Spontaneous Triploid and Intermediate Ploidy White Sturgeon in Commercial Aquaculture and Conservation Hatcheries: Potential Risks and Benefits

WESTERN REGIONAL AQUACULTURE CENTER

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Introduction

White sturgeon, *Acipenser transmontanus*, are considered evolutionary octoploids, with eight copies of each chromosome (8N). Spontaneous autopolyploids, or spontaneous triploids, originating from unintentional duplication of chromosome sets within the species, have a genome size 1.5 times larger (12N) and have been identified in both commercial aquaculture and conservation hatcheries.[1,2] Direct induction of triploidy using thermal, pressure, chemical, or electrical shock has beneficial aquaculture applications and has been used on numerous fish and shellfish species. However, since white sturgeon hatcheries were not intentionally inducing triploidy, the identification of spontaneous triploids was unforeseen. In addition, 12N white sturgeon females, which are not sterile, have been unintentionally crossed with 8N males to produce progenies of intermediate ploidy (10N). Throughout this research bulletin, we will refer to the normal ploidy white sturgeon as 8N, the triploids as 12N, and intermediate ploidy sturgeon as 10N.

Current Techniques for Determining Ploidy in Sturgeon

There are four techniques available to detect ploidy in sturgeon and other fishes: karyotyping, flow cytometry, Coulter® Counter, and blood smear analysis. Details of each technique and their advantages and disadvantages have been reviewed.[3,4]

Incidence of Spontaneous Triploids (12N)

Spontaneous triploid white sturgeon were identified on an aquaculture farm[5] and in several progenies derived from spawned wild-caught adults[1] in a conservation hatchery. What has been most remarkable is the highly variable incidence of 12Ns among different maternal families, ranging from 0% to >90%.[1,2,4,6]

However, spontaneous triploidy is not just a recently identified hatchery issue. As far back as 1992, researchers inadvertently found a spontaneous triploid in wild caught Lake sturgeon and Gulf sturgeon,[7] and subsequent investigations have identified spontaneous triploids in many other sturgeon species, including wild caught adults, captive broodstock on farms, and progenies from spawned wild-caught and captive broodstock.[4]

After the first 12Ns were identified at a commercial hatchery, discussions regarding their potential benefits were initiated, which resulted in a multi-state research project, "Determining causes, costs, and benefits of triploidization to improve sturgeon caviar production," funded by the Western Regional Aquaculture Center (WRAC).

This research bulletin addresses the potential causes of variable ploidy in sturgeon, the differences in sex ratio, growth, and physiological performances among 8N, 10N, and 12N white sturgeon; the potential benefits and risks of variable ploidies in sturgeon aquaculture and conservation hatcheries; and guidelines to reduce the frequency of spontaneous triploids.

Incidence of 12N Ploidy Broodstock

Determining the prevalence of female and male 12N broodstock on sturgeon farms in California and Idaho was one objective of this project. Over several years, on seven different farms, blood samples were collected and ploidy of all broodstock determined using flow cytometry or a Z2 Coulter® Particle Count and Size Analyzer (Beckman Coulter, Inc., Brea, CA). Four of the seven farms had at least one 12N, and out of the total 658 broodstock sampled, the overall proportion of 12N was 1.5% (n=10), and on the individual farms, the proportion of 12N varied from 0% to 3.4% (Table 1).

Incidence of Intermediate Ploidy (10N) Broodstock

A confounding issue was that a few mature 12N female white sturgeon, in years prior to broodstock ploidy verification, were inadvertently spawned on farms with normal 8N males to produce progeny of intermediate 10N ploidy. These intermediate ploidy sturgeon are viable, and some can reach sexual maturity, albeit delayed maturity in females when compared to normal 8N females.[8] Three

of the farms had at least two identified 10Ns, and out of the total 658 broodstock sampled, the proportion of 10Ns was 2.0% (n=13), and on the individual farms, it ranged from 0% to 10% (Table 1).

Causes of Spontaneous Triploidy

Induction of second polar body retention is routinely used to produce genetic triploid fish in many teleosts, and is also the most likely mechanism of spontaneous triploidy in white sturgeon hatcheries.[2] Second polar body retention has been successfully induced in white sturgeon, using a thermal shock applied at 12–15 minutes post-fertilization,[9] although currently, there are no hatcheries inducing triploidy.

