



## Original article

# Novel insights into the reproductive strategies of wild Chinese sturgeon (*Acipenser sinensis*) populations based on the kinship analysis

Dan Yu<sup>a,1</sup>, Xin Gao<sup>a,1</sup>, Zhongyuan Shen<sup>a,1</sup>, Masami Fujiwara<sup>b</sup>, Ping Yang<sup>a</sup>, Tao Chang<sup>a</sup>, Futie Zhang<sup>a</sup>, Xinghua Wu<sup>c</sup>, Zhonghua Duan<sup>a</sup>, Huanzhang Liu<sup>a,\*</sup>

<sup>a</sup> The Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China

<sup>b</sup> Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX, 77843-2258, USA

<sup>c</sup> Research Center for Yangtze River Ecological and Environmental Engineering, China Three Gorges Corporation, Beijing, 100038, China

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## ABSTRACT

Understanding the reproductive strategy of an organism is important in conservation ecology as it directly affects the population performance under changing environmental conditions. Chinese sturgeon (*Acipenser sinensis*) are the largest anadromous fish in the Yangtze River, China. Currently, the species has only one spawning ground and has failed to spawn in recent years, leading it to the brink of extinction. To develop effective conservation measures, a further understanding of its reproductive strategy is needed. In our study, we conducted kinship analyses by using mitochondrial and microsatellite DNA data from 216 wild juveniles collected over nine years (2006–2013, 2015) to understand the mating system, breeding interval, effective number of breeding adults, and reproductive success. The results from these analyses suggested polygynandry, with some parents contributing up to eight half-sibling juvenile genotypes. Although the spawning ground was restricted to a limited area, genetic diversity was maintained at a relatively high level (observed heterozygosity from 0.698 to 0.787 and expected heterozygosity from 0.763 to 0.787) and inbreeding coefficients in each year-class ranged from –1% to 9% (low to modest detrimental effects on offspring). A parental inference analysis revealed that Chinese sturgeon have a breeding interval of 2–6 years, indicating that it has the potential to feed, accumulate nutrition in the ocean, and then migrate back to the Yangtze River for iteroparous reproduction. The annual effective number of breeders in the Yangtze River ranged from 14 to 161 during the study period, and it decreased by 62.1% from the 2011–2014 year-classes. This sharp population decline likely contributes to the reproduction failure. However, the ratios of effective to census population size ( $N_e/N_c$ ) were all larger than 0.20 after the 2010 year-class, indicating relatively even reproductive success. Based on these results, a suggested approach to protect this species is to restock parent fish to increase the reproductive stock size and optimize the discharge of the Three Gorges Dam to reduce the unsuitable hydrological conditions and rehabilitate spawning ground habitats.

## 1. Introduction

Reproductive strategies, including mating systems, breeding intervals, and the ratio of effective to census population size ( $N_e/N_c$ ), affect the population growth rate of a species (Frankham, 1995; Avise et al., 2002). Therefore, understanding these strategies is important for comprehending the adaptive strategies of a species, explaining the reason behind its endangered status, and developing suitable conservation measures (Jakobsen et al., 2009). In particular, mating strategy affects

how species respond during and after periods of intense exploitation and habitat changes, and could be used to assess extinction risks and to choose conservation strategies (Rowe and Hutchings, 2003). Fish species often exhibit diverse mating systems, including self-fertilization, strict monogamy, polygyny, polyandry, and polygynandry, causing fish species to respond differently to various external conditions (Avise et al., 2002). Considerable efforts have been made to report variations in mating systems among diverse groups of fishes and to identify the factors leading to and maintaining the variations (Emlen and Oring, 1977; Ribolli et al.,

\* Corresponding author. No. 7 Donghu South Road, Wuchang District, Wuhan, 430072, China.

E-mail address: [hzliu@ihb.ac.cn](mailto:hzliu@ihb.ac.cn) (H. Liu).

<sup>1</sup> These authors contributed equally to this work.

2016; Franckowiak et al., 2017). However, it is often difficult to accurately identify animal mating behaviors in the absence of genetic data because of their complex behaviors and observability. For example, both males and females may take part in extrapair spawning (Wootton and Smith, 2014). Currently, the development of highly variable genetic markers and kinship analyses have contributed greatly to the study of mating systems in a wide range of species, revealing a higher degree of promiscuity than was inferred previously from behavioral observations alone (DeWoody and Avise, 2001; Gibbs and Weatherhead, 2001; Griffith et al., 2002).

Many animals are iteroparous capital-breeding species, which increases breeding probability throughout individual lifespans. But their breeding intervals, which are generally determined by the time required to recover from the cost incurred from previous breeding and to prepare for the next breeding by gaining resources, can be considerably variable (Church et al., 2007). Longer breeding intervals (>1 year) have primarily been linked with poor feeding conditions, resulting in insufficient energy accumulation (Rideout et al., 2005). For example, skipped spawning of Atlantic cod (*Gadus morhua*) was linked to poor environmental conditions (Rideout and Rose, 2006; Rideout et al., 2006; Skjæraasen et al., 2009). Similarly, a small proportion of white crappie (*Pomoxis annularis*) females that were starved or given an intermediate ration failed to produce vitellogenic oocytes, whereas females given a high ration successfully developed (Bunnell et al., 2007). Therefore, feeding conditions might be inferred from observed feeding intervals and the life history strategies of organisms. If an observed breeding interval is shorter than the typical period for the life history strategy, there should be sufficient energy accumulation. There is growing interest in studying the breeding intervals of birds (Verhulst et al., 1997), reptiles (Harris and Ludwig, 2004), and fishes (Rideout et al., 2005). To broaden the knowledge of animal reproductive strategies, more research regarding spawning patterns across taxa with various life history strategies, such as anadromous species, is needed.

For long-lived, iteroparous species, census population size ( $N_c$ ) is challenging to estimate in natural populations (Ferchaud et al., 2016; Ruzzante et al., 2016). Instead, many studies have focused on determining the effective population size ( $N_e$ ) and the effective number of breeding adults ( $N_b$ ).  $N_b$  represents an effective size for a single reproductive season, reflecting the parental contributions and changes in the inbreeding rate (Waples, 1990, 2002); it is thought to be a more accessible and valuable parameter for population monitoring and management than  $N_c$  or  $N_e$  (Waples, 2005; Luikart et al., 2010; Tallmon et al., 2010; Waples et al., 2018; Jones et al., 2019). Therefore,  $N_b$  estimated from genetic methods would be useful for tracking  $N_c$  if a correlation was observed between  $N_c$  and  $N_b$  (Duong et al., 2013; Ferchaud et al., 2016). In this case,  $N_b$  estimates provide a complementary metric or could be used in place of population size (Tallmon et al., 2010).

