Genetic diversity and reproductive success of a wild population of Chinese sturgeon (*Acipenser sinensis*) from the Yangtze River inferred from juveniles born in 2014

Zhong-Yuan Shen1,2, Dan Yu1, Xin Gao1, Fu-Tie Zhang1, Huan-Zhang Liu1,*

1 The Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China
2 University of Chinese Academy of Sciences, Beijing 100049, China

ABSTRACT

The Chinese sturgeon (*Acipenser sinensis* Gray, 1835) is a large anadromous fish species, which is under considerable threat due to dramatic declines in population numbers. In the current study, population genetic diversity and individual reproductive success were assessed using nuclear microsatellite markers (simple sequence repeat, SSR) and complete mitochondrial (mtDNA) genome analysis of juveniles born in 2014. Results showed the existence of size polymorphism in the mtDNA genome of Chinese sturgeon, which was caused by a repeat motif. Population genetic diversity was high based on both SSR (*Ho*: 0.728±0.211; *He*: 0.779±0.122) and mtDNA genome analyses (*H*: 0.876±0.0035; *Pi*: 0.0011±0.0010). A positive inbreeding coefficient (*FIS*: 0.066±0.143) was also found, indicating the occurrence of inbreeding. Reconstruction of sibling groups identified 11 mothers and 11 fathers involved in reproduction of Chinese sturgeons in 2014. Variance in individual reproductive success was not significant, with reproductive success of parent fish instead shown to be relatively even (*P*=0.997>0.05), thus suggesting the absence of sweepstakes reproductive success (SRS). These results indicate that, in regard to conservation, loss of genetic diversity due to the effects of SRS is not of particular concern. However, we must focus on having an adequate number of adults and suitable environmental conditions to ensure that fish can reproduce.

Keywords: Chinese sturgeon; Genetic diversity; Size polymorphism; Inbreeding; Reproductive success

INTRODUCTION

Genetic diversity assessment plays a significant role in conservation strategy planning for the long-term persistence of populations, particularly for threatened and endangered species. Populations that have experienced severe interference may undergo a significant fluctuation in genetic diversity, leading to possible population bottleneck, genetic drift, increasing rates of inbreeding, and fixation of mildly

Received: 20 December 2019; Accepted: 20 May 2020; Online: 02 June 2020

Foundation items: This study was funded by the National Key R & D Program of China (2018YFD0900801); Strategic Priority Research Program of Chinese Academy of Sciences (XDB31040000); Follow-Up Work of the Three Gorges Project (2136902); and Sino BON-Inland Water Fish Diversity Observation Network

*Corresponding author, E-mail: hzliu@ihb.ac.cn

DOI: 10.24272/j.issn.2095-8137.2020.011
deleterious mutations. This can, in turn, impact adaptive potential and increase the probability of extinction (Harrison et al., 2014; Luikart et al., 1998; Reed & Frankham, 2003; Willi et al., 2006). Christie et al. (2012) found that captive-born steelhead trout (Onchorrhyncus mykiss (Walbaum, 1792)) frequently show poor fitness when reintroduced into the wild under captive breeding programs. This is because fish from large families are more likely to mate with relatives, and subsequently exhibit lower per capita fitness due to inbreeding depression. In addition, da Paz Aguiar et al. (2018) and de Sá Teles Oliveira et al. (2019) found a higher level of genetic diversity (polymorphic ratio, heterozygosity, and number of alleles) in natural populations of tambaqui (Colossoma macropomum (Cuvier, 1816)), compared with farmed populations, suggesting genetic bottlenecks caused by confinement. Therefore, from a conservation perspective, it is important to assess the potential genetic problems that a species may experience and take suitable conservation measures.

Reproductive success is a crucial part of species life history, and understanding the mating strategies adopted by fish is critical for their continuation. With the rapid development of molecular technologies, individual reproductive success has been evaluated based on parenthood analysis and individual relatedness (Garant et al., 2001; Richard et al., 2013; Serbezov et al., 2010). Hedgecock (1994) and Hedgecock & Pudovkin (2011) found wide variations in reproductive success among individuals in many marine animal species due to a sweepsakes-like chance of matching reproductive activity (i.e., sweepsakes reproductive success, SRS). This theory suggests that most offspring are from relatively few parents, resulting in low genetic diversity and $N_e/N$ ratio ($N_e$: effective population size; $N$: population size). Several studies have shown that theory and empirical evaluations are in accordance with the expected effects of SRS based on the $N_e$ and $N_e/N$ ratio (Hedrick, 2005; Waples, 2016). However, Waples et al. (2018) reported a higher than expected $N_e/N$ ratio (about 0.5) against the SRS in southern bluefin tuna (Thunnus maccoyii (Castelnau, 1872)) using genetic and life history data (almost 13,000 fish collected over five years). Jones et al. (2019) also showed a higher $N_e/N$ (about 0.33) than expected in New Zealand snapper (Chrysophrys auratus (Foster, 1801)), suggesting the absence of SRS. These studies indicate that different mating strategies likely exist in different fish species.

