The sex ratio of wild Chinese alligators *Alligator sinensis*

Lan ZHAO¹, Hai-Qiong YANG¹, Li-Ming FANG², Guo-Liang PAN², Wei-Qiang ZOU², Da-Bin REN², Qiu-Hong WAN¹*, Sheng-Guo FANG¹*

¹The Key Laboratory of Conservation Biology for Endangered Wildlife of the Ministry of Education, State Conservation Center for Gene Resources of Endangered Wildlife, College of Life Sciences, Zhejiang University, Hangzhou 310058, China
²Changxing Yinjiabian Chinese Alligator Nature Reserve, Changxing 313100, Zhejiang, China

**Abstract** The Chinese alligator *Alligator sinensis* is one of the most endangered crocodilian species, and typically exhibits temperature-dependent sex determination. It is extremely important to clarify the sex structure of Chinese alligators to implement recovery projects successfully. However, the sex ratio of wild Chinese alligators remains unknown. In this study, we collected 28 years of sex ratio data from Chinese alligators residing in the natural and artificial habitats of Changxing Nature Reserve, China, and examined the differences in the sex ratio dynamics between these two populations. We observed that the sex ratio of wild Chinese alligators is 1 male to 4.507 females, which was significantly lower compared to that of the captive population (1 to 2.040; \( P < 0.001 \)), and is significantly different to previously documented sex ratios for this species (all \( P < 0.01 \)). Furthermore, we documented an annually stable (\( P = 1.000 \)) female-biased sex ratio for wild alligators at hatching [1 male to 4.507 females; 0.174 (0.167–0.182)], in contrast to a dramatically fluctuating sex ratio (\( P < 0.001 \)) in captivity [1 male to 1.674 females; 0.374 (0.246–0.593)], showing a potential mechanism for adjusting the sex structure. Finally, we found that the hatching sex ratios were similar to that of the population sex ratio (\( P = 0.748 \)), with little correlation to air temperature values in the 60–70 day incubation period during the breeding season (July and August; both \( P > 0.05 \)). Overall, this study indicates that the stabilized female-biased sex ratio of Changxing Chinese alligators might result from selection pressure caused by local mate competition and major inbreeding [*Current Zoology* 59 (6): 725–731, 2013].

**Keywords** Reptile, Female-biased, Temperature-dependent sex determination, Conservation

The Chinese alligator *Alligator sinensis* is a critically endangered species that is endemic to China, and is currently categorized as Accessory I in the Convention on International Trade in Endangered Species (http://www.cites.org/eng/app/appendices.php, last accessed in August 2013). Because this species is on the verge of extinction in the wild, the International Union for the Conservation of Nature recognized the wild Chinese alligator as being the most endangered of all 23 crocodilian species (http://www.iucnredlist.org/details/867/0, last accessed in August 2013). Historically, this species was distributed throughout the extensive wetland habitats of the lower Yangtze River (Chen, 1990; Thorbjarnarson et al., 2002). However, habitat loss and high levels of illegal hunting led to a sharp decrease in the size of the wild population (Thorbjarnarson et al., 2002). A recent field investigation indicated that the total number of wild Chinese alligators is probably fewer than 130 individuals, and that it is declining at an annual rate of 4%–6%. This serious population bottleneck was recently verified by scanning genome-wide single nucleotide polymorphisms (Wan et al., 2013). Hence, protecting this species from extinction, in parallel to recovering population numbers, remains a difficult, yet vital, task.

