

# WHITE STURGEON DOMESTIC BROODSTOCK MANAGEMENT

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1/ UC Davis represents 3 commercial sturgeon farms in California participating in the White Sturgeon Broodstock Development Program.

## **PROJECT OBJECTIVES**

The goal of this project is to develop management practices for domestic sturgeon broodstocks, and specific objectives are: 1) to investigate growth, sexual maturation, fecundity and gamete quality in broodstocks of California and Idaho; 2) to refine methodology of induced spawning; 3) to develop sperm cryopreservation; 4) to determine the effect of broodstock diets on growth and reproduction; 5) to elucidate the vertical transmission of sturgeon viruses and develop prevention methods; 6) to establish breeding objectives, broodfish selection, and initiate studies on genetic inheritance of important traits; 7) to develop a sturgeon broodstock manual for the aquaculture industry.

## **ANTICIPATED BENEFITS**

Domestication and commercial culture is the only plausible way to sustain production of sturgeon resources. Our project will support the developing sturgeon industry by establishing management guidelines for domestic broodstocks which will be compiled into a manual. The sturgeon industries established in California and Idaho will improve their capabilities of producing domestic fish for flesh and caviar markets, maintaining healthy and reproductively efficient broodstocks, and developing breeding programs that fit the industry needs.

## **PROGRESS AND PRINCIPAL ACCOMPLISHMENTS**

### *Objective 1. Growth and Maturation*

Annual collection of data on growth, maturation, and reproductive performance of sturgeon broodstocks continued in Idaho and California. Growth rates of broodstocks raised at constant temperature 15EC (Idaho) were approximately twice lower, compared to those raised at elevated temperatures 18-22EC (California). While, in California fifty percent of females undergo first ovarian vitellogenesis at age 7

years (mean body weight 25 kg), in Idaho, forty-five percent of the Sacramento River stock had vitellogenic ovaries at age 13-14 years (mean body weight 17 kg), and eighteen percent of the Columbia River stock had vitellogenic ovaries at age 11 years (mean body weight 27 kg). Three females in Idaho reached sexual maturity and one (Columbia River stock, body weight 25 kg) was the first to be successfully spawned on 7/16/98. Approximately 40,000 eggs were collected via cesarean section and fertilized by sperm from several 1990 Snake River males. Fertilization and hatching rates were 60% and 48%, respectively. Analysis of data indicates that fecundity increases significantly with age, body size, and repeated ovarian cycles. Estimated additional gain in fecundity due to iteroparity was approximately 20 th eggs at the second ovarian cycle and 100 th eggs at the third cycle. Data on reproductive performance of 19 iteroparous females spawned 2-4 times, revealed significant increases in egg diameter, fertilization and hatching rates due to iteroparity.

#### ***Objective 2. Induced Spawning***

Spawning trials with GnRH $\alpha$ , GnRH $\alpha$  + domperidone (DMP), and carp pituitary extract (CPE) were conducted during spring 1997. Females whose oocytes exhibited a 100% GVBD response in the bioassay were induced to ovulate in 3-19 days after sampling. They were injected IM with a priming (10% of total) and resolving (12 hours later) dose of the respective hormone. The most effective treatments were GnRH $\alpha$  + DMP and GnRH $\alpha$  alone, resulting in 75% and 54% hatchability, respectively. During spring 1998 we initiated spawning trials to optimize the dose of GnRH $\alpha$ . There were no significant differences in ovulation, fertility, neurulation and hatching rates between the 20 and 40Fg/kg treatment doses, suggesting that the currently used higher dose may be further reduced.

