PALLID STURGEON PROPAGATION - 2000
Garrison Dam NFH

Rob Holm

US Fish and Wildlife Service
Garrison Dam NFH, Riverdale ND

Background/Introduction

The Pallid Sturgeon Recovery Plan (1993) established guidance for collection of wild brood fish, propagation, research needs and reintroduction of progeny to accomplish recovery goals. This hatchery's role in the recovery effort centers around the spawning and rearing of larval pallids. 1997 marked the first year of Garrison Dam NFH's attempt to spawn a pallid sturgeon. Eggs were recovered from a single female but unfortunately an electrical short to the hatchery's boiler resulted in a complete loss. In June of 1998 two females were successfully spawned at Garrison Dam NFH. The newly hatched fry were flown to Gavins Point for rearing due to a lack of suitable facilities at Garrison Dam NFH. A viral outbreak on shovelnose sturgeon held at Gavins Point NFH and an outbreak on pallids held at Valley City NFH during the winter of 1998/1999 prompted a change of plans for the recovery effort. Garrison Dam NFH would need to gear up to propagate fingerling pallids while Gavins Point NFH was under quarantine. Progeny resulting from the 1999 spawn were held on station at Garrison Dam NFH until November 18th when they were moved to Valley City NFH for overwintering. The yearlings were moved back on May 4th where they were separated out by family lot with the orange and black family lots held in a 8 X 60 foot fiberglass raceway and the yellow, pink, and green families in 4 foot circular tanks. On July 7, 2000, the Montana Fish, Wildlife, and Parks Fish Health Lab confirmed the yearling pallids in the raceway were infected with an iridovirus. Samples sent to the University of California, Davis, were also confirmed positive. During that time frame pallid sturgeon broodstock were spawned at the hatchery and progeny were being successfully cultured. Seventeen family lots were attempted using the traditional approach with four females. Sixteen family lots were attempted using cryopreserved sperm from last year and this year's fish. The results of the family lots were varied, however, indicating a need for further study and the importance of establishing as many family lots as practical. Cryopreservation was also determined to be an unqualified success and will become a vital tool in the recovery effort.

Objectives

This year our goal is to locate as many gravid females as practical with the hopes of collecting and successfully spawning up to fourteen fish. In addition, Montana Fish Wildlife and Parks fisheries crews above Fort Peck Reservoir will attempt to collect milt from pallids captured at large for introducing the genetics of the Fort Peck fish into the recovery effort. Cryopreserved sperm from last years males will be used on a subsample of eggs to attempt fertilization. Several hormone strategies will be employed to improve our success. Domperidone, a substance that inhibits the negative effects of stress on ovulation, will be used in conjunction with reduced levels of our typical ovulation hormone, LH-RH. Carp pituitary will also be administered in place of LH-RH to determine it's success as compared to that of LH-RH. The male pallids will

receive the same treatment as in the past. We will be utilizing the catheterization method again this year for determining egg stage. We will be relying on palpation methods to collect eggs to avoid the risks of stress associated with cesarian or suction spawning. All eggs collected will be treated with 50 ppm Betadyne for 30 minutes following the deadhesion process. Fin punches and anal fin sections will be collected from all broodstock for iridovirus inspections and fish genetic profiling prior to spawning. Oxytetracycline will be administered as needed to combat any infections that develop and as standard protocol on all captured fish.

Fall Capture 1999

Results

No Fall capture was accomplished due to the lack of overwintering facilities at Garrison Dam NFH and a quarantine at the Gavins Point facility.