Reproductive success is affected by both variations in population size and diverse reproductive strategies and is closely related to variation in population size (Hedgecock and Pudovkin, 2011). For most populations, the ratio of  $N_e$  to  $N_c$  falls roughly in the range of 0.1–0.5. However, numerous estimates of “tiny”  $N_e/N_c$ , ranging from in the range  $10^{-3}$ – $10^{-6}$  have been reported in some high-fecundity marine species (Hauser and Carvalho, 2008; Hedgecock and Pudovkin, 2011). This could be an indication of sweepstakes reproductive success (SRS). The SRS hypothesis, owing to a sweepstakes-like chance of matching reproductive activity (Hedgecock, 1994), suggests that very low  $N_e/N_c$  ratios may be commonplace for these species (Turner et al., 2002; Hedrick, 2005; Waples, 2016). A species exhibiting SRS is expected to have a fraction of the genetic diversity that would be expected from their  $N_c$ , compared with non-SRS species. Sweepstakes reproductive success occurs because of large reproductive variance, meaning that few breeders “win” the reproductive race, while many more “lose”, resulting in low genetic diversity in natural populations. However, recent studies reported higher than expected  $N_e/N_c$  ratios in some marine fishes, including 0.5 with southern bluefin tuna (*Thunnus maccoyii*, Waples et al.,

2018) and 0.33 with New Zealand snapper (*Chrysophrys auratus*, Jones et al., 2019). These results suggested an absence of SRS despite exhibiting high fecundity and high early mortality. Using simulations, Waples (2016) also highlighted that extreme scenarios were required to reduce  $N_e/N_c$  below approximately 0.01.

It is broadly accepted that habitat loss can reduce population size and genetic diversity and thereby increase the inbreeding rate and extinction risk (Carvalho et al., 2019). In general, reductions in habitat area may increase mating among close relatives, which decreases the levels of genetic diversity and lead to reduced fitness (Charlesworth and Willis, 2009; Lange et al., 2013). However, Templeton et al. (1990) suggested that, despite the negative effects, genetic variation is not completely lost. In some cases, habitat fragmentation or loss can have neutral or even positive effects (Fahrig, 2003). For example, Carvalho et al. (2019) revealed that habitat loss driven by mining did not affect genetic diversity in two endemic herbs from Amazonian savannas. Therefore, understanding the genetic consequences of habitat loss is essential to the conservation of biological diversity.

Chinese sturgeon (*Acipenser sinensis* Gray 1835) are the largest anadromous migratory fish in the Yangtze River, and it was categorized as critically endangered in the International Union for Conservation of Nature (IUCN) Red List in 2010 (Gao et al., 2016). Chinese sturgeon are an iteroparous species with overlapping generations and a complex life history. During summer, mature males (8–26 years old) and females (13–34 years old) in reproductive condition (gonadal stage III) migrate from the mouth of the river (Shanghai) to the upper reaches (Yibin, Luzhou and Hejiang, etc) of the Yangtze River (The Changjiang Aquatic Resources Survey Group Sichuan Province, 1988). They normally stay over one year in the river and spawn during the next autumn (Gao et al., 2014). Therefore, every year, two cohorts of adults, the “new” and “old” cohorts, coexist in the Yangtze River. After breeding, the “old” cohort migrates quickly to the sea, and the “new” cohort remains in the Yangtze River for breeding in the following year. After spawning, adults return to the sea and the newly hatched larvae disperse along the river for refuge and feeding (Zhuang et al., 2002). This dispersal process lasts approximately 7–9 months in the river, and then they reach the estuary in April–August of the following year (Li et al., 2011). This reproductive strategy presents challenges in disentangling the mating systems through direct behavioral observations. Additionally, the construction of the Gezhouba Dam (GD) blocked their access to the upper spawning ground since 1981, and harvest pressures on the adults immediately after the dam construction led to a sharp decline in the reproductive stock. The reproduction of the Chinese sturgeon was also limited to a narrow reach located 3 km downstream of the GD (Gao et al., 2009), further limiting the carrying capacity of the spawning ground. Furthermore, impoundment of the Three Gorges Reservoir in the 2000s affected water temperature and discharge. Those changes caused 30–40-day delays in spawning dates of Chinese sturgeon because of the “temperature hysteresis effect”, in which cooling temperatures during October–November were impeded (Gao et al., 2016). In 2013, 2015, and 2017–2021, the sturgeon failed to spawn below the GD. Wei (2020) speculated that seriously delayed breeding in recent years likely resulted from poor feeding conditions in the ocean, but without evidence. Currently, the reproductive stock size has dramatically decreased and is currently below the minimum viable population size (Gao et al., 2016; Wang et al., 2019). As the population size declines, it became difficult to directly estimate adult abundance because they are too rare to detect. Therefore, determining the reproductive strategies of Chinese sturgeon and estimating effective number of breeding adults in the Yangtze River are difficult but crucial for understanding its current biological status (Belmar-Lucero et al., 2012).

Our study objective was to investigate the reproductive strategies of the Chinese sturgeon, including its mating system, mate number, and breeding interval, based on annual juvenile genetic data (2006–2013, 2015) and kinship analysis. In addition, we estimated the annual inbreeding coefficient and variance of  $N_b$  and  $N_e/N_c$ . Then, the SRS

hypothesis of Chinese sturgeon was tested. The results from this study provide important genetic information regarding the reproductive strategies of this species, which may be used to help guide conservation programs.

