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Review Article

Existing Status and Resurgence Strategies for Chinese Alligator (*Alligator sinensis*)

Iram Maqsood [1,2,3] and Ke Rong *[1,2]
[1]*College of Wildlife Resources, Northeast Forestry University, Hexing Road 59 Street, Xiang Fang District, Harbin City 150040, China*
[2]*Key Laboratory of Conservation Biology, National Forestry and Grassland Administration, Harbin 150040, China*
[3]*Department of Zoology, Shaheed Benazir Bhuto Women University, Peshawar, 25000*

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ABSTRACT

Recently merely two crocodile's species has been enjoying this universe, and one of them is Chinese alligator (*Alligator sinensis*) who is at the edge of extinction in nature. Approximately, 130-150 individuals left in wild. Habitat loss is considered as a major source of population decline of this precious species. Consequently, for the sake of *A. sinensis* conservation, China took steps with the top priority of habitat restoration, controlled management in captive breeding centers, as well as initiation of reintroduction into the wild. Captive breeding centers focused on livestock, controlled sex ratio, promoting growth characteristics, population genetics, and organ preservation. In addition to this, the habitat restoration program committed to improving the quality and habitat area. The reintroduction project illustrated that *A. Sinensis* bred in captivity well adapted to wild habitat and successfully reproducing their offspring's. Only healthy crocodiles were selected for release into the wild to renew natural populations. Chinese alligator currently under strict regulation over its commercial use and illegal hunting. All of these strategies give a revival hope to this world most endangered species and give a chance to be part of this ecosystem biodiversity in perspective future. China contributed greatly to conserve ecological and cultural roles of Chinese alligators and its sustainable utilization.

INTRODUCTION

The International Union for the Conservation of Nature predicted the wild Chinese alligator as being the most endangered among 23 crocodilian species and The Convention was held in international trade of world endangered species and regarded Chinese alligator as Accessory I endangered species (Chen *et al*., 2003). These alligators are presently prevalent to the lower Yangtze River in China and choose freshwater habitats such as streams, marshes, ponds, lakes, and rice paddies. Previously, during field investigation, some wild nests with less ability to produce viable progeny have been observed. Historically, these species have been dispersed throughout the extensive wetland habitats of the lower Yangtze River (Chen *et al*.,2003; Thorbjarnarson, 2002). It has estimated that wild habitat carried fewer than 200 individuals and more than 13,000 individuals under captive center management in Anhui and Zhejiang Provinces and that it is decreasing at 4%–6% annually. This severe population bottleneck has been verified by scanning genome-wide single nucleotide polymorphisms (Wan *et al*., 2013). According to Wildlife Conservation Society, a survey in Shanghai Wetland Park disclosed eggs of Chinese alligators were spotted in a nest and hatching has confirmed by baby alligators founded later (Scott, 2016). To raise the number of wild alligators, since 2003 the reserve has reintroduced a bunch of the reptiles to their natural habitat. After the uninterrupted four months of dormancy period, over 13,000 Yangtze alligators have spotted outside to make them enjoy the warm sunshine of spring in Xuancheng, East China's Anhui province, in order (Xinhua, 2017).

Until now Chinese alligators population affected by varied factors but mostly included habitat fragmentation and degradation, shooting, natural disasters, geographic separation, low productivity, and pollution. The supreme priority for the conservation of *A. sinensis* is habitat reestablishment to achieve the goal of the reintroduction of captive bred individuals. Additionally, the potential aftermath of environmental pollution and reduced genetic diversity of the wild population must be addressed (Ding *et al*., 2004). The SFA broadcasted the "China Action Plan

* Corresponding author: rongke@nefu.edu.cn
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for Conservation and Introduction of Chinese Alligator" in 2002 and catalog *A. sinensis* as one of 15 leading species of National Wildlife Conservation Project (Jiang, 2010). Since then, the Chinese Government has paid more attention to the protection and management of wild populations and their habitats, as well as speed up release projects. The reintroduction program initiated in 2001 by ANNRCA (Jiang *et al*., 2006).

