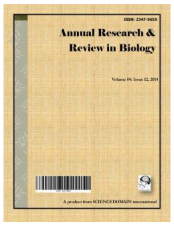

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Captive Breeding of Threatened Mammals Native to Southeast Asia – A Review on their Ex-situ Management, Implication and Reintroduction Guidelines

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Heavy deforestation and land use conversion in Southeast Asia caused most of the mammals to face the threat of extinction due to limited availability of suitable habitats, which jeopardizes their survival throughout the region. As the demand for certain body parts of threatened mammals increases, illegal poaching activities increase, and consequently their population continuously decreases. Protecting sustainable population numbers or supporting efforts to multiply the population of threatened mammals in their own natural habitats is very challenging, almost impossible until the threats in the wild are removed. Therefore, ex-situ conservation through captive breeding is another reliable method which already been practiced for years across the world. Nevertheless, transferring and raising these mammals in breeding centers requires proper guidelines to maintain their welfare and genetic variability. In this paper, we discussed threatened mammals native to Southeast Asian countries that are currently under captive breeding programs. A

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multi-disciplinary overview, including: group size and social structure; health, stress and mortality studies and; enclosure design and environmental enrichment, are key components of the best management and husbandry practices. The mammalian alleles may experience evolutionary change if the populations of endangered mammals are retained in captivity after few generations, and that could lead to genetic problems. Therefore, a proper gene 'flow' is crucial to maintain genetic variation within and between populations. Finally, an important tool for species conservation is reintroduction of well-managed captive breeding populations into the wild. A complete health screening, selection of sites and pre-release training prior to reintroduction are crucial and need to be addressed for these mammalian populations.

Keywords: Captive breeding; reintroduction; threatened mammal; ex-situ; wildlife conservation.

1. INTRODUCTION

Among the world's tropical regions, the Southeast Asian region comprises the countries of Cambodia, Laos, Myanmar (Burma), Thailand, Vietnam, Malaysia, Indonesia, Brunei, Philippines and Timor-Leste, are facing massive deforestation [1] (with Singapore the most heavily affected (>95%; [2])). This impacts the population of the endemic flora and fauna [1,2]. Heavy losses of native habitats and limited availability of suitable habitats in Southeast Asia have caused most of the mammals to face the threat of extinction and jeopardize their survival throughout their regions [3]. Based on the International Union for the Conservation of Nature (IUCN) Red List 2018, the numbers of 'Critically Endangered' mammals have increased dramatically from 169 to 201 species, and 'Endangered' mammals from 315 to 482 species since 1996, which are very alarming and need immediate action [4].

Various conservation efforts are being taken throughout these countries to protect and prevent these mammals from extinction in the near future through in-situ or ex-situ conservation [5,6]. In this paper, we will focus on ex-situ conservation strategies of several threatened mammals (*Panthera tigris*, *Elephas maximus*, *Bos gaurus*, *Dicerorhinus sumatrensis*, *Tapirus indicus*, *Pongo pygmaeus* and *Helarctos malayanus*) by providing a review and recommendation pertaining to their ecology, physiology, adaptation and husbandry from previous studies for the betterment of the species' captive breeding management and reintroduction processes throughout the Southeast Asian region.

The overview delineates four parts. First, we highlight the current mammal populations, their status, and potential threats in the wild that justifies captive breeding in Southeast Asia. Second, we will discuss the implication of and suggestions for management and husbandry in captivity. Third, we will discuss the adverse genetic issues that can result from captivity and which may affect the mammals' welfare. Finally, we will discuss guidelines for reintroduction of captive animals back into the wild with a hope towards restoring healthy and self-sustaining populations. Throughout this paper, we support our discussions with reference to published literatures on captive breeding and reintroduction using systematic search and review method. A total of 94 literature papers had been reviewed to justify the implications and guidelines on better captive management of seven species of threaten mammals in Southeast Asia countries.

2. CURRENT POPULATION, STATUS AND POTENTIAL THREATS TO THE MAMMALS

2.1 Tiger (*Panthera tigris*)

The tiger (*Panthera tigris*) has two extant subspecies in Southeast Asia, the Sumatran tiger (*Panthera tigris sumatrae*) found in Sumatra (Indonesia), and the Malayan tiger (*Panthera tigris jacksonii*), found in Peninsular, Malaysia [7,8]. Both the Sumatran tiger and Malayan tiger are classified as 'Critically Endangered' according the IUCN Red List due to an extreme decline in population [8,9,10], with only 400-500 individuals and 250-340 individuals, respectively remaining in the wild [9,10]. The main threat to the tigers is poaching, followed by habitat loss from illegal logging, palm oil harvesting, forest fires, the depletion of their prey, and human-tiger conflicts [8,11].