## 2. Materials and methods

### 2.1. Sampling

The sampling sites were located at Zhenjiang, Nantong, Changxing, and Chongming Islands in the lower reaches of the Yangtze River; these locations cover the entire river transect below the spawning area (GD; Fig. 1; Supplementary Table S1). Between 2006–2015, juvenile Chinese sturgeon descending the river for the first seaward migration were caught using long-handled nets along the river. Juvenile sampling began approximately 5–8 months after the first observed spawning event and lasted for approximately 40–60 days. Thus, these samples revealed the reproductive information in the previous year instead of the sampling year (Supplementary Table S1). A small piece of caudal fin was collected and stored in 95% ethanol. Many studies have demonstrated that restocked Chinese sturgeon contributed very little (i.e. 1.71%–3.03% during 1996–1998; 0.00–0.45% during 1998–2003; 0.00–3.80% in 1999) to the recruits in the lower reaches of the Yangtze River (Chang and Cao, 1999; Wei, 2003; Zhao, 2006). Additionally, we excluded four individuals that might have full-sib or half-sib relationships with some released fish (unpublished data). In total, 216 Chinese sturgeon wild juveniles were used in this study.

### 2.2. Genotyping

DNA was isolated and extracted using the high salt protocol with slight modifications (Aljanabi and Martinez, 1997). All samples were genotyped at two mitochondrial DNA (mtDNA) genes, Cyt *b* and COI (Supplementary Table S2), and 20 nuclear microsatellite loci (Supplementary Table S3). Polymerase chain reaction (PCR) was conducted using a Veriti™ 96-well thermal cycler and a Mastercycler nexus gradient PCR (Eppendorf). Conditions for the cycling reactions were detailed below. PCR was performed in volumes of 30  $\mu$ L for Cyt *b* and COI amplification and 20  $\mu$ L for microsatellite loci amplification, and reaction mixtures contained 30–50 ng template DNA, 0.5  $\mu$ L dNTP mixture (2.5 mmol/L each), 0.3 U Taq DNA Polymerase with MgSO<sub>4</sub> (2 mmol/L of Mg<sup>2+</sup>), 2  $\mu$ L 10  $\times$  Taq Buffer, 0.5  $\mu$ L of each primer (10  $\mu$ mol/L), and

deionized H<sub>2</sub>O. The PCR procedure for Cyt *b* and COI was as follows: initial denaturation at 94 °C for 3 min; followed by 35 cycles at 94 °C for 30 s, annealing temperature for 40 s, and 72 °C for 1 min; and then one cycle at 72 °C for 10 min. The PCR products were sent to commercial sequencing companies (Shanghai Sangon Biotech Inc.) for purification and sequencing. The PCR procedure for microsatellite loci was as follows: initial denaturation at 94 °C for 4 min; followed by 30 cycles at 94 °C for 45 s, annealing temperature for 45 s, and 72 °C for 1 min; and then one cycle at 72 °C for 10 min. The PCR products were then separated by capillary electrophoresis using a 3730 DNA Analyzer (Applied Biosystems). Alleles were scored using GeneMapper 5 with GeneScan 500 LIZ size standards (Applied Biosystems). Fragment sizing was confirmed by manual proofreading.

### 2.3. Genetic diversity, kinship analysis, and individual relatedness

Multiple alignments of mtDNA sequences were performed using CLUSTAL X (Thompson et al., 1997) and subsequently checked by eye in SEAVIEW v5 (Galtier et al., 1996). The number of haplotypes (*H*), haplotype diversity (*H<sub>i</sub>*), and nucleotide diversity (*P<sub>i</sub>*) were estimated in DNASP v5.10 (Librado and Rozas, 2009).

All microsatellite data were checked with Micro-Checker (Van Oosterhout et al., 2004) to exclude any null alleles and large allelic dropout. In polyploid species, a phenotypic heterozygote may have several possible underlying genotypes that vary in the number of copies of one or more alleles. In tetraploids, for example, the 3-band phenotype ABC at a microsatellite locus can have three alternative genotypes ABBC, AABC, and ABCC. Sturgeons are typical polyploid species and most individuals of Chinese sturgeon amplified 4 alleles. Therefore, all datasets were processed using the tetrasomic inheritance model combing the number of copies of each allele after artificial correction. The total number of alleles (*A*), observed heterozygosity (*H<sub>o</sub>*), and unbiased expected heterozygosity (*H<sub>e</sub>*), as well as the inbreeding coefficient (*F<sub>IS</sub>*), were computed with AUTOTET (Thrall and Young, 2000). Polymorphic information content (*PIC*) was estimated using PICcalc (Nagy et al., 2012). Genepop 4.7 (Rousset, 2008) was employed to test the Hardy-Weinberg equilibrium using the following Markov chain parameters: 10,000 dememorization steps, 1000 batches and 10,000 iterations per batch.

For the standard methods for kinship analysis, polyploid codominant genotypes were transformed into pseudo-diploid-dominant genotypes according to Wang and Scribner (2014). Allele scores (base pairs) at a codominant locus were converted to dominant loci, where the presence

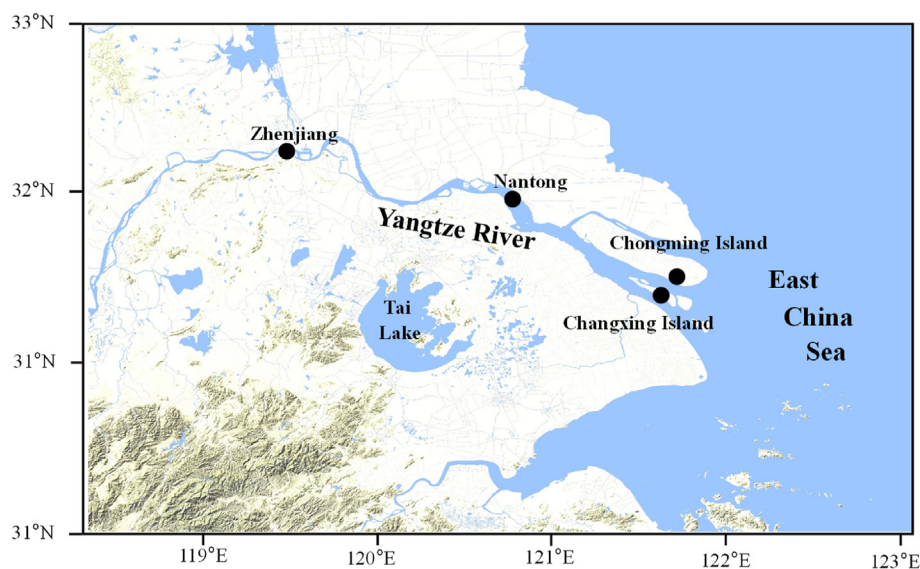


Fig. 1. The sampling sites of Chinese sturgeon juveniles from the lower reaches of the Yangtze River.



of the band was coded by 1 and absence was coded by 2. For example, a phenotype that has bands at the 4th, 7th, and 9th allele at a polysomic locus with 10 alleles would be converted to an array of phenotypes (2, 2, 2, 1, 2, 2, 1, 2) at 10 dominant marker “loci.” A total of 221 loci were produced across 216 juveniles.