Although conservation efforts for this species in China began in 1979, efforts have focused on the wild population only since 2000. Some of the following conservation strategies are under consideration until now.

ESTABLISHMENT OF CAPTIVE BREEDING CENTERS

Chinese alligator conservationist has focused on captive breeding and established centers in Anhui and Zhejiang Provinces (Wan *et al*., 1998). The Anhui Research Center for Chinese Alligator Reproduction (ARCCAR) is the biggest facility, housing over 13,000 *A. sinensis* and assisting as the administrative center for alligator conservation in Anhui Province, controlling 5-county and 13 protected sites that covered all the remaining regions with wild Chinese alligators. In spite of this, breeding centers have been set up at Qiongshan City, National Forest Park of Qiandaohu, Doumen County, and Jiangyin City. Additionally, numerous small-scale safari parks, museums, and captive-breeding farms also maintain *A. sinensis* (*e.g.,* Chongqing and Shanghai Municipalities). Chinese alligators breeding has also been practicing in the United States at the Bronx Zoo (New York), St. Augustine Alligator Farm (Florida), and Rockefeller Refuge (Louisiana). Specimens are seized in zoos and private holdings outside of China, and the index is well-maintained for the USA and Europe (Jensch, 2008). Guangzhou motive has issued at the International Workshop on Captive Breeding and Commerce Management in Crocodylia in order to support captive breeding and commerce running of crocodilians in China (SFA, 2002). To provide good living condition and ease the stress of the large captive population, State Forestry Administration (SFA) of China and Anhui Provincial Government co-financed ARCCAR to construct well-facilitated breeding areas. Meanwhile, in 2003 the SFA and Changxing Government co-financed for CBRCCA to facilitate infrastructure and wetland restoration (Jiang, 2010). Captive breeding centers have focused on many aspects of Chinese alligators, which are as follows:

Sexual development of Chinese alligator

In addition to population survival and development, the sex ratio is a major issue of mate competition and mate selection among all species (Oring and Emlen, 1977; Hurst and Dyson, 2004). Preference towards one sex in a small isolated population influence the extinction of a species. Therefore, the appropriate sex ratio for Chinese alligator should maintain in order to recover wild populations. Numerous studies reported that inbreeding depression is a major problem for population recovery as it disturbed natural male-to-female sex ratio of Chinese alligator (Wang and Ding, 2004; Zhang *et al*., 2006; Wang *et al*.,2006). For several decades, the Chinese alligator has been subject to population bottlenecks. Genome-wide single nucleotide polymorphism advocated major inbreeding within populations (Wan *et al*., 2013). To maximize the number of offspring under inbreeding pressure, more females produce than males (Hamilton, 1967), which could lessen the effect of low survival rates on overall population development. The female-biased sex ratio for regardless of mate-competition requires a polygynous mating system to achieve an efficient reproduction (Lance *et al*., 2009). To overcome sex ratio, the improvements have been achieved in sex development of Chinese alligator by using a reptile scale incubator under automatic temperature and humidity recorders with highest survival rate. Incubation at 29 degrees produces female with 91.67% survival rate and Incubation at 34 degrees produces male with 87.50% survival rate (Fang and Fang, 2015).

Investigation of hormonal development

In recent years, reproductive biology got consideration by the various researcher to examine the mechanism of reproductive hormones. Zhang *et al.* (2015) have investigated the changes in expression of FSHß during the reproductive cycle. He explored the mechanisms that regulate the reproduction of the Chinese alligator and it might play an important role in promoting ovarian development. It might be a contribution towards producing Recombinant Chinese alligator FSHß, which could assist in artificial breeding aimed to increase its captive reproductive efficiency. Another fact-finding revealed that ESR1 mRNA plays an important role in the mediation of estrogenic multiple reproductive effects in Chinese alligator because the highest level has recorded in the ovary as compared to other organs (Zhang *et al*., 2016). Consideration toward growth development also plays a key contribution in Chinese alligator management.