The Chinese sturgeon (Acipenser sinensis Gray, 1835) is a large anadromous fish, which inhabits coastal areas in eastern and southeastern China (including the Yellow Sea, East China Sea, and Taiwan Strait) and breeds in freshwater of outflowing rivers (Wei, 2019). Unfortunately, most populations of Chinese sturgeon have disappeared due to impact from human activities, including habitat deterioration, overfishing, and climate change. Currently, only one small population located in the Yangtze River in known (Chang et al., 2017; Gao et al., 2016; Wei, 2019). Construction of the Gezhou Dam (GD) in 1981 and the Three Gorges Dam (TGD) in 2003 along the Yangtze River resulted in the deterioration of environmental conditions suitable for Chinese sturgeon reproduction (Chang et al., 2017; Dai et al., 2014; Kynard et al., 1995; Wei et al., 1997). For example, Gao et al. (2009) estimated that the GD reduced the population size of Chinese sturgeon by 83%. After the TGD impoundment, the mean number of adults after spawning decreased by 64.4% (2004–2013) compared with that before impoundment (Gao et al., 2016). The continual decline in population size has resulted in the Chinese sturgeon being listed as Critically Endangered on the International Union for Conservation of Nature Red List in 2010.

Several studies on mating strategy, population structure, genetic diversity, and kinship have been conducted on the Chinese sturgeon. In terms of mating strategy, based on reproductive marks, Yu et al. (1986) stated that the breeding interval of Chinese sturgeon was at least five to seven years. Wei et al. (2005) found that the female to male sex ratio was 5.86:1 in a breeding population in 2003. Zhang et al. (2003) reported on genetic variation among pooled Chinese sturgeon based on mean haplotype and nucleotide diversities, which were 0.949±0.010 and 0.011±0.006, respectively. Zhao et al. (2015) reported that Chinese sturgeon kinship could be identified based on analysis of three tetraploid microsatellite loci. In recent years, Chinese sturgeons have faced an even more severe situation. In 2013, the fish failed to spawn in the field, and failed again in 2015, 2017, 2018, and possibly 2019. At present, it is not known whether this species exhibits an SRS mating strategy, which could lead to low population genetic diversity.

In the current study, we estimated genetic diversity based on juveniles born in 2014 using nuclear microsatellite markers (simple sequence repeat, SSR) and complete mitochondrial (mtDNA) genome. We then analyzed sibling relatedness among wild Chinese sturgeon juveniles to estimate the number of parents involved in reproduction in 2014. Finally, we analyzed uniformity among parents and offspring and explored the reproductive success of the mating strategy of wild Chinese sturgeon. Our intention was to suggest future conservation strategies for this critically endangered species.

MATERIALS AND METHODS

Statement on animal subjects

All experimental protocols involving fish in this study were approved by the Ethics Committee for Animal Experiments of the Institute of Hydrobiology, Chinese Academy of Sciences. The methods used in this study were conducted in accordance with the Laboratory Animal Management Principles of China.

Sample collection and genotyping

Fifteen wild juveniles of A. sinensis (27.45–229.62 mm total length) were captured and stored in 95% ethanol in the lower reaches of Yangtze River (one in Zhenjiang, eight in Nantong, six in Changxing Island) in late May 2015. Muscle tissues were collected from individual fish and stored in 95% ethanol and refrigerated at −20 °C for genetic analysis. Total genomic
DNA was extracted from the muscle tissues using a high salt protocol with slight modifications (Aljanabi & Martinez, 1997).

We used complete mtDNA and 21 nuclear microsatellite markers to estimate genetic diversity, inbreeding, individual relatedness, and candidate parentage identification. The complete mtDNA primers (Liao et al., 2016) are listed in Supplementary Table S1. We developed microsatellite primer sets, including h423 (forward primer 5'-GCCTTGCTTGGAGCTTTTC-3' and reverse primer 5'-CAGAGGCATCACCTCCATTTC-3') and 20 other pairs (GenBank), as presented in Supplementary Table S2. Polymerase chain reaction (PCR) was performed in volumes of 20 μL (SSR) or 30 μL (mtDNA) containing 30–50 ng template DNA, 0.5 μL dNTP mixture (2.5 mmol/L each), 0.3 U Taq DNA Polymerase with MgSO₄ (2 mmol/L of Mg²⁺), 2 μL 10×Taq Buffer, 0.5 μL each primer (10 μmol/L), and deionized H₂O. The PCR profile for mtDNA was: 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; then one cycle at 72 °C for 10 min. The PCR profile for SSR was: 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; then one cycle at 72 °C for 10 min. The PCR products were electrophoresed on 1.0% non-denaturing polyacrylamide gels, purified with a DNA Agarose Gel Extraction Kit (Omega, USA), and analysed on an ABI 3730 automated genetic analyzer.