During the WRAC study, two potential causes of second polar body retention were studied. The initial cause was hypothesized to be post-ovulatory aging, as it has been shown to increase rates of triploidy in numerous fishes, including bester sturgeon (*Huso huso* x *Acipenser ruthenus*), and if multiple white sturgeon females are ovulating at the same time, the delayed collection of ovulated eggs from some females could impact 12N production.[6]

Table 1. Ploidy analysis of California and Idaho captive white sturgeon broodstock during 2015–2022 at seven different aquaculture farms. Ploidy was determined by flow cytometry and/or a Z2 Coulter® Particle Count and Size Analyzer.

Farm	Sampled (month/year)	#	≈ Mean W (kg)	# 8N (%)	# 12N (%) (Sex=M/F))	# 10N (%) (Sex=M/F)
#1	3/21 & 2/22	144	31	135 (93.7%)	1-M (0.7%)	1-F (0.7%) & 7-M (4.9%)
#2	3/17	30	38	27 (90%)	0	3-F (10%)
#3	3/15 & 9/15	147	46	140 (95.2%)	5-F (3.4%)	2-M (1.4%)
#4	11/18 & 1/19	41	35	40 (97%)	1-F (3%)	0
#5	3/18 & 3/20	105	35	102 (97%)	3-F (3%)	0
#6	2/16 & 11/16	134	28	134 (100%)	0	0
#7	12/18	57	39	57 (100%)	0	0
TOTALS		658		635 (96.5%)	10 (1.5%)	13 (2.0%)

Our study found that post-ovulatory aging of eggs in vitro for 6–8 hours only increased the incidence of spontaneous triploidy in 4 out of 10 females, and the proportions of 12Ns produced was not excessive (4%, 5%, 6%, and 15%).[6] Since these experimental times were the maximum amount of aging time potentially occurring on the farms, another cause of spontaneous triploidy was investigated.

The second potential cause of 12N production was the mechanical shock of eggs during the one hour de-adhesion process, which begins after 2 minutes of egg fertilization.[6] Proportions of 12Ns in fertilized eggs exposed to a continuous vigorous de-adhesion treatment were significantly greater than that in farm controls or the gentle de-adhesion treatment in 75% of the females. Results were highly variable between females (1% to 92% 12N).[6] Our study also found cumulative effects of post-ovulatory aging and mechanical shock, which significantly increased the proportion of 12Ns relative to the controls and gentle treatments. It was hypothesized that disruption of the spindle apparatus occurred from the repeated shock of sudden acceleration and deceleration of eggs colliding with each other and the sides of the bowl when de-adhesion is too vigorous, and thus the 2nd polar body is retained.

Another potential cause for 12N production could be that some females have a genetic propensity to produce spontaneous triploids, as was reported in a study on common carp (*Cyprinus carpio*).[10] During our study, one female white sturgeon was spawned in both 2017 and 2019, and a high proportion of spontaneous triploids was found in the experimental vigorous treatment for each year—80% and 64%, respectively.[6] However, the gentle treatment in 2019 had only 4% triploids, compared to 52% in 2017. Additional research is needed to further test this hypothesis of genetic propensity to spontaneous triploidy in white sturgeon.

The very high variability in the proportion of 12Ns among families observed in both aquaculture and conservation hatcheries is most likely related to egg quality.[6] Egg quality has been reported as the probable cause of increased triploidy in other sturgeon species.[11,12] Egg quality in fishes is associated with environmental, biological, and genetic factors,[13] and the most widely discussed reasons are husbandry practices. Husbandry practices are those that are managed on farm and include the ovarian stage at the time of spawning, spawning induction techniques, and post-ovulatory aging of eggs.[13,14,15,16,17] It appears that high quality white sturgeon eggs can better withstand some post-

ovulatory aging and mechanical shock with 0% or very low proportions of spontaneous triploidy, but for lower quality eggs, aging must be avoided and de-adhesion must be very gentle to have significantly lower proportions of 12N progeny.[6]

Risks and Benefits of Spontaneous Triploid and Intermediate Ploidy Sturgeon

For Aquaculturists

Because 12N white sturgeon were found to be fertile, we evaluated the utility of triploid females as beneficial for aquaculture. These benefits include: having a female-biased sex ratio; maturity at a similar size and age as normal 8N females, with a comparable caviar yield; and most importantly, having the highly desired larger eggs (caviar), as genome size is clearly related to egg size in fishes.[18,19]

