Review Paper
Captive breeding of threatened mammals native to Southeast Asia – A review on their ex-situ management, implication and reintroduction guidelines.

ABSTRACT
Heavy deforestation and land use conversion in Southeast Asia caused most of the mammals facing the threat of extinction due to limited availability of suitable habitats that jeopardize their survival throughout their regions. As the demand for certain body parts of threatened mammals is on a treadmill, the illegal poaching activities increasing and consequently their population size continuously decreasing. Protecting a sustainable population numbers or the efforts to multiply the population of threatened mammals in their own natural habitats is very challenging or 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 need a proper guideline to maintain their welfare and genetic variabilities. In this paper, we discussed on the threatened mammals native to Southeast Asia countries that are currently under captive breeding programme. Multi-disciplinary overview including group size and social structure; health, stress and mortality studies; and enclosure design and environmental enrichment are the key components for the best management and husbandry practices. The allele may experience evolutionary change if the population retained in captivity after few generations that could lead to genetic problems. Hence, a proper gene flow is crucial to maintain genetic variation within and between populations. Lastly, an important tool for species conservation is reintroduction of well-managed captive breeding populations into the wild. Therefore, a complete health screening, selection of sites and pre-release training prior to reintroduction are something crucial and need to be addressed.

Keywords: Captive breeding, Reintroduction, Threatened Mammal, Ex-situ, Wildlife conservation

INTRODUCTION
Among the world’s tropical regions, Southeast Asia that comprises the countries of Cambodia, Laos, Myanmar, Thailand, Vietnam, Malaysia, Indonesia, Brunei, Philippines and Timor-Leste are facing a massive deforestation [1] (with Singapore the most heavily affected (>95%; [2])), where this impacting the population of their endemic floras and faunas. Heavy losses of native habitats and limited availability of suitable habitats in Southeast Asia caused most of the mammals facing the threat of extinction that 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’ mammal from 315 to 482 species since year 1996 which is very alarming and need immediate actions [4].
Various conservation efforts are being taken throughout the countries to protect and prevent these mammals from extinction in 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 Southeast Asia regions.

The overview delineates four parts. First we highlight on the current population, status and potential threats of the selected mammals in the wild that reasoned for captive breeding in Southeast Asia regions. Second, we will discuss on implication and suggestions on management and husbandry in captivity. Third, on the adverse genetic issues in captivity that may affect the mammals’ welfare and lastly, the guidelines for reintroduction of captive animals back into the wild with a hope to restore healthy and self-sustaining populations.

CURRENT POPULATION, STATUS AND POTENTIAL THREATS OF THE MAMMALS

Tiger (Panthera tigris)

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

Asian Elephant (Elephas maximus)

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

Gaur (Bos gaurus)

Currently, there are 273 to 333 individuals in Peninsular Malaysia [15], Laos approximately 1000 individuals [16], Thailand 100 individuals at Khao Yai National Park [16] and about 198-239 individuals remaining at Kulburi National Park [17] and at Vietnam, a total of 121 individuals were recorded at Ea So Nature Reserve and Yok Don and Cat Tien National Parks [18]. The current population of Gaur in Myanmar is poorly known; however an old survey that carried out twenty years back had estimated about 100-200 individuals [16]. Habitat loss and poaching for meat are the two main threats that caused the population to decline throughout most of its range [19].
Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

The population is estimated to be only 170 to 230 individuals remaining at Bukit Barisan Selatan, Way Kambas, and Gunung Leuser National Park, Sumatera [20]. In Malaysia, few studies back in 1975 to 1981 estimated 50 to 75 individuals occurred in ten isolated areas in Peninsular Malaysia (e.g., 20 to 25 individuals at Endau-Rompin National Park, 8 to 12 individuals at Taman Negara Pahang and 4 to 6 individuals at Sungai Dusun; [21]) and around 50 individuals in Tabin National Park and Danum Valley in Sabah [20]. Sadly, this critically endangered species is most likely to be extinct from wild in Peninsular Malaysia after no evidence of the species’ presences in the wild since the last capture in year 2007 [22]. The rapid decline in population has been associated with continuous hunting for their horns [20,23]. The horns been vastly used by Chinese doctors and pharmacist in their traditional medicine to treat fever, rheumatism, gout, typhoid, headaches, carbuncles, vomiting, food poisoning and snakebites, and for their traditional custom practices such as treating devil possession and hallucinations [20,23].

