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Short communication

Incidence of spontaneous autopolyploidy in cultured populations of white sturgeon, *Acipenser transmontanus*

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ABSTRACT

Many fish of aquacultural importance including sturgeon and paddlefish have a history of polyploidy in their evolutionary lineages. Spontaneous autopolyploids have been detected in several sturgeon species in culture despite the fact that the induction of triploidy is not a management tool in farming sturgeon for meat and caviar. In 2010, we discovered two female spontaneous autopolyploid white sturgeon (*Acipenser transmontanus*) on a commercial caviar farm that had successfully produced offspring of intermediate ploidy in crosses with normal males. Here we use flow cytometry and microsatellite genotyping to screen a second aquaculture program for the presence of spontaneous autopolyploids. Flow cytometry of female parents and their progeny in the 2011 year class created by the Kootenai River white sturgeon conservation aquaculture program revealed that wild caught females used as dams possessed normal ploidy (8N). Five of ten families sampled from the 2011 year class contained spontaneous autopolyploids possessing DNA content consistent with dodecaploidy (12N). The percent of autopolyploid individuals sampled ranged from 0 to 33% per family, and the total number of spontaneous autopolyploids in the 2011 year class was 12/150 (8%). Microsatellites were not useful in detecting spontaneous autopolyploids in the conservation aquaculture program due to low levels of genetic diversity in the endangered Kootenai River population. The two families with the highest incidence of spontaneous autopolyploids (33% and 26% of offspring sampled) were sired by a single male, suggesting a paternal effect on the generation of spontaneous autopolyploids in white sturgeon.

1. Introduction

Recent phylogenetic discoveries indicate that a whole genome duplication event occurred prior to the radiation of the ray finned fishes ~320–350 million years ago (Van de Peer, 2004; Vandepoele et al., 2004). Therefore, many fish species of aquacultural importance have experienced one or more polyploid events in their evolutionary history. Sturgeon and paddlefish, commonly farmed for meat and caviar, experienced one to several additional genome duplication events during the evolution of the Acipenseriform lineage (Peng et al., 2007). Although the genomes of other evolutionary polyploid fishes such as salmonids exhibit only residual polysomy (Allendorf and Thorgaard, 1984), many sturgeon species show evidence of functional polyploidy (Fontana, 2002).

Spontaneous autopolyploidy, or spontaneous genome duplication within a species in the absence of hybridization, has been detected in aquaculture programs for several sturgeon species. Spontaneous triploids with a genome size 1.5 times larger than the normal genome size has been reported for bester (*Huso huso* × *Acipenser ruthenus*), white sturgeon (*Acipenser transmontanus*), Siberian sturgeon (*Acipenser baerii*), sterlet (*A. ruthenus*), and Sakhalin sturgeon (*Acipenser mikadoi*) (Drauch Schreier et al., 2011; Havelka et al., 2013; Omoto et al., 2005; Pšenička et al., 2011; Zhou et al., 2011, 2013). Although the induction of triploidy is a tool commonly used in fish and shellfish culture to improve product quality (Piferrer et al., 2009), sturgeon farmers do not manipulate genome size and therefore genome duplications within individuals are unintentional and undesirable.

In many fish species, triploidy results in sterility or otherwise negatively affects fertility (reviewed in Piferrer et al., 2009), which would be detrimental for a fish farm that maintained a self-perpetuating broodstock. A larger genome size may increase ovum size or nucleus size and consequently modify caviar characteristics (Otto, 2007). Endangered populations or species of sturgeon, including white, lake and shortnose sturgeon, rely on conservation aquaculture for their persistence (Ireland et al., 2002; Peterson et al., 2006; Smith et al., 2002a, 2002b) and may be affected if spontaneous autopolyploids released from hatchery programs exhibited reduced individual fitness due to lowered fertility in F_2 or later generations. Therefore, characterization of the rate at which spontaneous autopolyploids are produced in sturgeon culture is imperative for commercial and conservation applications.

The white sturgeon is an evolutionary octoploid (8N), possessing ~240 chromosomes (Birstein, 2005; Hedrick et al., 1991). While conducting an inheritance study using crosses from a white sturgeon

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commercial caviar farm in California, we detected two spontaneous autopolyploid (12N) females that had mated with normal (8N) males that produced viable offspring of intermediate ploidy (putative 10N; Drauch Schreier et al., 2011). Although the spontaneous autopolyploid females appeared to exhibit normal fertility, the reproductive success of their offspring is unknown. The presence of 10N individuals in a captive broodstock could undermine the continuity of a commercial aquaculture population.

