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Short communication

Evidence for female heterogametic sex determination in paddlefish *Polyodon spathula* based on gynogenesis

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ABSTRACT

A cohort of gynogenote paddlefish was stocked in a 26-ha lake and harvested nine years later. The sex ratio of these mature fish based on gonadal examination indicated that paddlefish female sex determination is heterogametic rather than the previously reported homogamety. Both sexually mature males and females were present ($n = 177$). The observed sex ratio of 19.8% male: 80.2% female is consistent with the female heterogamety model and strengthens the emerging pattern in Acipenseriform fishes.

1. Introduction

Some of the most valuable commercial fishes in the world are the Acipenseriforms (sturgeons and paddlefish). Their high quality meat has been in demand for centuries, but the darkly pigmented eggs are even more treasured as caviar. These fishes are long-lived and grow to large sizes, two characteristics that account for their vulnerability to overfishing. The Caspian Sea fishery has been the source of the majority of the caviar traded internationally; however, this fishery has been drastically over-exploited recently and is now being managed under CITES (Convention for International Trade in Endangered Species) regulations (Mims et al., 2009). Culture of Acipenseriform fishes could reduce pressure by providing a supplemental supply of these products, and all-female culture would facilitate meeting the demand for caviar.

Culture techniques for the American paddlefish *Polyodon spathula* have been primarily applied to restocking programs (Graham, 1986; Paukert and Scholten, 2009). Propagation for food fish production and development of a program for all-female culture have been the long-term goals of a series of investigations in the U.S. (Mims and Shelton, 2005). In this program, special methods have been developed that manipulate the genetic and phenotypic sex through gynogenetic induction and steroid-induced sex reversal, respectively (Mims et al., 1997; Shelton, 2006; Shelton and Mims, 2003; Shelton et al., 1997). Sex determination in vertebrates is characterized by genetic models of either female homogamety (XX) and male heterogamety (XY) or male homogamety (ZZ) and female heterogamety (WZ) — both systems are present in fishes (Devlin and Nagahama, 2002; Pandian, 1999). An earlier hypothesis was that paddlefish sex determination was based on homogametic females, so that gynogenetic induction would produce only female progeny (Mims and Shelton, 2005).

The gynogenetic treatment protocol was developed in 1994 and 1995 (Mims et al., 1997). Initial evaluation of progeny sex in this study was done with histological examination of 70-week-old juvenile control and gynogenote paddlefish. The sex ratios of control progeny from the 1994 and 1995 year classes were 24♀:26♂ and 27♀:23♂, respectively. A cohort of gynogenote progeny was also examined at 70 weeks-of-age, and all 45 were identified as females, largely based on the developing lamellae in the anatomically differentiating gonads. However, a group of maturing gynogenote paddlefish was sampled 10 years later and contained a significant number of males. Therefore, we have revised our original hypothesis of female homogamety and modified our long-term management objectives; further, it is important to correct our earlier erroneous report.

2. Materials and methods

Mature female paddlefish were ovulated using an injection of 100 μg/kg of LH-RHa, and gynogenetic development was induced by activating the eggs with heterologous, genetically inactivated spermatozoa (Mims and Shelton, 2005; Mims et al., 1997). UV-treated spermatozoa from shovelnose sturgeon *Scaphirhynchus platorynchus* were used to activate the ovulated paddlefish eggs. The eggs were incubated for 18 min after activation at 18 °C ($0.26\tau_0$), then transferred to water of 35 °C for 2 min to interrupt the expulsion of the 2nd

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meiotic polar body (Mims et al., 1997; Shelton, 2006; Shelton et al., 1997). This protocol ensured that all diploid paddlefish larvae would have only a maternal-genome.

In the present study, the gynogenotes were produced in 1996, grown in ponds as in the 1994 and 1995 year classes, then at the end of a 5-month nursery phase, they were transferred to a larger lake for growout. The 26-ha strip-pit lake was located on private land in southern Indiana; no paddlefish had previously been stocked, and there was no connection to other surface waters. Three-hundred gynogenotes at an average size of 30 cm TL were stocked at 11/ha. Fish were harvested nine years later over a 3-day period (20–22 February 2005) using six 100-m, 12.5-cm bar-mesh gill nets. Fish were weighed, measured and gonadal condition was examined macroscopically in the field.