Malayan Tapir (*Tapirus indicus*)

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

Bornean Orangutan (*Pongo pygmaeus*)

The Bornean orangutan, *Pongo pygmaeus* have three subspecies in Malaysia and Indonesia; Northwest Bornean orangutan (*Pongo pygmaeus pygmaeus*) occurs in Sarawak and West Kalimantan, Southwest Bornean orangutan (*Pongo pygmaeus wurmbii*) occurs in West Kalimantan and Central Kalimantan and Northeast Bornean orangutan (*Pongo pygmaeus morio*) occurs in North and East Kalimantan and Sabah [26]. The estimated population size of the Northwest Bornean orangutan subspecies is 2,000-2,500 individuals in West Kalimantan and 1,143-1,761 individuals in Sarawak. The Southwest Bornean orangutan population in Central and West Kalimantan is estimated to be around 34,975 individuals and the estimated population of Northeast Bornean orangutan is 4,825 individuals in East Kalimantan and 11,017 individuals in Sabah [27]. Major threat for this species is the destruction of their natural habitat for massive development of oil-palm plantation, illegal logging and illegal hunting [26,27,28]. Orangutans are also killed for several reasons such as for traditional medicine, to sell offspring, hunting for fun, for food, for self-defense; or when the orangutan perceived as a pest [29].

Malayan Sun Bear (*Helarctos malayanus*)

The Malayan Sun bear (*Helarctos malayanus*) also known as ‘honey bear’ or ‘beruang matahari’ is found in tropical forest of Southeast Asia region that includes; Brunei, Cambodia, Indonesia; Laos, Malaysia; Myanmar, Thailand and Vietnam and extinct in Singapore [30,31]. The population is estimated between 1.13 to 1.57 bears/km² in Taman Negara and 0.128 bears/km² in Ulu Segama Forest Reserve [32]. The population estimation in other Southeast Asia countries is poorly known due to lack of studies. The major threats of this species are habitat loss and poaching. Malayan Sun bear’s meat and body parts are highly consumed in Japan and Korea, whereas their gall bladders and bile extraction are...
commercially demanded in countries like Myanmar, Thailand, Laos, Cambodia, Vietnam and East Malaysia for traditional Chinese medicine uses [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 are the key component to protect these species for further extinction. However, it is proved challenging primarily due to socioeconomic in origin, including population growth, poverty, shortage in conservation resources such as funding, and also corrupted national institutions [1]. Therefore, ex-situ conservation is essential to sustain and breed these mammals in captivity. However, the management and husbandry are important elements to be considered to achieve the purpose of ex-situ conservation.

**IMPLICATION AND SUGGESTIONS ON MANAGEMENT AND HUSBANDRY IN CAPTIVITY**

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

**Group Size and Social Structure**

Different mammalian species live in a different group size and composition; some live as solitary while others 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 predator, defense territory and foraging that are no longer a concern among captive mammals. However, the proportion of group size of too small or too large in captivity, can root to negative consequences on their behavior, welfare, and lifetime reproduction [35,36].

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

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

Sumatran rhinoceros, Malayan tapir, Bornean orangutan, Malayan Sun bear and tigers are solitary animals except during weaning period where the offspring accompanied by their dam until they become independent or a pair of adult female and male during mating encounter in the wild [35,38,39,40]. Housing Sumatran rhinoceros and Malayan tapir in captivity either solitary or in a pair are largely depended on the animals social behavior and temperament [39,40]. It is suggested that only an estrus female is introduced into a male enclosure to avoid serious injuries inflicted onto the females [41,42]. Juveniles can be kept with the dam until it reach puberty and need to be separated for breeding to avoid aggressive behavior of dam towards older calf after the born of new calf [40]. Certain solitary mammal, like Bornean
orangutans are flexible, living in group and shown successful social interaction among conspecific [35]. Overall, a careful management of social grouping of mammals is crucial to avoid problems such as delays in first age of reproduction, longer inter-birth intervals and premature death [13,35]. In the case of failure to promote natural reproduction in the captivity, captive centers should seek the national semen bank for administration of in-vitro fertilization. For example, Malaysia maintains a semen bank at the National Institute of Animal Biotechnology (NIAB), under Department of Veterinary Services [15]. Taman Safari Indonesia also setup a sperm bank since the almost all the rescued tigers are impossible to be reintroduced to the wild due to physical abnormalities and aged [43]. For Asian elephant’s semen, data supported that TEST + glycerol as an acceptable cryopreservation media for the establishment of sperm banks [44].