Population genetic diversity analysis
The mtDNA sequences were edited and assembled using the software package DNASTAR (Burland, 2001) and aligned using ClustalX 2.0 (Larkin et al., 2007). The aligned sequences were revised according to a published sequence (Liao et al., 2016) by manual correction with SEAVIEW v5 (Galtier et al., 1996) and spliced using the package Mesquite 2.75 (Maddison & Maddison, 2011). The overall base composition of the sequences was analyzed using MEGA v6.0 (Tamura et al., 2013). The number of haplotypes, haplotype diversity (H), nucleotide diversity (P), and other genetic information were estimated in DNASP v5.10 (Librado & Rozas, 2009).

Sturgeons are a polyplody species. Here, our microsatellite datasets with artificial correction were processed in the tetrasomic inheritance model. The data were checked with Micro-Checker (Van Oosterhout et al., 2004) to detect any null alleles and large allelic dropout. After that, the parameters of genetic diversity for each sampling, linkage disequilibrium between loci, and inbreeding in the population were estimated using AUTOTET (Thrall & Young, 2000). Polymorphic information content (PIC) was estimated using PICcalc (Nagy et al., 2012).

Parentage analysis and individual relatedness
Individual relatedness and parentage analysis among wild Chinese sturgeon juveniles were estimated using a simulation module with co-dominant microsatellite markers (Wang, 2004). Computations of individual relatedness and parentage identification were executed using COLONY 2.0 (Jones & Wang, 2010). To further improve the precision of identification, the complete mtDNA genome and inclusive and exclusive probability thresholds of parent clusters (exclusive probability thresholds of 0.12 and 0.25, combined with 0.40 inclusive probability; Thorstensen et al., 2019) were used to assist in candidate parent identification.

In addition, genetic distance was calculated using Populations v1.2.32 (Langella, 2000), and phylogenetic relationships were reconstructed using maximum-likelihood (ML) following the Tamura-Nei model and unweighted pair-group method with arithmetic mean (UPGMA) implemented in MEGA v6.0 (Tamura et al., 2013).

Assessment of reproductive success
The best configuration of paternal and maternal sibs given by COLONY 2.0 based on ML was used to obtain the number of candidate parents. Although mature Chinese sturgeons were not sampled, COLONY can infer their genotypes from the best configuration of paternal and maternal sibs when marker information is sufficient (Jones & Wang, 2010). The total number of candidate parents was used to estimate the relative contribution of mature candidate parents in 2014.

To assess the reproductive success of mature Chinese sturgeons, we statistically checked the differences among parents (both sexes) with different numbers of offspring, and the uniformity between total parents (both sexes) and juveniles using the Chi-square test in SPSS v25.

RESULTS
Assessment of population genetic diversity
The mtDNA genome of Chinese sturgeon demonstrated circular molecules from 16 524 to 16 688 base pairs (bp) in size (Supplementary Table S3). Four different sized mtDNA genomes (16 524 bp, 16 561 bp, 16 606 bp, 16 688 bp) were detected in this survey. DNA sequence analysis indicated that the size polymorphism occurred in the D-loop region near tRNA®™ due to a repeat motif containing 4.5 copies of an 82 bp unit (gacagtctttactcaatcaattctictacggccatcacacalaattgtgcgaggtactgaatttt). The maximum and minimum mtDNA gaps observed within the Chinese sturgeons were 164 bp and 82 bp in length, respectively.

Comparison of genome sequences resulted in the identification of eight mitochondrial haplotypes (Table 1). Haplotype diversity (H) and nucleotide diversity (P) were 0.8760 ±0.0035 and 0.0011±0.0010, respectively. Fifty-one nucleotide variable positions (Supplementary Table S4) were identified, including 16 singleton variable sites (accounting for 31.37%), 35 parsimony informative sites (accounting for 68.63%), and 204 alignment gaps or missing-information sites. Of the 51 nucleotide variable positions, 23 were located in the coding region, with five causing amino acid changes (i.e., asparagine to serine, methionine to isoleucine, isoleucine to leucine, proline to arginine, and leucine to glutamine). The control region sequences had the highest variation.
Null alleles were not found by Micro-Checker. There was no evidence of large allele dropout or scoring errors caused by stuttering. The 21 markers used in this study were found to be highly polymorphic and most indicated tetraploidy. A total of 185 alleles was detected, with the total number of alleles per individual at a locus ranging from 3 to 13 (Table 2). The mean±SE number of four allele genotypes at a locus was 12.095±2.606. The polymorphism information content (PIC) was between 0.409 and 0.867. Two loci (AS-035 and AS-043) deviated from the Hardy-Weinberg equilibrium (HWE).

