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Cover Photo

A wild yak

Credit: **Liu Wei, Northwest Institute of Plateau Biology, CAS**

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EDITOR'S NOTE

By Corinne Bailey, AWCSG Programme Officer

Welcome to the eighth issue of BULLetin, the newsletter of the IUCN SSC Asian Wild Cattle Specialist Group (AWCSG). In BULLetin, we present novel research on the ecology and conservation of Asian wild cattle species, and share stories about their conservation.

This issue profiles significant and diverse Asian Wild Cattle research, including multiple articles describing new genetic findings and research surrounding banteng, wild yak and the evolution and genomic resources of Asian cattle, as well as new insight into the utilisation of thermal drones to support population monitoring of banteng in Cambodia.

We are pleased to also feature a summary and analysis of forty years of *ex situ* conservation of Malayan gaur using studbook data, and also an important status update on tamaraw conservation in Mindoro. Thank you to all our contributors for submitting their research and updates to BULLetin so we can continue to share them with this community.

The ninth issue of BULLetin will be published early next year and we look forward to receiving your interesting articles and updates. Get in touch via social media, our website or contact me at c.bailey@chesterzoo.org.

In addition to banteng, we will be conducting Red List Assessments for certain Asian wild cattle species in the coming years. If you would like to be involved, please contact Tom Gray (tgray@wwf-tigers.org) or James Burton (jamesaburton@yahoo.co.uk). We will be reaching out to many of you to ask for your contributions to this process.

On August 13th, banteng and anoa will be celebrated during our fifth annual Action Indonesia Day. Find out more and see how you can join the global awareness raising efforts on the Action Indonesia GSMPs website: actionindonesiagsmp.org

I'm pleased to share with you that the tamaraw is now receiving even greater support and partnership from the European zoo community (EAZA). In September last year, the tamaraw was approved as a remote style EEP. This means tamaraw is an officially recognised program within EAZA, working with our in country partner the D'Aboville Foundation. This allows the opportunity for European zoos to give technical and financial support to the work in the Philippines. The new tamaraw EEP coordinator is Fiona Sach (Fiona.Sach@zsl.org).

Keep up to date with our activities and other Asian wild cattle news on our website (www.asianwildcattle.org) and social media (Facebook: IUCN Asian Wild Cattle Specialist Group, Twitter: @IUCN_WildCattle and Instagram: @iucn_wildcattle).

We hope you enjoy this issue, and thank you for your continued efforts in conserving Asian wild cattle.

NEWS AND UPDATES

Updating the IUCN Red List Assessment for Banteng

By Milou Groenenberg

Recent dramatic declines in the global stronghold population of banteng (*Bos javanicus*) in Eastern Cambodia (Griffin and Nuttall, 2020; Groenenberg et al., 2020; Groenenberg and Kandasamy, 2021; Groenenberg et al., 2023) have sparked an interest in conducting a re-assessment of the species' IUCN red list status. When the recent rate of banteng population decline in Eastern Cambodia is extrapolated over a three-generation period the estimated population size reduction is estimated to be a shocking 93 - 98%. This astonishing decline rate easily surpasses the threshold of 80% required for the critically endangered category. The Eastern Cambodia population made up roughly 60% of the global population and therefore has a major influence on the overall range-wide population trend. Could it be that this beautiful species is now critically endangered?

In order to find out, the AWCSG is embarking on a thorough global review of the species distribution, population status and trends. Banteng experts from Indonesia, Malaysia, Myanmar, Thailand, Viet Nam, China, Cambodia, and Lao PDR came together on a virtual call in October 2022. During the call the experts agreed on a process and way forward for the assessment. Countries agreed to focal points who will lead the process of gathering data from networks and engaging with governments and other stakeholders to gain meaningful input.

The first banteng red list assessment dates back to 1986 when the species was categorized as 'Vulnerable'. It maintained this status in the three subsequent assessments (1988, 1990, and 1994) but was uplisted to 'Endangered' in the year 2000, and maintained that category over subsequent assessments (2000, 2008, and 2016) (Gardner et al., 2016). Today's endangered status is based on a >50% population decline over a three-generation period. Although the last assessment was published in 2016, the assessment itself was conducted in 2014 and some of the data used for the assessment stems back as far as 22 years ago. The upcoming red-list (re)assessment will not only help conservationists and practitioners to correctly determine the species' risk of extinction, it will also improve global access to updated information on the species, and help to raise the profile of this emblematic species.

If you would like to contribute to the updated assessment, and have not been contacted yet, please click here to get in touch!

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Investigating the reticulated evolution and genomic resources of Asian *Bos*

By Rasmus Heller, Dept. of Biology, University of Copenhagen

Asia is arguably the biogeographic stronghold of the genus *Bos*, with seven nominal species existing in the region. Astonishingly, wild Asian *Bos* have been domesticated at least four times independently, and the region has therefore played an outsized role in the emergence of the livestock that people depend on globally today. Despite the importance of this genus in the evolution of livestock and the history of mankind, and the amount of research devoted to cattle and cattle genomics, it is surprising that so few genomic resources exist for the wild *Bos*. As of today, there is only a single published complete genome from wild gaur, and none for wild banteng. Only about a dozen genomes have been published from captive individuals of these taxa. Previous studies that investigated the sparse whole-genome data that does exist did not distinguish clearly between e.g. wild banteng and the domesticated version referred to as Bali cattle, despite their evolutionary relation being all but clear. Without genomic data from the wild populations, we have very limited insights into the taxonomy, genetic structure, phylogeography and adaptations of these important and endangered species. Furthermore, given that these wild *Bos* have admixed extensively with the domesticated *Bos* in Asia (Chen et al. 2018; Wu et al. 2018), there is an urgent need to
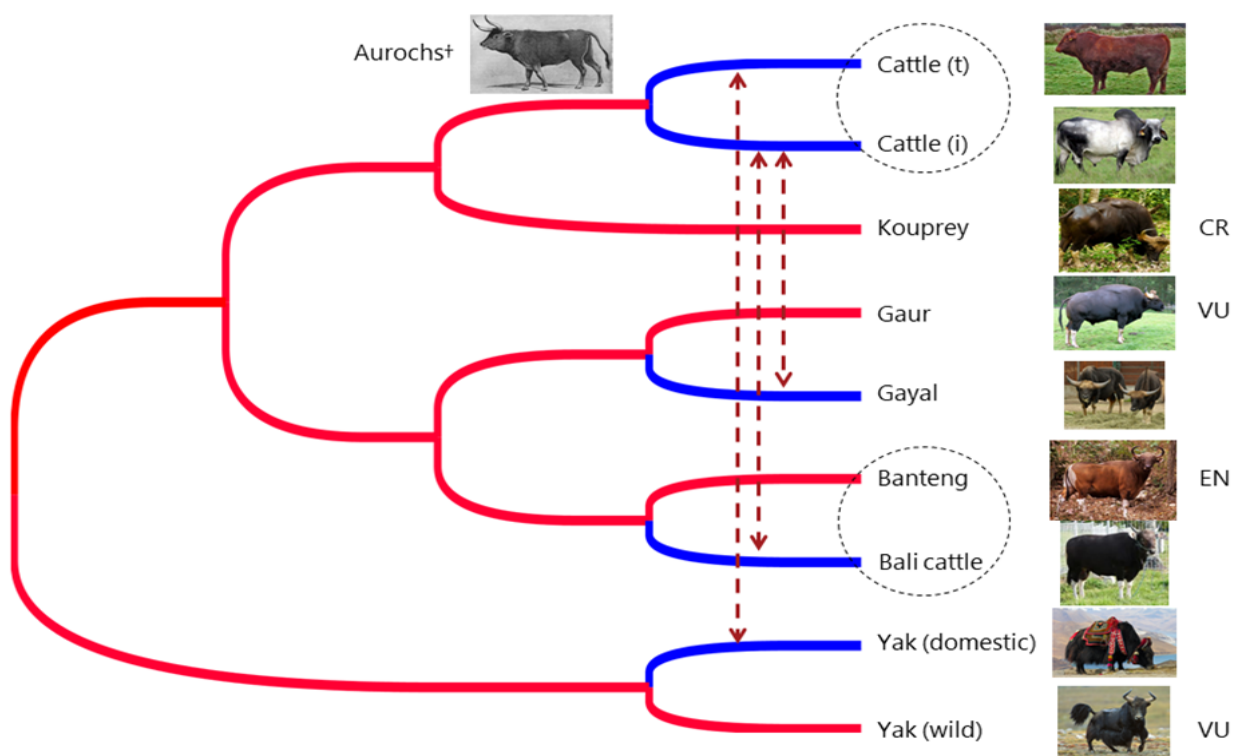
"The phylogeny of the Asian Bos lineages. Dashed circles group formally conspecific lineages. Branches in blue are domesticated lineages. Dashed arrows represent genetically inferred (known) gene flow events. Also depicted is the extinct aurochs, believed to be the ancestor of two independently domesticated cattle lineages, taurine (t) and indicine (i). Branch lengths are arbitrary and do not represent evolutionary divergence. In the right margin is the IUCN Red List category of each wild lineage: 'VU' = vulnerable; 'EN' = endangered and 'CR' = critically endangered. Modified from Wu et al., 2018."

understand better when, where and how this took place, and to determine which genetic variants they have contributed to the domestic Asian *Bos*. Besides its intrinsic biological interest, this is of direct relevance in livestock (especially cattle) genetics and in managing the genetic resources of livestock in Asia and elsewhere.

Since 2018 we have worked on two fronts to close the knowledge gaps caused by the lack of genomic resources from Asian *Bos*. In one project, we are working closely together with Indonesian researchers led by IPB University in Bogor to sequence and analyze complete genomes from several different Indonesian cattle breeds as well as several populations of Bali cattle, the domesticated banteng. We use this data to try to understand how different *Bos* species have contributed to Indonesian cattle, which have a uniquely admixed origin (Mohamadet al. 2009; Sudrajad et al. 2020). We also want to get an insight into the genetic diversity of the Javan banteng, and whether it is threatened by low genetic diversity or genetic swamping from cattle. In another project, we try to understand at a larger scale the reticulated evolution of Asian *Bos*, in particular where and when admixture between different species has taken place, and how it has affected the genetic variation within and between all the different species. The large degree of evolutionary reticulation could even have impacted the domestication processes themselves, by contributing genetic variation that could have been used in human-mediated selection for desirable traits at various times and in particular environments. This synthesis can only be achieved by having representative genomic data from all the important sub-groups of the different wild Asian *Bos*, and in particular the gaur and

banteng is lacking in this regard, with the almost non-existent availability of whole genome data from these species in the wild.

Clearly, more genomic data is needed for these important species! We are actively searching for collaborators in Southeast Asia with an interest in *Bos* genomics and with access to relevant samples. We are also interested in collaborators working on domesticated *Bos* (mainly cattle, Bali cattle and gayal) in Southeast Asia, as these populations are sure to each contain important information about the rich and complex evolutionary history of the genus - as well as unique genetic variation that needs to be characterized so we can better understand the under-appreciated genetic diversity that has entered domestic *Bos* from their wild sister species. Email me at rheller@bio.ku.dk if you are interested in our projects and/or would like to collaborate on these projects.

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Bornean banteng is closely related to Javan banteng

By Mikkel-Holger Sinding, Smurfit Institute of Genetics, Trinity College Dublin

Many would agree that the banteng (*Bos javanicus*), is one of the most interesting bos species alive. Its ecology, domestication and not at least evolutionary history is fascinating to a broad array of scientists. Its evolution is currently controversial based on mitochondrial genetic modelling, from which a truly intriguing lineage sorting is documented. Banteng in mainland Asia has a mitochondrial lineage, similar to that of the gaur (*B. gaurus*) from Peninsular Malaysia and Kouprey (*B. sauveli*) from Cambodia (Sinding et al. 2021). Then the Javan banteng have a private mitochondrial lineage, while the Bornean banteng lineage is nested within a diverse spectra of gaur lineages (Sinding et al. 2021).

This structure has been used to argue that maybe banteng's are more than one species. This was suggested with the publication of the first Bornean banteng mitochondrial genome by Ishige et al. 2016, with a robust placement outside that of mainland, as well as Javan banteng, hence potentially representing a distinct species.