**Health, Stress and Mortality**

Captive environment has significant impact on natural behaviors of wild animals [45,46] due to chronic visitors; restricted space and being manage by human [47]. Apparently, not all animals survive well in captivity and 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 health and mortality whereas frequent exposure to human (i.e., visitors) and lack or unsuitable enrichment leads to increase in stress level in captive animals.

Large mammals often have low reproductive rate with one offspring each birth and long gestation period. It is important for individuals to survive up to reproductive age to contribute their gene to the population. Introduction of mates to solitary mammal like Sumatran rhinoceros in zoos and breeding centers was reported with fewer/no chances of success in mating in wide range of countries [40]. This effort is however; often lead to injury and death due to their aggressiveness and stress.

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

Lack of knowledge on biosecurity measures, could lead to bacterial infections and eventually, deaths in captivity. Malacca Zoo, Malaysia carried out captive breeding for Sumatran rhinoceros back in 1984 and after Salmonellosis outbreak in 1985, all the captive rhinoceros transferred to Sungai Dusun Rhino Conservation Centre in Selangor. Even though, this center managed well with daily husbandry and monthly health care monitoring [50], the breeding management for 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 protozoan called Trypanosoma evansi causing fever, weakness, and lethargy that lead to weight loss and anemia, and by Escherichia coli and Klebsiella pneumoniae bacterial that infected abundantly on their vital organs [51,22]. The tragedy recurred again after seven years from 17th-29th September 2010 at Sungai Dusun, which by then had already been turned into a Malayan tapir breeding center, where seven tapirs died from Escherichia coli and Klebsiella pneumoniae bacterial infection in twelve days span and one tapir showed trypanosomes in the blood [22]. After two such incidents and with a proper handling and biosecurity knowledge, this center is now managing well with twelve Malayan tapirs. The orangutans are exposed to 11 to 47 different viruses in captivity, and this exposure and transmission occurs...
through food handling and other stressor such as human contact, overcrowding of visitors
and abnormal social structure [52].

Previous studies also reported on broken and inflamed skin with suppurations in rhinos,
even death after suffered with generalized cracked skin due to wallow. Following this, it was
suggested that the wallow have to 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°C) caused Malayan tapirs to suffer from dryness in captivity, and thus resulted in
frequent ingestion (drinking) and locomotion behavior which indicative of thermal stress [42].

Other captive mammal such as Sun bears is prone to develop dental pathology due to
longevity in captivity, inappropriate diet, trauma, and stereotypical bar biting [54]. Early
detection is important to minimize the negative welfare consequences [54]. Tiger may prone
for health problem such as an inadequate diet, dental disease, neoplasia, or tuberculosis,
thus, periodic weighing have to be taken to diagnose the issue that will resulted in excessive
weight loss even though tiger may appear 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]. Study reported that the stress
levels measured through fecal corticoids is relatively high in rhinoceros that are maintained
in enclosures that allowed visitors viewing [56]. High number of visitor had lowered the
activity of captive Malayan tapir [42]. A study on Malayan gaur behavior in the presence of
zoo visitors, showed a higher level of intragroup aggression and moving behavior, in contrast
more rests when no visitors thus, the presence of visitors is significantly influenced the
natural behavior of captive Malayan gaur that may have affect their welfare [57]. Visitor
numbers also affect endocrinological stress levels and behavior in orangutans [58].

Enclosure Design and Environmental Enrichment

Enclosure design, size and environmental enrichment are among the 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. For example, minimum outdoor exhibit
areas per rhinoceros is ranged from 771m² to 929m² and recommended indoor holding
areas range from 18m² to 30m² [40,60]. For tigers, the fence should be at least 5m high and
vertical except for the top 1m which should be turned into the exhibit at about a 45° angle.
This fence should be constructed of heavy gauge steel with equally strong support posts and
a concrete footing and bury the fence at least 1m angled toward the inside of the exhibit to
prevent digging under the fence [61]. The tiger enclosure should be 300m x 30m minimum
with a maximum number of two animals and for each extra animal an additional area of
20m² is needed [55]. Importantly, the exhibit must not be next/near prey animals [55]. For
tapirs, it is recommended that the animal is housed individually in an indoor exhibit with
minimum dimension of 3.6m x 4.5m and area of 17m² and 4.9m x 4.9m for female with
offspring, and outdoor exhibit measured at least 55.7m² [62]. Low fences in the outdoor
exhibit making tapirs susceptible for bullying by the visitors, therefore, higher fence needed
as such no visitor able to get in contact with the tapirs [42]. Tapirs are prone for chronic
lameness, arthritis and degenerative joint disease therefore; heating coils have to be buried
in the concrete floor to help prevent health issues [39].