The kinship analysis across Chinese sturgeon juveniles was conducted using an empirical module in COLONY (Jones and Wang, 2010). This software uses the full maximum-likelihood method based on multilocus data to simultaneously infer all possible relationships (parentage and sibship). It is appropriate for a polygamous mating system in which a year-class of juveniles can include full siblings, half-siblings, and unrelated individuals. Many studies have assessed this program's ability to accurately infer full-sibling, half-sibling, and unrelated dyads without the help of any parental information (Rodríguez-Barreto et al., 2013; Wang and Scribner, 2014; Hunter et al., 2020; Scribner et al., 2022). For example, simulations from Hunter et al. (2020) demonstrated sufficient power to accurately assign Lake Sturgeon (*Acipenser fulvescens*) larvae to full-sibling, half-sibling, and unrelated groups.

Accuracy in kinship analysis is dependent on the number of loci and the amount of information provided by the markers (Wang and Scribner, 2014). Before empirical data analyses, 100 replicate simulations were used to assess the power to infer familial relationships based on offspring genotypes alone using COLONY. For each simulation, a breeding matrix was created to represent the reproductive ecology of Chinese sturgeon. The size of each breeding matrix was dependent on the number of successfully breeding adults, offspring sample size and sex ratio. The number of adults was set to a medium level 50 (Hunter et al., 2020). Scribner et al. (2022) found splitting full-sibling samplings into half-siblings occurred less frequently at higher offspring sample sizes (i.e.  $n = 100, 200, \text{ or } 500$ ). As offspring sample size increases, the proportion of half-siblings correctly inferred plateaus ( $n = 200$ ). Additionally, the empirical offspring sample size was 216. Thus, the number of offspring was sampled as 200. The sex ratio (males to females) was set to 1:1 (Wei et al., 2019). Allele frequencies cannot be reliably estimated with polysomic datasets and they're insensitive to assumed allele frequency distributions (Hunter et al., 2020), allele frequencies were simulated from a uniform distribution for 221 loci. For all simulations, polygamous mating was allowed, no sibship prior was used, and class 1 and 2 error rates were 2% and 1% across all loci, respectively. The fulllikelihood method was used to reconstruct pedigrees with “high precision”. The best configuration pedigree for each simulation identified inferred full-sibling, half-sibling, and unrelated dyads which were compared to known (simulated) parents, allowing assessment of the power to correctly infer dyadic relationships.

In this study, juvenile analyses were also conducted at very high precision for the full likelihood calculation with no information on parental genotypes, assuming both male and female polygamy, no inbreeding, and unknown population allele frequencies. Three COLONY runs with different random numbers of seeds were used to check the reliability of the results. Only the relationships found in the three runs were kept in an inferred pedigree. Genotyping error rate was set to 0.01 for all loci. Full and half-sibships were inferred from the “best configuration” output files, which were more accurate than the results found by the pairwise analysis in the files with full- and half-sibling dyads (Wang, 2018). The best configuration of paternal and maternal sibs was used to obtain the number of candidate parents. Full- and half-sibling assignments were accepted upon a posterior probability exceeding 0.90 and 0.80, respectively (Herrera et al., 2016; Ribolli et al., 2020; Kupferschmid et al., 2022).

To assess the reproductive success of mature Chinese sturgeon, the uniformity test between candidate parents (inferred from kinship analysis) and offspring (sampled juveniles) was conducted using the Chi-square test in SPSS v25.

In addition, mtDNA that presented matrilineal inheritance characteristics was also used to compensate candidate parentage and sibship assignments with exclusive probability thresholds of 0.12 for paternity

and 0.25 for maternity as well as an inclusive probability threshold of 0.40 (Thorstensen et al., 2019) for all individuals involved in full- and half-sibships.

Individuals from the 2006 and 2010 year-classes were excluded from kinship analyses due to their small sample sizes ( $N < 10$ ).

#### 2.4. Estimates of $N_b$ and ratios of $N_e/N_c$

The LDNE program was used to estimate the effective number of breeders ( $N_{bLD}$ ) from each year-class (2005–2014) based on the linkage disequilibrium (LD) with the allele exclusion criterion  $P_{crit} = 0.05$  (Waples and Do, 2008; 2010). The equations from Waples et al. (2014) were used to obtain an estimate of  $N_{bLDadj}$ , which corrects bias due to overlapping generations using three simple life-history traits:

$$N_{bLDadj} = \frac{N_{bLD}}{(0.991 - 0.206 \times \text{Log}(A) + 0.256 \times \text{Log}(\alpha) + 0.137 \times \text{CVf})}$$

where  $A$ ,  $\alpha$ , and  $\text{CVf}$  correspond to adult life span, age at maturity of females and variation in age-specific fecundity, respectively.  $A = 10$ ,  $\alpha = 24$ , and  $\text{CVf} = 0.10$  were used in this study and were computed using the data published in Gao et al. (2009).

The program COLONY (Jones and Wang, 2010) was implemented to estimate the effective population size ( $N_e$ ) from each year-class (2005–2014) based on the assumptions of polygamous mating systems for both sexes, no inbreeding, and no prior information on candidate parents or sibship sizes ( $N_e$ , Garant et al., 2001; Richard et al., 2013). The ratio between  $N_e$  and  $N_c$  from hydroacoustic surveys (Gao et al., 2016) was also calculated ( $N_e/N_c$ ).