Explore development factors

The insulin-like growth factors (IGFs) play an essential role in growth and development (Moriyama *et al*., 2000; Ohlsson *et al*., 2009). It has already been established that IGF-I is predominately produced in the liver and release into the circulation (Hu *et al*., 2006; Ponce *et al*., 2008; Escobar *et al*., 2011; Zhang *et al*., 2013). Investigation illustrated that Chinese alligator IGF-I (caI GF-I) have a supreme role in the regulation of growth and development during the active and hibernating periods. Recombinant caI GF-I might be tested to increase the captive growth rate of the Chinese alligator (Xue *et al*., 2017). An investigation conducted during early postnatal growth in *Alligator sinensis*, proposed that Nerve growth factor (NGF) expression is involved in several structures of the partial central nervous system (Zheng *et al*., 2013). Zhang *et al*. (2015) advocated that B-cell activating factor (BAFF) has involved in survival and proliferation of B cells of *Alligator sinensis*. Further, the phylogenetic investigation revealed that *Alligator sinensis* BAFF gene is a sister group of birds and reptiles that indicate that they have evolved from a common ancestor.

Genetic resolution

Genetic status of captive populations and the remaining wild individuals has not been well described due to scarcity in the information of genetic markers, impeding the efforts of organizing a well-structured reintroduction strategy. Microsatellite markers could be advantageous in selecting of proper individuals for reintroduction plan, might advocate some important conservation queries. Xu *et al*. (2006) found 8 polymorphic loci in Chinese alligator with 26 alleles among 32 animals yielding an average of 3.25 alleles per polymorphic locus. He has illustrated that joint polymorphic loci could be sensitive markers in genetic diversity investigation and analogous inference within the Chinese alligator populations. Microsatellites as a class of highly variable genetic markers are widely distributed in eukaryotic genomes and have been used in numerous crocodilian population studies. Ten more polymorphic microsatellite loci were isolated from the Chinese alligator (*Alligator sinensis*). These unique molecular markers were suitable for population genetic studies and kinship investigation within *A. sinensis* population (Zhu *et al*., 2009). Furthermore, these ten microsatellite loci were tested for the selection of Individuals with maximum genetic differences from captive-bred Chinese alligators. It is very crucial to use an appropriate molecular marker to inspect the genetic diversity before reintroduction of an unrelated alligator into a particular wild area (Zhu *et al*., 2010). An important part of the Chinese alligator conservation project is the preservation of organs, tissues, sperm, oocytes, embryos and genomic DNA libraries. Furthermore, somatic cells have become an attractive resource for conserving rare and endangered species genetic materials with the flourish of Cytogenetic methods and induced pluripotent stem cell techniques. Researcher established three cell lines from liver, heart and muscle tissues of the Chinese alligator and preserved at –196°C, this contribution is a vital asset for the protection of this rare and critically endangered species (Zeng *et al*., 2011).