Both white sturgeon commercial farms and conservation aquaculture programs employ similar artificial spawning techniques and it is at this stage of culture that spontaneous autopolyploids are most likely to be produced. Could the unintentional induction of spontaneous autopolyploidy be occurring in other white sturgeon hatchery facilities, including conservation aquaculture programs? To determine whether the spontaneous autopolyploidy observed in the caviar farm was an isolated incident or was occurring on a larger scale in white sturgeon culture, we measured genome sizes of parents and offspring in controlled crosses made by a white sturgeon conservation aquaculture program in 2011. We utilized flow cytometry and microsatellite genotyping to test for spontaneous genome duplication in parents and progeny. Our detection of spontaneous autopolyploids in a second white sturgeon aquaculture program indicates that some aspect of artificial spawning is producing white sturgeon progeny of abnormal ploidy and we suggest that this phenomenon may be occurring in other aquaculture or conservation programs.

2. Materials and methods

2.1. Sample collection

We conducted genome size monitoring in the Kootenai River white sturgeon conservation aquaculture program to ascertain whether spontaneous autopolyploids were unknowingly being produced at this facility. Blood and/or fin tissue samples were taken from wild parents captured in 2011 and their offspring. Blood for flow cytometry was collected from six of seven wild Kootenai River white sturgeon females captured and held in the hatchery for use as broodstock in 2011. Fin tissue for genotyping was collected from all seven female broodstock. We were unable to obtain blood samples from wild males used as sires for the families we examined as none were held in the hatchery in 2011. However, we did obtain fin tissue from seven males used to create the families examined in the 2011 year class. A power analysis indicated that a minimum of ten samples was necessary to detect spontaneous autopolyploidy if it had occurred within a family. We collected both blood and fin tissues from 15 offspring from each of ten families in the 2011 year class, for a total of 150 samples. Sampling of offspring occurred just before their release in 2012.

Sexually mature wild white sturgeon females were captured in the Kootenai River by Kootenai River Tribal Hatchery (KRTH) biologists between March and May 2011 and held in the KRTH until spawning occurred. Egg samples were taken periodically from female white sturgeon in the hatchery to examine polarity index (PI), a metric of germinal vesicle migration that indicates ovulatory readiness. When a female's PI was ≤ 0.1, an injection of LHRHa was administered as per the recommendations of Conte (1988) to promote ovulation. When females were ready for hormone injection, KRTH biologists captured wild white sturgeon males (between May and June) and ~120 mL of milt was collected from each of these males in situ (C. Lewandowski, KRTH, pers. comm.). Milt from each male was stored in two separate plastic bags with oxygen (~60 mL/bag) and transported back to the hatchery held on ice and wet newspaper until females had ovulated (C. Lewandowski, KRTH, pers. comm.). Milt from each male (10 mL) was mixed in 1 L of water in separate batches of ~10,000 eggs from one or several females. Spawning conditions for the production of the 2011 year class are summarized in Table 1. Each full sibling family was reared separately in the KRTH until release in fall of 2012.

2.2. Flow cytometry

Blood for flow cytometry was collected and prepared according to the methods described by Blacklidge and Bidwell (1993) with modifications. Specifically, 250–500 μl of blood was drawn from six 2011 female broodstock by caudal vein puncture using 18 × 1-1/2 A needles primed with acid citrate dextrose (ACD). A similar procedure was followed for age-1 offspring, except 22 × 1 A needles were used. Blood was transferred to a 5 ml polystyrene tube containing 1 ml ACD and gently mixed. Samples were allowed to set overnight on ice in a 4 °C refrigerator. Approximately 25 μl of red blood cells (RBCs) were transferred to a new tube containing 1 ml ACD and gently mixed. The concentration of RBCs was calculated for each sample by cell counting using a hemacytometer and Trypan blue. The RBC volume that corresponded to 4.0×10^6 cells was transferred to a new tube containing 4 ml phosphate buffered saline and gently mixed. Approximately 500 μl of the cell suspension (~1.0×10^6 cells/ml) was transferred to a new tube and mixed with 1.0 ml Vindelov's propidium iodide solution (Vindelov's PI: 121 mg Tris, 58.4 mg NaCl, 1 mg RNase DNase-free, 5.01 mg propidium iodide, and 0.1 ml Nonidet P-40 in 100 ml distilled water) and incubated on ice in the dark for 30 min. Sample fluorescence was immediately analyzed using a FACSAria (BD Biosciences, San Jose, CA) at the University of Idaho at Moscow Optical Imaging Core Facility. Data were analyzed using FloJo software (Tree Star, San Carlos, CA).