#### ***Objective 3. Cryopreservation of Sperm***

The fertility of frozen sturgeon milt was examined using the percentage of fertilized eggs (4-cell stage) as the endpoint. The variables were: 1) the composition of the freezing solution; 2) the ratio of freezing solution to milt; 3) the size of the straw (0.5ml vs 5 ml) in which the milt was frozen, and 4) the solution used to activate the thawed sperm (hatchery water, 50mM Tris or 118mM Tris). For all treatments the sperm was frozen in a two-step method (cooling at a controlled rate of -30EC/minute to -70EC, followed by plunging the straws into liquid nitrogen). In most cases, the motility of the frozen and thawed sperm was very low and the fertility was zero. In a recent study, the motility of the thawed sperm was # 5%, but a few eggs were fertilized by sperm from 6 out of 9 straws. In this experiment, the sperm was frozen in 0.5ml straws and was thawed quickly (40EC water bath for 25 seconds) and activated with a 50mM Tris (pH=8.0) solution. The characteristics of sturgeon sperm/milt were found to be as follows: 1) seminal fluid pH = 8.48 " 0.11 (n=4, mean " sem); 2) sperm concentrations = range from 0.05 x 10<sup>9</sup> cells/ml to 6.40 x 10<sup>9</sup> cells/ml; 3) sperm ATP = 14.3 " 3.1 pmol ATP/10<sup>6</sup> sperm cells (n=9), range = 3.1 to 34.3. The measurements of sperm ATP in different atmospheres was undertaken to determine the rate of turnover of ATP in sturgeon sperm. Since cryopreservation is a potentially destructive process and we observe a significant reduction in the proportion of cells that are motile at post-thaw, measurement of cellular ATP will provide evidence on whether cryopreservation leads to a loss of ATP from the cells or an uncoupling of stored ATP from the flagellar compartment.

#### ***Objective 4. Effect of Diets on Growth and Maturation***

One hundred and two 1993 year class white sturgeon were initially sampled and transported from a farm to the UC Davis Putah Creek Aquaculture Facility. All the females were at a similar stage of gonadal development, and histological analyses revealed previtellogenic oocytes. The fish are currently being reared in six -20' diameter circular tanks (each with 15 females and 2 males) supplied with 50 gpm of well water (18 " 1EC), and are fed via automatic feeders at 0.3% body weight/day. Half the tanks receive the control diet and the other half the experimental diet. After six months the fish were re-sampled. Fish fed the control diet gained 3.14 kg in 6 months, compared to 3.69 kg in the test diet treatment. The growth data indicates that the fish have adapted to the new rearing environment and consume both diets adequately.

#### ***Objective 5. Vertical Transmission of Sturgeon Viruses and Development of Prevention Methods***

Broodstock parents were sampled and examined for WSIV and WSHV-2 using virus infectivity assays on white sturgeon cell cultures, as well as for anti-WSHV-2 antibodies using an ELISA serologic assay. No viruses were recovered from the 23 (10 female, 13 male) adults, while anti-WSHV-2 antibodies were found in all broodfish. The offspring were brought into a commercial hatchery, monitored, and four WSIV outbreaks were documented. Infectious disease modeling was conducted for the largest outbreak to determine whether the outbreaks are clustered in time and/or location of tanks in the hatchery, but no evidence of simultaneous temporal and spatial clustering was detected. This suggests that no tank-to-tank transmission was taking place. Furthermore, each outbreak of the disease was linked to the progeny of a specific mating. There are at least two possible reasons why the outbreaks occurred in certain groups and not others; 1) the virus was passed from an infected adult only to that progeny, or 2) that regardless of the source of the virus it did not spread because the other fish were resistant. Observations on the disease of these populations will continue. Progeny from each mating will be kept separately from the others when they are transferred to the grow-out facility. Exposure to the viruses and observation of the disease patterns will allow us to distinguish between the vertical transmission outbreak and the resistance hypothesis.

#### *Objective 6. Genetic Studies*

The genetic component has been focused on sex identification and evaluation of genetic variation within aquaculture stocks. Sex-specific pools of DNA from different genetic stocks (unrelated mature broodstock, white sturgeon full-sibs, hybrid sturgeon full-sibs) were analyzed using subtractive hybridization, representational difference analysis and bulk segregant analysis, but DNA sequences associated with sex were not identified. Gynogenetic progeny were produced and presence of both sexes was observed, indicating that white sturgeon has a female heterogametic (ZZ:ZW) genetic sex determination system. To evaluate genetic variation within farm stocks experiments have been initiated, including a series of dam by sire crosses in order to estimate parental effects and heritabilities of growth rates and WSHV-2 susceptibility. Genetic variation is being assessed by microsatellite and AFLP analysis of the individuals from several year classes in order to estimate the variation within and between year classes.