WETLAND STABILITY

There is a persistent need to solve many problems related to wetland conservation and biodiversity conservation in China (Wang *et al*., 2017). The first priority concern with the severe decrease in the area of wetlands. The estimated area was 660,000 km^2 in 2005 (Liu and Diamond, 2005) but in 2009 it has reported that it decreased to 359,478 km^2 (Niu *et al*., 2009), causing a sharp habitat destruction for numerous species. For instance, the Yangzi reserve and Freshwater National Nature Reserve play a principal role in the protection of Chinese alligator. Most of the central and southern China reserves with the maximum protection value were located in in the middle and lower reaches of the Yangtze River. The wetland ecosystems in these reserves are in the form of lakes, rivers, and marshes and representative for the diversity of threatened and endemic species. In southern Anhui Province, alligators are limited to a small number of agricultural ponds within a five-county region (Wan *et al*., 1998; Thorbjarnarson and Wang, 1999). Breeding centers, zoos in China and North America carry a reasonably large number of captive population (Behler, 1995; Wan *et al*., 1998). Various organizations involved in the restoration of wetlands or investigation of existing areas with a suitable living condition where captive-reared alligators can be released (Wan *et al*., 1998; Thorbjarnarson and Wang, 1999). In 2003, The UNDP-GEF Support the Effectiveness of the Protected Area System in Anhui Province, this project assist in Anhui Wetland project to restore habitat for the Yangtze alligators and other species. All these efforts contribute to management effectiveness of the Wetland Protected Area (WPA) systems in Anhui and emerging threats to the globally significant biodiversity and essential ecosystem. Wang *et al*. (2011) suggested that southern part of the pond might play a crucial role by creating buffer areas between farmland and the edge of the pond. The buffer areas should be covered with vegetation to ensure that the alligators bask out of the water and can conceal themselves from farming activities.

REINTRODUCTION OF CHINESE ALLIGATOR

International Workshop on Conservation and Reintroduction of Chinese Alligator was held in 2001 (SFA, 2002). The Anhui Yangtze Alligator NNR initiate its first step toward the captive-release program in 2002. In 2003, one of the protected sites Hong Xing Reservoir welcomed three captive-reared alligators. Another reintroduction site, Gaojingmiao Forest Farm (GFF), was evaluated as a reintroduction sit. The SFA has approved a Construction plan for Chinese alligators release in Zhejiang Province in October 2006 and introduced with 6, 6 and 9 alligators in 2006, 2007 and 2008 respectively. In 2008, one *A. sinensis* nest constituting 17 out of 19 fertile eggs were discovered at GFF by ANNRCA staff (Jiang, 2008). Further Chinese alligators from U.S. zoos have introduced to breeding centers in China to treasured genetic diversity in captive populations. Later in 2015, 6 more alligators from Anhui Chinese Alligator National Nature Reserve were transferred into Dongtan Wetland Park (www.sciencedaily.com/releases/2016/10/161026133227.htm). The second batch of 12 captive-bred Chinese alligators from Anhui National Nature Reserve for Chinese Alligators was released into the wild in Xuancheng (SFA, 2015). So far, the NNR has released approximately 100 alligators into the wild. All of these efforts indicated that captive-reared *A. sinensis* well adapted to restored habitats and retained breeding capacity in the wild. These statements signal a huge success for the species and for ongoing reintroduction efforts initiated by the different organization of china.

Nesting ecology

Previous studies about nesting ecology of *A. Sinensis* illustrated that nesting and egg-laying time were affected by the weather condition and moisture in the area (Jiang and Xia, 2005). Distance to water and thickness of vegetation canopy also affected the selection of nesting sites (Zhang *et al*., 2006). Sites with 63% to 88% vegetation coverage have observed more suitable for *A. sinensis* nesting (Zhou, 2007). The study has conducted in reintroduction locality for *A. sinensis* similar to natural habitat. Vegetation coverage was measured as a most important variable affecting the nesting of the Chinese alligator (Zhang *et al*., 2003; Jiang and Xia, 2005). Previously sunlight duration was not measured; however, sunlight duration has the greatest scores in PC1. Consequently, it has also considered as a principal variable affecting the nest-site choice in a natural habitat because the sunshine is a source for nest temperature (Magnusson and Lima, 1985; Chen and Li, 1979). Too high nest temperature lead to hatching failure and too little sunlight resulted in low temperature for successful incubation. Therefore, Chinese alligators preferred habitat with the correct level of sunlight duration for nesting. Additionally, nest temperature in a certain range is also proportional to incubation time (Zhou and Wang, 2000). Nesting ecology is an important judgment for the Chinese alligator reintroduction. It has estimated that a reintroduction area with sunlight duration of 5 h and nearest bank slope of 46° was more suitable for the Chinese alligators nesting (Jianjun *et al*., 2011).