2.2 Asian Elephant (*Elephas maximus*)

In Southeast Asia, the Asian elephant is widely distributed throughout Cambodia, China, Indonesia (Kalimantan and Sumatra), Laos, Malaysia (Peninsular Malaysia and Sabah), Myanmar (Burma), Thailand, and Vietnam [12]. The estimated population in Cambodia is 250-600, in Indonesia 2400-3400, in Laos 500–1000, in Myanmar (Burma) 4000–5000, in Thailand 2500–3200, in Malaysia 2100-3100, and in Vietnam 70-150 [12,13,14,15]. They are usually hunted for their ivory and skin, and often traded illegally for forestry products, and cultural ceremonies [12]. Massive habitat loss, degradation, and fragmentation have increased the conflict between humans and elephants [12,13]. Hundreds of elephants are being killed annually as a result of the damage they cause to agricultural crops [12,13].

2.3 Gaur / Indian Bison (*Bos gaurus*)

The Gaur is also known as 'Indian bison' in general, however, the subspecies *Bos gaurus hubbacki* is called as 'Seladang' or 'Malayan Gaur' in Malaysia. Presently, there are 273 to 333 Gaur in Peninsular Malaysia [15], in Laos approximately 1000 [16], in Thailand 100 at Khao Yai National Park [16], and approximately 198-239 remaining at Kuiburi National Park [17], and in Vietnam, a total of 121 were counted at Ea So Nature Reserve and Yok Don and Cat Tien National Parks [18]. The current population of Gaur in Myanmar (Burma) is poorly known; however, an old survey performed twenty years ago estimated approximately 100-200 Gaur [16]. Habitat loss and poaching for meat are the two main threats that caused the population to decline throughout most of its range [19].

2.4 Sumatran Rhinoceros *(Dicerorhinus sumatrensis)*

The population is estimated to be only 170 to 230 remaining in Bukit Barisan Selatan, Way Kambas, and Gunung Leuser National Park, Sumatra [20]. In Malaysia, a few studies conducted from 1975 to 1981 estimated 50 to 75 in ten isolated areas in Peninsular Malaysia (e.g., 20 to 25 in Endau-Rompin National Park, 8 to 12 in Taman Negara Pahang, and 4 to 6 in Sungai Dusun; [21]), and approximately 50 in Tabin National Park and Danum Valley in Sabah [20]. Sadly, this critically endangered species is most likely extinct in the wild in Peninsular Malaysia since there has been no evidence of the species' presence in the wild since the last capture in 2007 [22]. The rapid decline in population has been associated with continuous hunting for their horns [20,23]. The horns been used by Chinese doctors and pharmacists in their traditional medicine practice to treat fever, rheumatism, gout, typhoid, headaches, carbuncles (clusters of boils), nausea, food poisoning, and snakebites, and also for treating demonic possession and hallucinations [20,23].

2.5 Malayan Tapir (*Tapirus indicus*)

Malayan tapirs are native to Southeast Asia, ranging from Southern Myanmar (Burma), Thailand, Peninsular Malaysia, and Central and Southern Sumatra [24,25]. In Peninsular Malaysia, there are approximately 1,300 to 1,700 left in the wild [25]. Smaller populations were estimated in Sumatra (less than 400-500) and Thailand-Myanmar [Burma] (less than 250) [25]. This species is facing a continuous decline in its population due to ongoing deforestation that caused habitat loss and fragmentation, hunting pressure, and increasing road kills [25].

2.6 Bornean Orangutan (*Pongo pygmaeus*)

The Bornean orangutan, *Pongo pygmaeus*, has three subspecies in Malaysia and Indonesia, the Northwest Bornean orangutan (*Pongo pygmaeus pygmaeus*) is found in Sarawak and West Kalimantan, the Southwest Bornean orangutan (*Pongo pygmaeus wurmbii*) is found in West Kalimantan, and the Central Kalimantan and Northeast Bornean orangutan (*Pongo pygmaeus morio*) is found in North and East Kalimantan and Sabah [26]. The estimated population of the Northwest Bornean orangutan subspecies is 2,000-2,500 in West Kalimantan, and 1,143-1,761 in Sarawak [27]. The Southwest Bornean orangutan population in Central and West Kalimantan is estimated to be approximately 34,975, and the estimated population of the Northeast Bornean orangutan is 4,825 in East Kalimantan, and 11,017 in Sabah [27]. The major threat to this species is the destruction of their natural habitat to build massive developments associated with palm oil plantations, illegal logging, and illegal hunting [26,27,28]. Orangutans are also hunted for several reasons, such as for traditional medicine, selling their offspring, hunting for fun, for food, for self-defense, or when the orangutan is perceived to be a pest or threat [29].

2.7 Malayan Sun Bear (*Helarctos malayanus*)

The Malayan Sun bear (*Helarctos malayanus*), also known as 'honey bear' or 'beruang matahari', is found in the tropical forests of Southeast Asia which include, Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar (Burma), Thailand, and Vietnam, and is extinct in Singapore [30,31]. The population is estimated to be between 1.13 to 1.57 bears/km^2 in Taman Negara, and 0.128 bears/km^2 in the Ulu Segama Forest Reserve [32]. The population estimation in other Southeast Asian countries is poorly known due to the lack of population census studies. The major threats to this species are habitat loss and poaching [29,30]. The Malayan Sun bear's meat and body parts are highly consumed in Japan and Korea, while their gall bladders and bile extract are commercially used in countries like Myanmar (Burma), Thailand, Laos, Cambodia, Vietnam, and East Malaysia for traditional Chinese medicine [29,31,33,34].