Two drops of Trout Erythrocyte Nuclei (TEN; BioSure® Inc., Grass Valley, CA) were similarly prepared and included in the analysis as an internal DNA content standard; a value of 5.240 pg DNA/cell was assigned according to the specifications of the manufacturer. The DNA content of each experimental sample was estimated by comparing the resulting fluorescence to the fluorescence and genome size of the TEN control by the equation DNA (pg) = 5.24 × (S/T), where S and T

Table 1
Spawning conditions for the Kootenai River white sturgeon conservation aquaculture program 2011 year class. Families are named after the last four digits of the paternal parent PIT tag ID. Time to ovulation refers to the time elapsed between the injection of LHRHa and ovulation. Injection temp refers to the water temperature at the time of hormone injection and spawning temp refers to water temperature at fertilization.

Date	Family	Time to ovulation (h)	Injection temp (°C)	Spawning temp (°C)	Spontaneous autopolyploids?
5/13/2011	B4DA[ab]	50	8–14	14	Y
6/1/2011	156A	24.5	10–14	14	Y
	156A-2	24.5	10–14	14	Y
6/3/2011	6870	22	9–14	14	N
6/8/2011	5A41	31	11–14	14	N
6/14/11	2434	21.5	14	14	Y
	4C2E	21.5	14	14	N
6/15/11	2434-2	21	14	14	Y
	4C2E-2	21	14	14	N

[a] The female parent of this family experienced ambient water temperatures in the Kootenai River during spawning induction while other females experienced intentionally increased water temperatures to accelerate ovulation.
[b] Blood was unavailable from this female.

Table 2
Number of normal (8N) and spontaneous autopolyploid (12N) individuals per family in the Kootenai River 2011 conservation aquaculture year class and corresponding nuclear DNA content (pg). Five sampled families contained one to several spontaneous autopolyploids.

Family	Male parent	Female parent	No. 8N individuals	8N range (pg)	8N mean (pg)	No. 12N individuals	12N Range (pg)	12N Mean (pg)
6870	6870	2923	15	7.4–8.5	8.2	0	–	–
6870-2	6870	AC47	15	8.2–9.1	8.7	0	–	–
5A41	5A41	AC47	15	7.7–8.8	8.4	0	–	–
156A	156A	5E6D	11	8.3–8.8	8.6	4	13.0–13.2	12.4
156A-2	156A	E703	10	8.2–8.6	8.4	5	11.9–13.6	13.2
2434	2434	AEFC	14	8.0–9.2	8.5	1	12.3	–
2434-2	2434	2E93	14	8.1–9.1	8.5	1	12.8	–
4C2E	4C2E	AEFC	15	8.1–9.0	8.7	0	–	–
4C2E-2	4C2E	2E93	15	8.2–8.9	8.5	0	–	–
B4DA	B4DA	C6EB	14	7.8–8.8	8.5	1	12.3	–

correspond to the mean fluorescence values of the white sturgeon RBCs and TEN respectively.

2.3. Microsatellite genotyping

We wished to determine if microsatellites could be used to detect spontaneous autopolyploids in the Kootenai River conservation aquaculture program. DNA was extracted from white sturgeon fin tissue using a BioRobot Universal System (Qiagen). PCR was conducted with fluorescently labeled primers for thirteen microsatellite loci: AciG 2, AciG 35, AciG 52, AciG 53, AciG 110, AciG 140, As015, Atr 105, Atr 107, Atr 109, Atr 117, Atr 1101, Atr 1173 (Börk et al., 2008; Rodzen and May, 2002; Zhu et al., 2005). PCR reactions and thermal profiles for these loci have been published elsewhere (Drauch Schreier et al., 2012). PCR product was combined with 8.85 μl of highly deionized formamide (Gel Company) and 0.15 μl of Rox 400 HD size standard (Life Technologies). Genotyping was conducted on a 3730xl Genetic Analyzer (Life Technologies) using GeneMapper v.4.0 software (Life Technologies).