However, mitochondrial lineage sorting may be very different from autosomal structure. A relevant example is in *Bison*, where the wisent (*Bison bonasus*) mitochondrial lineage is a sister to aurochs/cattle (*B. primigenius/taurus*), while the American bison (*B. bison*) mitochondrial lineage is a sister to yak (*B. mutus*). Still at autosomal level, the two bison lineages are clearly closely related (Wang et al. 2018). Therefore, evaluation based on autosomal variation is necessary to evaluate if the Bornean banteng is a distinct species or a form of banteng.
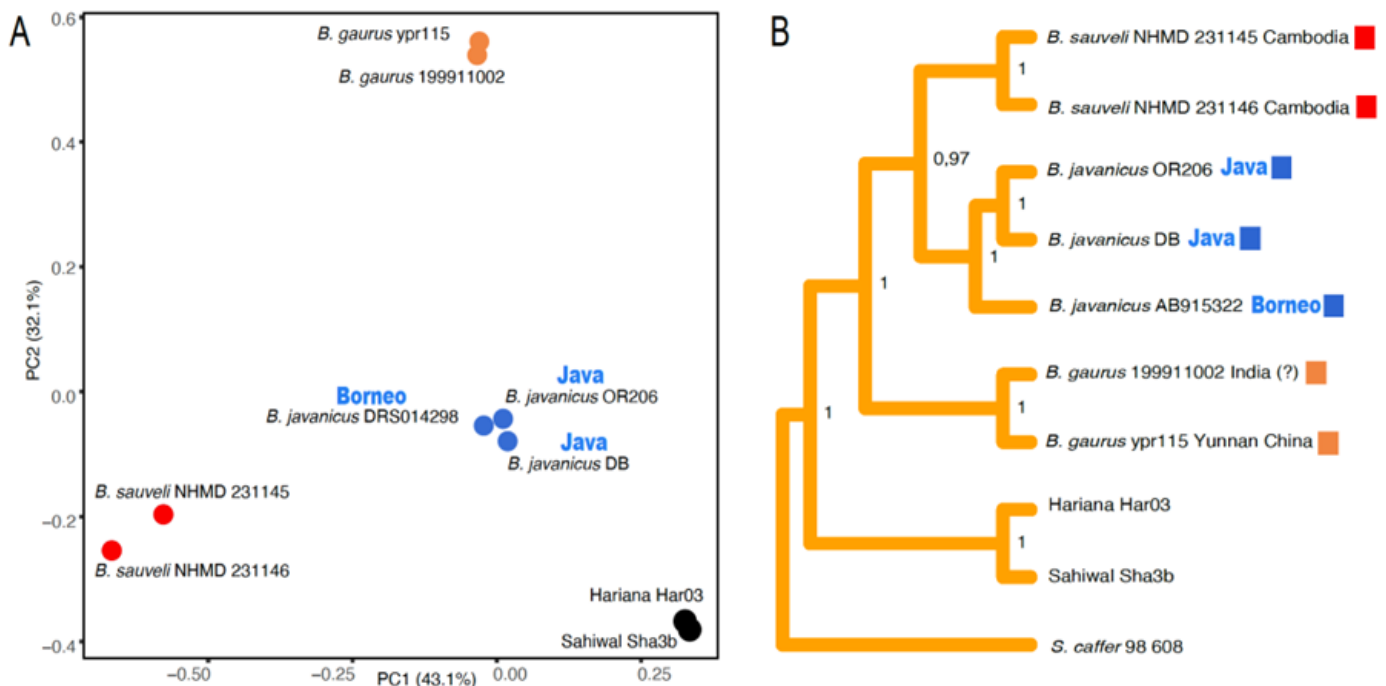

Figure 1. (A) PCA of full nuclear genomes. (B) Nuclear genome phylogeny estimated by ASTRAL-III. The tree is rooted to the African buffalo (*Syncerus caffer*). Numbers placed at branch nodes represent clade supports expressed in posterior probabilities and computed by RAxML-ng using 100 replicates in Astral-III.

Conveniently, the Bornean banteng mitochondrial genome sequence was obtained by assembling the mitochondria from shotgun sequences of a Bornean specimen. The read archive was released along with the paper and is publicly available on NCBI (SRA: DRA00172) (Ishige et al. 2016).

Based on this archive my co-authors and I were able to map a 0,2x nuclear genome of the Bornean banteng and place it into autosomal based species context together with other relevant *Bos* genomes. The work was recently published in Sun et al. 2022 and in short, we find that the Bornean banteng at autosomal level is closely related to Javan banteng (fig 1), supporting that the Bornean banteng indeed belongs to the species banteng. Despite this, we highlight that these results by no means decrease the conservation value of the Bornean banteng, but rather allow focus and coherence by clarifying species affiliation.

Also, this work is based on the few limited (unrelated) genomes available, clearly there is a great need of geographically diverse gaur and banteng genomes, to gain further depth in analysis. I have obtained funding for more work in *Bos* genomics and am looking for potential collaborators with samples. If you are interested and have access to banteng and gaur tissue (non-faecal), please feel free to contact me on email (mhssinding@gmail.com).

The incoherence in banteng structure observed in mitochondrial contra autosomal variation is truly interesting. Whatever the explanation, it hints at a fascinating evolutionary history, as with the bison's, we can only hope more research will uncover this story and its subtleties.

Read the full paper:

Sun, Xin, Marta Maria Ciucani, Jacob Agerbo Rasmussen, M. Thomas P. Gilbert, and Mikkel-Holger S. Sinding. "Genomic evidence refutes the hypothesis that the Bornean banteng is a distinct species." BMC Ecology and Evolution 22, no. 1 (2022): 1-4. https://doi.org/10.1186/s12862-022-02062-1

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Genetic and cellular insights into yak adaptation to the high-altitude environment

By Qi-En Yang, Xue Gao, Rui-Dong Wan, Northwest Institute of Plateau Biology, Chinese Academy of Sciences

The yak (*Bos grunniens*) is one of the largest ruminants living in the Qinghai-Tibetan Plateau (QTP) with the highest average altitude in the world and is physiologically adapted to hypoxic and cold environments. Domestic yaks normally live between 3000 and 5000 m above sea level, while wild yaks inhabit elevations from 4000 to 6000 m on the QTP. Although significant morphologic differences exist between wild yaks and domestic yaks, both species share genetic features of high-altitude adaptation and are considered excellent models for studying hypoxia tolerance in large mammals. Recent studies have shown that structural variants (SVs) are widely present in genomes and provide an extensive source of genetic variation for identifying candidate genes involved in the regulation of critical biological processes. Unfortunately, due to quality-related issues of the yak reference genome, SVs and SV-related genes in wild yak and domestic yak have yet to be mapped and compared with those in the cattle genome in detail.

We assembled reference genomes of domestic and wild yaks using ONT, Hi-C and Illumina data. The domestic yak genome was sequenced and assembled to obtain a 2.61 Gb genome with 30 chromosomes. The scaffold N50 length was 104.02 Mb, and the contig N50 length was 44.91 Mb. Annotation of the domestic yak genome showed that 45.67% of the genome was repetitive, and 23,143 protein-coding genes were predicted. Functional annotation showed that 95.3% of the coding genes had corresponding functions. The wild yak genome was sequenced and assembled to obtain a 2.63 Gb genome and 30 chromosomes. The scaffold N50 length was

Wild yaks (Credit: Liu Wei, Northwest Institute of Plateau Biology, CAS)

103.90 Mb, and the contig N50 length was 38.28 Mb. The annotation of the wild yak genome showed that 45.81% of the wild yak genome was repetitive, with a total of 22,931 protein-coding genes predicted for the genome. Based on the high-quality genomes, a structural variation map of the yak genome was constructed using the genome. Among the SVs obtained based on the three methods, the structural variants obtained based on the long-read data and the matching strategy are the most reasonable method, and the obtained SVs represent the complete genetic map of SVs in the yak genome. Insertions and deletions were the two major types, and more SVs were in intergenic and intronic regions of the gene. Candidate structural variants (High-F_{ST} SVs) for adaptation in yaks were obtained by counting population differentiation indices (F_{ST}) between yaks and cattle, involving 6733 genes, of which 897 genes carrying High-F_{ST} SVs located in exonic and promoter regions were annotated. Finally, transcriptomic, ATAC-seq, single-cell transcriptomic and histological methods were used to investigate the potential influences of SVs on the gene expression and adaptation of yaks to low oxygen. Structural variation combined with transcriptomic data revealed that the lung contains more differentially expressed genes carrying candidate SV (High-F_{ST} SVs & DEGs) and selected a candidate gene, *RORA*; five candidate transcription factors ARNT, GATA1, MAFG, KLF5, and HOXB5

associated with lung development and hypoxic adaptation were identified by combining ATAC-seq data and structural variations. Single-cell transcriptomic data from yaks and cattle both have four types of cell subpopulations: epithelial cells, endothelial cells, mesenchymal cells and immune cells, and a unique endothelial cell population was identified in the lung tissue of yaks.

The results of the integration analysis of multiple samples from cattle and yak suggest possible differentiation within the endothelial cell lineages. Collectively, we assembled high-quality reference genomes of domestic yak and wild yak and described the genomic SV landscape of yak. The findings of the present study provide valuable genetic resources for follow-up studies on yak genetics. These data also revealed the relationship between genomic SVs and yak adaptation, offering a new perspective on the genetic mechanisms of animal adaptation. (These studies were supported by the Natural Science Foundation of Qinghai, 2020-ZJ-902).

Read the full article:

Gao, X., Wang, S., Wang, YF. et al. Long read genome assemblies complemented by single cell RNA-sequencing reveal genetic and cellular mechanisms underlying the adaptive evolution of yak. Nat Commun 13, 4887 (2022). https://doi.org/10.1038/s41467-022-32164-9

Wild yaks (Credit: Xinmin Lian, Northwest Institute of Plateau Biology, CAS)

Demonstrating the use of thermal drones for the monitoring and protection of wild Banteng (*Bos javanicus*) in the Kampong Speu region of Cambodia.

By Naomi Davies Walsh[ab], Nick Marx[c] and Serge Wich[b,]
[a] Knowsley Safari, Merseyside, UK, [b]Liverpool John Moores University, Merseyside, UK, [c]Wildlife Alliance, Phnom Penh, Cambodia

Project Background

Illegal wildlife trade is an ongoing problem throughout Southeast Asia. Cambodia based NGO Wildlife Alliance (WA) aim to protect the country's rainforests, counteract the illegal wildlife trade through rescue and rehabilitation and provide education and support to local communities (Wildlife Alliance, 2021). Due to the 'high value' nature of the animals they aim to protect and rescue; WA's protected sites are a target for poachers. Although WA have significant and successful anti-poaching patrols in these areas, ranger deterrents have limitations in forest environments and at night, when it is easy for hunters to evade capture. To support their efforts, WA are now looking to explore new technology in the fight to protect Cambodia's wildlife.

Drones with thermal cameras have been shown to have potential as a deterrent for illegal hunting (Hambrecht et al., 2019; Doull et al., 2021), as they can capture real time images at night as well as from within vegetated areas where individuals are camouflaged, meaning that illegal hunters cannot rely on the cover of darkness, or a canopy, to evade detection. The presence of a drone in the area can also be a deterrent. This is especially true when conveyed via signage in the operational area, though often word of mouth is

just as effective, and subsequently reduces the personal risk for ranger teams when targeting illegal hunters (Olivares-Mendez et al., 2013; Wich and Piel, 2021). Although there are still challenges e.g., dense vegetation reducing thermal capacity, the ongoing development of the technology is promising.

The use of drones to detect animal presence is also increasing (Corcoran et al., 2021; Chalmers et al., 2021; Burke et al., 2019). Thermal technology can allow animal populations to be seen and recorded more effectively, as animals are not hidden when camouflaged against vegetation. It is also useful for crepuscular and
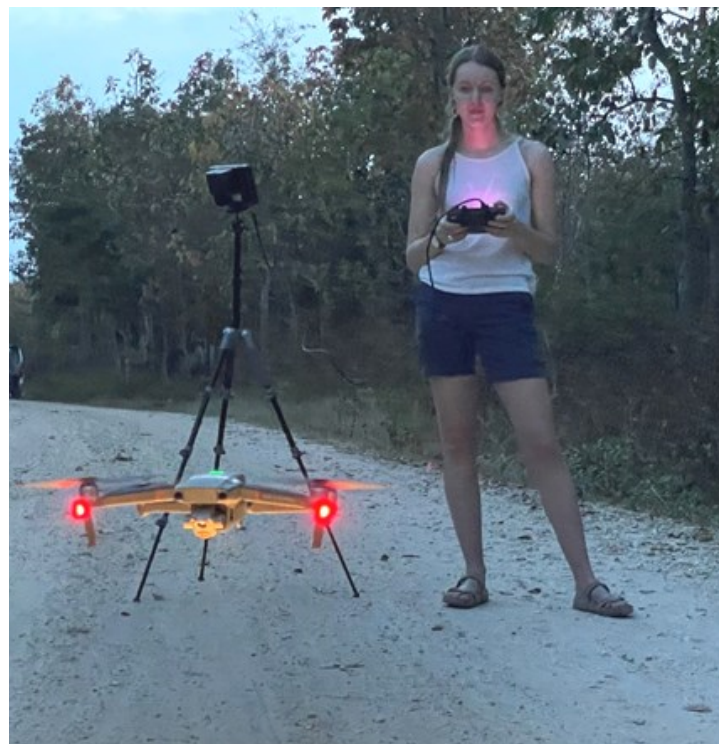

Figure 1. *Drone taking off at Phnom Tamao Wildlife Rescue Centre*

Figure 2. Still taken from drone footage demonstrating the boundary between the protected forest and sugar cane plantations.

nocturnal species (Wich and Piel, 2021). Drones have previously been used as effective monitoring tools for cattle and deer species (Rivas et al., 2018; Chretien et al., 2016) e.g., Banteng (*Bos javanicus*) and Gaur (*Bos Gaurus*). Regular population surveys of threatened species are also beneficial for conservation action planning, noting patterns that may detect threats to the population e.g., hunting and identifying where interventions are necessary. High tech and effective population monitoring can also contribute to ongoing research into a wide variety of species. The ability to monitor wild populations would enable more informed decisions regarding management of wild populations and release of captive individuals in the future. Thus, organisations like WA would be able to funnel resources in the most effective way to maximise conservation output.