Spring Capture 2000

Methods and Results

Between April 11 and April 17, pallids were captured near the confluence of the Missouri and Yellowstone Rivers using 6" X 10" mesh trammel nets. Most of the captured fish were concentrated at the confluence of the Missouri River and the Yellowstone River. They were apparently staging there waiting for an increase in flows to stimulate the spawning run. Sixteen of the captured fish were transported to the hatchery, five females and eleven suspected males. One of the females (1F4A436E66) had eggs that were immature and she was taken back on April 13th and released. A second female(7F7F065A3D) had mature eggs that apparently were in a state of resorption. When catheterized, a grey fluid specked with egg shells was recovered (Gregg Gamett, an employee of Stolt Seafarms of California, confirmed that they also encounter the greyish fluid on white sturgeon that are in the process of egg resorption. He further stated that the 'Salt and Pepper' eggs that are occasionally observed are in reality eggs that are undergoing resorption combined with immature eggs that are developing in the ovaries). A fish of undetermined gender (1F4B225A1A) was catherized to check for mature eggs. The fish appeared very old and weak and there was concern expressed as to whether it could survive the spawn event. Since it was confirmed not to have mature eggs, it was returned along with the female undergoing resorption to the confluence on April 16th. That left us with three confirmed females and ten males. One of the ten males(1F477B3A65) was a recapture used in last year's spawn. To resolve the issue of whether the males spawn on successive years or there is a pause between spawn cycles we held this fish. Milt from the fish was not planned to be used for creating progeny. The fish were injected either at the capture site or upon receipt at the hatchery with oxytetracycline. They were held at the hatchery in a twenty foot tanks at about 50 degrees Fahrenheit. Water temperatures were fluctuated periodically to mimic natural conditions. Salt at ½ % was applied routinely to the tanks.

There were 2-3 crews available for broodstock collection from Montana Game and Fish and the Missouri River FWMAO. A transport tank and netting assistance was also supplied by the Garrison Dam Fish Hatchery. This effort was timed well, river flows were low and fish capture was relatively routine. A chronology of events:

4/11/00 - Three crews arrive at Confluence at 10:00 am and set nets. Three females and three males are captured and taken to the hatchery. Of the three females, only one has viable eggs.

4/12/00 - Three crews are netting at 9:30 am. By noon five pallids are captured. One of the five is an older fish with a very flaccid abdomen. The other four are thought to be males.

4/13/00 -Three boats working from 9:30 to 11:00 am collected a male and female pallid. A suspected hybrid is also sent back to the hatchery.

4/17/00 -Two crews working from 3:30 to 4:30 collect five fish. Two suspected females are sent off to the hatchery. The larger of the two, a 61 pound fish, is confirmed at the hatchery as a male, the smaller a female. A smaller male is also taken back to the hatchery. Water temperature today dipped from ~50° to 46° F.

6/6/00 - Fin punches are collected on the broodstock and the tissue is sent off to Bozeman FHC for analysis. The three females are also staged to check maturation. Both progesterone and GV position tests were run. Female # 752 had a considerable amount of fat removed with the egg sample and had two distinct sizes of black eggs. Female #213 had a small section of fatty tissue and #55A had only eggs removed. Stomach abrasions were found on two fish (2202236E31 & 11571355A). Both fish also had swollen blood vessels around the vent and were treated topically with betadine and with an injection of LA-200 (oxytetracycline). That same day, the fisheries crew from Lewistown, MT, netting above Fort Peck, captured a male pallid (1F4A4B5973) and collected milt. The milt was stored in a ziplock bag shipped the following day to the hatchery in a chilled insulated box. Water temperature in the Fort Peck headwaters in the low 70°'s.

6/7/00 - Results of GV position test are determined. There appears to be movement of the nucleus to the periphery of the egg. The eggs appear ready for ovulation in two of the three females. Eggs from the progesterone assay are removed from the steroid solution, boiled, and placed in formalin to harden.

6/8/00 - The progesterone assay samples were removed from the formalin and sectioned. Female #55A had a nucleus present in 4 of 10 eggs, female # 213 had the nucleus present in 2 of 10 eggs, and female # 752 had no nucleus present in any of the 10 eggs sectioned. From these results we concluded #752 and # 213 were ready to spawn. Milt from 'Lew' taken on 6/6/00 was received at the hatchery in good shape. The sample was split into three bags, oxygen was added, and it was stored in the refrigerator. A drop was observed under the scope, excellent motility and high numbers of sperm present. Two additional males were captured above Fort Peck and milt was collected from both (41476A0462 & 1F48512325). Water temperature in the Fort Peck headwaters was 71°-72° F.

6/9/00 - Milt stored in the top bag in the refrigerator had begun to crystalize, a slush had formed. The temperature was lowered to a setting of "2" to prevent further freezing.

6/11/00 - Adventure Divers of Minot were in the tailrace and captured 12 shovelnose sturgeon. They had also observed a pallid a few days earlier and were given instructions to capture the fish if possible. The divers are able to pick these fish up by hand. To assist in the viral work, Ron

Hedrick plans on using stress tests performed on shovelnose progeny to attempt to express the iridovirus. Valley City NFH will propagate any shovelnose progeny produced. Spawning will take place at Garrison Dam NFH with the fish being held away from the hatchery proper in the west unit pond kettles.