### 3. Results

#### 3.1. Genetic diversity

For the concatenated sequences of Cyt *b* and COI genes, only nine haplotypes were identified among 216 Chinese sturgeon juveniles. The number of haplotypes ( $H$ ) ranged from one to seven in the nine year-classes. The mean  $\pm$  SD levels of haplotype diversity ( $H_i$ ) and nucleotide diversity ( $P_i$ ) in each year-class ranged from  $0.304 \pm 0.094$  to 0.520

**Table 1**

Summary of the population genetic diversity for Chinese sturgeon juveniles in the 2005–2014 year-classes.

Year class	$N$	$H$	Mitochondrial DNA		Microsatellite DNA			
			$H_i$ (mean $\pm$ SD)	$P_i$ (mean $\pm$ SD)	$A$	$H_o$	$H_e$	$F_{IS}$
2005	68	7	0.520 $\pm$ 0.056	0.0067 $\pm$ 0.0021	9.55	0.738	0.787	0.062
2006	5	1	0.000 $\pm$ 0.000	0.0000 $\pm$ 0.0000	–	–	–	–
2007	18	2	0.476 $\pm$ 0.008	0.0005 $\pm$ 0.0001	8.05	0.710	0.763	0.069
2008	38	4	0.323 $\pm$ 0.108	0.0005 $\pm$ 0.0006	9.48	0.746	0.773	0.035
2009	21	2	0.441 $\pm$ 0.010	0.0005 $\pm$ 0.0003	8.14	0.787	0.778	–0.011
2010	6	1	0.000 $\pm$ 0.000	0.0000 $\pm$ 0.0000	–	–	–	–
2011	30	2	0.304 $\pm$ 0.094	0.0004 $\pm$ 0.0003	9.29	0.727	0.785	0.073
2012	15	1	0.000 $\pm$ 0.000	0.0000 $\pm$ 0.0000	8.67	0.698	0.769	0.092
2014	15	2	0.440 $\pm$ 0.112	0.0005 $\pm$ 0.0004	8.81	0.728	0.779	0.066

$N$ : the number of sampled juveniles;  $H$ : the number of combinations of Cyt *b* and COI haplotypes;  $H_i$ : haplotype diversity;  $P_i$ : nucleotide diversity;  $A$ : number of alleles;  $H_o$ : observed heterozygosity;  $H_e$ : expected heterozygosity;  $F_{IS}$ : inbreeding coefficients calculated as  $1 - (H_o/H_e)$ .

$\pm 0.056$  and  $0.0000 \pm 0.0000$  to  $0.0067 \pm 0.0021$ , respectively (Table 1).

For microsatellite DNA data, there was no evidence of null alleles and large allelic dropout among the 216 juveniles. The polymorphism information contents (PICs) of 18 microsatellite loci were high except two loci (AS16-53 and Afu-19), ranging from 0.409 to 0.867 (mean  $\pm$  SD:  $0.747 \pm 0.140$ ; Supplementary Table S4). The mean number of alleles per locus ( $A$ ) ranged from 8.05 to 9.55 and the observed heterozygosity ( $H_o$ ) ranged from 0.698 to 0.787. The mean unbiased expected heterozygosity ( $H_e$ ) was high and stable across years, ranging from 0.763 to 0.787 (Table 1). The inbreeding coefficients ( $F_{IS}$ ) remained low to modest, ranging from  $-0.011$ – $0.092$  (Table 1).

### 3.2. Kinship assignment and genetic mating system

Simulation analyses indicated that most known full-siblings and half-siblings were correctly inferred. The correct assignment of full-siblings ranged from 91.96% to 100.00% (mean  $\pm$  SD:  $97.47\% \pm 1.76\%$ ), and half-siblings ranged from 90.64% to 100.00% (mean  $\pm$  SD:  $98.24\% \pm 1.35\%$ ). Additionally, the kinship analysis found no uncertainty in the assignment of full- and half-sibling relationships. From the 216 genotyped juveniles, the consensus pedigree identified 53 pairs of full-siblings. Most full-siblings were observed in the 2008 (14 pairs) and 2009 (28 pairs) year-classes. Two half-siblings were found in the 2005, 2009, 2011, and 2015 year-classes, and only one pair was found in the 2007 year-class (Table 2).

Kinship assignment identified 8–60 potential parents in each year-class, and the estimated sex ratio was approximately 1:1, except for the 2009 year-class (Table 3). Clear polygynandry tendencies were observed. Parental inference indicated that most individuals of one sex mated with two to nine different breeders, and most individuals of the opposite sex also mated with two to eight breeders (Supplementary Table S5). Additionally, maternal or paternal groups could be further inferred when combining mitochondrial gene information and exclusion methods. For instance, both halfsib individuals from the 2005 and the 2007 year-class had different mtDNA haplotypes, indicating that they inherited mitochondrial genes from different mothers and shared the same father (Table 4). For individuals of the 2009 year-class, two halfsibs (Juvenile 1 and 2) likely shared the same father, but the other two (Juvenile 2 and 3) shared the same mtDNA haplotype, meaning they had the same mother (Fig. 2).

### 3.3. Evidence of iteroparity

The interannual sibling relationships (3 full-siblings and 6 half-siblings) showed that the breeding intervals ranged from 2 to 6 years between the 2005–2014 year-classes (Table 4). There were two full-

**Table 2**  
Kinship assignment for Chinese sturgeon juveniles in the 2005–2014 year-classes.

Year-class	$N$	Fullsib pairs	% Fullsib	Halfsib pairs	% Halfsib	% Nonrelated
2005	68	5	14.71	2	5.88	79.41
2006	5	1	40.00	0	0.00	60.00
2007	18	1	11.11	1	11.11	77.78
2008	38	14	36.84	0	0.00	63.16
2009	21	28	71.43	2	14.29	14.28
2010	6	0	0.00	0	0.00	100.00
2011	30	0	0.00	2	13.33	86.67
2012	15	3	40.00	0	0.00	60.00
2014	15	1	13.33	2	26.67	60.00

$N$ : the number of sampled juveniles; Fullsib pairs: the number of full-sibling pairs inferred from the kinship analysis; % Fullsib: the number of individuals included in Fullsib pairs/ $N$ ; Halfsib pairs: the number of half-sibling pairs inferred from the kinship analysis; % Halfsib: the number of individuals included in Halfsib pairs/ $N$ ; % Nonrelated: the number of individuals excluded in Fullsib or Halfsib pairs/ $N$ .

sibling pairs between the 2005 and the 2008 year-class and one between the 2007 and the 2011 year-class. Both half-sibling pairs (the 2005–2007 year-class and the 2005–2011 year-class) presented the same pattern; one pair shared the same father, and the other shared the same mother (Table 4). The half-sibling pair between the 2008 and the 2014 year-class probably shared the same mother, while the other pair between the 2011 and the 2014 year-class shared the same father (Table 4).