We necropsied known 12N sturgeon (n=67) at age 2 years and did find a female biased sex ratio (2.2 female : 1 male), which could be beneficial for aquaculture. While processing age 8–9-year-old females for caviar, no incidental triploids were identified. However, during our study on older (10–16 years) females processed for caviar (n=198), we found 11% and 14% were 10N and 12N, respectively, indicating a delayed sexual maturity for both ploidies (Table 2). More interesting was that 75% of the late maturing females were actually normal 8N, indicating that other factors, besides ploidy, are affecting delayed maturity in white sturgeon. What percent of a year class that will have delayed maturity is not known as different year classes of immature females are usually combined to maintain optimal stocking densities in tanks, but it is estimated to be approximately 10% of any given year class of females. While finding mature females for caviar processing from the older age groups, we also processed some of the older immature females for ploidy (Table 3), and found 61% and 4% were 10N and 12N, respectively, and 35% were normal 8N females. In this study, the older 10Ns had the lowest proportion of maturing females and the highest proportion of delayed maturity, making them detrimental to the production scheme of a caviar farm.

The dominant feature of the ovaries from older immature females, regardless of ploidy, was the fatty ovigerous folds and very large lobes of adipose tissue (Figure 1). Upon closer examination of the individual folds, some ovaries had only abundant adipose tissue, and some had the occasional small, white early vitellogenic oocyte (Figure 2). Often, in

Table 2. Caviar processing characteristics of late maturing (ages 10–16 years), different ploidy females, and for comparison the more commonly harvested 8-year-old 8N females from a northern California farm. Data are body weight (BW), egg diameter, ovary weight (OW), GSI is gonadosomatic index (GW/BWx100), and caviar yield expressed as a percent of BW and OW. Ovaries are classified as high fat (caviar yield < 50% ovary weight), medium fat (caviar yield 50–65% ovary weight), and low fat (caviar yield > 65% ovary weight). The data are means ± standard deviation and different letters denote significant differences between ploidies (P < 0.05).

	8N n=149)	10N (n=22)	12N (n=27)	CA Farm (n=95)
Body Weight (kg)	49 ± 14A	59 ± 17B	45 ± 16A	37 ± 6
Egg Diameter (mm)	3.16 ± 0.23A	3.34 ± 0.50B	3.35 ± 0.36B	n/a
Ovary Weight (kg)	7.4 ± 3.1A	10.9 ± 4.4B	8.1 ± 4.6A	4.7 ± 1.2
GSI (%)	14.9 ± 4.0A	18.3 ± 4.4B	17.6 ± 5.2B	12.9 ± 2.1
Caviar Yield (% BW)	7.8 ± 3.3A	5.9 ± 3.3B	7.0 ± 2.5AB	8.1 ± 1.9
Caviar Yield (% OW)	52.1 ± 16.5A	33.7 ± 18.2B	42.0 ± 16.6B	63.0 ± 11.3
High Fat Ovaries (%)	43A	82B	70B	13
Medium Fat Ovaries (%)	30A	18B	22B	38
Low Fat Ovaries (%)	27A	0B	8B	49

Table 3. Ploidy analysis of older (age 10–16 years) immature California white sturgeon females. Data are numbers of fish with percent of the total in parentheses. Ploidy was determined by using a Z2 Coulter® Particle Count and Size Analyzer.

Sample Year	# 8N (%)	# 10N (%)	# 12N (%)	# Totals
2017	14 (42%)	19 (58%)	0	33
2018	6 (25%)	18 (75%)	0	24
2020	7 (35%)	10 (50%)	3 (4%)	20
Totals	27 (35%)	47 (61%)	3 (4%)	77

these immature older females, there were no small white oocytes, but only oogonia and primary oocytes surrounded by numerous adipocytes that can clearly be seen upon histological examination (Figure 3). The older mature female ovaries had densely packed, large black mature oocytes (Figure 4). The older immature females with the occasional early vitellogenic oocyte had very low testosterone and estrogen concentrations compared to female white sturgeon with normal gonadal development and few adipocytes. The low steroid concentrations are likely due to the very few ovarian follicles, which produce the steroids, compared to normal females with many ovarian follicles and higher steroid levels.