6/12/00 - Spawning is initiated with the injection of a warm-up dose of LH-RH (0.005 mg/kg) and the total dosage of Domperidone (3 mg/kg) into female # 752 at 7:45 pm.

6/13/00 - The resolving dose of LH-RH (0.045 mg/kg) was administered to female # 752 and four males were also injected at the 0.05mg/kg rate. The injections took place between 7:45-8:00 am. Milt from the two Montana fish collected on 6/8/00 was received at the hatchery. Male #41476A0462, 'Perot' was received in good shape in a 'zip-lock' bag. Male #1F48512325, 'Harvey' was shipped in plastic containers. The lids had come off the containers and milt was spilled all over the inside of the shipping box. We salvaged what we could and put both samples in the refrigerator. A sample was taken and observed under scope - motility was marginal on Perot at 35 %, and excellent in Harvey at 90% ??. The samples from Lew were also observed (including the crystalized bag) - motility was marginal at 20 % and 15 % in two of the bags, the third bag had 40% motility. Three more Fort Peck fish males were captured, one yesterday and two today, milt will be at the hatchery on the 15th. Approximately 12 hours after the resolving dose, at 9:00 pm, the female is actively swimming around periphery of tank. Ventilations appear more pronounced and frequent than with the males.

6/14/00 - Ovulation checks continue-12:00 am check reveals the female in the typical prespawn 'tail-up' position. All fish, both injected and non-injected, are fairly active. 4:05 am check-female and injected males swimming with the current and riding high in the water column. Fish appear attracted to the flashlight and are curious. Female is grasped by the tail to check for egg release. No eggs observed yet. 6:10 am check female found 'resting' near the center of the tank and is palpated for ovulation. Males still actively 'cruising' around tank. 8:00 am palpation reveals milky colored fluid - no eggs. 10:05 -female checked for eggs and milt is taken from males and observed for motility**.** At 11:00 am the female still is not releasing eggs. It is now 27 hours after the resolving dose. Ideally the females will ovulate at 24 hours after the resolving injection. The cloudy fluid is cause for concern. The decision is made to remove eggs via catheterization to determine condition. The female is inverted in the stretcher and a tube is inserted down the ducts. Clumps of eggs are removed. Apparently the eggs had hydrated in the ducts causing the vent to be plugged. Due to the deteriorated condition of the eggs, I would assume that this fish's ovulation response was actually triggered by the warm-up injection and consequently the eggs had been released on the evening of the 13th. The checks that evening did not reveal any eggs released because of the blockage in the vent. This was the first pallid to respond to the warmup injection at Garrison Dam NFH. It appears that either the female was exceptionally sensitive to the hormone injection or the anti-stress dopamine blocker, domperidone, was working - too good. With the eggs from this fish lost, we regrouped. The Fort Peck fisheries crew was still on the upper reservoir. They had indicated that they had collected a gravid female earlier in the week. The call was made to determine the likelihood of collecting a female. With the odds being poor at best - but the best in years, we loaded up the pallid distribution tank and headed for the headwaters. Later that afternoon, at 4:00 pm, female #

220F0F6213 was given a warmup shot of 0.082 mg of LH-RH (0.005 mg/kg) and a warmup dosage of 5 mg of domperidone (0.3 mg/kg). In order to utilize milt from the Montana males, we needed an ovulated female within a short time frame.