The sex ratio is an important component of mate competition and mate choice in all species, in addition to influencing population survival and development (Emlen and Oring, 1977; Dyson and Hurst, 2004). Excessive bias towards one sex in a population might even lead to the extinction of a species, particularly species that have small isolated populations. Therefore, it is important to maintain the appropriate sex ratio for wild Chinese alligator populations to facilitate population recovery. Nevertheless, detailed reports or consensuses about the optimal sex ratio, or wild population sex ratio, of Chinese alligators remain sparse. Chen et al. (1985) first reported the sex ratio of the Chinese alligator. The authors examined the wild population, and determined a male-to-female sex ratio of 1:5 (Chen et al., 1985, 2003). This sex ratio is not dissimilar to that of the American alligator *Alligator mississippiensis*, a species from the
same genus *Alligator*, for which wild sex ratio data were first reported in 1982 (Ferguson and Joanen, 1982). However, subsequent researchers found it difficult to count wild population numbers, or to distinguish the sex of wild alligators, because of the similar appearance between males and females (Li et al., 1996; Ding et al., 2001; Thorbjarnarson et al., 2002; Ding and Wang, 2004; Wu and Wang, 2004; Zhu et al., 2008). Even so, numerous studies, including a population viability analysis of wild Chinese alligators, have continued to use the 1:5 sex ratio (Ding and Wang, 2004; Wu, 2004; Wu and Wang, 2004). In another study, Zhu (1997) observed a wild population, and reported that the male-to-female sex ratios from 1992–1995 were 5:4, 4:3, 3:2, and 1:3, respectively, which contradicted the findings of Chen et al. (1985). However, the population Zhu (1997) sampled was isolated and fragmented, with a maximum population size of 13 individuals. Therefore, the sex ratios of Zhu (1997) might not be representative of the entire wild population sex ratio. More recently, Zhang et al. (2005) reported a male-to-female sex ratio of 1:4 in the Chinese alligator population in the firstly initiated breeding area of the Anhui Research Center of Chinese Alligator Reproduction (Anhui Center). Moreover, Wang et al. (2006) reasoned that the 1:4 ratio was close to the wild sex ratio, and used the report of Zhang et al. (2005) as a baseline to study the effect of aggregation formation during the Chinese alligator mating season. However, captive populations differ to wild populations in many respects, such as the age of sexual maturity and the mating system under manual intervention. Therefore, the sexual structure of captive populations cannot accurately represent the situation in the wild population.

Because of the broad variation in reported sex ratios for Chinese alligators, there is a clear requirement to examine the appropriate sex ratio of wild populations. Such information would contribute towards enhancing the reproductive effort of the Chinese alligator, and hence the survival of its populations, and the species as a whole. Here, we collected sex ratio data of Chinese alligators from the natural habitat of Changxing Yinjiabian Chinese Alligator Nature Reserve (Changxing Reserve) in China to serve as a reference for population recovery programs.

1 Materials and Methods

1.1 Study sites

The Changxing Reserve (30°93’ N, 119°73’ E) is located in the county of Changxing, Zhejiang Province, China. This nature reserve is an extension of the pristine habitat of the Chinese alligator. We named this natural habitat “Area C,” which covers an area of 5401.57 m², and contains a natural wetland. In the 1950s, Area C was the core distribution area of the Chinese alligator. From April 8, 1979, as alligator populations began to decline severely, Changxing Reserve focused on establishing and protecting Area C from habitat loss and illegal hunting. Because of these protective efforts, the original habitat structure of Area C has been retained (Fig. 1).

1.2 Data collection

The incubation period of the Chinese alligator lasts 60–70 days, spanning July-August (Chen et al., 2003). To determine the sex ratio of the Chinese alligator in the wild, the eggs were collected every year since 1984 from all alligator nests laid within Area C, at about 1 week before they were due to hatch. The sex of the Chinese alligator is determined by the temperature during egg incubation, low temperatures produce females, while high temperatures produce males; the proportion of males increases as the temperature rises from 27°C to 36°C (Chen et al., 2003). The temperature-sensitive period (TSP) is undefined in the Chinese alligator; however, the TSP of American alligator has been reported to occur between 30 and 45 days of incubation.