The implementation of effective conservation strategies to reduce habitat loss and other major threats in the wild for native Southeast Asia mammals throughout their entire range is the key component in protecting these species for further extinction [3,5,6]. However, conservation strategies have proved challenging primarily due to socioeconomic issues, including population growth, poverty, shortage of funding for conservation resources, and corrupt national institutions [1]. Therefore, ex-situ conservation is essential in sustaining and breeding these mammals in captivity [6]. However, species management and husbandry are important elements to be considered in achieving ex-situ conservation [6].

3. IMPLICATION AND SUGGESTIONS ON SPECIES MANAGEMENT AND HUSBANDRY IN CAPTIVITY

Species management and husbandry in captivity play vital roles for the well-being and welfare of the captive animals [6]. A multi-disciplinary overview, including group size and social structure, health, stress and mortality, the suggested enclosure design, and environmental enrichment for the best species management and husbandry approaches for these seven threatened mammals are further discussed below. The existing captive breeding facilities for these mammals are presented in Table 1.

3.1 Group Size and Social Structure

Different mammalian species live in different group sizes and compositions, some live in a solitary mode, while others live in groups [35]. Most mammals form social groups through a certain degree of affinity or bonding [36]. In the wild, living in a group is important to avoid predators, defend territory, and forage for food, which is not a concern for captive mammals [36]. However, the group size either being too small or too large in captivity, can lead to negative consequences on their behavior, welfare, and lifetime reproduction [35,36].

The Gaur has three different social structures in the wild, living in solitude, living in bull groups (adult males forming bachelor herds), and mixed herds [19]. The mixed herds of Gaur consist of adult females, juveniles, and calves, containing up to 47 individuals in a group, which are generally lead by females [19].

Elephants exhibit a matriarchal social structure in the wild, consisting of adult females from several generations [37]. Therefore, female elephants are usually housed together in captivity, although the group size is smaller than in the wild, while adult males are generally housed separately [37]. The risks of death to the captive elephant are relatively low compared to in the wild [37]. Nevertheless, the effect of low populations in social structures leads to inbreeding, depression, and causes the captive population growth to be slower than expected [13,19,35,37].

The Sumatran rhinoceros, Malayan tapir, Bornean orangutan, Malayan Sun bear, and tigers are solitary animals, except during the weaning period, where the offspring are accompanied by their dam until they become independent, or an adult female and male 'pair up' during a mating encounter in the wild [35,38,39,40]. Housing Sumatran rhinoceros and Malayan tapir in captivity, either solitary or in pairs, are largely depended on the animals' social behavior and temperament [39,40]. It is suggested that an estrus female is introduced to a male in an enclosure to avoid serious injuries that can be inflicted on the females [41,42]. Juveniles can be kept with the dam until they reach puberty and need to be separated for breeding and to avoid aggressive behavior of the dam towards older calves after a new calf is born [40]. Certain solitary mammals, like Bornean orangutans, are flexible, since they can live in groups and have demonstrated successful social interaction among members of the same species [35]. Overall, careful management of the social grouping of mammals is crucial to avoid problems such as delays in the first age of reproduction, longer inter-birth intervals, and

Table 1. Description of the seven selected threatened mammal species undergoing captive breeding in Southeast Asia

Species	Common Name	Status (IUCN 2018)	Captive Breeding Centre
Panthera tigris sumatrae	Sumatran tiger	Critically Endangered	1) Sumatra Tiger Captive Breeding Centre (PPHS), Taman Safari, Indonesia
Panthera tigris jacksonii	Malayan Tiger	Critically Endangered	2) Sungkai National Wildlife Rescue Centre, Perak
Elephas maximus	Asian Elephant	Endangered	1) National Elephant Conservation Centre, Kuala Gandah Pahang Malaysia. 2) Sungai Ketiar Elephant Sanctuary, Terengganu Malaysia 3) Lok Kawi Wildlife Park, Sabah Malaysia[4] 4) Thai Elephant Conservation Centre, Thailand 5) Elephant Conservation Centre, Sayabouri Loas
Bos gaurus	Gaur	Vulnerable	1) Wildlife Conservation Centre, Pahang Malaysia 2) Sungkai Wildlife Conservation Centre, Perak Malaysia
Dicerorhinus sumatrensis	Sumatran Rhinoceros	Critically Endangered	1) Tabin Wildlife Reserve, Sabah Malaysia 2) Sumatran Rhino Sanctuary, Indonesia
Tapirus indicus	Malayan Tapir	Endangered	1) Sungai Dusun Wildlife Reserve, Selangor Malaysia
Pongo pygmaeus	Bornean Orangutan	Critically Endangered	2) Sepilok Orang-Utan Rehabilitation Centre, Sabah Malaysia 3) Semenggoh Orang-Utan Rehabilitation Centre, Sarawak Malaysia 4) Matang Wildlife Centre, Sarawak Malaysia 5) Bukit Merah Orang Utan Island Foundation, Perak Malaysia 6) Tanjung Puting National Park in Central Kalimantan Indonesia
Helarctos malayanus	Malayan Sun bear	Vulnerable	1) Bornean Sun Bear Conservation Centre, Sarawak Malaysia 2) Moon Bear Rescue Centre, Tam Dao Vietnam 3) Sun Bear Sanctuary, Indonesia 4) KWPLH Sun Bear Centre, Indonesia 5) Phnom Tamao Wildlife Rescue Centre, Combodia