Captive environment 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. This
constraint space need to be change because typically wild elephant walks 30 miles per day,
however, it is impossible for zoo or captive breeding centers to allocate such large space for
elephant roaming [65]. It is therefore, recommended to setup natural abrasive surfaces for their foot health and provides regular exercise or activities by the well trained keepers to help them to lower weight and maintain 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 in captivity. Pitsko (2003) has suggested a wide variety of techniques of environmental enrichment for tiger such as their food can be hidden throughout exhibit areas for them to tempt it to perform hunting behaviors, wood blocks or logs can be given to satisfy scratching behavior when trees are not available, stimulating scents can be spread throughout enclosures and sterile concrete enclosures can be replaced with natural substrate and vegetation. Such method can also be applied to other big cats in captivity. For Malayan tapir as well for Sumatran rhinoceros, it is important to equip wallows, pools, sand pits, rubbing posts and other items that provide opportunities to perform activities similarly in the wild as well to regulate their body temperature [24,40,42]. Increasing the amounts of shade could help to control temperature and prevent over-heating that would lead to heat stress in tapirs and rhinos.

Smaller enclosures are restricted in term of spatial use and lack of enrichment, thus it is important to provide large or recommended enclosures with species specific enrichment that mimics the wild environment for the mammals in captivity for their welfare.

ADVERSE GENETICAL ISSUES IN CAPTIVITY

Genetic and inbreeding depression

Ex-situ conservation (i.e., captive breeding) provides a favorable and stable environment that can offer a relaxed selection on allele 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 mutation 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 include 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, $|s| < 1/2Ne$, 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. Detrimental variation often brought into the population by mutation or gene flow and sometimes increased by drift that has a negative effect on fitness. High level of detrimental variation can contribute to inbreeding depression when the probabilities of homozygous for detrimental alleles are increased during population decline [70]. Adaptive variation affects fitness and helps populations to respond to environmental challenges [70].

Captive breeding program is an alternative strategy to restore the declining population of endangered species, however, prolonged program may cause more deleterious genetic mutations that could lead to inbreeding depression (e.g., reduced survival probability, reproductive success rate [72] and loss of genetic diversity [67]). It was evidenced that 70% of gaur's born in captivity since 1956 were inbred with the average inbreeding coefficient per year ranged from 0.139 to 0.234 [73]. Inbred offspring aged 6 months or below faced higher mortality rate compared to non-inbred offspring [73]. Inbreeding depression and loss of genetic diversity increase 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 on populations that likely to be used for reintroduction into the wild but it is somehow difficult to minimize in small population [66,74].

Genetic diversity can be measured as quantitative trait variation, allelic diversity and heterozygosity. Quantitative trait variation is 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. 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 are due to recessive lethal alleles [72]. This happens through the process called genetic drift when both allelic diversity and heterozygosity are lost in small population [75,76]. A study in orangutan estimated that small populations with below than 300 individuals tend to loss 10% of their genetic diversity, thus are at high risk of extinction after 1000 years due to inbreeding [72]. Possibly, if orangutans are free from any external threat, their population can grow at only 2% annually, hence a small loss of individual means a lot in their population size [72].

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

Strategies of genetic management

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

It is important to retain founders’ genetic diversity unvaried to serve as genetic reservoir for the species in captive which is crucial during reintroduction process [75]. In the cases where species need longer captive breeding process to achieve targeted population size, it is suggested to use genetically independent method [68], for example, ‘landscape genetic method’ to sustain their genetic diversity and variation [71]. Neutral and adaptive gene components that can be affected by landscape and environmental variables are called as landscape genetic [71]. Landscape genetic method is a useful method to promote landscape connectivity for species of conservation that lost habitat due to fragmentation [71]. It provides a direct relationship between population and landscape structure such as the 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. Researchers are able to differentiate Malayan tiger from another subspecies of tiger based on 3 unique microsatellite alleles, 5 subspecies-specific mtDNA haplotypes and 3 MHC DRB alleles [69,79]. The distinction among tiger species are 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 are estimated only 1000 tigers that limited to Amur, Indian and Sumatran tiger subspecies [69]. This amount needs to be expanded to other endangered tiger subspecies as well using molecular genetic conservation.

