

# WHITE STURGEON DOMESTIC BROODSTOCK MANAGEMENT

<b>REPORTING PERIOD</b>	4/01/99–3/31/00		
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<b>FUNDING LEVEL</b>	\$100,000		
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<sup>1</sup> UC Davis represents 4 commercial sturgeon farms in California participating in the White Sturgeon Broodstock Development Program.

## PROJECT OBJECTIVES

The goal is to develop management practices that will improve reproduction, growth and health of domestic sturgeon broodstocks. The 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 egg quality; 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. This project supports the developing sturgeon industry by establishing management guidelines for domestic broodstocks which will be compiled into a manual. The sturgeon industries of California and Idaho will improve their capabilities to produce flesh and caviar for food markets, to maintain healthy and reproductively efficient broodstocks, and to develop breeding programs that fit the industry needs.

## PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

### *Objective 1. Growth, Maturation, and Reproductive Performance*

Collection of data on growth, maturation, and reproductive performance of sturgeon broodstocks continued in Idaho and California. Rearing temperature and fish genetic origin cause significant variation in growth and maturation rates of sturgeon broodstocks. In California (18–22°C) fifty percent of females exhibited ovarian vitellogenesis at age 7 years, while a similar development was observed at age 12–14

years in Idaho (15°C). During the last two years, seven females in Idaho broodstock have completed vitellogenesis, but the majority underwent ovarian regression before spawning and only one female was spawned repeatedly, with a biennial interval. The constant 15°C holding temperature may cause the frequent occurrence of ovarian regression prior to final maturation. We conducted a field experiment to elucidate the optimum timing for transfer of broodfish to cold water and the capacity of transferred females to maintain reproductive competence. Observations on oocyte development and plasma sex steroid profiles indicate that the temperature-sensitive phase coincides with transition to final ovarian maturation and the timing of temperature impact on ovarian development may be dependent on the female's endogenous reproductive rhythm. Reproductive performance of repeatedly spawned females was evaluated in four California broodstocks (20 females, age 7-17 years, with biennial breeding intervals). Iteroparity significantly enhanced all reproductive traits (egg size, embryo survival, and the production of larvae per female) except the number of ova collected and fertilization rate.

#### *Objective 2. Methodology of Induced Spawning*

We continued testing the efficacy of the 20 µg/kg GnRHa dose for induction of ovulation (Syndel Laboratories Ltd., Toronto, Canada sponsors this INAD). Females treated with this dose of GnRHa exhibit high ovulation rate and produce high quality eggs, but the management stress on one farm contributed to an overall modest ovulation rate and variable embryo survival to hatching. Multiple management stresses affected reproductive performance of sturgeon females on this farm.

#### *Objective 3. Sperm Cryopreservation*

During June 2000 a successful cryopreservation of white sturgeon sperm was demonstrated at Bonners Ferry, Idaho from a Kootenai River white sturgeon female, the fertility of sperm frozen in 0.5 mL straws from three different males ranged from 54 to 62% (freezing solution contained 30 mM TRIS-HCl and 5 % methanol; cooling curve was as follows: 4° C to -18° C at -4.5 degrees/min, -18° C to -70° C at -18 degrees/min, plunge into liquid nitrogen). Since these results were positive and since freezing sturgeon milt in 0.5 mL straws will have limited application, a field trial was initiated at the Kootenai Tribal Hatchery in Bonners Ferry with frozen semen from five males, but no success in application of cryopreservation to hatchery production ("en mass" fertilization and incubation) has been achieved at this time.

#### *Objective 4. Effects of Diets on Growth, Ovarian Maturation, and Egg Quality*

Adult sturgeon are being reared in six - 20' diameter circular tanks (each with 15 females and two males) supplied with 50 gpm of well water (18± 1°C), and are fed by automatic feeders, with three tanks receiving the control diet and the other three, the experimental diet. No significant difference in body size between control and experimental diets was detected after 28 months of rearing. Approximately 40% of the females in each tank were ripe in the spring of 2000 (at age 7 years) and were returned to the farm for caviar production, where they were sampled for yield of roe and tissue for laboratory analyses. Ovary and residual gonad tissue weights reflected the small difference in weight and length gain in the two diets, with the fish in the control group having slightly larger ovaries than the fish fed the experimental diet. There was a significant difference in the yield of caviar, expressed as a percentage of ovary weight, with a lower yield of finished caviar in the control group. Twenty seven percent of the fish in control diet were classified as "difficult to process" due to excessive ovary fat, compared to only 5.7% of the fish fed the experimental diet.