### Inbreeding

The inbreeding coefficients (FIS) of most markers were relatively low, ranging from −0.136 to 0.474, with a mean±SE of 0.066±0.143 (Table 2). The negative and positive FIS values were roughly equal, with 11 negative FIS and 10 positive FIS values. The average number of alleles of 0.728±0.211 and 0.779±0.122, respectively. The observed heterozygosity (He) and expected heterozygosity (H0) were 0.728±0.211 and 0.779±0.122, respectively. The mean±SE number of four allele genotypes at a locus was 8.810±2.713. The mean±SE number of alleles per individual at a locus (Ai) ranged from 1.467 to 3.867 (Table 2). The mean±SE value of Ai was 2.832±0.772. Results indicated that population homozygote excess may exist, to some extent, thus suggesting the occurrence of inbreeding.

### Number of parents involved in reproduction

Sibling relationship analysis identified four full-sibling groups, but no half-sibling groups. We also identified 11 different mothers and 11 different fathers of Chinese sturgeon (Table 3), with a male to female ratio of ~1:1.

We calculated the pairwise distances among juveniles based on SSR and mtDNA data and reconstructed a dendrogram using UPGMA and ML of relatedness based on the distance matrix (Figure 1). In addition, UPGMA (Supplementary Figure S1) analysis of mtDNA genome data was also performed as an assistant tree. The trees yielded good agreement regarding topological relationships. The trees

| Haplotype | Sample ID | Sample No. (n) | GenBank accession Nos. |
|-----------|-----------|----------------|------------------------|
| H1        | Y13       | 1              | MT272689               |
| H2        | Y2, Y5, Y7, Y12 | 4          | MT272690               |
| H3        | Y9        | 1              | MT276291               |
| H4        | Y1, Y8, Y14, Y15 | 4         | MT276292               |
| H5        | Y6        | 1              | MT276293               |
| H6        | Y3, Y4    | 2              | MT276294               |
| H7        | Y10       | 1              | MT276295               |
| H8        | Y11       | 1              | MT276296               |

### Table 2 Marker information for 21 microsatellite loci analyzed in Chinese sturgeon

| Locus | A | AI | G | Ho | He | FIS | PIC |
|-------|---|----|---|----|----|-----|-----|
| Afu-68| 10| 3.133| 12| 0.789| 0.836| 0.056| 0.818|
| AS-033| 9 | 2.400| 12| 0.622| 0.718| 0.133| 0.687|
| AS-035| 11| 1.933| 13| 0.522| 0.834| 0.374| 0.815|
| AS-102| 9 | 2.267| 15| 0.611| 0.832| 0.266| 0.812|
| AS02-7| 8 | 2.933| 12| 0.733| 0.711| -0.031| 0.683|
| AS-048| 7 | 2.200| 11| 0.556| 0.759| 0.268| 0.726|
| AS-050| 4 | 2.333| 8 | 0.678| 0.613| -0.106| 0.534|
| H423   | 6 | 2.667| 9 | 0.778| 0.743| -0.046| 0.703|
| Spt-100| 11| 3.600| 14| 0.933| 0.874| -0.067| 0.862|
| AS01-4| 12| 3.333| 15| 0.889| 0.873| -0.018| 0.860|
| AS03-13| 8 | 3.667| 12| 0.933| 0.822| -0.136| 0.799|
| AS08-23| 9 | 2.733| 12| 0.644| 0.719| 0.104| 0.689|
| AS14-48| 13| 3.667| 15| 0.944| 0.879| -0.075| 0.867|
| AS16-53| 4 | 1.467| 6 | 0.256| 0.486| 0.474| 0.409|
| Afu-19 | 3 | 1.533| 7 | 0.322| 0.482| 0.331| 0.432|
| AS06-20| 10| 3.800| 13| 0.967| 0.861| -0.123| 0.846|
| AS09-38| 9 | 3.800| 13| 0.956| 0.851| -0.123| 0.833|
| AS-018 | 12| 2.400| 15| 0.700| 0.878| 0.203| 0.866|
| AS-043| 10| 2.200| 13| 0.567| 0.857| 0.339| 0.842|
| AS-082| 10| 3.533| 13| 0.911| 0.877| -0.039| 0.865|
| AST1-43| 10| 3.867| 14| 0.978| 0.864| -0.132| 0.849|
| Means: | 8.81| 2.832| 12.095| 0.728| 0.779| 0.066| 0.752|
| StDev: | 2.713| 0.772| 2.606| 0.211| 0.122| 0.143| 0.140|

A: Number of alleles at locus; AI: Average number of alleles per individual at locus; G: Number of four allele genotypes at locus; Ho: Observed heterozygosity at locus; He: Expected heterozygosity; FIS: Fixation coefficients calculated as 1−(Ho/He); PIC: Polymorphism information content.

426 www.zoores.ac.cn
also showed similar best configuration of sibling relationships.

**Reproductive success analysis**

Sibling relationship analysis indicated that at least 11 different adult females and 11 different adult males bred successfully in 2014. Furthermore, the haplotypes of the mtDNA genomes implied that at least eight different adult females (or more) bred successfully. The fifteen Chinese sturgeon juveniles were from 11 different pedigrees (Table 3). The pedigrees “P1 (father ‘F1’ and mother ‘M1’), “P3 (father ‘F3’ and mother ‘M3’), “P4 (father ‘F4’ and mother ‘M4’), and “P6 (father ‘F6’ and mother ‘M6’) had two juvenile offspring, and the others had one juvenile offspring (Table 3).