The 10N and 12N females, on average, did have larger eggs than 8N females (Table 2), although it was a very small sample size compared to the thousands of 8N caviar females processed annually by the farms. Caviar yield, expressed as a percent body weight, was similar between 8N and 12N females but the 10N females had a lower yield. When yield was expressed as a percent of ovary weight, it was lower for both 10N and 12N females (Table 2). These lower yields were due to the high amount of adipose tissue in the ovaries. Both the 10N and 12N females had higher proportions of high fat ovaries, and very low proportions of low fat ovaries (Table 2). In addition, the older 8N females had lower caviar yields and higher proportions of high fat ovaries, when compared to the more typical 8-year-old 8N females processed for caviar (Table 2). In California, the ages at which caviar is usually harvested from any given year class is age 7 through age 10. It appears that females with delayed maturity have fattier ovaries, regardless of ploidy, resulting in lower caviar yields and increased labor during caviar processing. Since this study, California white sturgeon farms now tend to process immature females (no obvious small white vitellogenic eggs) for meat once they reach age 10, and this appears to be a good guideline to follow. However, white sturgeon farms in Idaho with slower growth in the cooler water need to establish their own maximum age for processing caviar (≈age 16–17).

Even though spontaneous triploids have a female-biased sex ratio and larger eggs, they may not be ideal for caviar production due to what appears to be delayed maturation, fattier ovaries, and lower caviar yields. Recently however, the youngest verified 12N female of known age was age 8, with 3.8 mm eggs and lean ovaries. Obviously, a much larger, long-term study with triploid females is required to determine what proportion of females mature at what age range and to compare their caviar yield metrics and egg size

Figure 1. Ovaries from a 13-year-old 10N white sturgeon. Forceps are laying on the fatty ovigerous folds that are attached to the large lobes of adipose tissue (smooth yellow, with pigment spots).
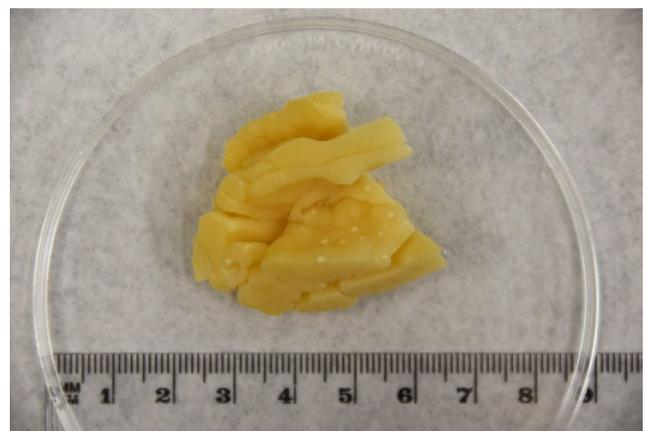

Figure 2. Pieces of ovaries from 13-year-old (top) and 14-year-old (bottom) 10N females. The top ovary has only fatty ovigerous folds, while the bottom ovary has a few scattered early vitellogenic white oocytes, surrounded by abundant adipose tissue.
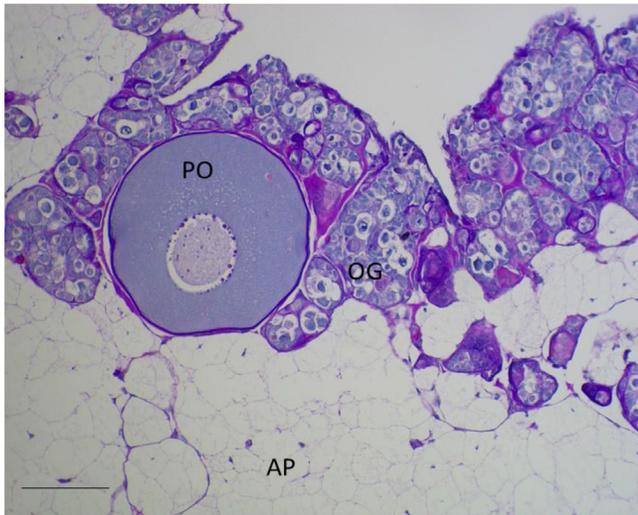
Figure 3. Histology of an immature ovary from an 8.5-year-old 10N female, stained with periodic acid schiff reagent. The ovigerous folds have clusters of oogonia (OG) in late meiotic stages around the periphery, with abundant adipocytes (AP) making up the majority of the ovigerous fold. An occasional primary oocyte (PO) in the endogenous growth phase is also seen. Scale bar is 100 microns.
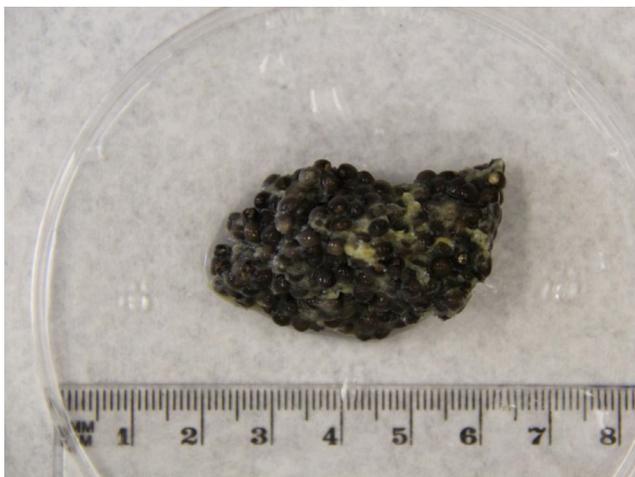