![Fig. 1 The natural habitat of the Chinese alligator (Area C)](image-url)
(i.e., the middle third of incubation) for intermediate temperatures (Lang and Andrews, 1994). Hua et al. (2004) compared different stages of embryonic development between Chinese and American alligators, and discovered that, at 30–45 days of incubation, the embryos of the American alligator incubated at 30°C corresponded to 30–39 days incubation of Chinese alligator embryos incubated at 29°C. This finding indicates that species in the genus *Alligator* exhibit similar TSP patterns. Therefore, all eggs collected in this study had, theoretically, been subjected to this hypothesized TSP stage in the wild, at which sex is determined before natural hatching. The eggs were incubated in artificial hatchery rooms for the last week of incubation, and were then raised until a certain age to allow gender identification by external observation (see Sexual identification section). After gender determination, the hatchlings were released back into the natural habitat.

The founders, 3 males and 8 females, began to reproduce and produce offspring in 1984, with reproduction occurring in 14 discontinuous years from 1984 to 2011. From 1984 to 2011, a total of 2,539 eggs were collected from natural Area C, of which 1,287 eggs were incubated until hatching. A total of 951 individuals survived until sexing (at 5–6 years old or at 2–3 months old), of which 414 (5–6 year-old) alligators were sexed using standard methods before 1997; hence, juveniles had to be raised until 5 or 6 years of age for gender determination, and were then released after sexing. However, after 1997, we have been able to identify the sex of juveniles by examining the genitalia in the cloaca at 2–3 months after hatching (Fig. 2). The male clitero-penis is typically red, round, and large in comparison to the female organ, which is white, sharper, and smaller (Webb et al., 1984; Allsteadt and Lang, 1995). Thereafter, we consistently recorded gender information for 3-month-old hatchlings only.

1.3  Sexual identification

Alligators have few external or secondary sexual characteristics; therefore, sex was determined by the examination of the cloaca, as described by Chabreck (1963). The digitus V of one hand is inserted into the cloaca, and is then moved interiorly through the cavity. If the alligator is a male, the penis is felt by moving the finger from side to side along the ventral floor of the cloaca (Chabreck, 1963). This method was used to sex subadults and adults before 1997; hence, juveniles had to be raised until 5 or 6 years of age for gender determination, and were then released after sexing. However, after 1997, we have been able to identify the sex of juveniles by examining the genitalia in the cloaca at 2–3 months after hatching (Fig. 2). The male clitero-penis is typically red, round, and large in comparison to the female organ, which is white, sharper, and smaller (Webb et al., 1984; Allsteadt and Lang, 1995). Thereafter, we consistently recorded gender information for 3-month-old hatchlings only.

1.4  Statistical analyses

We analyzed the sex ratio data using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and Origin 8.0 (OriginLab, Northampton, MA, USA). The sex ratio data for this species are binomially distributed (Hardy, 2002); therefore, we used quartiles for non-normally distributed data, median [the first (Q1) – third (Q3) quartiles], to describe variation in our sex ratio data. The quartiles are usually divided into Q0, Q1, Q2, Q3, and Q4, which represent the minimum, 25th percentile, median, 75th percentile, and maximum, respectively. Then, we employed the likelihood ratio chi-squared test to determine whether stable population and hatching sex ratios are maintained in Changxing Chinese alligator.
tors. Finally, we conducted Pearson's chi-squared test to determine whether there are any significant differences in the sex ratio between this work and previous studies, between natural and artificial habitats, and between hatchling and overall population sets.