Variation of reproductive success was tested by two statistical methods. The significance test was carried out between parents (both sexes) with different numbers of offspring, with no significant differences found among these parents (both sexes) (P=0.366>0.05, Table 4). The heterogeneity or uniformity test between parents (both sexes) and offspring showed good uniformity (P=0.997>0.05, Table 4). This indicated that reproductive success in mature Chinese sturgeons (both sexes) was relatively even.

**DISCUSSION**

**Genetic diversity and possible maintenance mechanism**

Assessing the potential status of population genetic diversity is crucial for the conservation of endangered species. As inferred from juveniles born in 2014, the Chinese sturgeon population showed high genetic diversity in the mtDNA genome (H: 0.876±0.003; Pi: 0.001±0.001) and relatively high genetic diversity based on the SSR data (Ho: 0.728±0.211; He: 0.779±0.122; PIC: higher than 0.409). These values are only slightly lower than those (H: 0.949±0.010; Pi: 0.01±0.006) previously reported in Chinese sturgeon in the mtDNA genome (Zhang et al., 2003), and higher than those (Ho: higher than 0.686; PIC: higher than 0.49) in closely related Acipenser baeri Brandt, 1869, based on SSR data (Fopp-Bayat, 2010). Therefore, these mtDNA

| Ped | Sample ID | Prob (Inc.) | Prob (Exc.) | Father ID | Mother ID |
|-----|-----------|-------------|-------------|-----------|-----------|
| P1  | Y1, Y15   | 0.5792      | 0.1870      | F1        | M1        |
| P2  | Y2        | 1.0000      | 0.2038      | F2        | M2        |
| P3  | Y3, Y4    | 0.9614      | 0.8860      | F3        | M3        |
| P4  | Y5, Y7    | 0.3449      | 0.2400      | F4        | M4        |
| P5  | Y6        | 1.0000      | 0.1183      | F5        | M5        |
| P6  | Y8, Y14   | 0.8546      | 0.3053      | F6        | M6        |
| P7  | Y9        | 1.0000      | 0.2170      | F7        | M7        |
| P8  | Y10       | 1.0000      | 0.8552      | F8        | M8        |
| P9  | Y11       | 1.0000      | 0.7014      | F9        | M9        |
| P10 | Y12       | 1.0000      | 0.5750      | F10       | M10       |
| P11 | Y13       | 1.0000      | 0.8168      | F11       | M11       |

Ped: Pedigree index; Sample ID: IDs of all offspring members of this pedigree; Prob (Inc.): Inclusive probabilities of this pedigree; Prob (Exc.): Exclusive probabilities of this pedigree; Father ID: Candidate father ID in this pedigree; Mother ID: Candidate mother ID in this pedigree.

**Figure 1** Phylogenetic tree based on SSR and mtDNA genome data using two methods

A: Phylogenetic tree based on 21 microsatellite loci data using unweighted pair-group method; B: Maximum-likelihood phylogenetic tree based on mtDNA genome sequences of juvenile Chinese sturgeons.
and SSR results indicate that population genetic diversity of Chinese sturgeon, as inferred from juveniles born in 2014, has not experienced a recent rapid decline. Several possible mechanisms could account for the maintenance of this high level of genetic diversity, despite the rapid population decline of Chinese sturgeon in the Yangtze River. Firstly, mtDNA genome size polymorphism (26.7% in our samples), which was caused by a repeat motif containing 4.5 copies of a 82 bp unit (approximately 82–164 bp), was found among individuals, as also reported in a previous study on Chinese sturgeon (Zhang et al., 1999). Thus, we inferred that size polymorphism could contribute to the high haplotype diversity, as reported in Drosophila mauritiana (Solignac et al., 1983), Rana esculenta (Monnerot et al., 1984), Amia calva (Bentzen et al., 1989), Alosa sapidissima Wilson, 1811 (Brown et al., 1992), and Acipenser transmontanus Richardson, 1836 (Brown et al., 1992). Moreover, the SSR results suggest that Chinese sturgeon must have abundant molecular variations in individual-based tetraploidy and a relatively large number of alleles (total number of alleles: 185; mean G: 12.095±2.606). These abundant variations may lead to a relatively high level of Ho and He in juveniles via reproductive inheritance of their parents. In addition, Chinese sturgeon do not breed each year. Males and females have different maturity intervals, which may help them mate with different individuals from different family lines, and thus increase genetic diversity.