Banteng in Kampong Speu, Cambodia

One of the many community projects WA supports is a local effort to protect an area of forest in the Kampong Speu province. Local families in the area started protecting the forest and its wildlife in 2003. Despite this, a lack of outside support has resulted in the area of forest reducing over time, gradually being replaced with sugar cane plantations. Efforts to protect the banteng have resulted in conflict between the community and sugar cane plantation owners due to the tendency of the banteng to leave the protected forest and graze on the sugar cane. WA became involved in the project in 2018, supporting a community ranger team to protect the banteng that reside in the restricted forest. Snares are frequently removed from the area and an ongoing camera trap study aims to gain further insight into wildlife in the area, including the banteng herd. The camera traps in operation have photographed calves, suggesting the population is recruiting, although there isn't yet enough data to produce a population estimate (Marx, 2023).

2022 Field Trip:

An opportunity was identified to trial thermal drone technology as a resource for WA to protect their release sites from poaching activity, as well as a population monitoring tool for focal species, including the banteng herd in Kampong Speu. Professor Serge Wich and colleagues at Liverpool John Moores University (LJMU) are internationally renowned for their work on conservation drones. They focus on using drones equipped with thermal cameras to aid population monitoring, inform conservation action and prevent illegal activities such as poaching, and have been using Knowsley Safari (KS) as a test site since 2016.

In order to assess the suitability of the technology, a team consisting of conservation scientists and keepers from Knowsley Safari, and Professor Serge Wich and Katie Doull, LJMU, travelled to Cambodia in February 2022 . Phnom

Tamao Wildlife Rescue Centre (PTWRC) facilitated initial drone tests. This location provided an ideal opportunity to test the technology at a known site with captive animals to ensure availability and validity of data before heading into the field. The drone was trialled at different times of day, including early morning and late at night to assess the effect of temperature on the ability to detect presence of different species. Following this testing phase, it was concluded for the tropical Cambodian climate, flying the drone before 7am and after 6pm gave the best results. Both RGB and thermal data were successfully collected on Banteng and Gaur at different times of day, providing confidence that those species would be detected in the field.

The team then travelled to Kampong Speu province, with an aim to locate the Banteng and demonstrate the capacity of a thermal drone in ongoing monitoring of the animals to make
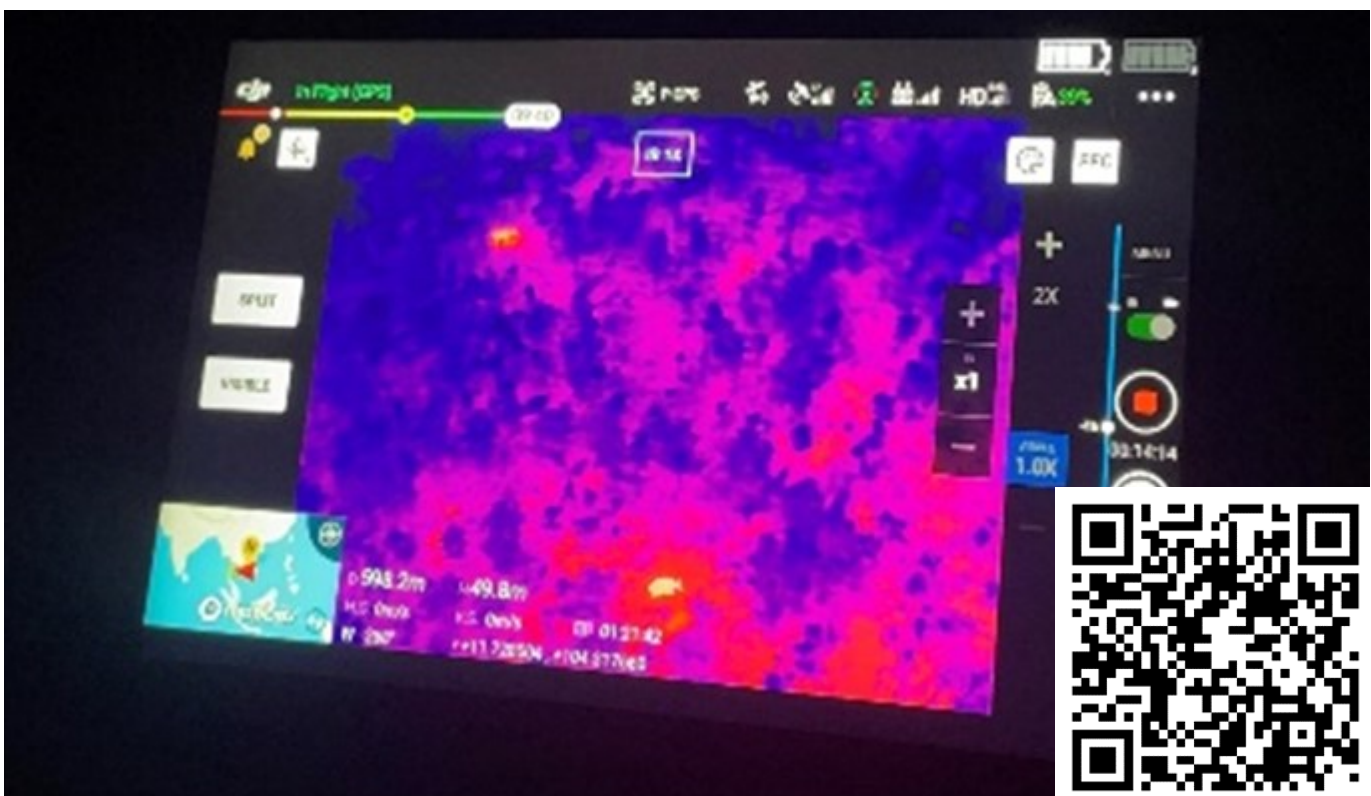

Figure 3. (R)QR code linking a youtube video with thermal footage of Banteng in Kampong Speu Province and (L)Two banteng visible after using a thermal drone to locate them

evidence-based conservation management decisions. Findings from PTWRC were used to locate the banteng. Flights were conducted with support from the ranger team during early evenings and early mornings over three days.

Heavy rains interfered with two of the six scheduled flight sessions, however the banteng were successfully located on days 2 and 3, demonstrating the capacity of thermal drones for further monitoring of this population. On the final morning, a herd of 15-20 banteng were located during the final flying session, demonstrating the potential of recurring flights to collect data to calculate a population estimate. Furthermore, the team were able to demonstrate the ability of the thermal drone to identify human presence using the individuals assembled on site, highlighting the additional potential for thermal drones as a poaching deterrent in that area.

The future

After seeing the thermal drone in action, Wildlife Alliance are now looking to obtain one to aid in their conservation efforts. The drone would be used to both deter illegal activity within their release sites and assist in the monitoring of wild populations of species they strive to protect. Once the technology is procured, staff at Knowsley Safari and Liverpool John Moores University will support with research design and analysis and consult when exploring the use of the technology with new species or in new locations. In the meantime, the community ranger team, with support from Wildlife Alliance, will continue collecting snares and patrolling the forest to protect not only the Banteng, but all species that reside there.

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RESEARCH

Four years after the Population and Habitat Viability Assessment, what do we know about the status of tamaraw (*Bubalus mindorensis)* in Mindoro?

Status of research and findings and implication for conservation.

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Introduction

The tamaraw (*Bubalus mindorensis*), or Mindoro dwarf buffalo, is endemic to the island of Mindoro in the Philippines and was once widespread across the island (Custodio et al. 1996). However, the species is now restricted to only a few isolated locations and is listed as Critically Endangered on *The IUCN Red List of Threatened Species* (Boyles et al. 2016). The **Tamaraw Conservation Program (TCP)**, a flagship program of the Philippine Department of Environment and Natural Resources (DENR), is in charge of coordinating and consolidating efforts in the implementation of the **Tamaraw Conservation and Management Action Plan (TCMAP)** that was formulated following a Population and Habitat Viability Assessment (PHVA) in 2018. The TCMAP provides a road map outlining actions for each of the four sub-populations known to persist on the island at the time of the PHVA workshop. Here, we present

new information on the status of each population as of 2023 and discuss how finding or changes in recent years may impact tamaraw conservation more broadly.

Mts Iglit-Baco Natural Park – A population that is smaller than previously thought and contracting in range.

Mts Iglit-Baco Natural Park (MIBNP) was first created as a game refuge in 1969, and then proclaimed as National Park the following year in order to protect the tamaraw. Domestic cattle ranching was phased out prior to protected area gazettement. The protected area covers 106,655.62 hectares of difficult terrain in the south central part of the island. The current tamaraw population of MIBNP, the largest of all known sub-populations, is entirely located within the territories of the Taobuid indigenous peoples whose ancestral domain shares boundaries with

the Park. By-catch and intentional killing for traditional purposes from Taobuid remain one of the causes of death of tamaraw, while poaching from outsiders has been a long-standing threat to the species.

The tamaraw population at MIBNP has been monitored on a yearly basis since 2000 with a method referred to as multiple vantage point estimation. Each year tamaraw are simultaneously counted from 19 vantage points distributed over a 2,200 ha area (called the "count zone"). The count zone is part of a core area that is regularly patrolled by rangers and where all tamaraws are confined. Vantage points are located in strategically located areas that give good field of view, and each observer is tasked with recording tamaraw within a specific area of the count zone. Because the multiple vantage point estimation method is based on direct observations, this approach requires observers to be able clearly to see tamaraw – which is difficult under natural conditions in

MIBNP, due to the high grass that covers most of the count zone. As a result, every year the local authorities burn the grassland within the sampling area, with the primary aim of increasing visibility so that tamaraw can be sighted, and a secondary aim to attract animals to new grass shoots that sprout following burning. Counts are repeated eight times (four consecutive mornings and evening sessions of 90 minutes each at dusk and dawn). At the end of the counting process, counts of the same animals between vantage points and different sessions are removed from the total count. The process of removing multiple counts is called the consolidation of tamaraw numbers. The final estimated population abundance from this approach is therefore the total number of animals counted during the consecutive sessions minus animals that were "double counted" during all sessions and considered different. The final number (Fig.1) is presented as the official tamaraw population size in MIBNP, and is used by authorities and conservation organizations.
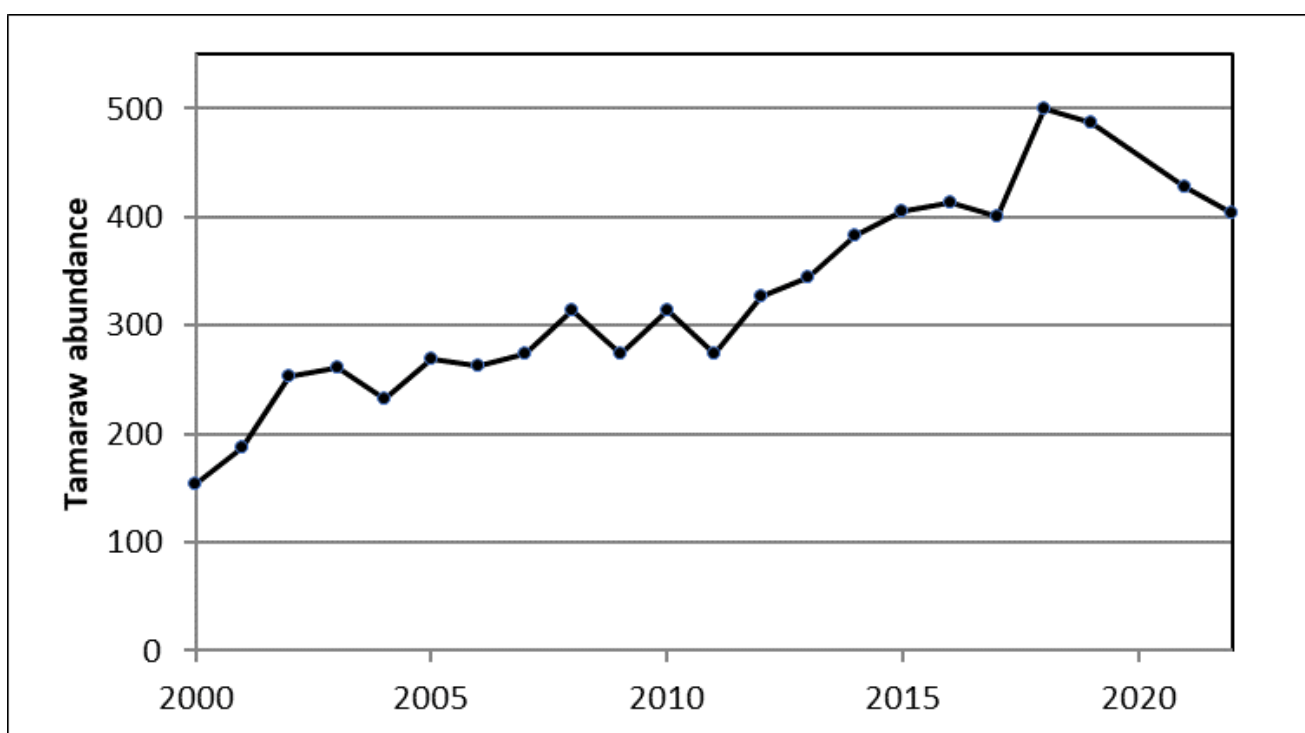