We calculated the number of alleles per locus for all 2011 broodstock and age-1 fish, assuming that spontaneous autopolyploid individuals may have >4 or >8 alleles at four and eight copy loci, respectively (Drauch Schreier et al., 2011). We also looked for differences in the mean number of alleles per locus possessed by 8N offspring and 12N autopolyploids in families 156A and 156A-2. Next, we calculated the number of alleles (across all loci) detected in normal 8N and putative 12N offspring in the two families containing several spontaneous autopolyploid offspring (156A, 156A-2). Two sample *t*-tests were used to examine differences in mean numbers of alleles per locus and mean total number of alleles between 8N and 12N individuals, with the harmonic mean utilized to account for differences in sample size between 8N and 12N individuals (Sokal and Rohlf, 1981). A sequential Bonferroni correction was applied to account for multiple comparisons when examining differences at each of the thirteen loci (Rice, 1989).

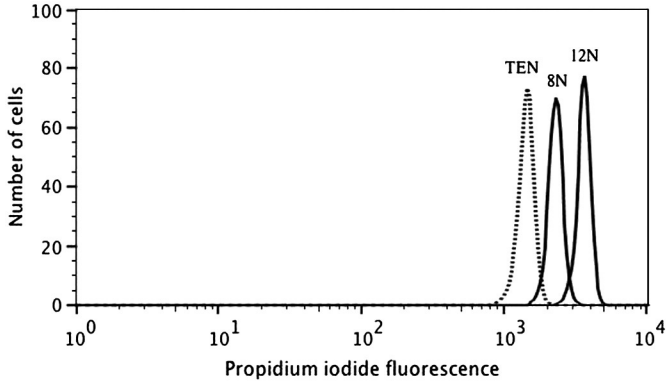

Fig. 1. Flow cytometry histogram illustrating the number of cells counted (*Y*-axis) and corresponding fluorescence (*X*-axis) of the TEN control (dotted line) and a putative 8N and 12N individual.

3. Results

3.1. Flow cytometry

Measurements of mean nuclear DNA content were used to make inferences regarding total genome size and ploidy class of white sturgeon families (representative flow cytometry histogram of relative levels of DNA fluorescence are shown in Fig. 1). The six female wild white sturgeon parents we were able to examine exhibited normal genome sizes (8.7, 8.8, 8.8, 8.9, 9.0, 9.2; mean 8.9 pg). Individuals belonging to five 2011 year class families (6870, 5A41, 6870-2, 4C2E, and 4C2E-2) also displayed genome sizes consistent with that of octoploid-derived (8N) sturgeon species (7.4–8.1 pg; mean 8.5 pg; Table 2; Drauch Schreier et al., 2011). Several individuals of the five remaining families (156A, 156A-2, 2434, 2434-2, and B4DA) showed evidence of a genome size greater than 8N, consistent with that of the dodecaploid-derived (12N) species (11.9–13.6 pg; mean 12.7 pg; Table 2; Blacklidge and Bidwell, 1993). In both cases in which male 156A was a parent, a high proportion of spontaneous autopolyploids were produced (33% and 26% in families 156A and 156A-2 respectively; Table 2). Dodecaploid genome size was also detected in both families in which male 2434 was a parent (Table 2).

3.2. Microsatellite genotyping

Microsatellites were not effective at detecting spontaneous autopolyploids in the conservation aquaculture program. No individuals possessed >4 or >8 alleles at four copy or eight copy microsatellites, respectively. Prior to applying the sequential Bonferroni correction, the number of loci at which spontaneous autopolyploids possessed a significantly greater mean number of alleles per locus than siblings of normal ploidy was 8/13 and 4/13 for families 156A and 156A-2, respectively. When the correction for multiple comparisons was applied, the number of loci with significant differences was reduced to 4/13 and 3/13 (Fig. 2). In family 156A, 12N individuals possessed a greater mean total number of alleles than 8N individuals but this difference was not significant at $\alpha = 0.05$ (44.2 and 39.8 alleles, respectively; two tailed, df = 13, P = 0.09). A significantly greater number of mean total alleles was revealed in 12N individuals in family 156A-2 relative to 8N individuals (42.2 and 37.4 alleles, respectively; $\alpha = 0.05$; two tailed, df = 13, P = 0.02).