premature death [13,35]. In the case of failure in promoting natural reproduction in captivity, captive centers should develop and use a national semen bank to facilitate in-vitro fertilization [15]. For example, Malaysia maintains a semen bank at the National Institute of Animal Biotechnology (NIAB), under the Department of Veterinary Services [15]. Taman Safari Indonesia also setup a sperm bank, since the almost all the rescued tigers are difficult to reintroduce into the wild due to physical abnormalities and age [43]. For Asian elephant semen, data supported that glycerol and raffinose as an acceptable cryopreservation media for the establishment of sperm banks [44].

3.2 Health, Stress and Mortality

Captive environments have a significant impact on natural behaviors of wild animals [45,46], due to constant visitors, restricted space, and being managed by humans [47]. Apparently, not all animals survive well in captivity, and are prone to poor health, repetitive stereotypic behaviors, and breeding difficulties [46,48]. Inbreeding and infectious diseases are examples of problems in captivity that lead to negative health and mortality outcomes, while frequent exposure to humans (i.e., visitors) and lack of or unsuitable enrichment (lack of stimulating environments that enhances the quality of animal care and encourage naturalistic behaviors), leads to increase in stress level in captive animals [40,42].

Large mammals often have low reproductive rates, with one offspring during each birth event, and long gestation periods [13,39,40,41]. It is important for individual mammals to survive to reproductive age to contribute their genes to the population. Introduction of mates to solitary mammals like Sumatran rhinoceros' in zoos and breeding centers, was reported to have few or no chances of success in mating in a wide range of countries [40]. Captive breeding efforts, however, often leads to injury and death due to the animals' aggressiveness and stress [40].

Cocks (2007) has examined the factors affecting the health and mortality of female orangutans in captivity, such as their primiparous age, and their inter-birth interval and weight, which appears to be critical [49]. Females should not breed until they are 12 to 15 years old because breeding at a younger age increases the risk of maternal death [49]. Mean inter-birth intervals, more than 4 years apart, have a higher survival rate than those with mean inter-birth intervals of less than 4 years apart, and obese females weighing 76 to 95 kg are more prone to early mortality in captivity [49].

Lack of knowledge on biosecurity measures, could lead to bacterial infections and eventually, mammalian deaths in captivity [50,51]. The Malacca Zoo, Malaysia, carried out captive breeding for the Sumatran rhinoceros back in 1984, and after the *Salmonellosis* outbreak in 1985, all the captive rhinoceros were transferred to the Sungai Dusun Rhino Conservation Center in Selangor [50]. Even though, this center managed the daily husbandry and monthly health care monitoring for the animals well [50], the breeding management for the Sumatran rhinoceros at Sungai Dusun ended in 2003 after all the captive rhinoceros died in a span of 18 days due to an infection by a protozoan called *Trypanosoma evansi*, which caused fever, weakness, and lethargy that lead to weight loss and anemia, and from *Escherichia coli* (E.coli) and *Klebsiella pneumoniae* bacterial infections that infected their vital organs [51,22]. The tragedy occurred again after seven years (from September 17th-September 29th 2010 at Sungai Dusun), which by then had already been turned into a Malayan tapir breeding center, where seven tapirs died from *Escherichia coli* (E.coli) and *Klebsiella pneumonia* bacterial infection during the twelve day span, and one tapir displayed *Trypanosomes* in its blood [22]. After two such incidents, and with proper handling and biosecurity training, this center is now managing very well with twelve Malayan tapirs [pers.obs]. Orangutans are exposed to 11 to 47 different viruses in captivity, and this exposure and transmission occurs through food handling and other stressors such as human contact, overcrowding from visitors, and abnormal social structures [52].