Figure 1. *Historical results of the annual tamaraw (Bubalus mindorensis) population count showing the total number of animals estimated after consolidation each year at Mounts Iglit-Baco Natural Park on Mindoro Island, Philippines. Note that there was no count in 2020 because of the COVID-19 pandemic.*

The multiple vantage point time series of tamaraw abundance has been valuable in measuring the population trend in MIBNP and assessing the effectiveness of protection measures. Bonenfant et al. 2023 used this data to estimate an average growth rate of 0.06% over the past 22 years. Moreover, analysis of the annual count data from 2003 to 2022 showed strong spatial structuring within the count zone, with the population growth close to +10% in the central vantage points located nearby ranger's base camps, and a growth rate of −5% at the periphery of the count area (Bonenfant et al. 2023). These results provide insights into the progressive contraction of the tamaraw distribution in the cores zone of MIBNP – an observation that has also been suggested by rangers working in the park. It is likely that tamaraw range is contracting because anthropogenic pressures are forcing the species to concentrate in high densities in areas where the presence of rangers is more deterrent to poachers and where Taobuid IPs have no permanent activities, creating a source sink dynamic (Fig.2). Despite a general increase of the population since the beginning of the census (163 in 2000, 396 in 2022), the multiple vantage point data highlights a progressive decrease of the average growth rate in time, suggesting significant density-dependence at the population level. If true, this means that the current abundance is close to the carrying capacity, with animals competing for space and resources in a smaller and smaller area.
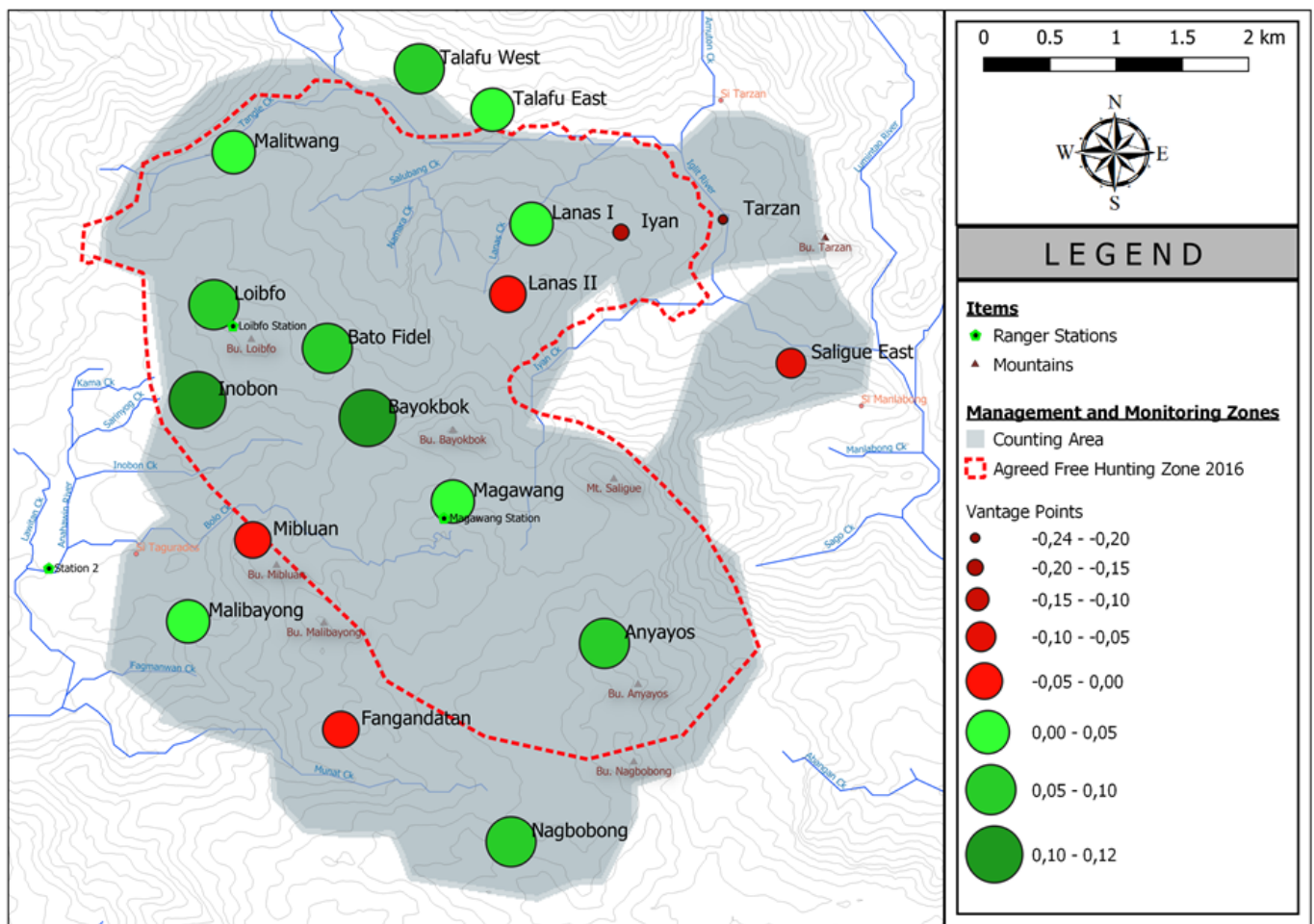

Figure 2. *Spatial variations in the local growth rate of tamaraw (Bubalus mindorensis) abundance in Mounts Iglit-Baco Natural Park on Mindoro Island, Philippines, from years 2000 to 2021 (taken from Bonenfant et al, 2023).*

The design of the multiple vantage points method is similar to other census methods used for other wildlife species around the world. However, because detection probability is not estimated, the annual tamaraw population count operation is unable to provide an absolute number or density of tamaraw, but instead provides an indicator of relative abundance. In practice, this means that it can provide trends in population abundance over time, but not the number of tamaraw in the MIBNP population.

A closer look at the multiple vantage points method and its protocol reveals several issues:

First, the density of animals returned by the annual tamaraw count raises some questions. Official results of the annual count suggest that the calculated density of animals in the count zone is much higher than any other value found in the literature for ruminant species of similar body size (table 1, p27). Based on results of the count for the period 2019-2022, more than 400 animals would live in an area of 2200 ha. This corresponds to a density of around 20 animals per km², which is rare for a wild herbivore species of this size and ecology. Estimated numbers in the vantage points located at the center of the count zone, where most sightings are recorded, return an even more biologically unrealistic density estimate of more than 50 animals per km², which is similar to densities found in the domestic cattle ranching industry.

Second, there are problems with the cumulative nature of the multiple vantage point count estimator, where participants attempt to sort out new tamaraw from animals observed in previous sessions. Such protocol means that the probability of errors increases with the number of sessions, while the total number of animals

becomes directly impacted by the number of sessions conducted; the more the number of count sessions, the larger the final result.

Third, the intrinsic subjective nature of the simultaneous multiple vantage point count method creates sources of variability at all phases of the operation, from observations in the field (variability in segregation of sexes and age classes, skills in spotting of animals between observers, determining whether animals have been previously recorded or not) to the consolidation step (choice of consolidation method, removing of possible multiple counts, people leading the operation). It is likely that changing observers or repeating the data consolidation process would lead to a different abundance estimation of tamaraws each time, thus limiting the ability to compare count years between each other.

As a result of these issues, it has been suggested that the annual tamaraw population count could have been overestimating the true number of tamaraw present at MIBNP, possibly hiding a long-standing stagnation of the population or a more concerning decline in recent years. Indeed, an overview of the raw data shows that the total number of animals sighted, before consolidation, seems actually to have reached a peak in the mid-2010s (Fig.3). The last annual count conducted in April 2023 goes in that direction with a substantial drop in the number of animal counted after consolidation with only 325 this year compare to 396 animals in 2022. The overestimation of the tamaraw population in MIBNP is corroborated by the results of a recently implemented and robust population size estimation analysis using the independent double observer estimator method carried out in 2022 and 2023 by the D'ABOVILLE

Foundation and Demo Farm Inc. (DAF) together with the TCP and the park office. The method, which aims to correct the bias of the multiple vantage point approach by calculating the probability of detection of tamaraw, suggests a population nearly half the size of the results of the annual count (2022: [163-200], 2023: [135-180]).Interestingly, the ratio between the results of the double observer estimator and the annual count for both years are similar (2022: [0.41 – 0.5]; 2023: [041 – 0.55]). This is suggesting that both methods, hence imperfect in estimating the true number of animals, are consistent in sustaining that the decrease in the number of animals observed reflects a true decline of the population in the recent years.

From a conservation perspective, a much lower tamaraw population size than what was envisioned means that the long-term viability of the species at MIBNP has probably been over-optimistic. Based on previous estimations and the data available at that time, the population viability analyses published by the Population and Habitat Viability Analysis (PHVA) assumed an initial population size of 400–500 animals and a conservative annual growth rate of 0.04 (Lee et al. 2019). Because the estimated time to extinction in PHVA models is a direct function of initial population size, it is likely that, with an initial population size of approximately 200 instead of 400, the projected extinction time is substantially shorter than the >100 year result that was previously estimated (Lee et al. 2019), even with a corrected growth rate reevaluated at 0,06. We recommend that the PHVA models are re-run with the updated population size information. In general, the new population size estimate strengthens the concerns raised at the PHVA workshop in 2018 about the viability of the MIBNP population, and stresses the urgency of implementing the measures and strategies outlined in the Tamaraw Conservation and Management Action Plan.

Another consequence of the revised population estimate in MIBNP is that it may cause conservationists to reassess the feasibility of
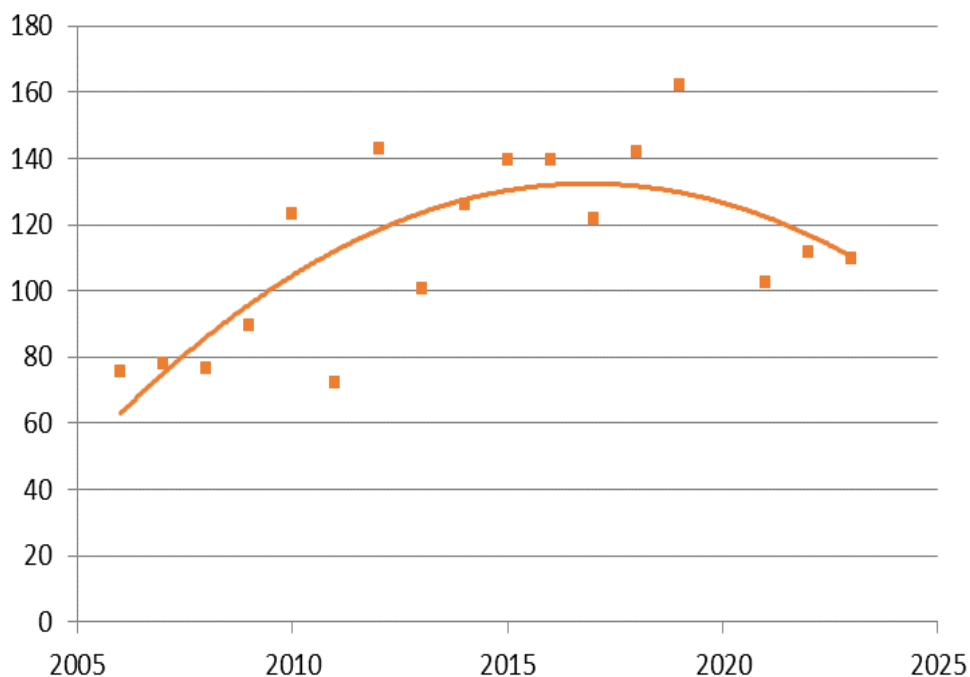

Figure 3. *Evolution of the average number of sightings per session from the raw data (total number of tamaraw sighted) during the annual tamaraw population count between 2006 and 2023, Mts Iglit-Baco Natural Park on Mindoro Island, Philippines.*

using this population as a potential source for translocation or *ex situ* intervention. The number of animals that could be removed without jeopardizing the viability of the MIBNP population will be smaller with a population of 200 rather than 400 individuals. This, in turn, may impact the ability of using the MIBNP population as a source for reinforcement of smaller populations such as Aruyan-Malati. However, any decisions about *ex situ* interventions in the MIBNP population will require robust analyses and in-depth discussions with local stakeholders.

Finally, the fact that negative density dependence is still occurring within such a small population highlights the need to increase the space available for the species where it could disperse and reproduce safely so as to see the population increase up to a more viable number of animals. As a matter of fact, the 1600ha "No Hunting Agreement Zone" of 2016, where Taobuid and TCP agreed that no traps would be set inside, is far too small to secure a viable population with full reproduction potential. An expansion of such agreement would be crucial for the conservation of the species.