Witnessing animal health

Before releasing captive animals, it is very important to examine the health of captive breed animals (Plowright, 1988; May, 1991; Kock and Woodford, 1991; Mills, 1999). Previously, bacteriological methods and molecular biology methods have used to identify bacterial genera revealed that captive alligator was a source of introducing pathogenic bacteria into the wild environment and could be endangering the native wild populations. It was strongly advised that the bacteria from the cloaca of Chinese alligators should be checked before release and reintroduction into the wild in order to avoid potential ecological harm (Ma *et al*., 2008). Consequently, only healthy captive-bred alligators were selected and release into the wild to renew the natural populations. Additionally, PHCs are endocrine-disrupting compounds, which can be maternally transferred to developing alligator eggs, is a major source for reduced hatchling success, promote embryonic mortality (Guillette *et al*., 2000; Rauschenberger *et al*., 2004a; b; 2007; Stoker *et al*., 2011). Evidence has provided for PHC presence in captive Chinese alligators and has investigated that these chemicals can be maternally transferred to the eggs. Thus, it is important to monitor the concentrations of PHCs in Chinese alligators before reintroduction into the wild. This investigation cooperates in alligator conservation plan because reptile species in the early stages of life are very sensitive to these chemicals. He found that DDTs were the most dominant among the investigated PHCs in Chinese alligators with mean contributions of >90%. Eggs examination of captive Chinese alligators revealed exceeded levels of DDTs and PCBs l caused impair reptile reproduction (Ting *et al*., 2014).

ENVIRONMENTAL POLLUTION INVESTIGATION

China is facing increasing problems with a variety of environmental contaminants including PFASs (Bao *et al*., 2010). Captive animals near urban industrialized areas have experienced contaminants, adversely affecting their health. Currently assessed PFASs exposure in Chinese alligators from a conservation center situated in an urbanized region of China. Further, gender and age-specific PFASs accumulation were investigated and discovered the sources of PFAS contamination. PFAS pollution is common in eastern China and prevalent the lower reaches of the Yangtze River (So *et al*., 2007; Yeung *et al*., 2008). Exposure to these chemicals has been investigated by

Serum examination of Chinese alligators, which revealed various levels of PFASs. It was also observed that level of PFTeDA, PFDoDA, PFUnDA, PFDA, and PFNA decreased with the increase in age (Jianshe *et al*., 2013).

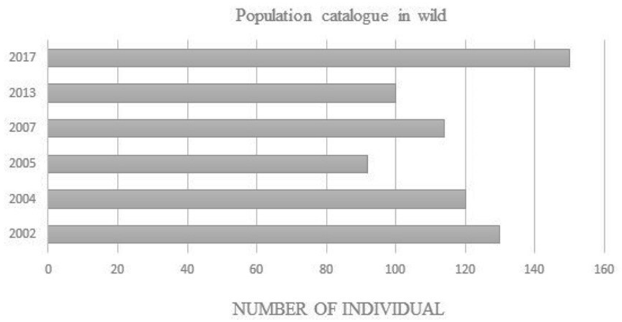

Fig. 1. Estimated number of wild Chinese alligators after captive release program.

