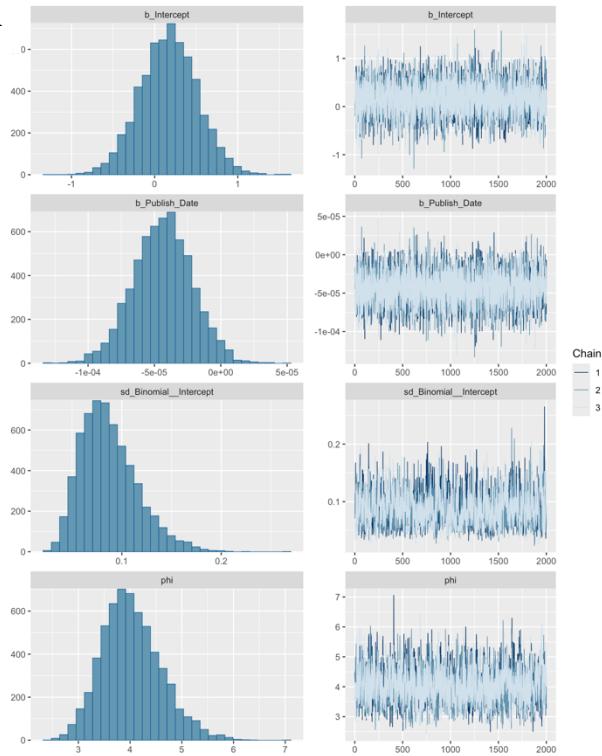
+ Add row + Add date range Advanced search

Clear Search

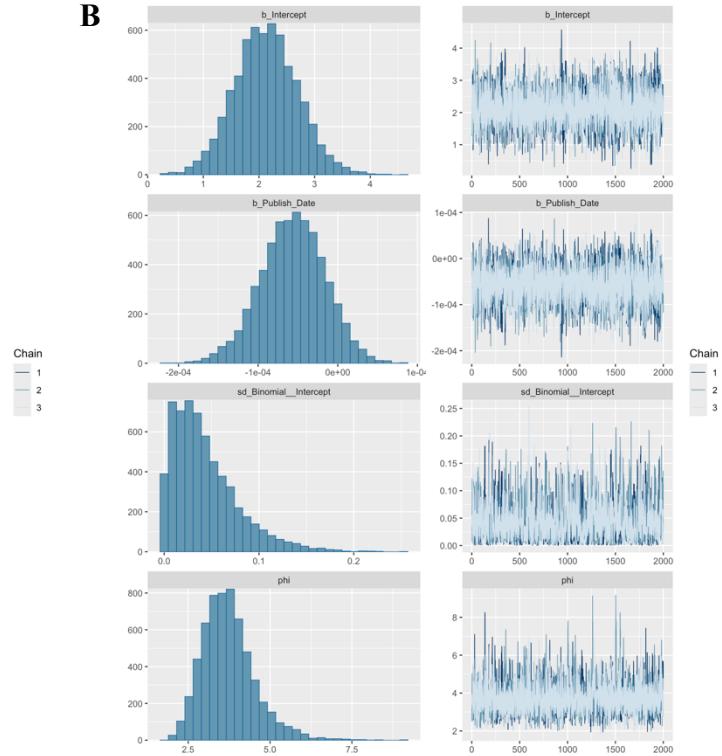
Figure A. Sample search on the Web of Science with the Lion.