Figure 4. Ovary section from a mature 13-year-old 8N female. Closely packed, darkly pigmented oocytes, approximately 3.2 mm in diameter, with only very small amounts of yellowish adipose/ovarian tissue.

with same age 8N female cohorts. Another interesting aquaculture study would be to use heat shock on fertilized 12N female eggs to produce 16N progeny and determine how this further increase in ploidy would affect growth, survival, maturation, egg size, and caviar yield.

For Conservation Hatcheries

Spontaneous triploids and intermediate ploidy sturgeon have no potential benefits for conservation hatcheries. Since they are not sterile, the major risk is that an unidentified 12N could be released into the wild and cross with a normal 8N, resulting in 10N progeny, which could ultimately produce 9N (10N x 8N) and 11N (10N x 12N) aneuploid progenies. Aneuploid individuals would likely have reduced viability and fertility due to unbalanced chromosome numbers and impaired chromosome pairing at meiosis.[4] All these abnormal ploidies could cause serious repercussions to long-term conservation goals and recovery of an endangered sturgeon population. Reducing the risk of 10Ns can be done by determining ploidy of all broodstock and not spawning any 12N individuals. Certifying 8N ploidy of every hatchery sturgeon prior to release would further reduce the risk to existing populations. Depending on the specific conservation program and its financial and personnel resources, different management strategies may be developed to reduce or eliminate release of spontaneous triploids.

Physiology of 8N, 10N and 12N White Sturgeon

Recent availability of spontaneous triploids has also allowed several juvenile physiology studies to be completed during this project. Differences in metabolic enzyme activity in response to warm acclimation and acute stress suggest juvenile 12N white sturgeon may have a reduced cellular metabolic capacity under stress, which may impact performance of 12N sturgeon in suboptimal conditions.[20] And while both 8N and 12N white sturgeon recover similarly to exhaustive exercise, 12N sturgeon exhibited lower aerobic scope, which is the available energy of an organism for processes beyond maintenance.[21] This could be part of the reason juvenile (2-month post-hatch) 12N white sturgeon had lower growth rates, lower feed efficiencies, and a higher percentage of deformities compared to 8N sturgeon during a 15-week grow-out study.[22] Further studies at different life stages are needed to determine if this lower juvenile growth rate continues through puberty and first maturation, as our

preliminary caviar study showed the late age 12N and 8N females had a similar body size (Table 2).

Regarding intermediate ploidy sturgeon, on one farm during spring 2013, two 12N females were inadvertently induced to ovulate and were crossed with pooled 8N milt, and after hatch, the 10Ns made up approximately one-third of that year class. Because some fertilized eggs of one of these females were obtained and incubated at UC Davis, it allowed us to conduct a physiology study comparing stress and metabolism between juvenile 10Ns and normal 8N white sturgeon.[23] It was found that 8Ns and 10Ns were similar in baseline immune and stress parameters and responded comparably to warm temperature acclimation. However, some differences in cellular enzymes indicated that 10N white sturgeon had a lower cellular aerobic capacity compared to 8N sturgeon.