6/15/00 - At 10:00 am female #213 was given the resolving dose (0.738 mg LH-RH and 45 mg domperidone) and milt was collected from the males injected initially for female #752. At about 10:30, the Montana fisheries crew has captured a short, very plump female pallid near the site of the Slippery Ann Campground. The fish, tag # 411D235BOE, is a 'new' fish and is christened with the name 'Alice.' At 11:00 the fish is injected with the warmup dosage of LH-RH (@ 0.005 mg/kg) and domperidone (@ 0.37 mg/kg). Shortly after 12:00 with no other pallids captured we headed back to the hatchery. We also collected two 5+ pound shovelnose females for the stress testing broodstock. Shortly after departure with the injected pallid, the u-bolt holding the rear axle of the trailer broke causing a few tense moments. After assessing the damage, a few calls were made, and with the help of a small town garage not far away we were able to piece the trailer together and limp on home. While repairing the axle, a call from the hatchery indicated the female injected with the warmup dose 23 ½ hours earlier had ovulated. The 3:30 pm check revealed eggs flowing. This piece of information backs up what we assumed happened with the first female, Ovulation was proceeding with the warm up injection. The three additional milt samples taken on June 12th and 13th also arrived. Two of the samples had very low viability, less than 5%, and one sample, 'Pomp' , # 411D40360E, was good at 65%. Spawning began at 3:45 with crosses first made with 'Harvey' fertilizing 160 mls of eggs and 'Pomp' fertilizing an additional 120 mls. At 5:15 only a small amount of eggs, 42.5 mls., were recovered. The eggs were turned over to the 'Cryoperservation Team' to run their tests. At this time three additional males were injected to combine with 'Alice' now in transit to the hatchery. The three males selected were those that were not recommended to be crossed by Bernie May in the genetics lab at the University of California, Davis. With this being a Fort Peck female, the genetics would be assumed different at this point and we had wanted to utilize all males captured if possible. An hour had gone by and the fish was palpated again, this time 25 mls were recovered and fertilized with 'Perot'. The volume of eggs being recovered is not as good as hoped, given the initial take of 280 mls. At 8:15 pm another small take of 50 mls, then at 10:35 an additional 70 mls. Both takes are fertilized with 'Perot' to help build up a family lot. At just after 12:00 am the trailer with "Alice" arrived at the hatchery. The fish is injected with the final dose of LH-RH (0.45 mg/kg) and domperidone (1.10 mg/kg), off loaded into a 20 foot tank and the spawning continues with another 43 mls taken. This time the eggs are fertilized with male #1F4849755B. At 1:15 another 61 mls are recovered with the aid of the catheter and crossed with #55B. The last four takes over the next 2 ½ hours result in an additional 145.5 mls of eggs which were fertilized with male # 1F4A143350. See Table 2 for a summary of female #220F06213.

6/16/00 - Alice, the third female, was observed for signs of ovulation beginning at 7:30 the morning of June 16. Checks were performed every two hours until 3:00 pm when three eggs were expressed. An hour later 50 eggs were expressed from this fish. At the recommendation of Serge Doroshov these eggs were placed in a petri dish unfertilized. Serge had indicated that viable eggs will orient themselves with the animal pole pointed upright. He further stated that the vegetal pole is more adhesive and attachment in this manner allows the micropiles which are located in the animal pole to be oriented in a position where they are more readily accessible to fertilization. Another possibility suggested by Dettlaff in the publication, Sturgeon Fishes, is that the ova rotates within the outer shell to position the animal pole upward. In either case, it

indicates a viable ova. The eggs in the dish oriented with the animal pole positioned upright indicating that ovulation was occurring. At 5:30 the fish was again checked, this time only fluid was expressed. At 7:30 when no eggs were expressed, a tube was inserted. A few hundred eggs flowed into the tube initially then the flow stopped. A finger was inserted in the vent alongside the tube to prevent the duct from collapsing. Again the flow of eggs began. A half hour later the process was repeated. As long as the duct was held open, ovulated eggs would flow through the inserted tube. The process was repeated at hourly intervals until 2:00 am when we had made crosses with every available male. Ovulation on this fish had begun at 3:00 pm and lasted until at least 2:00 am, 11 hours in duration. Although egg viability had deteriorated towards the end, fry were produced from all but 1 cross (a family produced with poor quality sperm at the last egg take). Ovulation of this fish began 28 hours after the initial injection. After egg collections had ended the fish was injected with LA-200.

6/18/00 - Treated eggs from 'Alice' with 65 ppm malachite green for 15 minutes and moved jars to tanks. Alice died this evening apparently from the stress of spawning. Internal examinations didn't reveal any unusual problems. Externally the fish was covered with reddned splotches.

6/19/00 - Eggs from female 213 are 85 % hatched by 7:15 am. Fungused eggs are removed from the jars.