Previous studies also reported broken and inflamed skin with abscesses in rhinos, even death after suffered with generalized cracked skin due to wallowing behavior [53]. Following this, it was suggested that the (mud) wallow must be changed every three months to maintain the quality and to prevent inflamed skin in rhinos [53]. Low humidity (55-69%) and warmer temperatures (31-33^{0}C) caused Malayan tapirs to suffer from dryness in captivity, resulting in frequent ingestion (drinking) and locomotion behavior, which is indicative of thermal stress [42]. Other captive mammals such as Malayan Sun bears are prone to develop dental pathologies due to long-term captivity, inappropriate diet, trauma, and stereotypical (enclosure) bar biting [54]. Early detection is important to minimize these negative consequences [54]. Tigers are prone to health problems from an inadequate diet, dental disease, neoplasia, or tuberculosis, therefore, periodic weighing have to be performed to diagnose the issues resulting in excessive weight loss, even though tiger may appear to be 'normal' [55].

The presence of human/visitors is potentially stressful for wild mammals in captivity, especially when there is no opportunity to hide or escape [40]. Studies have reported that the stress levels measured through fecal corticoids is relatively high in rhinoceros that are maintained in enclosures which allowed visitor viewing [56]. A high number of visitors reduced the activity of captive Malayan tapirs [42]. A study of Gaurs' behavior in the presence of zoo visitors, showed a higher level of intragroup aggression and moving behavior, in contrast with more resting when no visitors were present, thus, the presence of visitors significantly influenced the natural behavior of the captive Gaur that may have affected their welfare [57]. Visitor numbers also affect endocrinological stress levels and behavior in orangutans [58].

3.3 Enclosure Design and Environmental Enrichment

Enclosure design, size and environmental enrichment are among the animal husbandry principles that augment the quality of animal care, which are crucial for psychological and physiological well-being of captive animals [59]. Mammalian sociality shows enormous variation, thus well-planned enclosures are required for each mammal [59]. For example, minimum outdoor exhibit areas per rhinoceros ranges from 771 m^2 to 929 m^2, and recommended indoor holding areas range from 18 m^2 to 30 m^2 [40,60]. For tigers, the fence should be at least 5 m high and vertical, except for the top 1 m, which should be tilted inward, into the exhibit, at a 45^0 angle [55]. The fence should be constructed of heavy gauge steel, with equally strong support posts, and a concrete footing, and the fence should be buried at least 1 m deep and angled toward the interior of the exhibit to prevent digging under the fence [61]. The tiger enclosure should be 300 m x 30 m minimum, with a maximum number of two animals, and for each extra animal an additional area of 20 m^2 is needed [55]. Importantly, the exhibit must not be next to/near prey animals [55]. For tapirs, it is recommended that the animal is housed individually in an indoor exhibit with minimum dimension of 3.6 m x 4.5 m and an area of 17 m^2, and 4.9 m x 4.9 m for females with offspring, and the outdoor exhibit should be at least 55.7 m^2 [62]. Low fences in outdoor exhibits make tapirs susceptible to bullying by visitors, therefore, higher fences are needed to ensure that no visitor can get in contact with the tapirs [42]. Tapirs are prone to chronic lameness (abnormal gait or stance), arthritis and other degenerative joint diseases, therefore heating coils should be buried in the concrete floor to help prevent health issues [39].

Captive environments should be sufficiently large to allow a full range of locomotion activities, including walking, climbing, swimming, or burrowing as appropriate to the species concerned [63,64]. Elephants are generally kept in an enclosure as small as 2,200 square feet [65]. This constrained space needs to be changed because the typical wild elephant walks 30 miles per day, however, it is impossible for a zoo or captive breeding center to allocate such large spaces for elephant roaming [65]. It is therefore, recommended to setup natural abrasive surfaces for their foot health and provides regular exercise or activities to assist them in losing some weights and maintain their strength and flexibility [65].

Malayan tigers (*Panthera tigris*) have large home ranges in the wild and natural predatory hunting behaviors that are difficult to cater to in captivity [55]. Pitsko (2003) has suggested a wide variety of techniques of environmental enrichment for tigers such as hiding their food throughout exhibit areas to train them to perform hunting behaviors [63]. Wood blocks or logs can be provided to satisfy scratching behavior when trees are not available [63]. Stimulating scents can be spread throughout enclosures, and sterile concrete enclosures can be replaced with natural substrate and vegetation [63]. Such methods can also be applied to other big cats in captivity [63]. For Malayan tapirs and for the Sumatran rhinoceros, it is important to provide wallows, pools, sand pits, rubbing posts and other items that provide opportunities for them to perform activities that they would in the wild and to regulate their body temperature [24,40,42]. Increasing the amounts of shade could help to control their temperature and prevent over-heating that would lead to heat stress in tapirs and rhinos [24,40,42].

Smaller enclosures are restricted in terms of spatial use and lack of enrichment; thus it is important to provide large enclosures with species-specific enrichment that mimics the wild environment for the mammals in captivity [55,59,60,62].