Aruyan-Malati – A small population in decline

The Aruyan-Malati forest area, in the municipality of Sablayan in Occidental Mindoro, has long been known to hold tamaraw. The Aruyan-Malati region is believed to have harboured a substantial number of tamaraws in the 1980s; indeed, 20 animals were captured there between 1982 and 1994 to establish the tamaraw gene pool farm. The goal of the gene pool farm was to create an insurance population for the species, but ultimately this was unsuccessful, due to difficulties with managing and breeding the

species. Kalibasib, the only captive-born tamaraw reaching adult age, died in 2021 without leaving any decedents. In 1998, a maximum of 26 tamaraws was estimated in Aruyan-Malati during a rapid population assessment conducted by TCP using indirect signs of presence and direct sightings. (TCP records, unpublished). By 2015, the population was estimated to be no more than 15 animals by using a similar method (DENR TCP report, 2015). As a consequence of this finding, local authorities initiated the filing of 3,000 ha within the Aruyan-Malati region to be declared as "critical habitat", a protection category under Philippine laws that provides some level of resource and protection (albeit less than what is given under full protected area status). The TCMAP supported this initiative, calling for urgent actions in Aruyan-Malati before the tamaraw population declined further. As a first step, it was recommended to conduct a proper baseline survey on the small population.

From 2020-2022, DAF and the TCP conducted a systematic camera trap survey in Aruyan-Malati with the goal of assessing tamaraw population status. The survey followed a grid-based design, with stations spaced approximately 300m apart

Tamaraw captured on camera trap in Aruyan– Malati

across the core forest area of Aruyan-Malati, and active in the field for 4-5 weeks. Twenty camera traps were first deployed for five months in 2020, covering 80 locations and 700ha, undertaking four rounds. It was followed by another deployment of twenty devices for another five months in 2021-22 to complete the sampling area, based on information collected during the first phase. A total of 1000ha was sampled over the two years, defined thanks to prior foot surveys and consultation with Taobuid communities residing in the region and sharing their living space with the species. In seven stations the cameras malfunctioned or were stolen, providing a total of 73 active stations and 1,889 camera trap nights. Tamaraw were recorded in only six stations, with 51 events (independence threshold = one hour) (Fig. 4). At least four different individuals can be distinguished from the photos, based on physical characteristics and age.

Although the camera-trapping survey was unable to provide an estimate of tamaraw abundance, the findings are, overall, in accordance with the TCMAP estimates of no more than 15 animals – and the number may be in the single digits. Equally alarming is the fact that tamaraw were only recorded over an area of restricted range of approximately 170 ha at the time of the study. Together, the small population size and small area of occurrence reinforce the notion that the Aruyan-Malati population is currently not viable and will disappear in the near future without urgent conservation actions that will likely involve *ex situ* reinforcement. We also urge local officials to accelerate the approvement of the proposal to declare Aruyan-Malati as critical habitat.

Mt Calavite Wildlife Sanctuary – Still no evidence of tamaraw

Historically, tamaraws were also present in Mt. Calavite Wildlife Sanctuary (MCWS), a protected area in the Municipality of Paluan in the extreme northern part of Occidental Mindoro. In 1987,
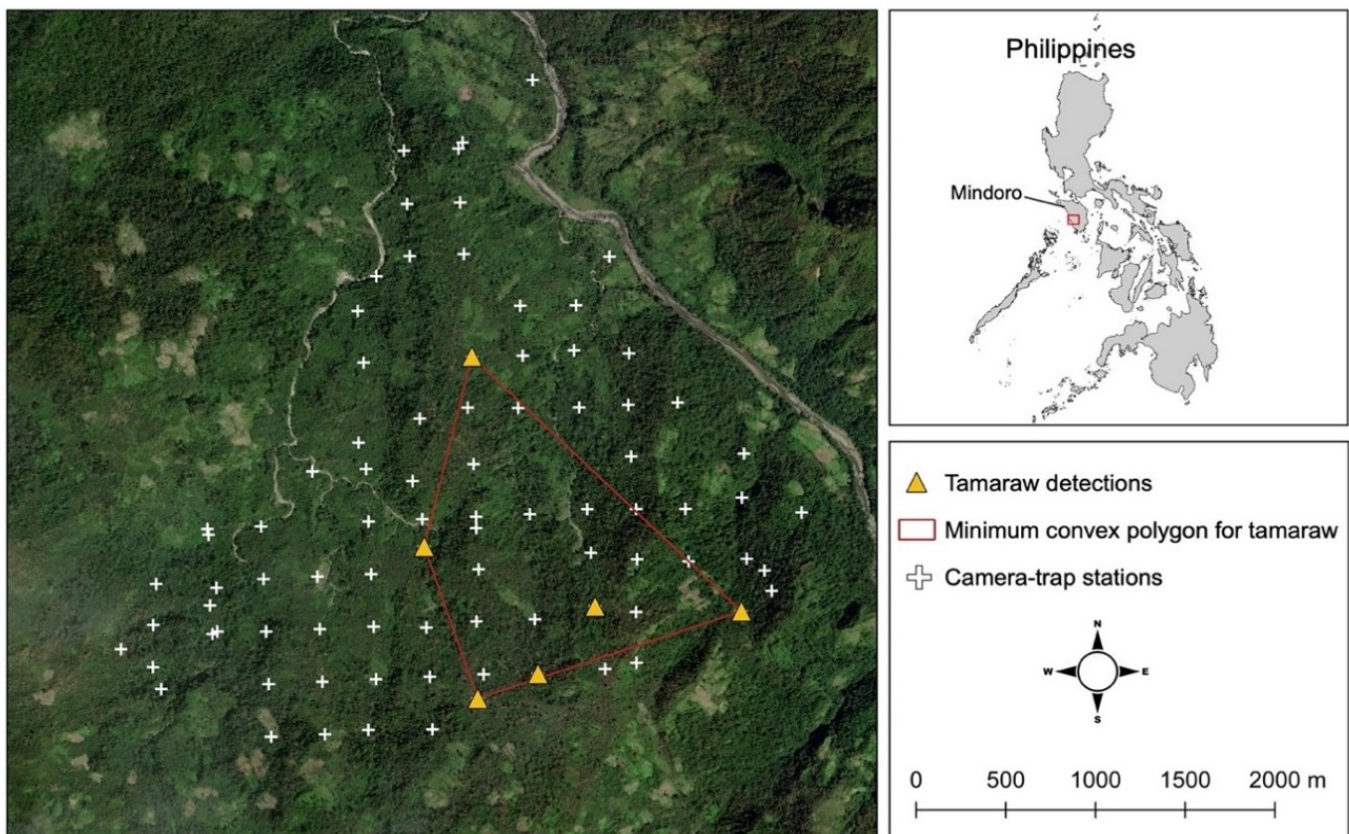

Figure 4: Aruyan Malati camera trap results: detections of tamaraw 2020-2022

Petocz (1989) estimated the tamaraw population in MCWS to be 45 individuals (though the method used is unclear). However, during surveys conducted in 1994, only a single animal was sighted, with some additional evidence of tamaraw from tracks and dung (de Leon et al. 1996). More recent surveys conducted in 2013 and 2014 failed to record tamaraw sightings or sign (Balete et al. 2013; Ishihara et al. 2014). The tamaraw population in MCWS was considered possibly extirpated until 2019, when a tamaraw survey conducted by the Mindoro Biodiversity Conservation Foundation Inc. (MBCFI), in collaboration with the DENR TCP and DAF, reported signs of tamaraw presence based on dung and tracks, and a sighting of a possible juvenile male. Based on this preliminary information, researchers estimated that the number of tamaraw in the site could be between 4 and 6 individuals (MBCFI 2019, Tabaranza et al., 2021), though this was little more than a guess. However, no sign of tamaraw presence could be photo documented, while the possible presence of feral carabaos or water buffalo (*Bubalus bubalis*) in the protected area brings doubt to these conclusions.

It is imperative to conduct robust and intensive surveys to assess whether tamaraw occur in MCWS, and if so, its population size and distribution. The project MATAPAT (**M**ultidisciplinary **A**pproaches for **Ta**maraw **P**rotection **A**gainst **T**hreat) is a collaborative research work between the University of Santo Tomas (UST), DAF, PAMO MCWS and the DENR-TCP. The main purpose of the project is to establish information on the tamaraw population of MCWS using foot surveys, camera-trapping, and habitat suitability analysis. In addition, the project also aims to obtain data on other threatened medium to large-sized mammals such as the Mindoro warty pig (*Sus oliveri* VU; Schütz 2016) and the Philippine brown deer (*Rusa marianna* VU; Mackinnon et al. 2015).

From July to November 2021, two foot surveys were conducted to (1) obtain information on the likely current distribution of tamaraw through direct and indirect indications of presence, and (2) to identify areas to be included in subsequent camera trapping. In total, the surveys covered 32 kilometres walked with a sampling area of approximately 543ha size. No actual sightings were reported, but possible indirect signs of tamaraw were recorded, which includes hoof and dung (Fig. 1).

Feral carabao captured on camera trap in Mt. Calavite

Follow-up camera-trapping was conducted between December 2021 and will be completed by third quarter 2023 (one round is missing at time of this article). The sampling area for the camera trapping is covering 1,300 hectares or 7% of the total protected area. The survey design is following the protocol used in Aruyan-Malati, with stations spaced approximately 300m apart in a grid, and cameras deployed in successive phases (Fig. 2). In total, the survey will cover 120 camera stations across six phases. At the end of the fifth phase, no tamaraw were recorded, but feral carabaos were captured on multiple occasions (image on previous page).

Could tamaraw still exist in MCWS? Our results, although not definitive, suggest that the species is either extirpated or, if present, then occurs at extremely low numbers. The presence of feral carabaos is adding further doubt to the findings of most recent surveys, while local IP communities confess that they haven't seen the species for many years now (personal communication). However, some extremely remote areas of the park remain unsurveyed, and future work should target these areas and adjacent mountainous areas of the Park to understand if a remnant tamaraw population could survive in MCWS.

Meta-population research across the Mindoro central spine – new evidence of tamaraw found in 2022-23

The rediscovery of the tamaraw in the upper Amnay watershed region in 2018, on the border between both Occidental and Oriental provinces (Schütz, 2019), provided hope that the species could still persist outside of the three populations mentioned above. Spurred on by this finding,

conservation stakeholders formalized a goal to locate all remaining sub-populations that could survive within the island's central spine, and link additional sub-populations to the wider meta-population conservation approach defined in the TCMAP.

Several additional tamaraw surveys were organized in the Mindoro inner region between 2021 and 2023 with the objective of assessing potential tamaraw sites. Prior to field verification survey, Interviews with local indigenous people communities were conducted and completed with a rapid remote sensing data analysis to locate most potential areas based on socio-ecological parameters (land cover, IP settlement, land-use type and fire event). Although these surveys did not result in any direct sightings, tamaraw tracks were observed outside of the previously confirmed areas of 2018 in at least two different locations. More interestingly, a group of 5 hoofmarks was spotted in a remote region located nearby the Northern border of MIBNP. In addition, convincing recent tamaraw sighting reports were obtained from indigenous peoples whose ancestral domain stretches across these remote upland areas of inner Mindoro, while several of them, living in the upland, suggest the presence of tamaraws in areas not yet visited by the team.

Taken together, this information suggests that the distribution of the tamaraw population within the upper Amnay region could cover a much larger distribution than previously thought, extending across much of the island's central mountain spine, including MIBNP (fig. 5), or be composed of several subpopulations. Additional efforts are needed to understand tamaraw occurrence in the full region.
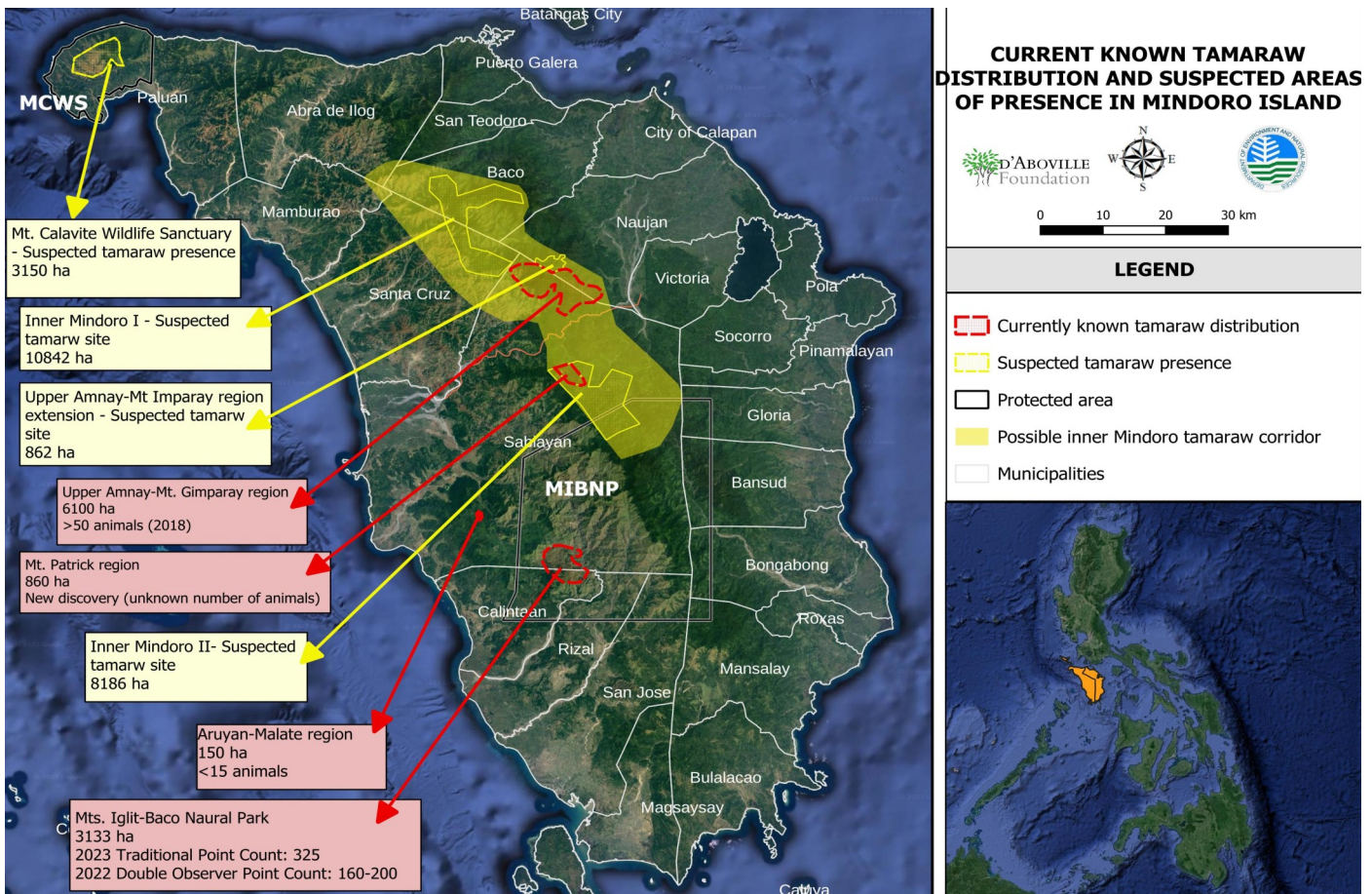
Figure 5: Map of Mindoro showing the known tamaraw sites, actual suspected areas of presence and potential area of occurrence of the species