6/20/00 - Eggs from Alice have nearly completed hatching. The final female, #55A, was injected the afternoon with the initial dose of 0.005 mg/kg of LH-RH in combination with 0.15 mg/kg of domperidone. The following morning the resolving dose of 0.045 mg/kg LH-RH along with 1.78 mg/kg domperidone is administered. The rate of injection of domperidone was suggested at 3mg/kg total dosage. The rate of injected was reduced to what was available , 1.93 mg/kg total dosage. The female was checked for signs of ovulation periodically through the night and every two hours starting at 6:00 am until eggs were expressed. At 4:00 pm on the 22nd of June approximately 20 eggs were collected, fertilized with milt from males # 350 and # E31, and incubated in a petri dish. Within ~15 minutes the eggs had oriented with the animal pole in an upward position indicating that fertilization had occurred and the eggs were viable. If this was the beginning of ovulation it is 20-24 hours later than the other two fish. An hour later the fish was again palpated, this time approximately 200 eggs recovered. These eggs were fertilized with male # E31. At 6:00 the fish produced another 150 eggs and the eggs were fertilized with the same male. Several of the eggs look mottled and some are disc shaped. There is concern that the fish may have gone past its prime but concerns over the health of the female prevent us from any invasive attempts to recover the eggs. At 7:05 the fish produced only 23 eggs which were fertilized as before. At 8:00 pm no eggs are recovered. Initial cleavage is observed on the eggs taken at 5:00. At 9:30 pm the fish is palpated and no eggs are observed. Five and a half hours have elapsed since the recovery of the first eggs. After the loss of Alice to the stress of spawning we had resigned to spawning this fish using only palpation methods. Now that we were not having results with palpation there was a new concern over these eggs going past their prime. The fish is placed on a stretcher and a 1/4" hose is inserted in the vent to rupture the mullerian duct. A 1/2" diameter hose is slid over the smaller hose to direct the larger hose into the opening. The smaller hose is removed and eggs begin to flow into the tube where they are collected. The fish is positioned on it's belly to allow it's own weight to push the eggs from the ceolomic cavity. Approximately 6000 eggs were collected and fertilized with two males. The egg flow halted but am unsure if this was a result of not having additional ovulated eggs present or if ovarian tissue had blocked the opening of the tube. Many of the collected eggs were flattened and some ruptured, possibly smashed between the two tubes initially. At 11:05 palpation was tried again,

350 eggs were collected. The fish was immediately positioned on the stretcher and the tube was inserted. An additional 24,500 eggs were collected and fertilized with the same two males. The egg quality is very poor with most eggs either flaccid or broken. Male motility at this time was 95% for #E31 and 85% for male #350. From the 31,400 eggs collected we had a total hatch of 639 prelarvae. Within a few days post-hatch all of the larvae died. See Table 4. for a summary of the spawning times and results.

Propagation Chronology - 1999 and 2000 Progeny

11/18/99 - 1999 progeny sent to Valley City NFH

2/8/00 - Fish sampled at VCNFH show severe *amoebic* gill disease. No virus evident.

5/4/00- Received 1999 progeny from Valley City NFH. Feeding Biodiet 1.5 mm.

5/5/00 - Fish concentrated in tail end of raceway 24.

5/8/00 - Moved feeders to back of raceway and began feeding at night.

5/12/00 - Converting from Biodiet to Rangen feed.

5/14/00 - Mortalities are occurring in the four foot tanks (Pink, Green, and Yellow family lots)

5/15/00 - Began oxytetracycline treatments 10 ppm in four foot tanks.

5/16/00 - Added spray bar to tank 24, fish moved to head end of tank. (Nitrogen levels elevated) Treating four foot tanks with 15 ppm OTC. Mortalities are still occuring.

5/17/00 - Treated four foot tanks with 20 ppm OTC

5/18/00 - Treated four foot tanks with 20 ppm OTC and added packed columns. Water temp 62 F. Samples sent to Bozeman FHC (no virus found).

5/19/00 - Shut down spray bar and added packed column to tank 24. Fish moved to tail end of raceway. Covers added to tail end. Mortalities have stopped in circular four foot tanks. Apparently either the treatments were effective or the packed columns alleviated the stress.

6/7/00 - Fish from 1999 year class (tank 24) sent to Bozeman FHC. No mortalities occurring in the Pink, Green, or Yellow tanks.

6/16/00 - Fish back on Biodiet 1.3 mm feed. Fish did not accept the Rangen #4 salmon diet very well. No more wasted feed.

6/19/00 - Beginning of increased mortality (one per day) in 1999 progeny in tank 24. Robust fish are dying. Water temperature 70 ° F . Nitrogen gas at 112.9 % saturation. Water supply switched to the Salmon Building where there are degassing capabilities.