Appendix B

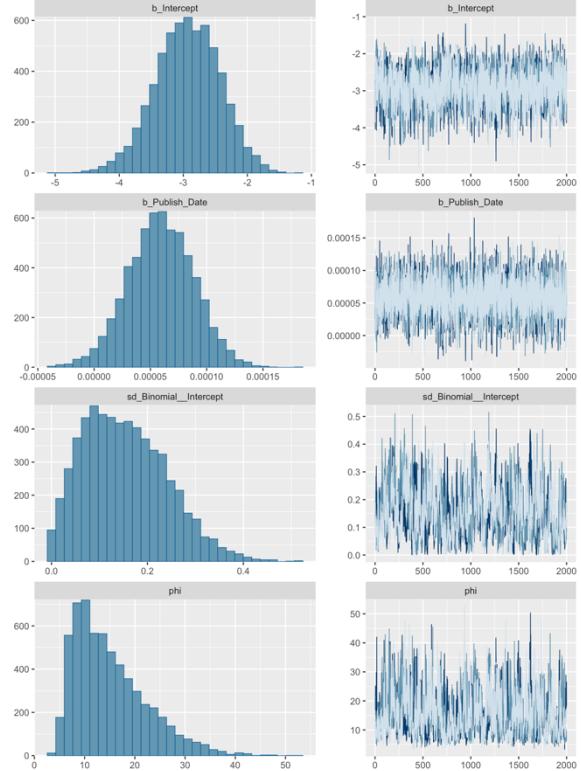
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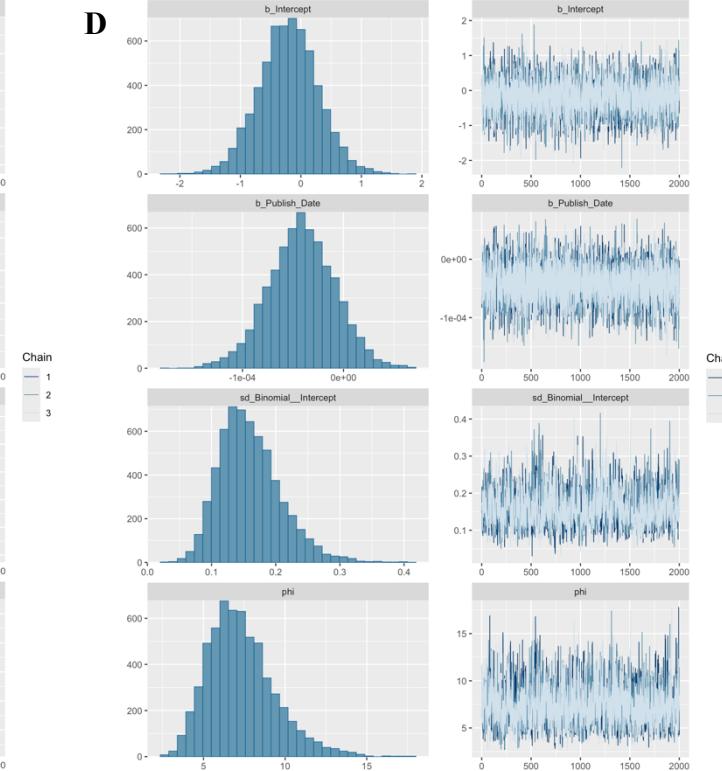
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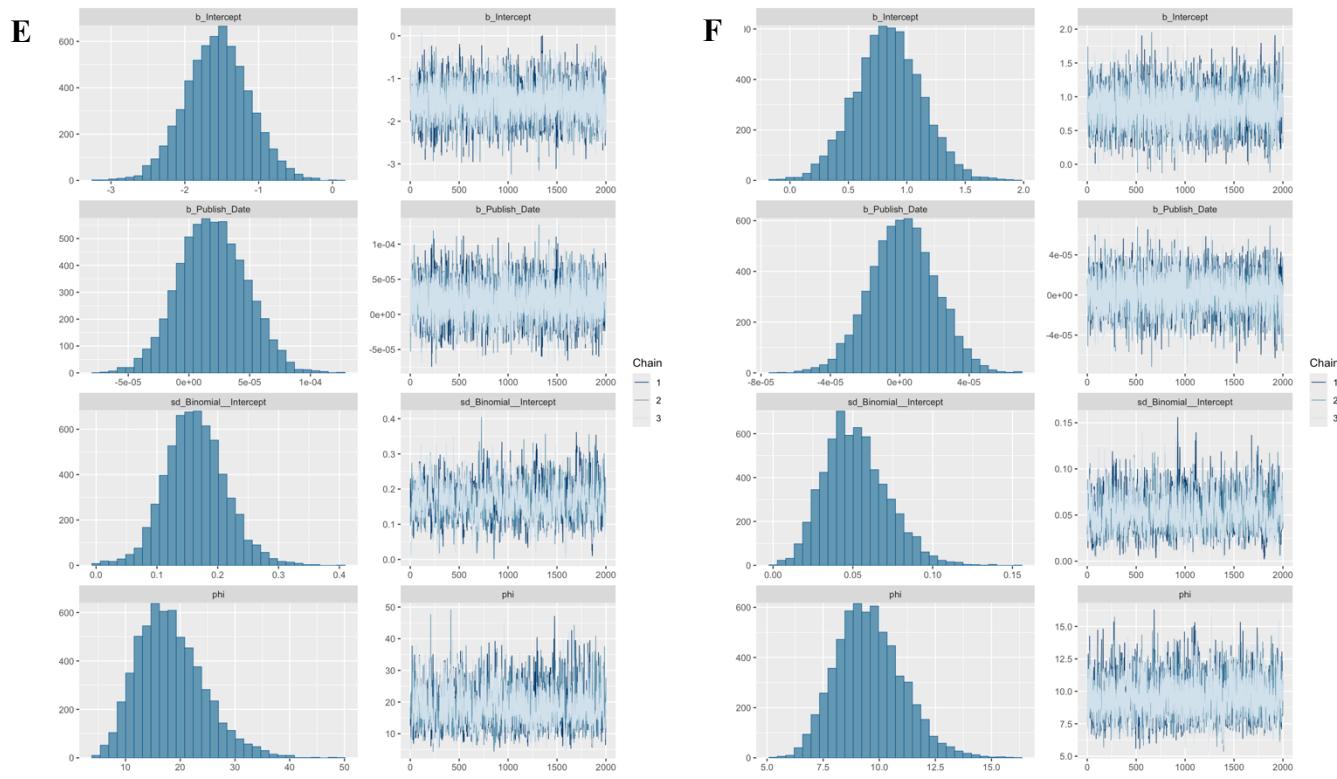