POPULATION MONITORING AND CATALOG

Breeding centers have been proven successful in the management of captive Chinese alligators and the carried more than 10,000 individuals at the Anhui Research Center for Chinese Alligator Reproduction (ARCCAR) (Thorbjarnarson, 2002; Chen *et al*., 2003). Even though successful achievement in a captive population of the ANNRCA, the wild population has continued to decline with the estimation of <25% of the 1980s. Recorded data in 2002 indicated that total wild population had declined from approximately 1000 alligators in the late 1970s to ≥130 (Thorbjarnarson, 2002). Further observations in 2004 revealed a continuous decline in wild population with the total number of fewer than 120 individuals in China (Ding *et al*., 2004). In 2005, investigations indicated a slight drop in an approximate number of 92-114 wild individuals (Jiang *et al*., 2006; Wu *et al*., 2008), and most sites were found with the stable wild population. Further monitoring indicated successful breeding in 2004 and 2005 results in increasing population in Hong Xing, Zhucun, Zhuangtou and Heyi (Jiang *et al*., 2006). By 2007, entire stocks exceeded 630 alligators, with 100-200 hatchlings produced annually (Wu *et al*., 2008). To find out their activity area and movement pattern different methods like Radio telemetry, direct observation by binoculars in daytime and spotlight night counting in nighttime has used. Observation illustrated that activity varied per individual but the male has a relative larger activity area than females. It has observed that sometimes, captive release alligators showed conflict with native ones and prefer bank edge with vegetation coverage to live (Ding *et al*., 2003). It has documented in 2013 that there were ~100 wild Chinese alligators and ~10,000 captive individuals in Anhui and Zhejiang Provinces (Wan *et al*., 2013).

Wildlife Conservation Society disclosed that eggs of Chinese alligators spotted recently in a nest of Shanghai Wetland Park have hatched and that baby alligators have been detected (Scott, 2016). Currently, It has estimated that there are more than 13,000 alligators in captive and approximately 130 to 150 individual in wild (Fig. 1) (Xinhua, 2017).

IMPEDE ILLEGAL HUNTING AND COMMERCE FOR CHINESE ALLIGATORS

As one of the highly endangered crocodilians in the world, Chinese alligators have been secured with the maximum effort of Chinese and international law over the past years (Wan *et al*., 1998). The captive-bred population in Anhui Research Center for Chinese Alligator Reproduction (ARCCAR) has successfully raised over 13,000 alligators under the breeding program (Xinhua, 2017). By 1992 export of second filial (F2) generation of alligators have been legalized by CITES, further commercial development and application of Chinese alligators have allowed in China. Previously, permitted commercial meat from farmed Chinese alligators have appeared in the market (Wu and Chen, 1999). However, State Forestry Administration of China (SFA) only authorized four restaurants in whole china to trade meat of Chinese alligators. Identification by morphological characteristics does not work because of Resemblances between the Chinese alligator meat and other animal meat. Molecular technology by polymerase chain reaction (PCR) provides a power tool to identify Chinese alligator meat, as well as prevent illegal trade of Chinese alligators and the hunting of some of this critical endangered wild animal (McVeigh *et al*., 1991; Meyer *et al*.,1995; Koh *et al*., 1998; Sanjuan and Comesana, 2002; Abdulmawjood and Buelte, 2002; Chang *et al*., 2003; Rodriguez *et al*., 2003). Additionally, single one PCR step with species-specific PCR primers has been used to identify the Chinese alligator meat by the multiplex PCR. This method has proved very powerful in identification of meat and other goods from Chinese alligators (Rodriguez *et al*., 2003).

CONCLUSIONS

Despite the world, most endangered species, researcher have gained successful achievements in managing this precious species. Pollution control should be the first priority followed the habitat reestablishment and reintroduction of captive bred individuals. Although great success has been acquired in captive and wild alligator population since there is a need to pay more attention toward the protection and management of wild populations, as well as release projects. Captive-bred centers are struggling hard in order to achieve genetic diversity by releasing genetically unrelated individual into wild. Moreover, as it is well known that researchers can control sex development of Chinese alligator in controlled conditions but still there is a need to develop more tactics to control sex development in wild. The goals of Conservation and Reintroduction of Chinese Alligator project, Anhui Wetland project and Preservation Project for Chinese alligator 'organs, tissues, sperm, oocytes, embryos and genomic DNA libraries are huge hope for Chinese alligator survival on this planet.

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Statement of conflict of interest

Authors declare that there is no conflict of interest.

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