We also monitored 10N gametogenesis in this year class, and the males were maturing normally at age 4.5 years (10–22 kg) and would have completed spermatogenesis during the upcoming spring, at age 5 years. However, females, beginning at age 2, were found to have reduced ovary size and delayed oogenesis, compared to 8N females. The most recent sampling was at age 8.5 years (mean BW ≈ 36 kg), and histological analysis of the ovarian tissue revealed all females were still at a similar stage of delayed maturation (Figure 3). Briefly, there were clusters of oogonia in late meiotic stages, and larger oocytes at the chromatin-nucleolus stage that were close to forming a basal lamina and entering the endogenous growth stage, and all the females had the occasional larger oocyte in the early endogenous growth phase. All 10N females had many

adipocytes surrounding the regions of ovarian cells. In comparison, most normal (8N) females at age 8.5 years would have ovaries with numerous and obvious (to the naked eye) medium white oocytes to larger brown pigmenting oocytes (mid- to late stages of vitellogenesis) or mature black oocytes, in the final stages of maturation.

Reducing the Incidence of Spontaneous Triploids

Based on the research conducted during this and past WRAC-supported white sturgeon projects, the following guidelines are recommended for obtaining high quality eggs from females and reducing 12N production in hatcheries, with more details provided in a recent review.[4]

1. Induce to spawn only 8N broodstock. Any identified 10N or 12N broodstock during ploidy evaluation should be culled or tagged for future research.
2. Maintain optimal broodstock culture conditions. Broodstock should be maintained in their own tank(s) with good water quality parameters, including winter vernalization (10–11°C) and a slow warming and holding at spawning temperature (14–15°C) during spring. They should only be disturbed for sampling to determine stage of maturity.
3. Induce spawning at the optimal stage of maturity. The stage of final maturity in female sturgeon is expressed as the polarization index (PI), or the distance of the germinal vesicle from the animal pole divided by the egg diameter along the animal-vegetal axis.[24] The optimal PI for captive white sturgeon is between 0.06–0.08,[24] and it has been reported that a PI of less than 0.05 could be overripe for some sturgeon[25, 26] and may lead to an increase in spontaneous triploids. However, if a conservation hatchery program recovers a female from the river at a low PI (<0.05), spawning induction soon after capture would likely be highly successful, as she has been exposed to environmental cues and is more naturally progressing through final maturation.
4. Start injections soon after the last stage of maturity (PI) check. When the optimal stage of maturity has been reached, a female white sturgeon should be induced to spawn within 7–14 days of that last PI check, while being held at water temperatures ≈ 15 °C.
5. Hold an injected female and male(s) together. Males and females are usually kept separately during spawning induction for convenience. Females alone may not display spawning behavior, and unreleased ovulated

eggs can accumulate in the body cavity, resulting in in vivo aging of oocytes. In fact, over the years of spawning on numerous farms, it has been observed at least a dozen times that some females held alone have fully ovulated but did not release any eggs.[27] Although no directed study has examined whether a higher incidence of spontaneous triploidy occurred in the progeny of these females, it makes common sense to try and mimic what occurs in nature. The holding of males and females together could help initiate spontaneous ovulation and spermiation through chemocommunication,[28] and other pheromonal communication that regulates reproductive behavior.[29,30,31,32] Wild-caught broodstock captured on known spawning grounds and injected within days or a couple weeks after capture are likely cued to spawn and holding them separately, or together, may not be a critical issue.

6. Minimize post-ovulatory aging. At commercial aquaculture hatcheries, once the first ovulated eggs (oviposition) are observed on the bottom of the spawning tank, the collection of eggs via caesarian-section should occur within 1–4 hours. The holding tank needs to be checked hourly, and checking should begin a couple hours before anticipated oviposition. If, upon first check, there are thousands of eggs on the bottom of the holding tank, you can only guess when oviposition began. At conservation hatcheries, the first stripping of eggs should be attempted about 2 hours after first oviposition. Some females readily release eggs at the first strip, while some need to be rested and re-stripped. Depending on the female, stripping can occur several more times, but should be stopped at approximately 6 hours after initial oviposition.