2 Results

We compared the sex ratio data before 1997 and after 1997, and found no significant difference ($\chi^2 = 0.015, df = 1, P = 0.932$), indicating that the different sexing methods had no effect on sex ratio data. Fig. 3 shows the sex ratio dynamic of the Changxing natural population from 1984 to 2011. The initial sex ratio was 0.273 (i.e., 1:2.667 male-to-female sex ratio), which was manually controlled. As the population size grew, the proportion of males in the population changed from 0.273 to 0.171 (i.e., 1:4.507 male-to-female sex ratio) during 1984 to 1986. From 1986 to 2009, the proportion of males was almost consistently maintained at 0.183 (i.e., 1:4.507 male-to-female sex ratio; Fig. 3). Because of an increase in the population density, the reserve managers removed 20 males and 60 females from the natural habitat to artificial habitats in May 2010; hence, the proportion of wild males declined to 0.146 (i.e., 1:5.846 male-to-female sex ratio), obviously deviating from the 0.183 sex ratio (Fig. 3). However, the sex ratio of newborn wild juveniles in 2010 was 0.171 (i.e., 1:4.507 male-to-female sex ratio); thus, the population sex ratio shifted to 0.156 (i.e., 1:4.507 male-to-female sex ratio), showing a tendency to recover towards the standard 0.183 level. In the natural habitat, the proportions of males in the both overall population [0.183 (0.173–0.189)] and at hatching [0.174 (0.167–0.182)] were clearly stable (Fig. 4). In contrast, these proportions were quite varied in the artificial habitats (Fig. 4), showing large ranges for the overall population and at hatching, i.e., 0.329 (0.256–0.390) and 0.374 (0.246–0.593), respectively. We found that the hatchling and overall population sex ratios of wild Chinese alligators were both statistically stable among years (overall population: $\chi^2 = 5.160, df = 27, P = 1.000$; at hatching: $\chi^2 = 1.165, df = 13, P = 1.000$). In comparison, we found that the hatchling and overall population sex ratios of artificial breeding individuals were significantly
different (overall population: $\chi^2 = 596.687$, df = 58, $P = 0.001$; at hatching: $\chi^2 = 335.864$, df = 27, $P = 0.000$).

The overall population (1:4.507) and hatching (1:4.747) sex ratios of wild Chinese alligators were both significantly biased compared to the expected 1:1 male-to-female sex ratio (overall population: $\chi^2 = 2579.0$, df = 1, $P < 0.001$; at hatching: $\chi^2 = 400.3$, df = 1, $P < 0.001$), suggesting that the Changxing natural population exhibits an extremely female-biased sex structure. We further found that the 1:4.507 ratio significantly deviates from the 1:5 male-to-female sex ratio reported by Chen et al. (1985) ($\chi^2 = 6.946$, df = 1, $P = 0.009$), and also from the 1:4 of Anhui alligators (Zhang et al. 2005) ($\chi^2 = 13.141$, df = 1, $P < 0.001$). In addition, the 1:4.507 sex ratio was highly divergent to the 5:4, 4:3, 3:2, and 1:3 ratios that were observed by Zhu (1997) in a small group of 13 alligators.

We found a significantly lower sex ratio in natural populations compared to the artificial habitats at both the overall population ($\chi^2 = 274.027$, df = 1, $P = 0.000$) and at hatching ($\chi^2 = 67.166$, df = 1, $P < 0.001$) level. Meanwhile, we detected no significant difference between the overall population and hatching sex ratios in natural ($\chi^2 = 0.112$, df = 1, $P = 0.748$) or artificial ($\chi^2 = 3.387$, df = 1, $P = 0.066$) habitats, indicating that the hatching sex ratio is an important factor that stabilizes the sex structure of the Chinese alligator population. Thus, we examined the correlation between the proportion of males at hatching and the mean ambient temperature in the incubation period (July–August). We found that air temperature minimally influenced alligator sex ratio (Fig. 5; Spearman correlation test, $P > 0.05$). Consequently, this extremely steady hatching sex ratio may indicate that wild Chinese alligators select suitable nest sites in certain habitats to adjust and optimize the population sex structure.

3 Discussion

Data from mammals show that natural selection favors the ability of parents to vary offspring sex ratio (Trivers and Willard, 1973). A study on the diamondback terrapin demonstrated that females have the ability to identify environments that have a higher probability of producing the sex that would generate the most maternal benefit (Roosenburg, 1996). Our data indicate that the Chinese alligator might also possess this ability. Regardless of significant annual variations in ambient temperature during egg incubation (July: 26–31°C, August: 25.8–30°C) from 1984 to 2010, the proportion of males at hatching [0.174 (0.167–0.182)] remained consistently stable in the natural population ($P = 1.000$), which was in direct contrast to the highly fluctuating sex ratios of captive hatchlings ($P < 0.001$) (Fig. 3 and 4). In addition, we found little evidence of air temperature during the reproduction season influencing alligator sex ratio (Fig. 5; $P > 0.05$).