### 3.4. Annual estimates of $N_b$ and $N_e/N_c$

The annual estimates of  $N_{bLDadj}$  (the biased  $N_{bD}$  estimates based on the life-history traits) ranged from 14 to 161 in the seven year-classes (Table 5). The  $N_e$  ranged from 9 to 48, thus the ratio of  $N_e/N_c$  remained relatively stable (0.23–0.62) throughout the study period.

### 3.5. Reproductive success analysis

The kinship analysis assigned juveniles from 13 to 60 different pedigrees in each year-class. The uniformity test was performed between juveniles and candidate parents year by year. The results showed that the reproductive success of Chinese sturgeon was unstable between the 2005 and 2009 year-classes ( $0 < P < 1$ ). For instance, it deviated from uniformity ( $P = 0$ , Table 5) in the 2009 year-class, while it was even in the 2010 year-class ( $P = 1$ , Table 5). However, the variation in reproductive success became small after the 2010 year-classes ( $P > 0.9$ ).

## 4. Discussion

### 4.1. Genetic mating system

Kinship analysis of Chinese sturgeon based on microsatellite genotypes of juveniles was performed to gain novel insights into the reproductive strategies of Chinese sturgeon. The results revealed a multi-male and multi-female mating process during a breeding season (Fig. 2), which represents the first report of polygynandry in this species. Chinese sturgeon spawned one or two times every year in October–November from 1982 to 2002 below Gezhouba Dam (Wei, 2003). Two spawning events were recorded in 15 years (71.4%) and one spawning activity occurred in 6 years (28.6%). Two batches of eggs increased the possibility that eggs from each female could be fertilized by multiple males in most of the years. In addition, Chinese sturgeon had spawning aggregations. Therefore, Wei (2003) speculated that Chinese sturgeon adopt a polyandry mating system since some male individuals remained at the spawning ground for approximately 12–21 days after the first spawning event. In the present study, our results verified his idea.

Polygynandry has been detected in other migratory species, including Atlantic salmon (*Salmo salar*) (de Mestral et al., 2012), brook trout (*Salvelinus fontinalis*) (Kanno et al., 2011), lake sturgeon (*Acipenser fulvescens*) (Bruch and Binkowski, 2002), and shortnose sturgeon (*Acipenser brevirostrum*) (Kieffer and Kynard, 1996). Because of the relatively large number of eggs (average fecundity of 645,000 eggs/female) released by a female Chinese sturgeon, polyandry can indirectly augment the fitness of females by increasing the genetic variability of the progeny (Coleman and Jones, 2011), likely improving the offspring survival rate and population viability. Similarly, polygyny can allow smaller males, even half the body weight of females, to have more access to females and increase the reproductive fitness of males by allowing the paternity of eggs from multiple females (Ribolli et al., 2016). Therefore, polygynandry is an advantageous reproductive strategy for this species.

### 4.2. Patterns of genetic diversity

Despite its declined populations size and restricted spawning ground, our results indicated that the overall genetic diversity of Chinese sturgeon juveniles (2005–2014) has been maintained at a consistently high

**Table 3**  
Estimated breeding population size of Chinese sturgeon in the Yangtze River in the 2005–2014 year-classes.

Year-class	N	Candidate mothers	P(mothers/ offsprings)	Candidate fathers	P(fathers/ offsprings)	Total number of parent	Sex-ratio
2005	68	31	0.28	29	0.01	60	1.07
2006	5	4	0.96	4	0.96	8	1.00
2007	18	9	0.34	11	0.95	20	0.82
2008	38	14	0.02	15	0.06	29	0.93
2009	21	5	0.00	12	0.80	17	0.42
2010	6	6	1.00	6	1.00	12	1.00
2011	30	16	0.95	15	0.94	31	1.07
2012	15	8	0.92	9	0.95	17	0.89
2014	15	10	1.00	9	1.00	19	1.11

N: the number of sampled juveniles; Candidate mothers: The number of candidate mothers inferred from the kinship analysis; P(mothers/offsprings): Probability of uniformity test between candidate mothers and corresponding offsprings; Candidate fathers: The number of candidate fathers inferred from the kinship analysis; P(fathers/offsprings): Probability of uniformity test between candidate fathers and corresponding offsprings.

**Table 4**  
Breeding interval of Chinese sturgeon between reproductive events based on kinship assignment.

Year-class	Full siblings	Half siblings	Breeding interval	Parent identification
2005–2008	2 pairs	–	3	–
2007–2011	1 pair	–	4	–
2005–2007	–	2 pair	2	1st pair: Same father 2nd pair: Same mother
2005–2011	–	2 pair	6	1st pair: Same father 2nd pair: Same mother
2008–2014	–	1 pair	6	Same mother
2011–2014	–	1 pair	3	Same father

“–”: data absent.

level ( $H_e$ : 0.763–0.787 for microsatellite data) when compared with previous studies ( $H_e$ : 0.733–0.873; [Xin, 2015](#)). Meanwhile, the annual inbreeding coefficient also stayed at a low to modest level, which supports the result of [Zhu et al. \(2006\)](#).