Conclusion

Four years after the formulation of the Tamaraw Conservation and Management Action Plan, we have a better picture of the conservation status of the species across Mindoro. Unfortunately, new information has painted a bleaker picture of tamaraw status than in 2018. Recent camera-trapping evidence suggests that the two small and isolated sub-populations of Aruyan-Malati and MCWS have little chance of survival without translocation actions for population reinforcement. However and given the substantial challenges around such active conservation measures, this option may not be realistic in the short-term.

Moreover, because the revised population estimate has put the MIBNP population at approximately 200 individuals – half the size of the number used in the PHVA modelling – the assumption that the tamaraw population of MIBNP could be a safe source of animals for a translocation or *ex situ* activities may need to be reconsidered. Furthermore, the MIBNP population is also undergoing a problem with density-dependence, which translates into a decrease of the growth rate and means that the population will likely continue to contract unless the species is given more space to disperse and reproduce safely beyond its current limits.

New records of tamaraw along the inner Mindoro spine provide hopeful news amid this overall quite pessimistic picture. In fact, with the revised population estimate for MIBNP, it is possible that the central Mindoro population or sub-populations may represent the best hope for the

long-term survival of the tamaraw, even if densities in upland forest habitat are lower than in the grassland dominating landscape of the core area at MIBNP.

To save the tamaraw from extinction it is critical to implement the conservation strategies outlined in the TCMAP. Within that context, the TCP, with the support of local and international partners, is planning to launch an island-wide work-plan of activities to improve protection efficiency in all sites while exploring the feasibility of conducting *ex situ* interventions. In addition, the authorities of MIBNP have engaged in a consultation process with residing IPs communities to expand the 1600ha. No Hunting Zone agreement that was agreed in 2016, building a larger "wildlife safe reproduction zone" using the customary laws of the Taobuid.

If successful, these conservation actions will be able to reverse the trend and avoid the need for emergency *ex situ* program.

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Table.1: Comparison of the density of various species of ungulates related to their body size

Species	Location	Body mass (kg)	Density (/km²)	Reference
Okapia johnstoni (Okapi)	Afro-tropical region	250	0-4	Fa & Purvis (1997)
Tragelaphus spekeii	Afro-tropical region	100	55	Fa & Purvis (1997)
Sincerus caffer nanus (African forest Buffalo)	Afro-tropical region	285	1-4	Fa & Purvis (1997)
Sincerus caffer (African buffalo)	Ma-an National Park (Cameroon)	500	0,03	Bekhuis, De Jong & Prins (2008)
	Virunga National Park (Congo)		12,3	1959 (Cited by Bourlière 1962)
	Queen Elizabeth National Park (Uganda)		7,2	Bere (1960) (Cited by Bourlière 1962)
	Serengeti (Tanzania)		0,2	Grzimek (1958) (Cited by Bourlière 1962)
Kobus ellipsiprymnus (waterbuck)	Virunga National Park (Congo)	150	1,26	1959 (Cited by Bourlière 2022)
	Queen Elizabeth National Park (Uganda)		1,4	Bere (1960) (Cited by Bourlière 1962)
	Nairobi National Park (Kenia)		1,1	Bere (1960) (Cited by Bourlière 1962)
Connochaetes taurinus (Blue wildebest)	Nairobi National Park (Kenia)	200	23,8	Bere (1960) (Cited by Bourlière 1962)
	Serengeti (Tanzania)		9,9	Grzimek (1958) (Cited by Bourlière 1962)
Taurotragus oryx (Eland)	Nairobi National Park (Kenia)	300	0,5	Bere (1969) (Cited by Bourlière 1962)
	Serengeti (Tanzania)		0,2	Grzimek (1958) (Cited by
Tragelaphus strepsiceros (Greater Kudu)	Southern Rhodesia (Zimbabwe)	250	1,3	Dasmann & Mossman (Cited by Bourlière 1962)
Bos gaurus (Gaur)	Kuiburi National Park	1000	2,5	Tanasarnpaiboon (2016)
Bubalus depressicornis (Anoa)	Tanjung Peropa Wildlife Preserve (Indonesia)	225	0,9	Mustari (2003)
Bubalus bubalis (feral watter buffalo)	Norther territory (Australia)	800	up to 34	Australia government (2011)

Ex-situ Conservation of Malayan Gaur (*Bos gaurus hubbacki*): A Forty-year Summary of Breeding Performance and Lifespan

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Abstract

Studbook data of captive Malayan gaur managed in two Wildlife Conservation Centres (WCC) by the Department of Wildlife and National Parks, Malaysia (PERHILITAN) were analysed for the period 1982-2022. The maximum individual lifespan recorded was 26.2 years for a female and 20.9 years for a male. The average lifespan over four decades decreased from 18.1 years to 9.4 years. The mean primiparity is 3.96 years, which is older than other closely related species. The fecundity of the ten most prolific cows averaged 10.2 calves. Although 53% of cows remained reproductive beyond ten years of age, only 11.6 % produced ≥ 10 calves each, with the remaining cows averaging four calves each in their life-time. The poor breeding performance over the years is closely related to the unsound captive environment, including management practices, human resources, and husbandry. There was a high mortality rate prior to adulthood, with perinatal and yearling mortality accounting for 74% of the total mortality. Traumatic injuries, snake bites, infectious diseases, glyphosate poisoning, mycosis, endoparasites (including protozoa), old age and stressor myopathy contributed to the death of this species in WCC. No congenital defects were observed in all cases. The findings indicated a very strong need to minimize reproductive inefficiencies whilst improving perinatal and neonatal care. Reducing stress and more stringent biosecurity can help reduce mortality in yearlings and adults. Although there is a lack of evidence of genetic problems in the herd, the need for a new founder is crucial, to improve the genetic qualities of the Malayan gaur in captivity.

Keywords: ex-situ conservation, life history, Malayan gaur, Seladang, sustainable captive management

Introduction

The Malayan gaur (*Bos gaurus hubbacki*), locally known as seladang, and the southern-most form of the species, occurring in the humid equatorial zone, is a member of the tribe Bovini, which includes cattle, bison, yak, buffalo, and saola. Thus, in captive conditions, its management, husbandry and mortality would be expected to be comparable to beef and dairy cattle farmed throughout the world. The species is listed as vulnerable in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (Duckworth et al. 2016). In Peninsular Malaysia,

it is totally protected under Wildlife Conservation Act 2010 [Act 716]. Previously, the Malayan gaur population was widespread in Peninsular Malaysia and was once a popular target of big game hunters from the late nineteenth century (Hubback, 1937; Foendander, 1952; Khan 2014; Khan 2016). Their dwindling population was recognised in the 1960s - 70s (Hislop, 1961; Stevens 1968; Khan 1977; Yusof 1981; Conry 1989). In 1982, the Department of Wildlife and National Parks (PERHILITAN) embarked on *ex-situ* conservation of Malayan gaur (Othman 1999; Khan 2016). Fig.1 shows a small herd of Malayan gaur in a paddock.

Life history analysis of captive populations is essential to provide informed decisions on the genetics, demographics as well as management and husbandry of captive populations of wild cattle. The first published analysis of Indian gaur life history covered wild herds in South India and captive gaur from the largest captive populations at Mysore Zoo and Omaha's Henry Doorly Zoo (Ahrestani et al. 2011). This study provides the first analysis of the life history of captive Malayan gaur, focusing on lifespan, reproductive performance, and survivability.

MATERIALS AND METHODS

The captive Malayan gaur population was established and is managed by PERHILITAN at two 'Wildlife Conservation Centres' (WCC), one in Jenderak Selatan, Pahang (GPS location 3° 38'19.6"N 102°18'24.3"E), the other in Sungkai, Perak (GPS location 4°02'07.8"N 101°21'56.6"E). The Jenderak Selatan WCC is located within the 62,395 hectare Krau Wildlife Reserve while the Sungkai WCC is located within the 2,468 hectare Sungkai Wildlife Reserve. The studbook containing the raw data of 227 individual gaurs was used as the data source for the life-history analysis. The founders of the entire captive population consist of four males and two females, all wild-caught, and the remainder

Figure 1. A family of Malayan gaur at Wildlife Conservation Centre in Malaysia.

(n = 221) were born in captivity between 1982 and 2022. The data recorded for each individual includes identification, sex, house name, origin (wild-caught or captive-born), sire, dam, date of birth (or date of capture for wild-caught), age, transfer location, date of death or last known date alive, and cause of death.

The age of the captive-born is known from the exact date of birth observed by the keepers. All the wild-caught individuals were young calves (Khan 2014) and their age was estimated to be two months old, based on their physical characteristics. The date of birth (estimated for the wild-caught individuals, precise for all captive-born individuals) was used to calculate the first parturition and lifespan. The Population Management x (PMx) software (Lacy et al. 2011) was used to analyse and estimate life expectancy and survivorship. For the life expectancy estimation, individuals that did not survive the first 30 days of life were excluded from further analysis to minimize the influence of neonate mortality. The age-specific survivorship (Lx) which illustrates the probability of surviving from birth to various ages for captive Malayan gaur was also estimated using PMx. Unlike domestic cattle, the captive Malayan gaur have been semi-intensively farmed and, the young remained with the mother for a much longer period than is usual for captive bovids. Mortality was divided into perinatal (within 48 hours after birth), neonatal (2-30 days), calf (1-6 months), weaners (6-12 months), yearlings and adults.

Results

The earliest primiparity recorded was at two years and two months (2.22 years), the latest at 8 years, with a mean primiparity of 3.96 years. Of 43 captive-born females that produced offspring, 25.6 % had primiparity under three years, and 55.8 % had primiparity between age 3 and 5. The remaining had primiparity above the age of 5 years. Except for one cow (SJ00012), which
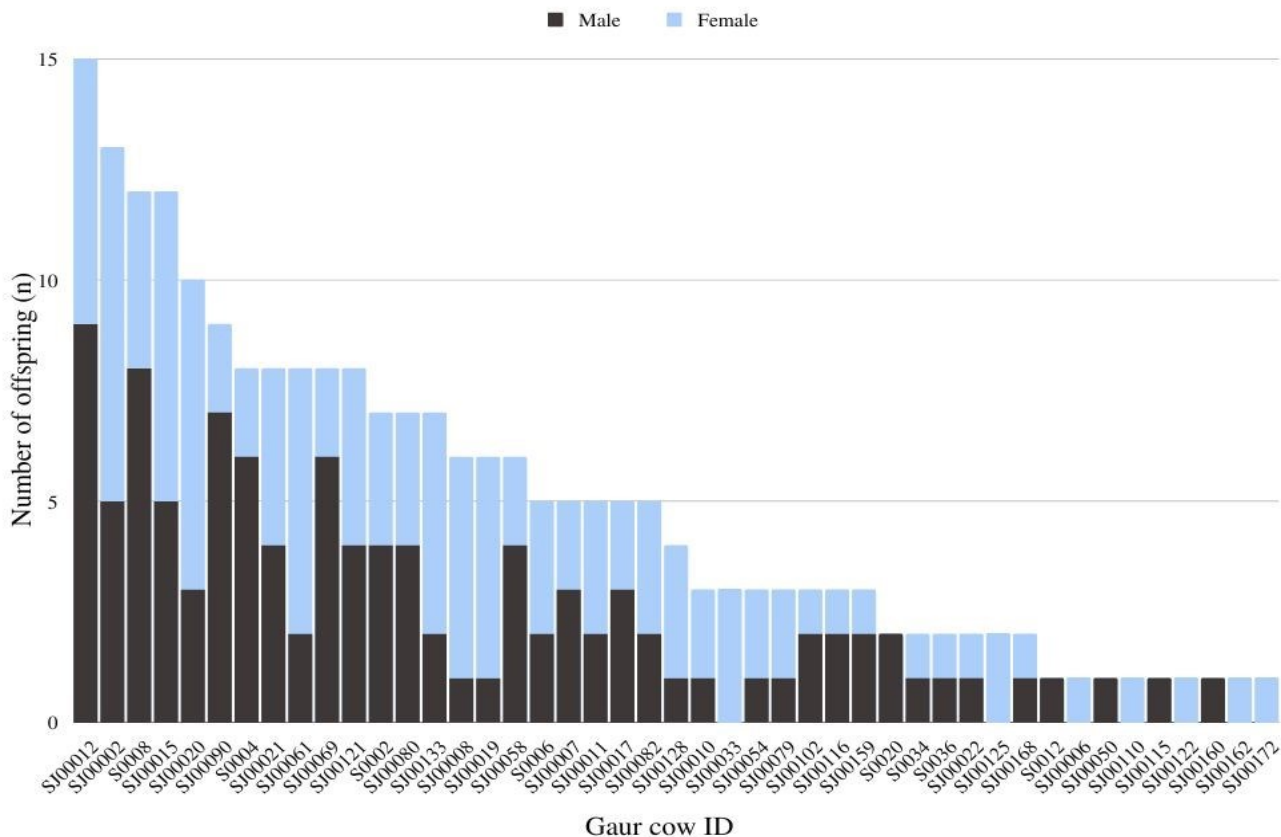

Figure 2: *The number of Malayan gaur calves produced at the Wildlife Conservation Centres in 1985-2022*

gave birth to her last offspring at the age of 21, no cow gave birth after the age of 19 years. The exception was the eighth calf of the founder pair and gave birth to 15 offspring (9♂, 6♀). This individual was the oldest Malayan gaur to live in captivity and succumbed to respiratory failure at the age of 26 years, 2 months, and 13 days.