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 to detect inbreeding [77]. It is because the analysis based on molecular markers allows reconstruction of pedigree relationship of captive individuals with unknown history. In addition, the allele sharing pattern can help in identifying the putative parents-offspring pair [80].

Promoting gene flow among different populations is one of the ways to avoid inbreeding and to maintain large population size, however, it is often impossible to maintain large populations in captive breeding programs given the limited resources available [81]. Therefore, development of genetic resources bank offers new solutions to facilitate the genetic management of endangered species for maintaining genetic diversity by preservation of semen, oocytes, embryos and other tissues [81]. Advantage of this technology is that it can help in preserving the maximum of genetic diversity in endangered species which may be used for many years even after the death of an animal [81]. The other recommendation for genetic management in captive breeding program is to obtain data of natural genetic structure of wild population of endangered species including those extinct in wild using museum materials [78]. It is also important to frequently reassess the genetic structure of captive populations after some generations [78].

**REINTRODUCTION PROCESS**

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

**Health Screening**

Health screening should be a fundamental part in reintroduction programs. It is crucial for species to be free from pathogen to reduce the risk of disease transmission to all the extant individuals in the wild [82]. Animals which are targeted for reintroduction have to go through a minimum of 30 to 60 days of quarantine under supervision of a veterinarian [83]. During this time the animals are subjected to a complete physical and clinical examinations, laboratory test and vaccination, based on their medical history and birth origin either wild or captive [83,84]. Feces samples have to be taken to determine any gastrointestinal parasitic infections (endoparasite) in order suitable anthelmintic can be administered to remove or control endoparasites, such as liver flukes in tapirs and Entamoeba sp. in primates [83]. Other test should be perform to detect the fecal virus particle such as by using electron microscopy and Elisa test for rotavirus or trypanosomes sp and etc [83,84]. It is also important that all animals tested positive to/or carry ectoparasite like sarcoptic mange, screw
worm, warble fly and tick infestation, should be given appropriate treatments before release into the wild. All therapeutic drugs should stop one week prior to introduction process to prevent drug resistant in the wild [83].

Antibody titrations need to be measured before the reintroduction to protect and achieve the satisfied immunity against diseases. Necessary vaccinations should be performed according to specified protocols. Various animal’s health screening protocols have explained in Woodford (2000) guideline entitled ‘Quarantine and Health Screening Protocols for Wildlife Prior to Translocation and Release into the Wild’ for references.

Soft Release and Trainings

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

A soft release area for arboreal species like orangutan or other species like Sun bear should be similar to wild habitat with big trees and vegetation and all other facilities for them to climb, rest, forage and making nests before they are released to forest [86]. All these assessments and trainings need to be executed 6 months prior to release [83]. For solitary mammals such as rhinoceros, group size need to be considered if the animal to be reintroduced into an area which 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. 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 [40].

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

Releasing Sites

Restoring habitats for the species or make appropriate selection of sites to release is important to lower the challenges that the species might face in the wild. Certain animals can only survive well in their natural habitats hence with an extra initiative towards continues habitat protection, better protection for poaching and public education are much required. For example, no data of captively bred tigers (Panthera tigris jacksoni) have been reintroduced into the wild in Malaysia from the ex-situ conservation project (protecting the critically endangered species outside of their natural habitat). Due to the loss of tiger habitats which subsequently caused a drastic population decline, the Malaysian’s Department of Wildlife and National Park (DWNP) Park established a project to save tigers population through in-situ conservation called as National Tiger Conservation Action Plan (NTCAP) 2008-2020. Three areas have been identified as totally protected priority areas for tiger
Similarly there were no captive bred elephants released into the wild in Malaysia before. 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. Through the translocation program, over 600 wild elephants have been captured between year 1974 and 2010 in which most of them have relocated to major conservation areas such as Taman Negara National Park and the surrounding forests (Pulau Besar and Sungai Ketiar), Belum-Temengor, and Endau Rompin. Apart from that, three captive bred gaur (one male and two females) from Jenderek Conservation Centre released to Krau Wildlife Reserve tagged with radio collar to monitor their movements. Whereas, the Malayan tapir released in Sungai Dusun Forest and Malayan Sun bear in Tabin Wildlife Reserve, Deramakot Forest Reserve, Danum Valley Conservation Area and Ulu Segama-Malua Forest Reserve.