4. ADVERSE GENETICAL ISSUES IN CAPTIVITY

4.1 Genetic and Inbreeding Depression

Ex-situ conservation (i.e., captive breeding) provides a favourable and stable environment that can offer a relaxed selection of alleles, however, it may promote evolutionary changes if the population is retained in captivity after a few generations [66,67,68,69]. Most of the endangered mammals in captivity are facing different type of genetic problems such as inbreeding depression, accumulation of deleterious mutations caused by genetic drift, loss of genetic diversity and genetic adaptation [66,68,70]. A proper gene flow is crucial to maintain genetic variation within and between populations [70,71]. There are three types of genetic variation in nature including neutral, detrimental, and adaptive [70]. Neutral genetic variation has a small selection coefficient relative to population size that reflects the levels of detrimental and adaptive genetic variation such that, $|s| < 1/2$ Ne, where s is either the selective disadvantage of a detrimental genetic variant or the selective advantage of an adaptive genetic variant, and Ne is the effective population size [70]. Detrimental variation is often brought into the population by mutation or gene flow, and sometimes increased by genetic drift that has a negative effect on fitness [70]. High levels of detrimental variation can contribute to inbreeding depression when the probabilities of homozygous detrimental alleles are increased during population decline [70]. Adaptive variation affects fitness and helps populations to respond to environmental challenges [70].

Captive breeding programs are an alternative strategy to restore the declining population of endangered species, however, prolonged breeding programs may cause more deleterious genetic mutations that could lead to inbreeding depression (e.g., reduced survival probability, reduced reproductive success rate [72], and a loss of genetic diversity [67]). It was noted that 70% of gaurs born in captivity since 1956 were inbred, with the average inbreeding coefficient per year ranging from 0.139 to 0.234 [73]. Inbred offspring aged 6 months or younger faced higher mortality rates compared to non-inbred offspring [73]. Inbreeding depression and loss of genetic diversity increases the risk of extinction of captive populations [74]. Nevertheless, the deleterious mutations can be removed by natural selection by minimizing the genetic adaptation to captivity especially in populations that are likely to be used for reintroduction into the wild, but it is somehow difficult to minimize genetic adaptation to captivity in small populations [66,74].

Genetic diversity can be measured as quantitative trait variations, allelic diversity, and heterozygosity [75,76]. Quantitative trait variations are related to overall fitness (e.g., survival probability and reproductive success rate) of individuals involving many loci rather than one [75]. Quantitative traits vary among individuals due to genetic and environmental differences [75]. Allelic diversity refers to the number of different alleles at any given locus, whereas heterozygosity is the percentage of loci that are heterozygous in a population or individual [72,75]. Inbred individuals have a low level of heterozygosity at genome-wide loci [76]. The consequence of inbreeding in a population with 1000 or fewer individuals is due to recessive lethal alleles [72]. This happens through the process called genetic drift, when both allelic diversity and heterozygosity are lost in small populations [75,76]. A study of orangutans estimated that small populations of fewer than 300 individuals tend to lose 10% of their genetic diversity, thus are at a high risk of extinction after 1000 years due inbreeding [72]. Potentially, if orangutans are free from any external threat, their population can grow at a 2% rate annually, hence a small loss of individuals means a lot in terms of their population [72].

Inbreeding and inbreeding depression also may vary in populations depending on life history traits, habitat and environmental conditions [77]. There is evidence from primate studies that inbreeding depression is more severe in females compared to males and their survival rates are lower than non-inbred females [77]. Each parent has a 50% chance of contributing either of its alleles at each locus to an offspring [75]. Sometimes when more or fewer alleles are passed to offspring, this will cause genetic drift [75]. A study of South China tigers found that the average number of alleles per locus is 4.24 ± 1.03, but an effective number of alleles is only 2.53 ± 0.91, because 21 alleles which carried by early breeders at 13 loci are absent in potential breeders [76]. Thus it is very important to have appropriate gene flow maintenance to prevent genetic issues due to small populations in captivity [70,71].

4.2 Strategies of Genetic Management

Inbreeding and loss of genetic diversity can be minimized in captive breeding management through population management with an effective population being maintained with at least 100 individuals [78]. Introduction of more individuals is necessary, but only after their genotypes have been taken into account to identify the relatedness to other captive individuals [78]. It is estimated that a minimum of 15 unrelated initial animals (founders) are required to maintain the genetic diversity in captivity [78]. However, a better genetic management technique is regularly introducing individuals from the wild into captivity to prevent the increase of captive generations, reduce adaptation to captivity [66,68], and to replace missing genetic lineages [79].

It is important to retain the founders' genetic diversity (unvaried) to serve as a genetic reservoir for the species in captivity, which is crucial during reintroduction process [75]. In cases where species need a longer captive breeding process to achieve targeted population size, it is suggested that a genetically independent method [68], for example, the 'landscape genetic method', be used to sustain their genetic diversity and variation [71]. Neutral and adaptive gene components that can be affected by landscape and environmental variables are called landscape genetics [71]. The landscape genetic method is a useful method in promoting landscape connectivity for conservation of species that lost their habitat due to fragmentation [71]. It provides a direct relationship between the population and the landscape structure such as movement, gene flow, and potentially adaptation [71].

