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

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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

deleterious mutations. This can, in turn, impact adaptive potential and increase the probability of extinction (Harrisson et al., 2014; Luikart et al., 1998; Reed & Frankham, 2003; Willi et al., 2006). Christie et al. (2012) found that captive-born steelhead trout (*Oncorhynchus 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 parentage 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 sweepstakes-like chance of matching reproductive activity (i.e., sweepstakes 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'-GCCTTGCTTTGAGCTGTTTC-3' and reverse primer 5'-CAGAGGCATCACCTCCATTT-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 MgSO$_4$ (2 mmol/L of Mg^{2+}), 2 µL 10×Taq Buffer, 0.5 µL each primer (10 µmol/L), and deionized H$_2$O. 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% nondenaturing 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 (*Pi*), and other genetic information were estimated in DNASP v5.10 (Librado & Rozas, 2009).

Sturgeons are a polyploidy 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^{pro} due to a repeat motif containing 4.5 copies of an 82 bp unit (gacatgctatgtttaatccacattaatttctagccaccataccataatgttc gcaggtacattagattgttcaagtacataa). 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 (*Pi*) were 0.876 0 ±0.003 5 and 0.001 1±0.001 0, 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.

Table 1 Summary statistics for complete mitochondrial genome in Chinese sturgeon juveniles

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

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 marker ranging from 3 to 13 (Table 2). The mean±*SE* allele number observed was 8.810±2.713. The mean±*SE* levels of observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were 0.728±0.211 and 0.779±0.122, respectively. 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 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

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
Spl-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
AS11-43	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.

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±0.0035; Pi: 0.0011±0.0010) 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

Table 3 Information on pedigree based on sibling relationships in wild juvenile Chinese sturgeon

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	0.6546	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.
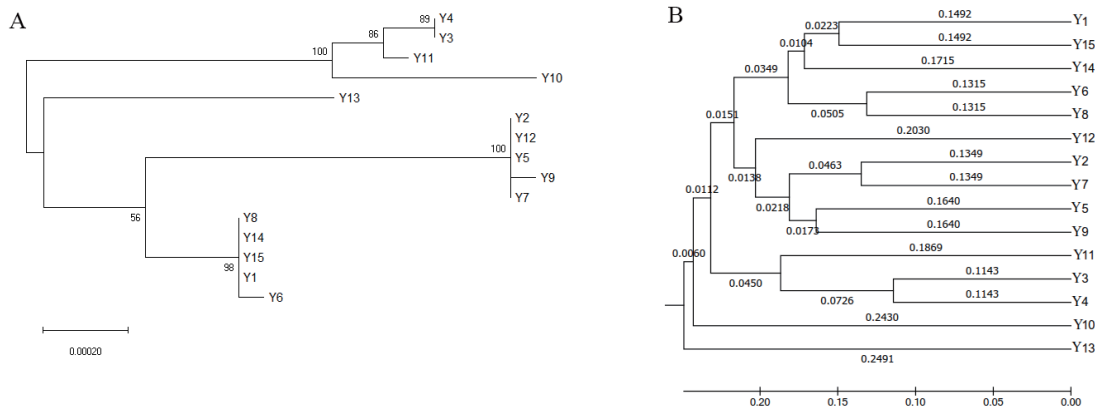

Figure 1 Phylogenetic tree based on SSR and mtDNA genome data using two methods

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.

Table 4 Parameters of significance tests among parents with two offspring and one offspring, and uniformity test between parent and offspring

Statistical method	N	X^2	P
Significance test between parent with different offspring	11	0.818	0.366
Uniformity test between parent and offspring	15	1.876	0.977

N: Number of samples analyzed in two statistical methods; X^2: Test statistic *Chi*-square value; P: Probability of test significance and uniformity.

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* L., 1766 (Bermingham et al., 1986), *Alosa sapidissima* Wilson, 1811 (Bentzen et al., 1989), 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; Mitton, 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.

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