#### *Objective 7. CD-ROM White Sturgeon Broodstock Manual*

The text from the original publication "Hatchery Manual for the White Sturgeon *Acipenser transmontanus* Richardson: with Application to other North American Acipenseridae" (Conte et al., 1988) was produced using an IBM word processor and offset printing. We scanned the text to create digitized files. Existing 35-mm slides of sturgeon aquaculture are being reviewed to identify usable images and subject matter in which new images will be created. Peer reviewed and grey-literature reports on white sturgeon broodstock activities are being collected. Efforts have been initiated (preproposal and letters of support) to obtain services and resources of the University of California Division of Agriculture and Natural Resources (DANR) Visual Media and Publications Unit. These resources include professional still and video photographers, film processors and editors, computerized graphing and morphing technology, text and hyperlink editors, final CD production, sales and distribution support.

### **USEFULNESS OF FINDINGS**

Industry progress in domestication of white sturgeon underscores the needs for broodstock management. Observations on growth and ovarian development of broodstocks in California and Idaho revealed effects of rearing temperature, broodfish origin, and farm husbandry. Accumulated data on California stocks and current observations in Idaho will elucidate broodstock phenotypes and effects of environmental and husbandry factors on broodstock performance. The increase of fecundity and egg size with iteroparity is of major interest to sturgeon breeders and the caviar industry. Application of GnRH $\alpha$  for the induction of ovulation, as well as the GVBD bioassay and prespawning cold water treatment, significantly improved fertilization and hatching rates and made sturgeon spawning more reliable and cost-effective. While our efforts have not resulted yet in a practical method for sperm cryopreservation, the development of new tests for sperm viability and freezing solutions will permit us to establish cryopreservation technique for its use in sturgeon breeding. The research on viral disease

provides evidence of vertical transmission of WSIV (and most likely WSHV-2) and indicates that preventive measures must be developed. The prevention program will have to begin with the development of broodstocks free of the virus, with the offspring reared in a virus-free environment or vaccinated to prevent the disease. Experiments have been initiated to examine inheritance of commercially important traits in sturgeon, including juvenile growth and susceptibility to WSHV-2 infection. Recent research on sex genetics has led to the discovery of a potential ZZ:ZW sex determination system in white sturgeon, using transmission experiments with gynogens, triploids and normal diploids. Sub-adults from these groups are currently reared for breeding. If the WW Asuper@ females are present among the gynogens, they will be expected to produce all-female offspring when crossed with normal males.

### **WORK PLANNED FOR NEXT YEAR**

Collection and analysis of data on growth and maturation of sturgeon broodstock as well as spawning performance of iteroparous females will continue. Spawning induction trials will examine the effects of different GnRH $\alpha$  doses on ovulation and fertility, in comparison with Ovaprim (mixture of GnRH $\alpha$  and DMP, produced by Syndel, Inc., which has recently began the INAD process). In sperm cryopreservation studies we will examine different rates of thawing and the type of activator solution used. In addition, the buffering capacity of sturgeon seminal plasma and the role of seminal plasma pH on the level of ATP in sperm and sperm motility will be examined. Semi-annual sampling of sturgeon on the diet study is scheduled, and the percent of maturing individuals will be compared between diets. Disease research will focus on the development of PCR methods to detect the viruses in adult sturgeon. Purification of the virus nucleic acid for cloning and sequencing will also begin. After initial sequences are obtained, primer sets for testing of detection in experimentally-infected compared to uninfected juvenile fish will be developed. To examine the genetic inheritance of commercially important traits in sturgeon a 3 dam x 3 sire full factorial cross, reared under similar conditions, will be analyzed for growth rates and WSHV-2 susceptibility. Sampling of broodfish on farms that have maintained spawning records will also allow us to evaluate the genetic diversity between year classes by estimating the relative proportions of individual families. Microsatellites or AFLP analyses will be used to test the hypotheses that these ratios have been maintained over time or if there has been selection for certain families based on growth rate or size of individuals. Future sturgeon manual CD-ROM plans include submission of the final proposal to DANR for inclusion of the project in the University publications activity plan. Meetings will be held with researchers to plan a final working outline of the text format and to define the working titles and subtitles for the hyperlink function. Still and video photography on broodstock activities, equipment and facilities will begin, and writing of sectional drafts of text material will be initiated.