Traditionally, equalization of family size (EFS) is recommended to reduce loss of genetic variation, inbreeding, and inbreeding depression in captivity [66]. Presently, various molecular genetic methods are being used to reduce the genetic consequences in captivity [74,75,78]. Researchers can differentiate the Malayan tiger from other subspecies of tiger based on 3 unique microsatellite alleles, 5 subspecies-specific mtDNA (mitochondrial DNA) haplotypes, and 3 Major Histocompatibility Complex (MHC)-DRB alleles [69,79]. The distinction among tiger species is due to rapid change of habitat and the effect of genetic drift [69]. Currently, the efforts to increase tiger populations, by using molecular genetic conservation to maintain genetic diversity in captive programs, have estimated that only 1000 tigers limited to the Amur, Indian, and Sumatran tiger subspecies have been treated using this method [69]. This needs to be expanded to other endangered tiger subspecies.

Inbreeding analyses should involve either pedigree construction, or heterozygosity at microsatellite loci, or both, because it allows researchers to estimate the real 'kinship' between individuals that reproduce in a given population [77]. However, heterozygosity measured using microsatellite loci appears one of the best current alternatives to pedigree construction in detecting inbreeding [77]. This is because the analysis is based on molecular markers and allows reconstruction of the pedigree relationship of captive individuals with an unknown history [77,80]. In addition, the allele sharing pattern can help in identifying the putative parents-offspring pairing [80].

Promoting gene flow among different populations is one of the ways to avoid inbreeding and maintain large populations, however, it is often impossible to maintain large populations in captive breeding programs given the limited resources available [81]. Therefore, development of genetic resource banks offer a new solution to facilitate the genetic management of endangered species by maintaining genetic diversity through preservation of semen, oocytes, embryos, and other tissues [81]. The advantage of this technology is that it can help preserve the maximum genetic diversity in endangered species and can be used for many years, even after the death of the animals [81]. The other recommendation for genetic management in a captive breeding program is to obtain data of the natural genetic structure of wild populations of endangered species, including those extinct in the wild, using museum materials [78]. It is also important to frequently reassess the genetic structure of captive populations after a few generations [78].

5. REINTRODUCTION PROCESS

An important tool for species conservation is the reintroduction of well-managed captive breeding populations into the wild [6]. A proper health screening, selection of sites and pre-release training prior to reintroduction are crucial and need to be addressed [6].

5.1 Health Screening

Health screenings should be a fundamental part in reintroduction programs [82,83]. It is crucial for species to be free from pathogens to reduce the risk of disease transmission to all the extant individuals in the wild [82]. Animals which are targeted for reintroduction must go through a minimum of 30 to 60 days of quarantine under the supervision of a veterinarian [83]. During this time, the animals are subjected to complete physical and clinical examinations, laboratory tests, and vaccinations, based on their medical history and birth origin, whether wild or captive [83,84]. Fecal samples must be taken to determine if any gastrointestinal parasitic infections (endoparasite) are present in order to administer a suitable anthelmintic to remove or control endoparasites, such as liver flukes in tapirs, and *Entamoeba sp.* in primates [83]. Other tests should be performed to detect fecal virus particles by using electron microscopy and ELISA tests for rotavirus or *trypanosomes sp.,* etc. [83,84]. It is also important that all animals that tested positive for/or carry ectoparasites, like sarcoptic mange, screw worms, warble flies and ticks, should be given appropriate treatments before being released into the wild [83]. All therapeutic drugs should be stopped one week prior to the introduction process to prevent drug resistance in the wild [83].

Antibody titrations need to be measured before reintroduction to achieve immunity against diseases [83]. Necessary vaccinations should be performed according to specified protocols [83]. Various animal health screening protocols have been described in the Woodford (2000) guideline entitled, 'Quarantine and Health Screening Protocols for Wildlife Prior to Translocation and Release into the Wild' [83].

5.2 Soft Release and Trainings

Soft release means pre-release of animal into an area that mimics the wild environment before it is reintroduced into the wild [85]. When animals are either born or have been in captivity for a long time, their level of habituation towards captive situations tend to be very high, therefore careful assessments (such as hunting behaviors, ability to compete for food and space, and performing natural activities) need to be made before they are released into the wild [85].

A soft release area for arboreal species like the orangutan or other species like the Malayan Sun bear should be like their wild habitats, with big trees, vegetation, and facilities for them to climb, rest, forage, and making nest before they are released to forest [86]. These assessments and trainings need to be executed 6 months prior to release [83]. For solitary mammals such as rhinoceros, group size needs to be considered if the animal is to be reintroduced into an area, which is already occupied by the species, to avoid serious aggression and mortality [40].

Independent foraging is vital for animals' survival in the wild, gaining experience in finding their own food is particularly important for Malayan tapirs in ex-situ breeding programs to enable them to be released successfully into wild [40]. Hence, a wide variety of plants need to be provided, and the leaves should be spread around the enclosure, rather than piled in a fixed placed, to enhance anticipation in foraging and feeding behavior [42].