4. Discussion

4.1. Discovery of spontaneous autopolyploids

The genome sizes reported here for white sturgeon fall within the range reported by others in the literature. Blacklidge and Bidwell (1993) report a mean nuclear DNA content of white sturgeon of 9.463 ± 0.043 pg, very similar to what we report for 2011 female broodstock. The 2011 year class offspring had slightly lower mean DNA content than the 2011 broodstock and that reported for white

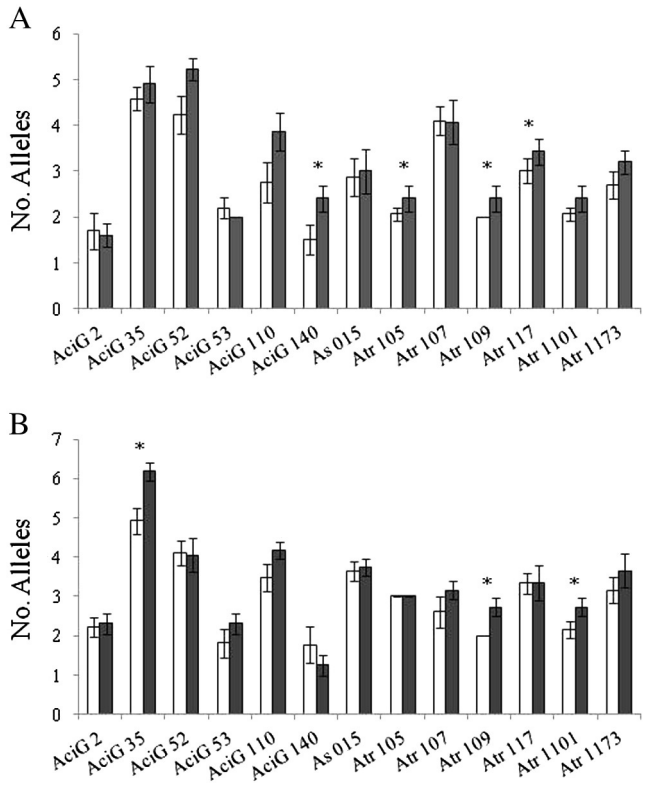

Fig. 2. Comparison of numbers of microsatellite alleles per locus between normal ploidy fish (white bars) and spontaneous autopolyploids (gray bars) in families A) 156A and B) 156A-2. Asterisks denote loci at which spontaneous autopolyploids had a significantly greater number of alleles after a sequential Bonferroni correction of $\alpha = 0.05$.

sturgeon in Blacklidge and Bidwell (1993). However, the 2011 year class DNA content was similar to the values reported for 8N white sturgeon sampled randomly at a caviar farm (8.5–9.2 pg; mean 8.8 pg; Drauch Schreier et al., 2011) and those reported by Zhou et al. (2011) (8.7–9.1; mean 9.0 pg). Although flow cytometry for both the 2011 broodstock and 2011 year class offspring were conducted at the same core facility, the samples were analyzed on separate days with separate batches of propidium iodide. Others have observed variability in DNA content measurements within ploidy classes in samples analyzed at different times. Significant interday variation in the magnitude of the coefficient of variance (CV) around cell cycle peaks in a flow cytometric analysis of largemouth bass DNA content was reported by Fisher et al. (1994). In accordance with Fisher et al. (1994), we attribute the differences in DNA content between 8N parents and 8N offspring analyzed on separate dates to slight differences in instrument parameters and staining procedures among days.

The spontaneous autopolyploids detected in five different families had similar genome sizes that were approximately 1.5 times that of their 8N siblings (Table 2). Genome sizes of spontaneous autopolyploid white sturgeon were similar to those reported for spontaneous autopolyploid Sakhalin sturgeon (12.7–13.0 pg; mean 12.8 pg; Zhou et al., 2013) as well as the evolutionary dodecaploid (12N) shortnose sturgeon (*Acipenser brevirostrum*), which possesses 360 chromosomes (mean 13.1 pg; Blacklidge and Bidwell, 1993). The randomly sampled 10N individuals detected on the caviar farm possessed a genome size intermediate to the putative 8N and 12N individuals reported here (10.4–11.4 pg; mean 10.9 pg; Drauch Schreier et al., 2011).

4.2. Microsatellite detection of spontaneous autopolyploids

Our data indicate that microsatellites are not useful for detecting spontaneous autopolyploids in the Kootenai River white sturgeon population. Previously we successfully detected both 12N and 10N individuals with microsatellites in a caviar farm population that had a fairly high level of genetic diversity (Drauch Schreier et al., 2011). The Kootenai River white sturgeon population, which is the broodstock source of the conservation aquaculture program, has a very low level of genetic diversity relative to other white sturgeon populations due to a founder effect and isolation from gene flow for 10,000 yrs (Drauch Schreier et al., 2012; Northcote, 1973). Instead of possessing >4 or >8 different alleles at four and eight copy microsatellites, spontaneous autopolyploids from the Kootenai River have multiple copies of the same alleles making them virtually indistinguishable from normal 8N individuals using microsatellite fragment analysis. Direct genome size analysis through flow cytometry or karyotyping may be necessary to identify spontaneous autopolyploids in aquaculture populations.