Figure B. Posterior prediction distributions of beta regression models. (A)normal sperm (B)intact acrosome (C)head abnormalities (D) mid-piece abnormalities(E) tail abnormalities (F) motility

Appendix C

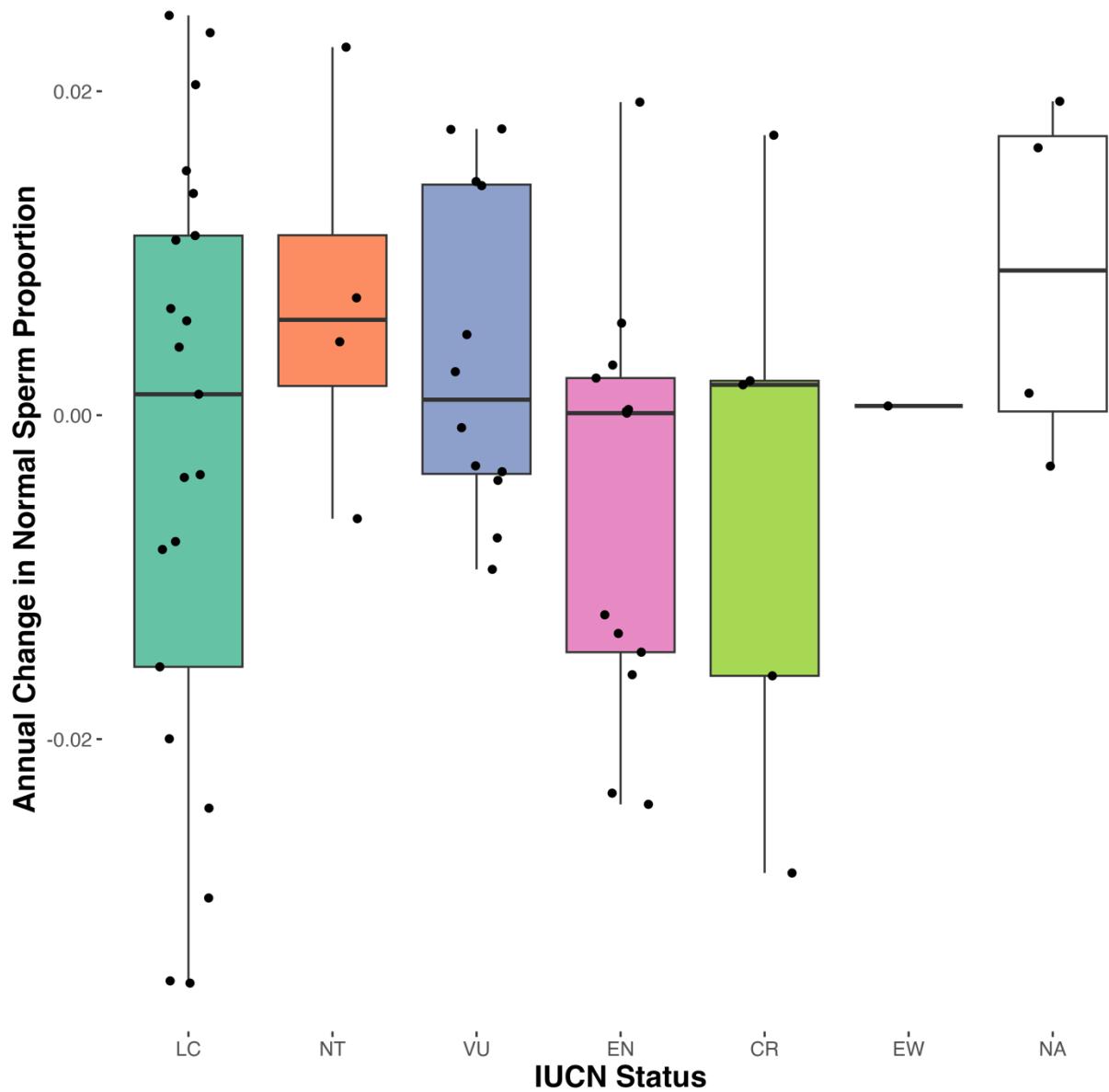


Figure C. Boxplot of estimated annual change in normal sperm proportion for species grouped by IUCN status. IUCN in order of increasing endangered status. NA represents species with no data available on the IUCN status.