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

Two epidemiology studies examined the problems due to runting or stunting of white sturgeon during production following WSIV outbreaks. These studies conclude that runts, once recovered from the ill effects of virus, grow at rates comparable to fish that were never runts. A preliminary financial analysis showed that if the price of sturgeon exceeds \$2 per kg, than saving and growing these recovered runts

is advisable. A third epidemiological study examined risk factors associated with WSIV outbreak, and parentage and hatchery management stressors were revealed as key risk factors associated with WSIV outbreaks. The development of a PCR test for the detection of WSIV in tissues of juvenile and adult sturgeon continues. We finished sequencing the major capsid protein (MCP) of WSIV, but the PCR is still unable to adequately detect the viral DNA in infected tissues. High G/C content makes amplification of this region of the genome problematic and thus a poor target for a diagnostic PCR. We have used co-solvents in the PCR mixture such as formamide, DMSO and TMAC with re-optimization of annealing conditions. However, in the presence of host DNA, the amplification is still suboptimal for a diagnostic PCR. Therefore, we have shifted our attention to other WSIV genes including the DNA polymerase, the ribonucleotide reductase small subunit, ATPase, elongation initiation factor- $\alpha$  and helicase for the development of diagnostic primers. This work has been started, using the sequence information from related iridoviruses (RSIV, EHNV and FV3).

#### *Objective 6. Genetic Studies*

The genetic component of sturgeon broodstock management has been focused on sex identification. We reported earlier that white sturgeon has a female heterogametic (ZZ {male}: ZW {female}) genetic sex determination system, and one can produce ZZ males, WW "super-females" and/or ZW females, using gynogenesis. If the WW females are viable they could be used for monosex production of females in caviar-oriented farms. In 1995 we produced gynogen sturgeon and eighteen of these gynogens have been kept and presently weigh an average of 11.5 kg and are 111 cm in length. These fish were recently sexed and their sex ratio was 72% females and 28% male. Since no sex specific markers have been identified in sturgeon, progeny testing is the only mechanism to determine if the WW "super-females" are present in this stock. We examined earlier a region of the Chromobox-helicase-DNA-binding gene (CHD-W) in white sturgeon and utilized PCR primers that were used for sexing birds to amplify white sturgeon genomic DNA. Two PCR fragments were amplified in both male and female white sturgeon (WS615 and WS841) and they were cloned and sequenced. We concentrated on fragment WS841 and designed a new set of primers specific to this fragment. The recently sampled male and female gynogen DNA's were amplified using PCR and cut with the restriction enzymes *AluI* and *MaeII*, but no sex specific differences were detected. To facilitate DNA extraction from sturgeon blood under laboratory or field conditions we have adapted the S&S IsoCode-stick card system which presents a great advantage over conventional chemical system of extracting DNA because blood samples can be stored without refrigeration and the labor involved is minimal in obtaining DNA for PCR analysis.

#### *Objective 7. Develop a CD-ROM Sturgeon Broodstock Manual*

Information on the technologies necessary for the production of sturgeon broodstock was gathered from researchers and research reports and continues to be placed in digitized format. Using non-WRAC funds, a postgraduate researcher with advanced computer skills was hired and computer upgrades made that allow T-100 transmission between high graphic computers used in graphic productions. The additional expertise included developing techniques to compress high-resolution graphics by a factor seven which allows more extensive visuals, and animation technology is now being incorporated. Photographic materials are now being enhanced and edited for inclusion in the final selection. Permission to photograph broodstock activities at commercial facilities has been secured and sessions are being arranged. A joint Center for Aquatic Biology and Aquaculture, WRAC, and Columbia River Fish & Wildlife Authority sturgeon conference is being held at UC Davis in September, where sessions will be taped and transcribed, and some materials used in the CD-ROM presentation.