6/20/00 - Water temperature at 65° F and Nitrogen at 84.8 - 86.5 % saturation.

6/23/00 - Mortality increased to 9 fish/day. (Finished spawning pallids)

6/24/00 - Fish from 1999 year class (tank 24) sent back with Crystal to Bozeman FHC. Mortalities still limited to tank 24 - 7 orange and 2 black so far this month.

6/25/00 - Treated tank 24 with 10 ppm OTC. 9 morts

6/26/00 - Treated tank 24 with 20 ppm OTC. 13 morts. Fish sent live to Bozeman FHC.

6/27/00 - 44 morts.

6/29/00 - Two live fish from orange family (1999 progeny), 6 frozen morts and several bags of larval pallids sent to UC Davis for disease analysis. Treating with oxytetracycline.

7/6/00 - Feeding 1999 fish medicated feed (TM) at 4% body weight. Nitrogen levels between 100 - 102.5 % saturation. *Phone call from Jim Peterson informed that fish diagnosed as positive for virus in tank 24 (orange family) by Beth MacConnell from samples taken by Bozeman FHC on June 24.*

7/7/00 - *Fish sent to UC Davis on 6/29/00 confirmed positive for virus.*

7/10/00 - 3 Orange family fish sent to Beth MacConnell, one robust, two emaciated. *All three were infected with the iridovirus*. Two pallids (on ice) sent to UC Davis for viral research. Results: *both had a light viral infection*

7/12/00 - Two boxes of live yearling pallids (4 fish) along with 7 bags of morts sent to UC Davis for viral research

7/13/00 - Fresh dead pallids sent to UC Davis for viral research

7/17/00 - Temperature at 74.4 ° and Nitrogen at 101.7 %.

7/18/00 - Fry tanks have been cleaned daily using a siphon hose to remove excess feed and fecal material from tank bottoms. The waste has been discharged into a pan, then the live fry siphoned incidental to the cleaning were put back into the tank. Mortality may be associated with this practice and so it was discontinued in favor of draining tanks and using a screen to pick out the larger materials. (Mortalities after this date dropped dramatically.)

7/24/00 - Water temperature at 67° F and Nitrogen level at 103-105 % with the exception of the eight foot tanks where it is at 117 %.

7/26/00 - Emaciated yearling fish sent to Bozeman FHC and UC Davis for analysis. Virus found in 3 of 4 fish sent to Bozeman and all fish were starving. UC Davis was sent one fish each from the three family lots not diagnosed positive (yellow, green, pink) and several dead orange family fish.

8/2/00 - Water temperature at 65° F and Nitrogen gas at 107-108 %. YOY pallids in 2 tanks have increased mortalities (30+ per day)

8/3/00 - YOY pallids from FT 6 (BOE X 773) and FT-18 (BOE X 773) sent to Bozeman FHC for analysis. Diagnosed with severe amoebic gill disease. Fatty livers also noted.

8/9/00- Two YOY pallids each from FT-1 (213 X 60E), T-10 (BOE X 53A), FT-11 (BOE X 773), and FT-30 (213 X 325) and samples from suspected viral 1999 progeny taken by Beth at the hatchery for analysis. Results: *One fish from FT-11 positive for virus*, also was infected with amoeba. Livers of all fish borderline pathological (fatty). Yearlings sampled in Tank 24 were virus positive.

8/14/00 - YOY pallids from FT-15 (BOE X 552) and fin clips of six shovelnose and one pallid (Alice) sent to Beth MacConnel for disease analysis. Results: *YOY positive for virus*, severe fatty livers, amoeba present and severe fusion of gill filaments. No virus detected in fin clips.

8/21/00 - Amoeba infected fish from 2000 progeny (T-8, BOE X 350) sent to Jim Peterson and Beth MacConnell. Seven boxes of pallids, 100 YOY fish from FT-18, (formerly FT-11, BOE X 773) and 13 yearling (2 black, 11 orange) sent to UC Davis for viral research.
Treated YOY tanks with formalin at 200 and 150 ppm in a 1 hour bath to remove the parasite. 250 ppm for even 15 minutes appeared to be too strong.

8/22/00 - YOY from FT-10 (BOE X 55B), FT-18 (BOE X 773), FT-20 (BOE X A65), and FT-26 (BOE X 773) sent to Beth MacConnell for assessment. Tanks 10, 20 and 26 were negative for the virus, *tank 18 was positive*. All four samples had amoebic gill disease.