Consequence of Reintroduction

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

A systematic reintroduction program should include an Ecologist who is proficient in population biology and genetics, behavioral ecology and evolution. Whenever a reintroduction is needed, a proper training phase has to be prepared and provided to the captive animals prior to release. Unfortunately with thousands of released animals throughout the global, often the chances of success are low as the original threats of these threaten species are not fully removed. Henceforth, the preparation before reintroduction an animal into the wild, including a prior knowledge of the species and the assessment are very crucial for reintroduction success.

CONCLUSION

Many mammals are currently undergoing extinction process, including few species endemic to Southeast Asia, thus it is our responsibility to engage in conservation efforts to save these animals. Several threats for national biodiversity are due to land conversion, the pollution, climate change and encroachments (poaching for their meat and body parts for the purpose of traditional remedies). These threats led the animals population to decline and extinct. Thus, conservation efforts such as captive breeding serve as a tool for ex-situ conservation where the wild animals removed from their natural habitat and breed in zoos or other private facilities.

A proper guideline on the management of captive breeding is crucial for the welfare of the animals. Sustainable population needs to be achieved in breeding centers to then reintroduce to the wild. The males and females will either mate naturally in the captive or by collection and freezing semen, embryos or ova in liquid nitrogen of FAnGR and kept it for in-vitro fertilization. However, before this process the animals should undergone complete
genetic assessment and health screening to avoid inbreeding and promoting healthy genes to the wild.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Status (IUCN 2018)</th>
<th>Captive Breeding Centre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panthera tigris sumatrae</td>
<td>Sumatran tiger</td>
<td>Critically Endangered</td>
<td>1) Sumatra Tiger Captive Breeding Centre (PPHS), Taman Safari, Indonesia</td>
</tr>
<tr>
<td>Panthera tigris jacksonii</td>
<td>Malayan Tiger</td>
<td>Critically Endangered</td>
<td>2) Sungkai National Wildlife Rescue Centre, Perak</td>
</tr>
<tr>
<td>Elephas maximus</td>
<td>Asian Elephant</td>
<td>Endangered</td>
<td>1) National Elephant Conservation Centre, Kuala Gandah Pahang Malaysia.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2) Sungai Ketiar Elephant Sanctuary, Terengganu Malaysia</td>
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<td></td>
<td></td>
<td></td>
<td>3) Lok Kawi Wildlife Park, Sabah Malaysia</td>
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<td></td>
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<td>4) Thai Elephant Conservation Centre, Thailand</td>
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<td></td>
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<td>5) Elephant Conservation Centre, Sayabouri Laos</td>
</tr>
<tr>
<td>Bos gaurus</td>
<td>Gaur</td>
<td>Vulnerable</td>
<td>1) Wildlife Conservation Centre, Pahang Malaysia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2) Sungkai Wildlife Conservation Centre, Perak Malaysia</td>
</tr>
<tr>
<td>Diceros rhinoceros sumatrensis</td>
<td>Sumatran Rhinoceros</td>
<td>Critically Endangered</td>
<td>1) Tabin Wildlife Reserve, Sabah Malaysia</td>
</tr>
<tr>
<td>Tapirus indicus</td>
<td>Malayan Tapir</td>
<td>Endangered</td>
<td>1) Sungai Dusun Wildlife Reserve, Selangor Malaysia</td>
</tr>
<tr>
<td>Pongo pygmaeus</td>
<td>Bornean Orangutan</td>
<td>Critically Endangered</td>
<td>2) Sepilok Orang-Utan Rehabilitation Centre, Sabah Malaysia</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>3) Semenggoh Orang-Utan Rehabilitation Centre, Sarawak Malaysia</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>4) Matang Wildlife Centre, Sarawak Malaysia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5) Bukit Merah Orang Utan Island Foundation, Perak Malaysia</td>
</tr>
</tbody>
</table>


59. Shepherdson DJ. Tracing the path of environmental enrichment in zoos. 2nd nature: Environmental enrichment for captive animals. 1998.