Another study suggested that nest-site selection has a substantive maternal effect on offspring survival and the sex of the snapping turtle (Kolbe and Janzen, 2002). Animals might select nest sites with appropriate canopy density, flat inclines close to headwaters, among other factors. For instance, canopy density has been observed to serve as a major factor in the sites selected by Chinese alligators for nesting (Zhang et al., 2006). Therefore, in the current study, the stable sex ratios recorded over the last 28 years indicate that the nests were selected by adult females according to different thermal properties. This hypothesis supports existing information stating that female alligators seek out appropriate nest sites for 1–2 weeks after mating. Since the Chinese alligator is a temperature-dependent sex-determining animal, with air temperature having a minimal influence on sex ratio, nest site selection behavior might explain

Fig. 5  Proportion of males at hatching in relation to the mean air temperature in July (A) and August (B) of each year
the stabilized sex structure of this reptile. Moreover, Chinese alligators inhabiting completely artificial habitats might only have access to suboptimal (less suitable) nest-site incubation conditions, resulting in the observed discrepancy in the hatchling sex ratio to that of wild Chinese alligators (standard, 1:4.747).

In sexual species, random mating and reproduction should theoretically produce a 1:1 sex ratio (Fisher, 1930). This 1:1 theory primarily works under ideal conditions. In a structured population, low reproductive activity results in availability of limited mates, which would induce a female-biased sex ratio to reduce local mate (inter-brother) competition (Hamilton, 1967). In an animal with male-biased dispersal, the mothers would preferentially produce more sons than daughters (male-biased sex ratio) to avoid local resource competition (Charnov, 1982). In fact, a sexual breeding system is quite complicated, because it involves breeding form, chromosome inheritance, mate choice, inbreeding avoidance, parental investment, group profit, and so on (Trivers, 1972). In brief, a selection balance between minimized costs and maximized returns contributes to an optimized (female or male-skewed) sex ratio in a population. In this study, we observed an annually stable 1:4.507 male-to-female sex ratio in the Chinese alligator, which is significantly biased compared to the expected 1:1 ratio ($P < 0.001$). This finding indicates that a female-biased sex ratio has selective advantages in this reptile species. Adult wild alligators are solitary and inhabit small areas (usually within a range of decades of meters from their caves), with low activity ability (the legs are not strong enough for them to promote long-distance dispersal); consequently, individuals tend to only interact with their neighbors (Chen et al., 2003). The resulting population substructure should theoretically lead to a bias towards producing females (Hamilton, 1967), to minimize a waste of reproductive resources and maximize the fertilization success rate of the whole mating population. This phenomenon might explain why both Chinese and American alligators exhibit a female-biased sex ratio (Chen et al., 2003; Ferguson and Joanen, 1982, 1983).

The Chinese alligator is an endangered species that has been subject to population bottlenecks for several decades. Major inbreeding within populations has been verified by genome-wide single nucleotide polymorphism (Wan et al., 2013). Therefore, inbreeding depression is a major problem for population recovery. Under inbreeding pressure, animals would produce more females than males to maximize the number of offspring (Hamilton, 1967; Maynard Smith, 1978), which could minimize the influence of low survival rates on overall population development. The female-biased sex ratio for regardless of mate-competition or inbreeding avoidance requires a polygynous mating system to achieve an efficient reproduction, with this phenomenon of 1 male to multiple females being confirmed for both American (Lance et al., 2009) and Anhui Chinese alligators (Chen et al., 2003). Examination of microsatellite-based paternity testing by our research group also revealed 1 father across multiple clutches of Changxing hatchlings (unpublished). Therefore, the steady female-biased sex ratio observed in the Chinese alligators might be driven by various selection pressures, particularly local mate competition and major inbreeding, to maximize reproductive success and population growth rates, respectively.

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