Several possible mechanisms could account for the maintenance of this high level of genetic diversity and low level of inbreeding. Firstly, Chinese sturgeon is a polyploid species with abundant molecular variations. Polyploids generally maintain higher levels of heterozygosity and exhibit less inbreeding depression than diploid species because they have more chance of genome rearrangements ([Soltis and Soltis, 2000](#)). Secondly, a promiscuous mating system reduces inbreeding because promiscuity holds the benefit of mating with more potentially unrelated partners ([Jennions and Petrie, 2000](#); [Hoffman et al., 2007](#)). In addition, difference in the age of first sexual maturity of females and males reduces the risk of inbreeding and the decline of genetic diversity. Thirdly, although Chinese sturgeon has overlapping generations, mating between parents and offspring may be rare because inbreeding avoidance, such as

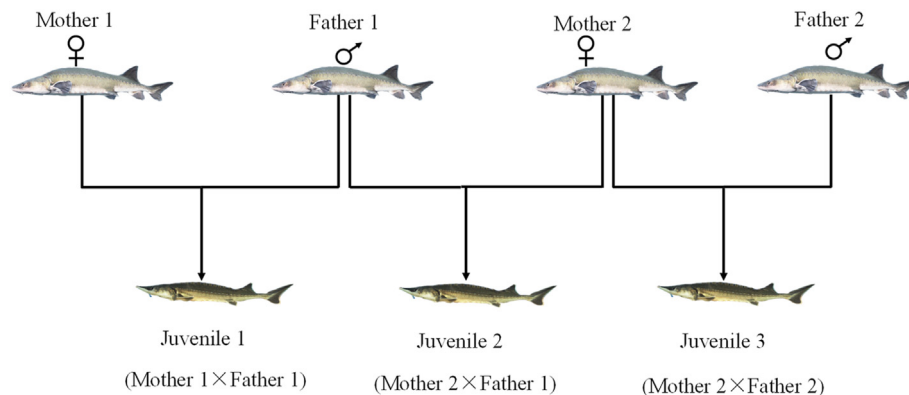
sex-biased dispersal and kin recognition, is universal in animals.

Although habitat loss usually has large negative effects on biodiversity, neutral or even positive effects were found in some species ([Leimu et al., 2006](#)). For example, long-term isolation has not negatively affected genetic diversity and inbreeding coefficients in the critically endangered Montseny brook newt (*Calotriton arnoldi*, [Valbuena-Ureña et al., 2017](#)). Similarly, habitat loss did not affect mate choice within populations of the promiscuous marsupial *Antechinus agilis* ([Banks et al., 2005](#)). These findings imply that the mating systems may not be evolutionarily labile traits, even when confronted with conditions that are likely to increase rates of inbreeding. Our study also shows that habitat loss does not result in negative genetic effects, such as the loss of genetic diversity and inbreeding on an evolutionary timescale.

#### 4.3. Short breeding intervals of Chinese sturgeon

Although spawning periodicity for the sexes is different, the same two previously mated adults should not spawn synchronously again (i.e. biennially) if the individuals adjust their reproductive strategies as the environment changes. Since the construction of the Gezhouba Dam (1981), Chinese sturgeon has only one narrow spawning ground in the Yangtze River, thus it is more likely for the same two adults to spawn synchronously.

In this study, the breeding intervals of Chinese sturgeon were 2–6 years based on genotypes of juveniles ([Table 4](#)). This was similar to other sturgeon species, for which spawning intervals ranged from 2 to 10 years. The inter-spawning intervals were generally 4 or 5 years in white sturgeon (*Acipenser transmontanus*) from the Kootenai River population ([Paragamian et al., 2005](#)). Under farm conditions, [Williot and Brun \(1998\)](#) showed that 25% of females of Siberian sturgeon (*Acipenser baerii*) reproduced annually, whereas in other situations, reproduction was either biennial (64%) or triennial (11%). In the Lena River population,



**Fig. 2.** Sibship assignments for Chinese sturgeon juveniles of the 2009 year-class. The pictures of Chinese sturgeon are cited from the manual of Species Identification for Cultured Sturgeons in China. ([CEF, 2016](#))

**Table 5**

Estimation of effective breeding numbers of Chinese sturgeon in the Yangtze River, and the ratio of effective population size to adult census breeding size in the 2005–2014 year-classes.

Year-class	$N$	$N_c$	$N_{bD}$	$N_{bLDadj}$	$N_e$	$N_e/N_c$
2005	68	78	104 (93–120)	107 (95–123)	48 (33–75)	0.62
2007	18	101	76 (52–132)	78 (53–135)	23 (13–46)	0.23
2008	38	97	34 (31–37)	35 (32–38)	22 (12–42)	0.23
2009	21	–	14 (12–16)	14 (12–16)	9 (4–24)	–
2011	30	72	157 (110–271)	161 (113–278)	41 (25–69)	0.57
2012	15	82	–	–	26 (13–61)	0.32
2014	15	–	59 (42–92)	61 (43–95)	30 (16–75)	–

$N$ : the number of sampled juveniles;  $N_c$ : the number of census breeding adults based on a hydroacoustic survey (Gao et al., 2016);  $N_{bD}$  was estimated from linkage disequilibrium;  $N_{bLDadj}$ : adjusted  $N_{bD}$ ;  $N_e$  was estimated from sibship assignment; “–”: data absent.

the breeding interval of wild Siberian sturgeon females was 4 years for approximately 50% of the fish; this interval can be reduced to 1 or 2 years under favorable rearing conditions, and half of the males within a year-class can reproduce every year. However, the breeding intervals detected in our study were shorter than the breeding intervals observed by Yu et al. (1986), who found the intervals of Chinese sturgeon to be at least 5–7 years based on gonadal reproductive traces. This discrepancy may have resulted from the difference in survey methods or potential analytical bias. We conducted simulation analyses to ensure our modeling accuracy, therefore, analytical bias in our study is less likely.

Previous studies have suggested that poor feeding conditions in the ocean contributes to breeding delays (Wei, 2020). In our study, however, we detected a 2–6-year breeding interval in Chinese sturgeon. The shortest breeding interval was only two years. For example, two half-siblings were found between the 2005 and 2007 year-classes. This suggests that the shortest feeding period in the ocean could be less than one year because of the time a spawned breeder spends migrating to the ocean after spawning (approximately 2–4 months, Wei et al., 2019), migrating back to the Yangtze River after feeding (approximately 2–4 months, Wei et al., 2019), and spending one year in the Yangtze River for breeding, leaving less than eight months spent in the ocean. Results reported here were consistent with the notion that Chinese sturgeon has a well-adapted migratory life history, and they can quickly feed, accumulate nutrition in the ocean, and stop feeding for reproduction in the river as a capital-breeding species. Therefore, marine conditions may not have caused delayed breeding in some years.