### **IMPACTS**

The aquaculture industry of the Western Region has made significant progress in the domestication of white sturgeon and is now able to replace products from the commercial fishery of depleted stocks with sustainable aquaculture production. Sturgeon farms are no longer dependent on wild sturgeon, and all commercial products (seedstock, food fish and caviar) during the next five years will be the second, third, and fourth generations of domesticated fish. The improvements in reproductive efficiency, diagnostic techniques for the assessment of reproductive development and health of broodstocks were the most significant contributing factors. The ongoing research on disease, nutrition, genetics and gamete cryopreservation will further enhance commercial sturgeon production.

**SUPPORT**

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT			TOTAL SUPPORT
		INDUSTRY	OTHER FEDERAL	TOTAL	
97	96,000	4,000	57,217	61,217	157,217
98	98,000	4,000	72,035	76,035	174,035
<b>TOTAL</b>	<b>194,000</b>	<b>8,000</b>	<b>129,252</b>	<b>137,252</b>	<b>331,252</b>

**PUBLICATIONS IN PRINT & MANUSCRIPTS**

- Aquaculture and Fisheries Program. 1997. The Tenth Annual Report of White Sturgeon Broodstock Development Program. University of California, Davis. 17 pp.
- Doroshov, S.I., G.P. Moberg and J.P. Van Eenennaam. 1997. Observations on the reproductive cycle of cultured white sturgeon *Acipenser transmontanus*. Environmental Biology of Fishes 48: 265-278.
- Doroshov, S.I., J.P. Van Eenennaam, E. DePeters and S. Taylor. 1998. Broodstock management and technology for the sturgeon and caviar industry. Final Report to the National Coastal Resources Research and Development Institute, Portland, Oregon. 57 pp.
- Van Eenennaam, A.L. 1997. Genetic analysis of the sex determination mechanism of white sturgeon (*Acipenser transmontanus* Richardson). Ph.D. Dissertation, University of California, Davis. 180p.
- Van Eenennaam, A.L., J.P. Van Eenennaam, J.F. Medrano and S.I. Doroshov. 1998. Evidence of female heterogametic genetic sex determination in white sturgeon. In press. J. of Heredity.
- Webb-Brewer, M.A.H., J.P. Van Eenennaam, S.I. Doroshov and G.P. Moberg. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. Submitted. Aquaculture.

**PAPERS PRESENTED**

- Adkison, M.A., C. Lee, N. Rooijackers and R.P. Hedrick. Investigations on the immune response of the white sturgeon (*Acipenser transmontanus*) and applicability to immunization against white sturgeon herpesvirus-2. 3rd International Symposium on Sturgeon, Piacenza, Italy, July 8-11, 1997.
- Amiri, B.M., T.E. Adams, S.I. Doroshov and G.P. Moberg. Use of mammalian gonadotropin-releasing hormone to characterize pituitary gonadotropin releasing hormone receptors in white sturgeon (*Acipenser transmontanus* Richardson). 3rd International Symposium on Sturgeon, Piacenza, Italy, July 8-11, 1997.
- Doroshov, S.I., J.P. Van Eenennaam and G.P. Moberg. Development of white sturgeon broodstock. 3rd International Symposium on Sturgeon, Piacenza, Italy, July 8-11, 1997.
- Doroshov, S.I. Progress in culture of sturgeon. The 127th Annual Meeting of the American Fisheries Society, Monterey, California, August, 24-28, 1997.
- Linares-Casenave, J., J.P. Van Eenennaam and S.I. Doroshov. Temperature-induced ovarian atresia in the white sturgeon, *Acipenser transmontanus*. 3rd International Symposium on Sturgeon, Piacenza, Italy, July 8-11, 1997.
- Nocillado, J.N., J.P. Van Eenennaam and S.I. Doroshov. Final ovarian maturation and egg quality in hormonally-induced white sturgeon (*Acipenser transmontanus*). 3rd International Symposium on Sturgeon, Piacenza, Italy, July 8-11, 1997.
- Hedrick, R., M. Adkinson, M. Georgiadis, L. Watson. Sturgeon viruses, impacts on aquaculture and recent appearances in Europe. The Fourth International Symposium on Viruses of Lower Vertebrates, 12-15 May, 1998. Weymouth, UK.
- Georgiadis, M.P., I.A. Gardner, T.E. Carpenter, W.O. Johnson, R.P. Hedrick. Applications of epidemiology in Aquaculture: controlling sturgeon viruses. Third International Symposium on Aquatic Animal Health. 30 Aug - 3 Sept, 1998. Baltimore, MD.