The founder pair of captive Malayan gaur produced 13 offspring, with only one death, shortly after birth. The majority of the remaining offspring (n = 10) lived for more than ten years. Since the initial establishment of the WCC in 1982, a total of 45 females (2 wild-caught and 43 captive-born) have produced a total of 221 offspring consisting of 107 males and 114 females (Fig. 2).

Records from the 43 cows who survived to adulthood indicated a marked decrease in the birth rate over the years. The ten most prolific cows produced an average of 10.2 cows in their lifetime. However, in the study, only five cows (11.1%) produced ≥ 10 calves throughout their lifespan. This constitutes a baseline for optimum fecundity for captive Malayan gaur. The remaining cows (n=38) produced an average of four calves (Fig. 3). The 24 cows (55.8%), that surpassed ten years lifespan, produced an average of 7 calves per cow. The average number of calves produced per cow, born within the periods 1991-2000, 2001-2010 and 2011-2020 were 6.6, 4.5, and 2.6 respectively.

The diseases diagnosed at the WCC, included traumatic injuries, envenomation (snake bite), infectious diseases (brucellosis, foot and mouth disease, lumpy skin disease, cowpox), poisoning (glyphosate herbicide), mycotic (aspergillosis), endoparasites (helminths, liver flukes, coccidiosis) and stressor myopathy. Most were fatal. No congenital defects were observed in all cases. Old age also contributed to the death in the WCC. Out of 221 offspring, 35.7 % (n = 79) were dead before adulthood (Fig. 4).

The average life expectancy was calculated to be 10.2 years for males and 9.6 years for females.
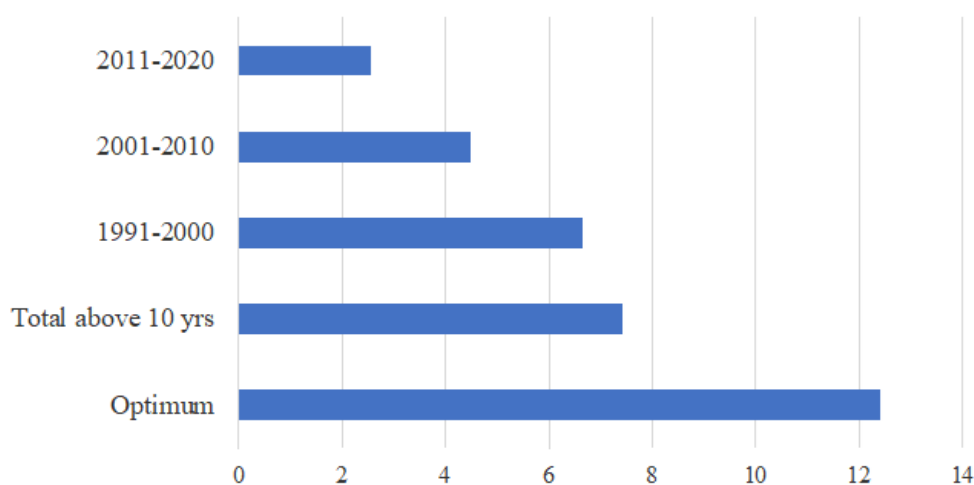

Figure 3. The optimum fecundity and average number of calves produced per natural lifespan of a Malayan gaur cow at WCC
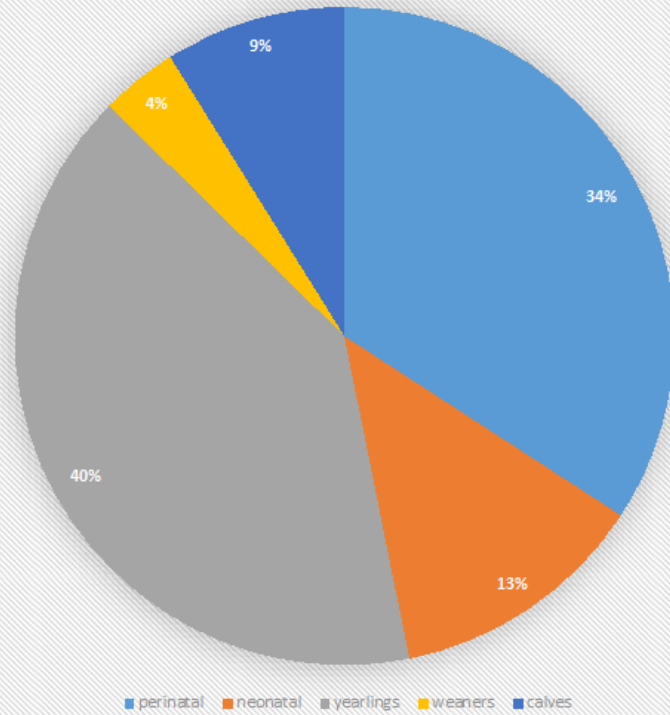
Figure 4. *Mortality before adulthood of captive-born Malayan gaur*

The age-specific survivorship (Lx) which illustrates the probability of surviving from birth to various ages for captive Malayan gaur was calculated using PMx (Fig. 5). One female is currently thriving beyond the age of 20 years. 10.5% (n=12) of captive-born females live beyond the age of 15 years, and the oldest recorded was 26.2 years. 10.3% (n=11) of males live beyond the age of 15 years, and the oldest recorded was 20.8 years. Currently, the oldest living male and female are 15.9 years and 22.5 years, respectively. The maximum lifespan for wild-caught males is 19.4 years as compared to 17.5 years in females. The minimum lifespan for wild-caught males and females is 9.5 and 8.6 years, respectively.
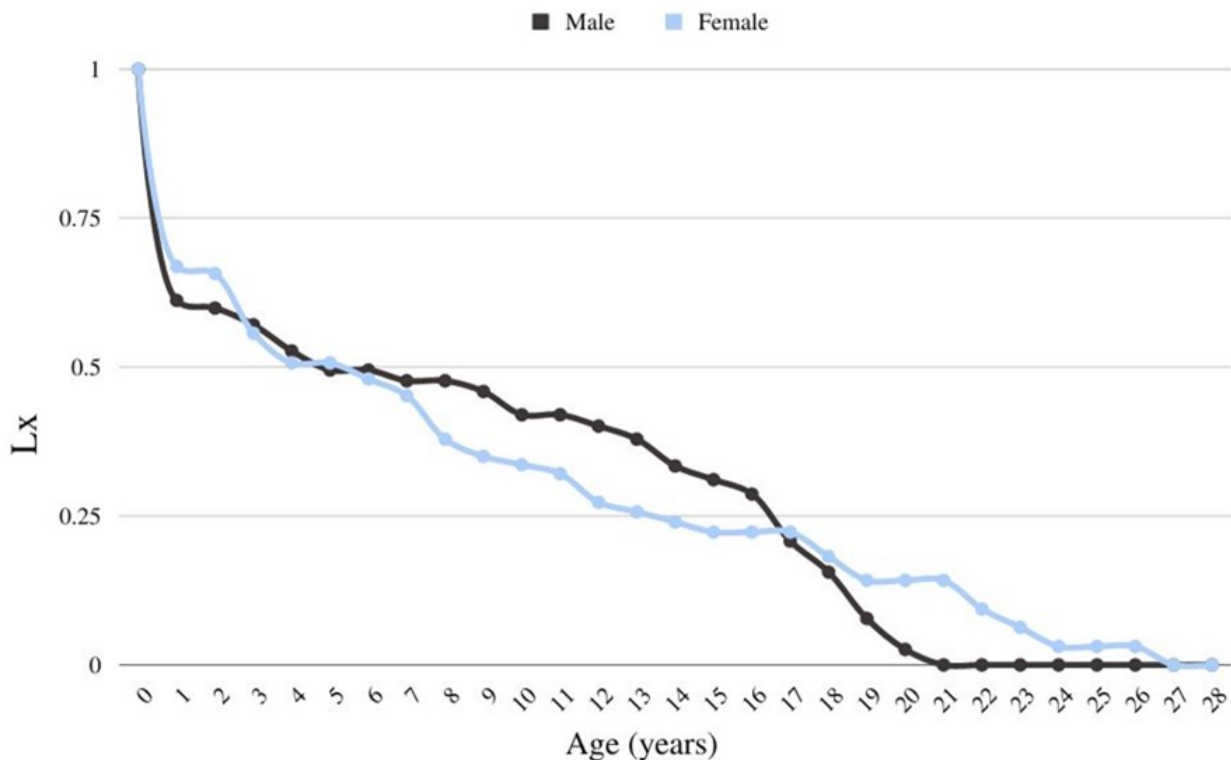

Figure 5. *Probability of surviving (Lx) from birth to various ages (years)*

Discussion

The wild population of Malayan gaur declined greatly during the twentieth century, due to a combination of habitat (grassland, predominantly along river valleys and in Orang Asli swidden farms) loss and poaching (Conry 1981; Payne et al. 2021). Recent observations on the extreme scatter and scarcity of clusters of wild Malayan gaur, coupled with very small and declining herd size, and apparent low fecundity, give rise to grave concern for the survival of this southern form of *Bos gaurus*. The wild clusters are clearly not increasing in size, strongly suggesting the deleterious effect of infertility or subfertility as a result of ageing and poor nutrition. Similar findings have been seen recently in *Bos javanicus lowi*, the Bornean form of the banteng, in Sabah, Malaysia (Zainal Zahari 2022; Zainal Zahari et al. in press). As shown by the above analysis, captive Malayan gaur numbers are also declining significantly over time, with high calf mortality, low fecundity, and short longevity. Inbreeding depression has been highlighted as a cause of death in neonates (Norsyamimi et al. 2016). However, after re-examining studbook data, history, autopsy reports, management systems, staffing, nutrition, husbandry, biosecurity, and genetics, a more accurate assessment can now be made.

A study of dairy and beef cattle in Finland in 1999-2006 reported congenital malformation in neonates, particularly heart defects (4%) and hydrocephalus (1%). Apart from the congenital anomalies, sporadic intrauterine infection and dystocia are the common causes of neonatal mortality in Finnish calves (Syrjälä et al. 2007). A study on Irish Holstein-Friesian dairy cows on the effect of inbreeding on milk production, calving performance, fertility and body conformation showed only a small impact. There were a 1%

higher incidence of stillbirth and 2% higher incidence of dystocia (Parland et al. 2007). In the case of Malayan gaur, more substantiated evidence is required to associate inbreeding depression with neonatal, perinatal, and yearling mortality. To date, there is a lack of evidence to associate the high mortalities with genetic problems.

The number of captive Malayan gaur increased over the years and reached a carrying capacity in the captive facilities around year 2000, but the available resources have remained unchanged. Calf mortality was observed to increase over the years. In addition, poor breeding performance, increasing age at primiparity and shortened life expectancy are factors of concern. The complete absence of congenital defects observed in all the captive Malayan gaur tends to argue against genetics (inbreeding depression) as a major source of concern. Instead, the small number of enclosures and of staff (support staff and veterinarian), and limited food resources, tend to point to management (husbandry, nutrition, biosecurity) as a greater reason for concern.

The present situation is serious but reversible. The problems need to be recognized at the departmental and national level and improving calf survival and optimizing breeding performance would require reprioritization. In the absence of a programme to improve food availability (managed pastures) for the wild Malayan gaur clusters in situ, the importance of ensuring and sustaining a well-managed captive population assumes ever greater importance. Failure to provide pastures for the wild clusters, coupled with a sub-optimally managed captive population, could put the Malayan gaur on the road to become the next species of large mammal to go extinct after the Sumatran rhinoceros.