#### 4.4. SRS was absent in the current Chinese sturgeon population

The SRS hypothesis posits a large variance in individual reproductive success, and the  $N_e/N_c$  ratio was usually much smaller than 0.01, even reaching the “tiny” level ( $N_e/N_c$ :  $10^{-3}$  to  $10^{-6}$ , Frankham, 1995; Hedgecock and Pudovkin, 2011). We found that the variance in reproductive success of Chinese sturgeon fluctuated temporally but the  $N_e/N_c$  remained relatively stable. Before 2010 (the 2005, 2008, and 2009 year-classes), although the variance in reproductive success was significantly different, the  $N_e/N_c$  ratios (0.23–0.62) were all higher than 0.01. In the 2006, 2007 year-classes and after the 2010 year-classes, the variance in reproductive success became smaller, but the  $N_e/N_c$  ratio was still larger than commonly expected (0.23–0.57) in marine species, indicating that Chinese sturgeon do not follow the SRS reproductive strategy, and that both males and females breed evenly. This temporal discrepancy was attributed to the variance in the number of successful

breeding adults, which was affected by environmental factors such as water discharge, flood events, and water temperature (Wei, 2003; Gao et al., 2009; Chang et al., 2017; Huang and Wang, 2018; Gao et al., 2019; Wei et al., 2019). In 2008, the Three Gorges Reservoir was filled to its highest level of 175 m, leading to a serious temperature hysteresis effect. The unstable reproductive successes in the 2005–2009 year-classes may have resulted from this temperature hysteresis effect. After 2010, juveniles were derived evenly from candidate parent clusters, both male and female, because the number of breeding adults increased (Gao et al., 2016). The relatively even reproductive successes in Chinese sturgeon indicate that many adults “win” the reproductive race and contribute their genes to the next generation. This was beneficial to maintaining high genetic diversity in this species and to keeping small variance in reproductive success compared to a SRS reproductive strategy. The high  $N_e/N_c$  ratio suggests the absence of SRS in the Chinese sturgeon population, which was previously detected in juveniles born in 2014 (Shen et al., 2020). A similar phenomenon was found in New Zealand snapper (*Chrysophrys auratus*, Jones et al., 2019) and southern bluefin tuna (*Thunnus maccoyii*, Waples et al., 2018). This high  $N_e/N_c$  ratio possibly resulted from the species’ polygamous mating system. Promiscuous mating, which was characteristic of aggregate spawning species, such as Chinese sturgeon, could decrease male and female variance in reproductive success (Pearse and Anderson, 2009; Duong et al., 2013), thereby buffering potential reductions in  $N_e/N_c$ . Additionally, Chinese sturgeon still maintained high genetic diversity even when the census population size was very low (50–100). Therefore, the high estimated  $N_e/N_c$  ratio in Chinese sturgeon is favorable news for the population because a small population size can maintain genetic diversity.

#### 4.5. Suggested conservation efforts

The extinction of wild Chinese sturgeon in the Yangtze River is imminent if current conditions persist. During our study, the annual effective number of breeders and effective population size of Chinese sturgeon in the Yangtze River ranged from 14 to 161 and 9–48, respectively (Table 5). These numbers are much lower than the recommended minimum number of 50 to avoid inbreeding (Franklin, 1980) and the minimum viable population size of 320 for Yangtze fishes (Wang et al., 2019). Also,  $N_e$  decreased by 62.1% from 2011 to 2014, although this decrease could be an artifact of spawning periodicity and spawning conditions. In 2018, 2019, and 2020, only 18, 10, and 9 individuals, respectively, were observed around the spawning ground (Bulletin on the Ecological and Environmental Monitoring Results of the Three Gorges Project). The population may experience demographic stochasticity and subsequently the Allee effect (Fujiwara, 2007). If there are no immediate and substantive recovery measures enacted to improve breeding adult survival and successful reproduction, this species will follow the extinction pattern of *Psephurus gladius* (Chinese paddlefish) and *Lipotes vexillifer* (Yangtze River dolphin) (Chen et al., 2020; Zhang et al., 2020).

Based on our results, we recommend more active artificial restocking with mature fish to increase the number of breeding Chinese sturgeon in the Yangtze River. Although more than seven million individuals have been continually released into the Yangtze River since 1984, these restocked fish comprised mostly juveniles or subadults, which contributed little to the total reproductive stock because of high mortality prior to breeding (Wei, 2020). To restore successful reproduction in a short period, we strongly suggest restocking an adequate number of parent fish. However, there are still many details to be considered, such as what kind of mature individuals to be released (newly matured or more experienced in breeding) as well as what season (spring, summer or autumn) and where (present spawning ground or other places) to release the fish.

To reduce the temperature hysteresis effect, it is imperative to improve the hydrological conditions by decreasing the water temperature and increasing high flows during the flood/spawning season. Our previous study (Chang et al., 2021) found that for many years the



environmental conditions were suitable for Chinese sturgeon spawning, which gives hope for sturgeon persistence. We also strongly recommend rehabilitating the spawning ground by improving the local topography and substrate (Wei, 2020). Therefore, multiple conservation actions must be taken simultaneously to protect this endangered species.

## 5. Conclusion

Chinese sturgeon is an iconic and endangered species in the Yangtze River. We demonstrated that it is a polygynandrous species and detected very short breeding intervals during its iteroparous life history over recent years. Although its reproductive stock size sharply declined, it maintained a relatively high level of genetic diversity and recruits evenly descended from the reproductive population. Combining the result of the high estimated  $N_e/N_c$  ratio, we conclude that Chinese sturgeon have adopted a non-SRS mating strategy, which has the potential to maintain high genetic diversity, and a large portion of the population is not needed for conservation compared with species exhibiting an SRS mating strategy. This is encouraging from a conservation standpoint and gives hope for saving this species. To restore successful reproduction in a limited timeframe, we strongly suggest (1) restocking parent fish, (2) increasing flood flows, (3) decreasing water temperatures, and (4) improving spawning ground substrate.

## Ethical statement

All experimental protocols were in accordance with the Laboratory Animal Management Principles of China. All applications and protocols for fish handling have been approved by the Ethics Committee for Animal Experiments of the Institute of Hydrobiology, Chinese Academy of Sciences.

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## Author's contribution

H.L. conceived and designed the research; C.T., F.Z., P.L., Z.D. collected the samples; Z.S. performed the experiments and analyzed the data. D.Y., X.G., and H.L. wrote the manuscript; F.M. revised the manuscript. All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.watbs.2023.100134>.

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