### **USEFULNESS OF FINDINGS**

Our annual field observations on farmed sturgeon broodstocks in California and Idaho reveal the labile nature of sturgeon growth and maturation in response to rearing temperature, broodfish origin, and farm husbandry. The continuing observations on broodstock performance will provide guidelines for

the best broodstock management practices. The enhanced reproductive performance of iteroparous female white sturgeon is advantageous to sturgeon culture, since the repeated breeding maximizes seed stock production and iteroparous females can be used as "multipliers" of genetic traits, such as egg size and disease resistance. Optimization of spawning methods (vernalization and GnRHa treatment) improved the efficacy of seed stock production but requires best stress-management practices on some of the farms. Spawning methods established for cultured white sturgeon have been successfully used for the first spawning of green sturgeon on the Klamath River. While the first success in fertilizing ova with cryopreserved sturgeon sperm was achieved, our efforts have not resulted yet in a practical method for sperm cryopreservation. The development of improved freezing solutions and freezing rates are needed to efficiently store white sturgeon genetic lines. The research on epidemiology of viral disease indicates that preventive measures must be developed to control vertical transmission of pathogens and disease outbreaks by improved management. Establishing a diagnostic test (PCR) will provide a tool to detect broodstock carriers and to control disease transmission in sturgeon farms. With concomitant development of preventive practices, the impact of viral disease on production can be reduced or eliminated. The DNA sex markers will be used in breeding programs with sturgeon broodstock and in development of monosex production for the caviar-producing sturgeon farms. The potential sex determining genes are currently being explored to identify genetic sex and gynogenetic super females. Fast and efficient growth and early maturation of sturgeon that produce high quality caviar is an important issue for the sturgeon industry. Our preliminary results demonstrate that a phase feeding approach can result in good weight gains and limit fat accumulation in the ovaries, which improves caviar processing, and egg quality.

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

We will continue collection and analysis of data on growth and maturation of Idaho sturgeon broodstock as well as spawning trials on farms to examine the efficacy of GnRHa at a dose of 20µg/kg for the INAD process. The last sampling in the sturgeon broodstock diet study is scheduled for March 2000, and we expect full sexual maturity in approximately 18 more females from each dietary treatment (80-90% of the initial stock), at which time fish will be harvested, tissue samples collected and caviar produced. Laboratory analysis of collected sturgeon tissue will continue. In the sperm cryopreservation study, milt will be derived from animals held at UC Davis and at the hatchery of the Kootenai Tribe and frozen in 5 mL straws using the identified cooling curve for 0.5 mL straws and its variations. Sperm derived from the Kootenai R. sturgeon will be frozen in 5 mL straws using the three best cooling curves, and will be used to fertilize eggs obtained from Kootenai River females. Based on the highest fertilization and hatching rates from three females, the best cooling procedure will be identified. Collaboration (at no cost to WRAC) with Dr. Donald Everson (SD State University) has been established to study sperm chromatin changes during freezing and thawing. Investigation on viral disease will focus on the development of a diagnostic PCR test to detect WSIV in juvenile and adult sturgeon. We will direct our efforts towards the DNA polymerase, the ribonucleotide reductase small subunit, ATPase, elongation initiation factor-alpha and helicase genes for the development of PCR primers. Sex genetic studies will focus on developing new sets of primers to amplify and analyze various genetic fragments associated with sex chromosomes, and to examine part of the CHD exon sequence. Future sturgeon manual CD-ROM plans will concentrate on staging selected photography at commercial facilities and inclusion of photography from other WRAC facility participants, continued interviewing of broodstock caretakers at the University and commercial facilities, and selection of management technologies for inclusion in the text presentations.

#### **IMPACTS**

The aquaculture industry has made significant progress in the domestication of white sturgeon, and wild-caught fishery products are now being replaced with sustainable aquaculture production. The improvements in reproductive efficiency, hatchery methodology and diagnostic techniques for the assessment of the reproductive state and health of the fish were the most significant contributing factors.

**SUPPORT**

FISCAL YEAR	WRAC-USDA FUNDS	OTHER SUPPORT			TOTAL SUPPORT
		INDUSTRY	OTHER FEDERAL	TOTAL	
97	100,000	3,000	21,735	24,735	124,735

The ongoing studies on molecular diagnostics of sturgeon viruses and sex, development of gamete cryopreservation techniques, and optimization of diets for sturgeon broodstock will significantly contribute to more advanced sturgeon production systems. All objectives of this projects are carried out in close collaboration with the commercial industry, and sturgeon culturists receive continuous feedback from the researchers.

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