A study of the Asian elephant in Thailand suggests that reintroduction procedures should practice to introducing adults with elephant calves to increase the chance of group formation and establishment of stable elephant herds [87]. This is because social bonding of the reintroduced elephants is not influenced by genetic relatedness, but rather by groups formed in association through the presence of an elephant calf [87].

5.3 Releasing Sites

Restoring habitats for species or making an appropriate selection of sites to release the endangered species is important to reduce the challenges that the species might face in the wild. Certain animals can only survive well in their natural habitats with an initiative toward continued habitat protection, better protection from poaching and public educations are required [6]. For example, there is no data of captively bred tigers (*Panthera tigris jacksoni*) who have been reintroduced into the wild in Malaysia, from the ex-situ conservation project [88]. Due to the loss of tiger habitats, which subsequently caused a drastic population decline, the Malaysian Department of Wildlife and National Parks (DWNP) established a project to save the tiger population through in-situ conservation called the National Tiger Conservation Action Plan (NTCAP) 2008-2020 [88]. Three areas have been identified as totally protected priority areas for tiger conservation, and they include the Belum-Temenggor Complex (3546 km^2), Taman Negara (4343 km^2), and the Endau-Rompin Complex (2389 km^2) [88].

Similarly, there were no captively bred elephants released into the wild in Malaysia [89]. However, DWNP established the Elephant Management Unit (EMU) with the objective of capturing and relocating elephants from areas of human-elephant conflict to more suitable habitats [89]. Through the translocation program, over 600 wild elephants were captured between 1974 and 2010, and most of them were relocated to major conservation areas such as the Taman Negara National Park and the surrounding forests (Pulau Besar and Sungai Ketiar), Belum-Temengor, and Endau Rompin [89]. Apart from that, three captive bred gaurs (one male and two females) from the Jenderek Conservation Center were released to the Krau Wildlife Reserve and tagged with radio collars to monitor their movements [90]. The Malayan tapir was released in the Sungai Dusun Forest [91], and the Malayan Sun bear in the Tabin Wildlife Reserve, the Deramakot Forest Reserve, the Danum Valley Conservation Area, and the Ulu Segama-Malua Forest Reserve [86].

5.4 Consequence of Reintroduction

Captive-bred animals are often prone to high mortality rates in the wild, and many species show poor reproduction, which contributes to the failure of reintroduction programs [40,48]. Low or modified temperament traits can reduce their anti-predator behaviors, and this could be one of the reasons for reintroduction failure [92]. Therefore, careful monitoring of the temperament traits is important for every species in captivity [92]. Reintroduction of solitary mammals as a group into the wild resulted in severe injuries the same species in the wild [40]. Therefore, it is suggested that feces (dung) from each animal to be released is spread around the release sites, and the largest and most aggressive animals be placed at extreme ends of the reserve to minimize aggressive contact [40].

A systematic reintroduction program should include an ecologist who is proficient in population biology and genetics, behavioral ecology, and evolution [93]. Whenever a reintroduction is required, a proper training program must be planned and provided to the captive animals prior to release [93]. Unfortunately, with thousands of released animals throughout the globe, the chances of success are low because the original threats to these threatened species are not fully removed [94]. Therefore, the preparation before reintroduction of an animal into the wild should include a prior knowledge of the species, and animal assessment which are very crucial to reintroduction success.

6. CONCLUSION

Threats to species biodiversity include land conversion, pollution, climate change and encroachments (poaching for meat and body parts and for the purpose of traditional remedies). These threats caused the animals' population decline and extinction. Thus, conservation efforts such as captive breeding serve as a tool for ex-situ conservation where the wild animals are removed from their natural habitat and bred in zoos or other private facilities. Proper guidelines for the management of captive breeding programs are crucial for the welfare of the animals. Sustainable populations can be achieved in breeding centers and then reintroduced to the wild. The males and females will either mate naturally in captivity or by collection and freezing semen, embryos, or ova, in liquid nitrogen for Farm Animal Genetic Resources (FAnGR) and maintained for in-vitro fertilization. However, before this process the animals should undergo complete genetic assessment and health screenings to avoid inbreeding and to promote healthy genes to the wild.

As many mammals are becoming extinct, including few species endemic to Southeast Asia, it is our responsibility to engage in conservation efforts to save these animals. At present, governmental organizations, Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), International Union for Conservation of Nature (IUCN) together with other non-governmental organizations (NGOs) such as the World Wildlife Fund (WWF), Borneo Orangutan Survival Foundation (Indonesia), Wildlife Alliance (Cambodia), Malaysian Nature Society (MNS), Save Vietnam's Wildlife, Bornean Rhino Alliance, Save Elephant Foundation (Thailand) and many more are working closely on various conservational projects to save the wildlife that are on the verge of extinction through research, education and outreach, rescue and combat wildlife trafficking, policy making and implementation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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