The average life expectancy (1982 – 2021), for captive Malayan gaur is 10.2 years for males and 9.6 years for females. According to Kohler et al. (2006), the average lifespan of captive mammals is roughly equal to half of the oldest age attained by that species. Life expectancy and longevity of captive ruminants are two useful parameters that may indicate the success of husbandry practices (Müller et al. 2011). However, when viewed every ten years (1982 – 2021), the average life span, showed a gradual shortening, from 18.1 years (1982-1991) to 17.3 years (1992-2001), 14.2 years (2002-2011) and 9.4 years (2012-2021). This decline is especially concerning in view of the observation of 59 mammalian species examined, including *B. gaurus*, 84% lived longer in captivity compared to their wild counterparts (Tidière et al. 2016).

The lifespan of the Malayan gaur in the wild has not been determined, but in this study, the wild-caught Malayan gaur males lived for 19.1 years and females, 17.2 years. In contrast, the oldest recorded captive-born Malayan gaur is 20.9 years for males and 26.2 years for females, indicating that male captive-born Malayan gaur have a shorter lifespan than females. This result is similar to captive Indian gaur in two zoos, where females live longer than males (Ahrestani et al. 2011). Eighteen captive Malayan gaur which were born between 1982 until 2001 lived beyond 15 years of age. In contrast, only eight individuals, born after 2002 survived beyond the age of 15 years. With the increase in the number of Malayan gaur bulls in a captive facility and no increase in facility size or resources, the bulls become very territorial. Clashes between adult and sub-adult bulls are often seen, some with serious wounds or death. Competition for food and space becomes apparent. These stresses would cause an animal to be immune-

compromised and becomes more susceptible to diseases, including helminthiasis and other infections (Kumar et al. 2017; Hodgson et al. 2005).

Calf survivability

A total of 221 calves were produced in the WCC by the end of 2022. Total calf mortality accounted for 37% of all recorded deaths in captivity (n=79). Of this, perinatal mortality constituted 34%. This is more than twice higher than the highest recorded internationally in the dairy industries, with the majority of countries, between 5-8% (John, 2013). The high incidence of perinatal mortality in the captive Malayan gaur is quite likely associated with anoxia or trauma, as most calving occurred at night or early morning inside the paddock and was never observed or attended by the keepers. In an international study, based on necropsy findings, the major causes of bovine perinatal mortality are dystocia (approximately 35%) and anoxia (approximately 30%), to a much lesser extent, infections (approximately 5%), congenital defects (approximately 5%) and 15% from other causes (John et al. 2019). Consistently high mortality rates and/or poor fertility may be an indication of failure in monitoring and/or acting on signals of animal performance. This general failure has a wide range of negative consequences for the welfare status of the animals on the farm. In addition, published literature on bovids shows significant influences of environmental factors on calf mortality (Mandal et al. 2019; Sandgren et al. 2009).

In Pakistan, the mortality in neonatal calves has mostly been attributed to infectious agents, immunodeficiency, seasonality, difficult parturition, and faulty management conditions

(Khan et al. 1991). Many research articles report abortion and perinatal mortality varying from 5 to 12% and 2 to 5%, respectively, which surprisingly, is regarded as representing a huge loss of calves (Moore et al. 2021). In comparison, calf mortality in Bali cattle, on Sumbawa Island, Indonesia, ranged from 10–27%, mostly in the older calves (Sriasih et al. 2021). Similarly, in Uruguay, the high preweaning dairy calf mortality risk is 15.2% (Caffarena et al. 2021). Therefore, the high percentage of calf mortality in the Malayan gaur in the WCC needs a serious intervention and the causes need to be redefined and not solely attributed to inbreeding depression.

Diseases of infectious origins were also diagnosed in the WCC. They caused mortality in adults, sub-adults and possibly new-born. They include bovine brucellosis in 2007. Bovine brucellosis is characterised by abortion, stillbirth, reduced milk production, weak fetus and infertility in both males and females (Mukhtar et al. 2013). The movement of infected captive Malayan gaur from an "infected herd" to another, which occurred in 2012, without prior evaluation, including serological test, could transfer the infection to other Malayan gaurs. Thus, all cases of abortion and stillbirth in captive Malayan gaur require thorough investigation. Foot and mouth disease (FMD) was diagnosed in 2008 but all captive Malayan gaur were vaccinated except for two individuals who did not survive. Another FMD outbreak occurred in 2016 but the outbreak was successfully contained without any death recorded (Merawin et al. 2017).

Five calves (2-27 days) in the captive population were trampled or killed by a bull. Two of the calves were one day old. Post-mortem findings included ruptured organs and fractures.

Traumatic injuries can be avoided by reducing the herd size (or separating the bulls) in the paddock during the third trimester of pregnancy. Pregnancy diagnosis, especially in the third trimester/late pregnancy can be made visually by an experienced manager. If heavily pregnant cows are isolated, they could be provided with better nutrition and be observed more closely prior to parturition.

The observed incidence of pneumonia and haemorrhagic enteritis is an indicator of infection by pathogens in the perinatal calves. One incident of septicaemia in a calf was also observed at the WCC. This occurred as a result of an ascending infection (bacteria), entering through the umbilical cord (navel), soon after birth. This would result in reduced appetite and fever and if left untreated will lead to death. A few perinatal calves were diagnosed with jaundice and anaemia. One of the differential diagnoses of jaundice in calves is leptospirosis. Coccidiosis was diagnosed and treated in the neonatal calf. Helminthiasis caused the death of two calves, aged one year and two months. Aspergillosis caused the death of a neonatal calf that was hand-raised in the WCC.

Reproductive performance

Published records of captive gaurs elsewhere show that this species reaches sexual maturity between the ages of 2 and 3 (Ahrestani et al. 2011; Castelló 2016). In this study, the earliest first parturition for captive female Malayan gaur was 2 years and 3 months (2.3 years). Only 11 out of 43 captive-born reproductive females had primiparity between the age of 2 and 3. The others were older. The gestation period of the Malayan gaur was estimated as nine months by Hubback (1937). Based on recorded observations at JSWCC from 1986-1995, the

gestation period is about ten months (Zainal Zahari Zainuddin, unpublished observation). Using this information, the earliest sexual maturity of female Malayan gaur in this study is estimated to be 17 months.

Diseases, both infectious and non-infectious will contribute to reproductive failures (Fuerst-Waltl & Fuerst 2010; Fuerst-Waltl & Sørensen 2010; Svensson et al. 2006; Lombard et al. 2007; Gulliksen et al. 2009). Animal welfare must be considered in every livestock production system. Reproductive efficiency is a direct indicator of the health and welfare situation of farm animals. Therefore, low reproductive rates (prolonged anestrus, low conception rates, high reproductive losses, and high percentage of assisted deliveries and/or dystocia) may indicate animal welfare problems (Dadin et al. 2021).

Data accumulated since 1982 indicate variability in breeding performance when compared between different generations. The main parameters observed in the Malayan gaur included delayed sexual maturity, late calving, long calving intervals, erratic conception rates, high stillbirth, and low fecundity. In captivity, the optimum fecundity in Malayan gaur averaged 12 calves/natural lifespan. This was based on the five cows that survived beyond the age of ten years and a calving interval of one calf/year. This benchmarking should allow WCC managers to track their progress and the success of any implemented changes on the farm. In the WCCs, low conception rate and short longevity resulted in overall poor breeding performance. Currently, 91% of Malayan gaur cows produced only 4 calves/cow/natural lifespan. In general, management practices and the farm environment play integral roles in determining optimum calf production (Schuster et al. 2020). The declining trend of calves/cow/lifespan in the WCCs, over more than three decades, indicates a lack of a breeding management strategy, insufficient human resources and suboptimum animal husbandry practices.

The optimum body weights of heifers are important prior to their first breeding. *Bos taurus* beef heifers need to attain 60% to 65% of their expected mature body weight, before the first breeding (Patterson et al. 1992; Gasser 2013). In comparison, Nelore heifers, with favourable genetic merit for age at first calving were observed to reach puberty at 80% to 107% of the expected mature body weight. These heifers attained puberty at 18 months of age (Ferraz et al. 2018). In the Malayan gaur, mature females weigh approximately 500-700kg (Ogilvie 1952; Johnston et al. 1994; Mamat-Hamidi et al. 2012). Thus, the weight at first breeding of the Malayan gaur should be within the range 300 – 480kg.

Stress is ultimately a major cause of reproductive failure. A dominant territorial bull will suppress reproduction in other bulls. In the WCC enclosures, the design is such that it only limits physical contact between bulls. However, the olfactory and vision senses are not limited by the horizontal bars that separate them. For cows, delayed resumption of ovulation and estrus can be due to metabolic-related stressors which in turn can be due to insufficient pre-calving nutrition and inadequate management practices, including alleviating stressors. The recommended body condition score (BCS) for bovids should be in the region of 2.75-3.0 (Crowe 2008). In the Bornean Banteng (*Bos javanicus lowi*), improving the nutrition in a wild herd resulted in a three-fold increase in the number of birth (Zainal Zahari et al. in press). Stress reduces fertility in cattle by interfering with the

reproductive hormones due to high levels of cortisol in the blood (Kumar et al. 2012; Wrzecińska et al. 2021). There is considerable evidence that acute stressors reduce GnRH and LH pulse frequency and delay ovulation (Suzuki et al. 2001; Peterson et al. 2006; Huszenicza et al. 2005). Local infection of the uterus in post-partum cows delays uterine involution, causes inflammation of the endometrium, reduces conception rate and may affect follicle growth, decrease estradiol secretion, and delay interval to first ovulation (Williams et al. 2007; Sheldon et al. 2002, 2008; Dadin et al. 2021).

Reintroduction Programme

Without reintroduction, conservation breeding may be seen as unsuccessful (Ebenhard 1995). PERHILITAN embarked on reintroduction with a pilot programme in 2010, in which two gaur individuals (1:1) were released. Unfortunately, the pilot program was not successful because the gaur starved to death. Additional reintroduction was planned and executed in the following years. As of 2022, 26 (16:10) captive-born Malayan gaur individuals have been relocated into wild habitats (PERHILITAN, unpublished information). One female individual released in 2012 was sighted with a male calf in January 2015 (PERHILITAN, unpublished information). However, unpublished reports suggest quite high levels of mortality in Malayan gaur released into the wild, perhaps because all cases were 'hard releases' (Paloma et al, 2021).

Nonetheless, even if a species is never reintroduced, conservation breeding programs can supply zoos with animals to display, thereby limiting the need to gather them from the wild (Ralls & Ballou 2013). Captive Malayan gaur have been transferred to zoos in Malaysia, including Melaka Zoo, Taiping Zoo, and Zoo Negara (National Zoo). Other than zoos in Malaysia, captive Malayan gaur have also been transferred to Khao Kheow Open Zoo in Thailand. Captive breeding programs also have considerable educational value because they can be utilized to educate zoo visitors about the benefits of preserving biodiversity and to boost enthusiasm for conservation (Ralls & Ballou 2013). Most importantly, a captive breeding programme provides an insurance against the potential risk of total extinction in the wild which, in view of the small and very scattered remaining wild clusters of Malayan gaur (Payne et al, 2021) is not an impossible scenario.

Genetics and captive breeding

Although the analysis presented here argues that a variety of management issues account for the observed poor and declining performance of the captive herd, genetics and the risks posed by inbreeding must not be ignored. Genetic diversity in any population comes from three sources: recombination, migration, and mutation (Griffiths et al. 2000). Since mutation rates are less likely to result in genetic variation in small captive populations, the genetic diversity of these populations is maintained by maximizing the genetic potential of the founders and immigrants (Ballou 1984). In captive populations, genetic diversity will inevitably be lost with each new generation (Crow & Kimura 1970). Introducing new bloodlines into the captive Malayan gaur population should be considered a priority, even though improvements in management and husbandry are the prime need. This is critical to maintain a high level of genetic variation and to ensure that the population in captivity is viable for reintroduction into the wild. Nonetheless, obtaining a wild gaur is no easy task; it has been 20 years since the last wild-caught gaur was

brought into the conservation centre in Malaysia. For the improved genetic sustainability of the captive breeding programme, assisted reproductive technology (ART) can be considered by collecting the sperm of bull gaurs in the wild, without the need to bring the animals into captivity, and apply in vitro fertilization with egg cells from captive cows. In addition, routine semen collection and cryopreservation from all the captive bull gaurs should be initiated on a large scale.

CONCLUSION

This study has furnished current information on captive gaurs in Malaysia for their future conservation. The life-history patterns of captive Malayan gaur should be similar to other bovids, but this analysis shows significant under-performance and weaknesses. Although some individuals lived more than 20 years and produced many calves, there are also very many individuals who failed to survive their first year of life. Moving forward, perinatal management is crucial to avoid future death after birth, trauma, anoxia or weakness. The high mortality of perinatal calves would suggest a need to improve attention to health, particularly of the dam and calf. Similarly, adequate nutrition to the pregnant cows will help to ensure a healthy calf. Prophylactic measures including the use of anthelmintics and anticoccidial medications must be implemented routinely. There is a need to ensure the paddocks are always clean, not overcrowded, and with ample dry land at all times, especially during rainy periods. Routine veterinary examination of the cow and calf can identify potential health issues. Calves that are neglected by the dam can be hand-raised to at least 4 months of age. In summary, improving captive Malayan gaur calf survivability requires re-prioritization of the management system.

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