7. De-adhesion should be slow and gentle. During de-adhesion, use a feather for small quantities of eggs, but a hand is usually most efficient for stirring the larger bowls of eggs collected at commercial aquaculture hatcheries. The first few minutes of de-adhesion may need to be continuous and a bit more than gentle, especially if there were any eggs that started to stick to the sides of the bowl, but after 3–4 minutes you can start to be very slow, and just very gently fold the eggs and silt from off the bottom of the bowl approximately every 5–10 seconds.[6] If you feel eggs beginning to clump together, then use a slow and gentle, but continuous, mixing. Often the eggs are most sticky during approximately the first five minutes of de-adhesion, and the more eggs you have in a bowl the more continuously

you will need to mix. The sensitive period for potential disruption of the spindle apparatus and 2nd polar body retention is approximately 12–15 minutes post-fertilization in white sturgeon.[9]

Photo: Fred Conte

Photo: Courtesy of Andrea Schreier

References

1. Schreier AD, May B, Gille DA. 2013. Incidence of spontaneous autopolyploidy in cultured populations of white sturgeon, *Acipenser transmontanus*. Aquaculture 416–417, 141–145.

2. Gille A, Famula T, May BP, Schreier AD. 2015. Evidence for a maternal origin of spontaneous autopolyploidy in cultured white sturgeon (*Acipenser transmontanus*). Aquaculture 435, 467–474.

3. Fiske JA, Van Eenennaam JP, Todgham AE, Young SP, Holem-Bell CE, Goodbla AM, Schreier AD. 2019. A comparison of methods for determining ploidy in white sturgeon (*Acipenser transmontanus*). Aquaculture 507, 435–442.

4. Schreier AD, Van Eenennaam JP, Anders P, Young SP, Crossman. 2021. Spontaneous autopolyploidy in the Acipenseriformes, with recommendations for management. Reviews in Fish and Fisheries. https://doi.org/10.1007/511160-021-09637-z

5. Drauch Schreier A, Gille D, Mahardja B, May B. 2011. Neutral markers confirm the octoploid origin and reveal spontaneous autopolyploidy in white sturgeon, *Acipenser transmontanus*. Journal of Applied Ichthyology 27, Suppl 2, 24–33.

6. Van Eenennaam JP, Fiske AJ, Leal MJ, Cooley-Rieders C, Todgham AE, Conte FS, Schreier AD. 2020. Mechanical shock during egg de-adhesion and post-ovulatory ageing contribute to spontaneous autopolyploidy in white sturgeon culture (*Acipenser transmontanus*). Aquaculture 515, 734530. https://doi.org/10.1016/j.aquaculture.2019.734530

7. Blacklidge KH, Bidwell A. 1993. Three ploidy levels indicated by genome quantification in Acipenseriformes of North America. Journal of Heredity 84, 427–430.

8. Schreier A, Van Eenennaam J, Anders P. 2018. A management plan for spontaneous autopolyploidy in cultured white sturgeon (*Acipenser transmontanus*) in the Lower Columbia & Snake River Impoundments (BPA Project 2007-155-00). Prepared for the Columbia River Inter-Tribal Fish Commission. 26 pp.

9. Van Eenennaam AL, Van Eenennaam JP, Medrano JF, Doroshov SI. 1996. Rapid verification of meiotic gyno-genesis and polyploidy in white sturgeon (*Acipenser transmontanus* Richardson). Aquaculture 147, 177–189.

10. Cherfas N, Gomelsky B, Ben-Dom N, Hulata G. 1995. Evidence for the heritable nature of spontaneous diploidization in common carp, *Cyprinus carpio* L., eggs. Aquaculture Research 26, 289–292.

11. Omoto N, Maebayashi M, Adachi S, Arai K, Yamauchi K. 2005. The influence of oocyte maturational stage on hatching and triploidy rates in hybrid (bester) sturgeon. Aquaculture, 245, 287–294.

12. Zhou H, Fujimoto S, Adachi S, Yamah, E, Arai K. 2011. Genome size variation estimated by flow cytometry in *Acipenser mikadoi*, *Huso dauricus* in relation to other species of Acipenseriformes. Journal of Applied Ichthyology 27, 484–491.