3. Results

Total harvest was 1765 kg, or a standing stock of 67.1 kg/ha; 181 fish ranged from 6.5 to 21.1 kg with a modal size of 12.2–14.5 kg. Survival from stocking was at least 60%, as some fish probably were not captured. Growth of these first generation gynogenotes (Gf_1) was comparable to that of other reservoir populations in Oklahoma and Missouri with similar growing seasons (Graham, 1986; Scarnecchia et al., 2011). It was expected that all fish would be females based on the putative XX-female genotype; however, a significant number (35) were males (Table 1). These were sexually mature and all had well-formed testes. Gonads of four fish were insufficiently developed to be sexed and are not included in the sex ratio data. A total of 142 fish were females, seventy-three had well formed ovaries with mature, pigmented eggs; however, sixty-nine had ovaries with well developed laminar folds, but without vitellogenic, or pigmented ova. Females begin to mature at 8–10 years in the mid-latitudes and all mature by 12–16 years-of-age, consequently, the undeveloped female gynogenotes in this study might have been approaching their initial maturation; the GSI for early maturing females is between 15 and 16%, but reaches 24–25% in 14–15 year-old-fish in this region (Scarnecchia et al., 2011). Mean gonadosomatic index (GSI) for mature gynogenetic females was 10.9%, or 7.5% for processed eggs; GSI was not calculated for the pre-vitellogenic females or males.

4. Discussion

The presence of males, and the sex ratio of 142 females: 35 males are inconsistent with the expected phenotypic sex of 2nd Pb-gynogenetic progeny of homogametic females (XX). On the other hand, gynogenetic progeny of a heterogametic female (WZ) would include some males, and further, the sex ratio would differ from a 1:1 [ZZ (males) and WW ('superfemales')] because of crossing over and recombination of the homologous chromosome fragments during the first meiotic cycle. The level of homozygosity in meiotic gynogenesis is limited by the frequency of crossing over (Recoubratsky et al., 2003). For heterozygous females (WZ) with a gene near the centromere, suppression of 2nd pb extrusion would result in predominately homozygous progeny, but as the distance between the centromere and the sex-determining element increases, a greater proportion of the progeny will be heterozygous (Thorgaard, 1983).

Table 1
Gonadal sex of 9-year-old gynogenetic paddlefish*.

	Mature ♀♀	Non-mature ♀♀	Total ♀♀	Total ♂♂
Number	73	69	142	35
Percent	41.2	39.0	80.2	19.8

* Stocked 300 gynogenotes @ 30 cm TL 6 September 1996 in 26.3 ha lake; harvested 181 fish 20-22 February 2005, four could not be sexed and are not included; mean Female weight = 14.5 kg, male weight = 12.3 kg.

Recent gynogenetic studies of sex determination in several Acipenseriform species have demonstrated that the sexual genotype of these sturgeon females is WZ. Van Eenennaam et al. (1999) reported that of 123 gynogenetic white sturgeon *Acipenser transmontanus*, 82% were females and 18% were males, suggesting that the sex-determining element was segregating independent of the centromere and that about 16% of the females might be WW. Omoto et al. (2005) found that in groups of gynogenetic progeny of bester (*Huso huso* female X *Acipenser ruthenus* male) 74–80% were females and 13–30% were males (4–6% could not be sexed). Flynn et al. (2006) reported that the sex ratio for gynogenetic progeny of shortnose sturgeon *Acipenser brevirostrum* was 65% females and 35% males. Recoubratsky et al. (2003) have conducted an extensive gynogenetic study for the Russian sturgeon *Acipenser gueldenstaedtii*, but sex ratio information has not yet been published. With the present data for paddlefish, it is clear that sex determination among Acipenseriforms conforms to the WZ-female model. Our earlier report that only females were identified in a gynogenetic group is troubling, but the sample size was small, and the gonads were examined early in the differentiating process. Ovaries in paddlefish are anatomically distinct at about 70-weeks of age, but testes are not as clearly identifiable; further, considering that in a sample size of 45, fewer than eight or nine males might be expected.

The proportion of gynogenetic male paddlefish in the present study (19.8%) was similar to those in the recent Acipenseriform studies. The females in this Gf_1 population should consist of WW and WZ genotypes, with the latter being the result of crossing over and recombination of chromosome fragments (Van Eenennaam et al., 1999); however, the fertility of females with these two genotypes is not yet known. The number of females observed with non-matured ovaries complicates interpretation, but it is probable that these might have developed vitellogenic eggs within the next few years (Scarnecchia et al., 2011); nevertheless, the data provide convincing evidence for heterogametic sexual genotype of female paddlefish. Identification and demonstration of the fertility of WW-females will be a major milestone to advance our monosex breeding program.

Gynogenote female progeny testing will be conducted during the 2012 spawning season. Identification of WZ- and WW-females will be examined; we will induce 2nd pb-gynogenesis using our established protocol to produce a second generation of gynogenotes (Gf_2). In our research program we have produced several year classes of gynogenetic (Gf_1) paddlefish, beginning in 1995 and continuing through 2009. We presently have two year classes (1997 and 2000) of sexually mature paddlefish that were produced through gynogenesis. Assuming fertility of both gynogenetic WZ and WW-females, gynogenetic progeny of WW-females will all be females, while progeny of WZ-females will include males in a similar proportion to that in the Gf_1 groups. While an alternative option to progeny test Gf_1 females is to cross test females with normal ZZ-males, the use of gynogenesis might produce a population of WW-female progeny, and these would be valuable broodstock later; potential inbreeding depression could be reduced in a production phase by using normal ZZ-males and these WW-females as broodstock.

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