4.3. Cause(s) of spontaneous autopolyploidy

There does not appear to be any clear relationship between environmental variables measured during 2011 spawning and the tendency for producing spontaneous autopolyploids (Table 1). Extremes of cold and warm temperatures may produce triploid fish by inhibiting the second meiotic division of oocytes and blocking extrusion of the second polar body (Piferrer et al., 2009). We found that spontaneous autopolyploids were produced on four of six spawning dates where females experienced water temperatures ranging from 8–14 °C, which were ambient Kootenai River temperatures (Table 1). On some spawning dates, all families we examined contained autopolyploids. Other dates produced some families containing autopolyploids and others not containing autopolyploids, indicating a parental effect on the incidence of spontaneous genome duplication (Table 1). Variability in triploid production among families in hatchery programs for European catfish (*Siluris glanis*; Várkonyi et al., 1998) and rainbow trout (*Oncorhynchus mykiss*; Haffray et al., 2012) support a parental effect on the generation of spontaneous autopolyploids.

Suppression of the second meiotic division and retention of the second polar body in oocytes due to post-ovulatory aging is a maternal effect that has been shown to produce spontaneous autopolyploids. Studies of the effects of post-ovulatory aging have shown a correlation between oocyte aging time and the incidence of spontaneous autotriploids in European catfish (Várkonyi et al., 1998), tench (*Tinca tinca*; Flajšhans et al., 2007), rainbow trout (Aegerter and Jalabert, 2004) and bester (Omoto et al., 2005). Although spontaneous autopolyploids were produced by the female white sturgeon parent with a prolonged time between hormonal induction and ovulation, females with much shorter times to ovulation also produced spontaneous autopolyploids (Table 1). Unexpectedly, our results suggest that the incidence of spontaneous autopolyploidy is to be linked to the male parent. Families 156A and 156A-2, which contained the greatest number of spontaneous autopolyploids, were produced by male 156A and two different female parents (5E6D, E703; Table 2). Families 2434 and 2434-2 represent another pair of paternal half-sibling families (one male, two females) in which spontaneous autopolyploids were detected (Table 2).

There are two possible mechanisms whereby spontaneous autopolyploidy may be instigated by the paternal parent. One is aging of sperm after ejaculation, although Yamazaki and Arai (1982; cited in Várkonyi et al., 1998) showed in rainbow trout that sperm may be aged up to 28 days and yet produce no unusual larvae when fertilizing freshly ovulated eggs. Milt from all male white sturgeon in this study was used within 48 h of collection so it is unlikely that sperm aging would be the cause of spontaneous autopolyploidy observed here. Another possibility is polyspermy. Sturgeon are unique among animals in that their eggs contain multiple micropyles at the animal pole, and therefore possess multiple places that sperm can penetrate the egg (Cherr and Clark, 1985). Theoretically, the cortical reaction that occurs in the egg after penetration of a single sperm should prevent other sperm from fertilizing an egg. However, unusually high concentrations

of sperm (10^6 sperm/egg) resulted in a low level (10%) of polyspermic fertilization of white sturgeon eggs in vitro (Cherr and Clark, 1985).

4.4. Conclusions

The incidence of spontaneous autopolyploidy in two separate white sturgeon aquaculture facilities implies that genome duplication in hatchery operations may be occurring for other Acipenseriform species. The apparent rarity of spontaneous autopolyploid fish in nature suggests that some aspect of artificial spawning may be responsible for producing spontaneous autopolyploids at a detectable rate. As spontaneous autopolyploid individuals of other fish species are often cryptic, with no phenotypic characteristics differentiating them from normal diploid conspecifics (Flajšhans et al., 2007; Leggatt and Iwama, 2004), we recommend that all commercial and conservation hatchery programs for sturgeon perform genome size screening to test for the production of spontaneous autopolyploids.

Contrary to expectation, our findings suggest that the production of spontaneous autopolyploids may be driven by a paternal effect in white sturgeon. Further experiments to determine the mechanism by which the male parent may induce autopolyploid production would allow white sturgeon aquaculture programs to develop preventative measures that would decrease spontaneous autopolyploid production.

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