Inbreeding analysis

For SSR analysis of tetraploid organisms, if the obtained value of AI is equal to 1, the individual is considered to be completely homozygous, whereas if the value is equal to 4, the individual is considered to be heterozygous. Based on our calculations, the AI of Chinese sturgeon was 2.832±0.772, well below the maximum theoretical number of 4. This suggests that the population may, to some extent, have a homozygote-bias. The positive FIS (0.066±0.143) also suggested such a bias in this species. Inbreeding is considered to have occurred when homozygote excess is detected in a species (Keller & Waller, 2002; Milton, 1997). Inbreeding can have a significant influence on genetic diversity, reproductive potency, survival, and resistance to disease and stress, and can result in the accumulation of deleterious mutations (Brook et al., 2002). The potential inbreeding detected in this study may increase the risk of extinction in this population of Chinese sturgeon.

Number of parents involved in reproduction

Previous studies have shown that the population size of Chinese sturgeon has decreased rapidly in the past several decades. For instance, Gao et al. (2009) estimated that the GD reduced the population size of Chinese sturgeon by 83%, and the mean number of adult sturgeon after spawning decreased by 64.4% following impoundment of the TGD (2004–2013) (Gao et al., 2016). According to our data, we determined that 11 Chinese sturgeon females and males (each) were involved in reproduction in 2014. This result is close to the limit of estimation (breeding size involved in reproduction: 13–60; unpublished data) by finding sturgeon eggs in the stomach and intestine of benthic carnivorous fish. It is also in accordance with the findings from a previous hydroacoustic survey (Chang et al., 2017), further suggesting that the population size of Chinese sturgeon is likely very small.

Reproductive success analysis

It has been reported that for marine fish and shellfish with high fecundity and high early mortality, most recruited young individuals in a given year come from very few parents (i.e., SRS). The SRS mating strategy has been found in at least 102 species based on Ne/N ratio estimates (Frankham, 1995). In the present study, we found no significant differences among candidate Chinese sturgeon parents who had different offspring (P=0.366>0.05), indicating that juveniles born in 2014 were from a number of parents instead of just a few. Our findings suggest that Chinese sturgeons do not follow SRS type reproduction, as found in the New Zealand snapper (Jones et al., 2019) and southern bluefin tuna (Waples et al., 2018). In addition, the heterogeneity also indicated that juveniles in 2014 were derived evenly from candidate parent clusters (both male and female) (P=0.997>0.05). This suggests that variance in reproductive success of wild Chinese sturgeon is very small.

The even reproductive success and small variance in Chinese sturgeon, rather than the SRS mating strategy, may be beneficial for the maintenance of genetic diversity. Based on the SRS strategy, only a few parents in a breeding population win the reproductive race, with many more losing (Jones et al., 2019). Therefore, population genetic diversity is not normally high. The relatively even reproductive success in Chinese sturgeon indicates that more adults win the reproductive race and contribute their genes to the next generation. Thus, this species may be able to maintain high genetic diversity, even when the population is small. This may also explain the relatively high level of genetic diversity found in the Chinese sturgeons in the present study as well as in previous research (Xin, 2015; Zhang et al., 2003).

Conservation implications

Based on juveniles born in 2014, we found that the wild Chinese sturgeon population has a high level of genetic diversity and recruits may be evenly derived from the reproductive population. This is good news from a
conservation standpoint because Chinese sturgeon may contain the necessary mechanism to maintain high genetic diversity via a non-SRS mating strategy. Therefore, regarding their conservation, loss of genetic diversity due to the effects of SRS may be of less concern. As long as the Chinese sturgeon can reproduce naturally, genetic diversity should be maintained at a high level. The major task for conservation is to ensure an adequate number of adult individuals and suitable environmental conditions for successful reproduction.

One limitation of our study is the small sample number (15 juveniles) for population genetic analysis. As the Chinese sturgeon population is dangerously small, it is not possible to collect many samples. Of particular concern, the population failed to breed in the field in 2013, 2015, 2017, 2018, and possibly 2019. Therefore, we explored all possible ways in which to utilize our limited material. We expanded our study to include complete mtDNA genome sequencing of all samples, increased the number of SSR markers, and added confidence thresholds for individual relatedness analysis. It has been suggested that these measures can complement the shortage of small samples (Jones & Wang, 2010; Thorstensen et al., 2019; Wang, 2004). As such, we believe that our methods and results are reliable and can provide a reference for better conservation of Chinese sturgeons.

SUPPLEMENTARY DATA
Supplementary data to this article can be found online.

COMPETING INTERESTS
The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS
H.Z.L. funded the project, designed the study, and revised the manuscript. Z.Y.S performed the laboratory work and wrote the manuscript. D.Y. and X.G. helped in data analysis. F.T.Z. provided information on Acipenser sinensis sampling. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS
We are grateful to Li-Xia Shi for collecting specimens. Many thanks go to Zhi Zhang, Xue Wang, and other colleagues for their help in the use of software.

REFERENCES
Aljanabi SM, Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25(22): 4692–4693.

Bentzen P, Brown GC, Leggett WC. 1989. Mitochondrial DNA polymorphism, population structure, and life history variation in American shad (*Alosa sapidissima*). *Canadian Journal of Fisheries and Aquatic Sciences*, 46(8): 1446–1454.