13. Brooks S, Tyler CR, Sumpter JP. 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7, 387–416.

14. Kjørsvik E, Mangor-Jensen A, Holmefjord I. 1990. Egg quality in fishes. Advances in Marine Biology 26, 71–113.

15. Bobe J, Labbé C. 2010. Egg and sperm quality in fish. General and Comparative Endocrinology 165, 535–548.

16. Migaud H, Bell G, Cabrita E, McAndrew B, Davie A, Bobe J, Herráez MP, Carrillo M. 2013. Gamete quality and broodstock management in temperate fish. Reviews in Aquaculture 5, S194–S223.

17. Samarin AM, Policar T, Lahnsteiner F. 2015. Fish oocyte ageing and its effect on egg quality. Review in Fisheries Science and Aquaculture 23, 30–314.

Photos: Andrea Schreier

18. Hardie TJ, Hebert PDN. 2004. Genome-size evolution in fishes. Canadian Journal of Fisheries and Aquatic Sciences 61, 1636–1646.

19. Smith EM, Gregory TR. 2009. Patterns of genome size diversity in the ray-finned fishes. Hydrobiologia 625, 1–25.

20. Leal MJ, Van Eenennaam JP, Schreier AD, Todgham AE. 2019. Triploidy in white sturgeon (*Acipenser transmontanus*): Effects of acute stress and warm acclimation on physiological performance. Comparative Biochemistry and Physiology A. 229, 10–17.

21. Leal MJ, Van Eenennaam JP, Schreier AD, Todgham AE. 2020. Diploid and triploid white sturgeon (*Acipenser transmontanus*) differ in magnitude but not kinetics of physiological responses to exhaustive exercise at ambient and elevated temperatures. Canadian Journal of Fisheries and Aquatic Sciences, s74, 666–673.

22. Leal MJ, Van Eenennaam JP, Schreier AD, Todgham AE. 2021. Reduced growth may be linked to lower aerobic scope in juvenile triploid white sturgeon (*Acipenser transmontanus*). Aquaculture 534, 736157. https://doi.org/10.1016/j.aquaculture.2020.736157

23. Leal MJ, Clark BE, Van Eenennaam JP, Schreier AD, Todgham AE. 2018. The effects of warm temperature acclimation on constitutive stress, immunity, and metabolism in white sturgeon (*Acipenser transmontanus*) of different ploidies. Comparative Biochemistry and Physiology. A. 224, 23–34.

24. Chapman FA, Van Eenennaam JP. 2007. Sturgeon Aquaculture – Specialized Techniques: Determining the Stage of Sexual Maturity in Female Sturgeon for Artificial Spawning: the Egg Polarization Index or PI (FA 153). University of Florida IFAS Extension, Gainesville, Florida.

25. Chebanov MS, Galich EV. 2011. Sturgeon hatchery manual. FAO Fisheries and Aquaculture. Technical Paper No. 558, Ankara, Turkey.

26. Williot P, Chebanov M. 2018. Controlled reproduction of farmed Siberian sturgeon *Acipenser baerii* Brandt. In: Williot, P, Nonnotte, G, Chebanov, M. (Eds.), The Siberian Sturgeon (*Acipenser baerii*, Brandt, 1869) Volume 2: Farming. Springer International Publishing, pp. 13–47.

27. Van Eenennaam JP, University of California Davis, unpublished data.

28. Kynard B, Hogan M. 2002. Attraction of prespawning male shortnose sturgeon *Acipenser brevirostrum* to the odor of prespawning females. Journal of Ichthyology 42, 205–209.

29. Bayunov LV. 2016. The effect of four C21-steroids on oocyte maturation in Siberian sturgeon (*Acipenser baerii* Brandt). Journal of Evolutionary Biochemistry and Physiology 52, 431–439.

30. Bayunova L, Semenkova T, Canario AVM, Gerasimov A, Barannikova I. 2011. Free and conjugated androgen and progestin levels in serum of stellate sturgeon (*Acipenser stellatus* Pallas) males treated with female coelomic fluid. Journal of Applied Ichthyology 27, 655–659.

31. Kasumyan AO, Mamedov ChA. 2011. Behavioral response of mature males of Acipenseridae to female sex pheromone. Journal of Ichthyology 51, 457–465.

32. Vizziano D, Barrio, F, Astigarraga I, Breton B, Williot P. 2006. Unusual conditions for Siberian sturgeon (*Acipenser baerii Brandt*) spawning. Journal of Applied Ichthyology 22 (Suppl. 1), 325–330.

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