Bermingham E, Lamb T, Avise JC. 1986. Size polymorphism and heteroplasmy in the mitochondrial DNA of lower vertebrates. *Journal of Heredity*, 77(4): 249–252.

Brook BW, Tonkyn DW, O’Grady JJ, Frankham R. 2002. Contribution of inbreeding to extinction risk in threatened species. *Conservation Ecology*, 6(1): 16.

Brown JR, Beckenbach AT, Smith MJ. 1992. Mitochondrial DNA length variation and Heteroplasmy in populations of white sturgeon (*Acipenser transmontanus*), *Genetics*, 132(1): 221–228.

Burland T G. 2001. DNASTAR’s Lasergene sequence analysis software. *Methods in Molecular Biology*, 132: 71–79.

Chang T, Lin PC, Gao X, Liu F, Duan ZH, Liu HZ. 2017. Using adaptive resolution imaging sonar to investigate Chinese sturgeon (*Acipenser sinensis* Gray, 1835) behaviour on its only spawning ground in the Yangtze River. *Journal of Applied Ichthyology*, 33(4): 681–688.

Christie MR, Marine ML, French RA, Blouin MS. 2012. Genetic adaptation to captivity may occur in a single generation. *Proceedings of the National Academy of Sciences of the United States of America*, 109(1): 238–242.

da Paz Aguilar J, Gomes PFF, Hamoy IG, dos Santos SEB, Schneider H, Sampalo I. 2018. Loss of genetic variability in the captive stocks of tambaqui, *Colossoma macropomum* (Cuvier, 1818), at breeding centres in Brazil, and their divergence from wild populations. *Aquaculture Research*, 49(5): 1914–1925.

Dai ZJ, Lin JT, Wei Q, Chen JY. 2014. Detection of the three gorges dam influence on the Changjiang (Yangtze River) submerged delta. *Scientific Reports*, 4: 6600.

de Sá Teles Oliveira C, Moreira RFC, Filho AAS, Fonteles SBA, Evangelista-Barreto NS. 2019. Genetic diversity in natural populations of *Colossomamacropomum* in the Brazilian Amazon region and in populations farmed in Northeast Brazil based on ISSR markers. *Aquaculture International*, 27(5): 1423–1434.

Fopp-Bayol D. 2010. Microsatellite DNA variation in the Siberian sturgeon, *Acipenser baeri* (*Actinopterygii, Acipenseriformes, Acipenseridae*), Cultured in a Polish fish Farm. *Acta Ichthyologica et Piscatoria*, 40(1): 21–25.

Frankham R. 1995. Effective population size/adult population size ratios in wildlife: a review. *Genetics Research*, 66(2): 95–107.

Gallier N, Gouy M, Gautier C. 1996. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Bioinformatics*, 12(6): 543–548.

Gao X, Brosse S, Chen YB, Lek S, Chang JB. 2009. Effects of damming on population sustainability of Chinese sturgeon, *Acipenser sinensis*: evaluation of optimal conservation measures. *Environmental Biology of Fishes*, 86(2): 325–336.

Gao X, Lin PC, Li MZ, Duan ZH, Liu HZ. 2016. Impact of the Three Gorges Dam on the spawning stock and natural reproduction of Chinese sturgeon in Changjiang River, China. *Chinese Journal of Oceanology and Limnology*, 34(5): 894–901.

Garant D, Dodson JJ, Bermatchez L. 2001. Genetic evaluation of mating system and determinants of individual reproductive success in Atlantic Salmon (*Salmo salar* L.). *Journal of Heredity*, 92(2): 137–145.

Harrison KA, Pavlova A, Telonis-Scott M, Sunnucks P. 2014. Using genomics to characterize evolutionary potential for conservation of wild populations. *Evolutionary Applications*, 7(9): 1008–1025.

Hedgecock D. 1994. Does variance in reproductive success limit effective population size of marine organisms?. In: Beaumont A. Genetics and Evolution of Aquatic Organisms. London: Chapman and Hall.
Hedgecock D, Pudovkin AI. 2011. Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. *Bulletin of Marine Science, 87*(4): 971–1002.

Hedrick P. 2005. Large variance in reproductive success and the N_e/N ratio. *Evolution, 59*(7): 1596–1599.

Jones AT, Lavery SD, Le Port A, Wang YG, Blower D, Ovenden J. 2019. Sweepstakes reproductive success is absent in a New Zealand snapper (*Chrysophrys auratus*) population protected from fishing despite "tiny" N_e/N ratios elsewhere. *Molecular Ecology, 28*(12): 2986–2995.

Jones OR, Wang JL. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources, 10*(3): 551–555.

Keller LF, Walter DM. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution, 17*(5): 230–241.

Kynard B, Wei QW, Ke FE. 1995. Use of ultrasonic telemetry to locate the spawning area of Chinese sturgeon. *Chinese Science Bulletin, 40*(6): 668–671.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics, 23*(21): 2947–2948.

Langella O. 2000. POPULATIONS 1.2: population genetic software, individuals or population distance, phylogenetic trees. http://bioinformatics.org/~tryphon/poplusions/.

Liao XL, Tian H, Zhu B, Chang JB. 2016. The complete mitochondrial genome of Chinese sturgeon (*Acipenser sinensis*). *Mitochondrial DNA Part A, 27*(1): 328–329.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics, 25*(11): 1451–1452.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity, 89*(3): 238–247.

Maddison DR, Maddison WP. 2011[2016-03-10]. Mesquite: a modular system for evolutionary analysis. Current release version: 3.61. http://mesquiteproject.org.

Mitton JB. 1997. Selection in Natural Populations. Oxford: Oxford University Press.

Monnerot M, Mounolou JC, Solignac M. 1984. Intra-individual length heterogeneity of Rana esculenta mitochondrial DNA. *Biologicals of the Cell, 52*(3): 213–218.

Nagy S, Poczaí P, Cernák I, Gorj AM, Hegedűs G, Taller J. 2012. PiCalc: an online program to calculate polymorphic information content for molecular genetic studies. *Biochemical Genetics, 50*(9–10): 670–672.

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. *Conservation Biology, 17*(1): 230–237.

Richard A, Dionne M, Wang JL, Bernatchez L. 2013. Does catch and release affect the mating system and individual reproductive success of wild Atlantic salmon (*Salmo salar*)? *Molecular Ecology, 22*(1): 187–200.

Serbezov D, Bernatchez L, Olsen EM, Vellestad LA. 2010. Matting patterns and determinants of individual reproductive success in brown trout (*Salmo trutta*) revealed by parentage analysis of an entire stream living population. *Molecular Ecology, 19*(15): 3193–3205.

Solignac M, Monnerot M, Mounolou JC. 1983. Mitochondrial DNA heteroplasy in Drosophila mauritiana. *Proceedings of the National Academy of Sciences of the United States of America, 80*(22): 6942–6946.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution, 30*(12): 2725–2729.

Thorstensen M, Bates P, Lepla K, Schreier A. 2019. To breed or not to breed? Maintaining genetic diversity in white sturgeon supplementation programs. *Conservation Genetics, 20*(5): 997–1007.

Thrall PH, Young A. 2000. Computer note. AUTOTET: a program for analysis of Autotetraploid genotypic data. *Journal of Heredity, 91*(4): 348–349.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes, 4*(3): 535–538.

Wang JL. 2004. Sibship reconstruction from genetic data with typing errors. *Genetics, 166*(4): 1963–1979.

Waples RS. 2016. Tiny estimates of the N_e/N ratio in marine fishes: are they real?. *Journal of Fish Biology, 89*(6): 2479–2504.

Waples RS, Grewe PM, Bravington MW, Hillary R, Feutry P. 2018. Robust estimates of a high N_e/N ratio in a top marine predator, southern bluefin tuna. *Science Advances, 4*(7): eaar7759.

Wei QW, Chen XH, Yang DG, Liu JY, Zhu YJ, Zheng WD. 2005. Variations in spawning stock structure of *Acipenser sinensis* within 24 years since damming of Gezhouba Dam. *Journal of Fishery Sciences of China, 12*(4): 452–457. (in Chinese)

Wei QW, Ke FE, Zhang JM, Zhuang P, Luo JD, Zhou RQ, Yang WH. 1997. Biology, fisheries, and conservation of sturgeons and paddlefish in China. *Environmental Biology of Fishes, 48*(1–4): 241–255.

Wei QW. 2019. Conservation Biology of Chinese Sturgeon (*Acipenser sinensis*). China: Science Press. (in Chinese)

Willi Y, Van Buskirk J, Hoffmann AA. 2006. Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics, 37*: 433–458.

Xin MM. 2015. Genetic Characteristics and Parentage Identification of Chinese Sturgeon (*Acipenser sinensis*) Based on SSR Markers. Master thesis, Southwest University, Chongqing, China.

YU ZT, Xu YG, Deng ZL. 1986. Reproductive ecology of Chinese sturgeon (*Acipenser sinensis*) in the lower reaches of Gezhouba water control project. In: Chinese Ichthyological Society. *Anthology of Ichthyology. China: Science Press. (in Chinese)*

Zhang SM, Deng H, Wang DQ, Zhang YP, Wu QJ. 1999. Mitochondrial DNA length variation and heteroplasy in Chinese sturgeon (*Acipenser sinensis*). *Acta Genetica Sinica, 26*(5): 489–496. (in Chinese)

Zhang SM, Wang DQ, Zhang YP. 2003. Mitochondrial DNA variation, effective female population size and population history of the endangered Chinese sturgeon, *Acipenser sinensis*. *Conservation Genetics, 4*(6): 673–683.

Zhao N, Qiao Y, Zhu B, Liao XL, Pan L, Chang JB. 2015. Identification ability of tetraploid microsatellite loci in parentage analysis. *Journal of Applied Ichthyology, 31*(4): 614–619.