8/22/00 - Amoeba infected YOY pallids (T-8, BOE X 350) and (FT-26, BOE X 773) sent to Steve Smith DVM at Virginia Polytech. Results: amoebic gill disease, fungal disease, and several species of bacteria.

9/5/00 - 40 YOY virus positive pallids sent to UC Davis to determine if YOY white sturgeon could become cross infected.

9/13/00 - Samples sent to Bozeman FHC from YOY pallids, FT 10 (BOE X 55B), FT 14 (BOE X 55B), FT 30 (213 X 325), T 2 (BOE X 55B), T 8 (BOE X 350), and T 9 (BOE X 55B) and yearling fish. Results: *iridovirus found in FT 14*, suspect virus in FT 10 and the Pink family, fatty livers and amoeba induced damage to gill tissue found in all YOY fish

9/22/00 - Ten of the twelve pallid broodstock were fitted with transmitters. The transmitters were a radio/sonic combination (CART tags) and were inserted through an incision made in the abdominal wall. The fish were administered an injection of Nuflor to combat infection. The broodstock were transferred back to the confluence on September 23-25th. The stitches were intact, and the fish appeared in good shape. Two fish were expelling a bloody fluid through the vent but no inflammation or redness was apparent. Vent area of male #E31 had what appeared to be swollen blood vessels when it was captured earlier this spring. The condition remained the same. All fish were located after restocking and had moved up into the Yellowstone River remaining near the confluence.

10/5/00 - Samples taken from tanks with low level mortalities. Tanks sampled include FT 2, 7, 10, 27, 28 and T 2 and T 9.

10/12/00 - All 1999 progeny were destroyed. Samples of all family lots were collected by the Bozeman FHC along with samples from most tanks of the 2000 year class.

10/23/00 - Began mixing biodiet feed with Silver Cup feed.

10/26/00 - Results back from 10/5 samples. Both of the 4 foot tanks negative (T 2 - BOE X 55B and T 9 - BOE X 55B). All five of the 30 inch tanks positive (FT 2 - BOE X 94B, 7 - BOE X 55B, 10 - BOE X 55B, 27 - BOE X 552, and 28 - BOE X 552). No amoeba were found on gills and gill tissue looked normal. Livers improved from previous exams.

Table 1. Milt Motility

TAG NUMBER	RIBBON COLOR TANK / NAME	CRYO SPERM AMOUNT	% MOTILITY (6/13/00)	% MOTILITY (6/14/00)	% MOTILITY (6/15/00)	% MOTILITY (6/16/00) 12:00 / 9:30	% MOTILITY (6/17/00)	% MOTILITY (6/22/00) 4:00 pm
1F4849755B	S GREEN			90	95	85		
7F7B081579	S BLUE			<1	40	85		
1F4A004552	S BLUE/GREEN	10- 0.5 ML		90	35			
1F4A143350	N BLUE	10- 0.5 ML		95	90	90		85
7F7F054773	N ORANGE	2-5 ML 10- 0.5 ML				90		
1F4A33194B	N GREEN	2-5 ML 10- 0.5 ML				95		
115712453A	N ORANGE/BLUE	10- 0.5 ML				35 / 85		
7F7D487531	LENNY				5			
7F7E6C1A60	CLARK				5			
411D40360E	POMP				65	65		
41476A0462	PEROT		35		35 / 20		35	
1FAA4B5973	LEW		20 / 15 / 40		0 / 5 / 15		10	
1F48512325	HARVEY		90		40			
2202236E31		10- 0.5 ML						95
115525534A								
1F477B3A65	99 MALES	2-5 ML 11-0.5 ML						
115552116A	99 MALES	1-0.5 ML						
1F482F3F2B	99 MALES	1-0.5 ML						
1F4A27214F	99 MALES	1-0.5 ML						

Table 2. Female # 220F0F6213 Spawning Results (6/15-16/00)

FEMALE # 220F0F6213									
TIME / DATE	MALE #	MLS EGGS	# EGGS	% EGGS VIABLE	% MILT MOTILITY	HATCHED FRY	# FRY @ 1 WEEK	% SURV TO A WEEK	# FISH 10/1/00
3:45 pm 6/15	1F48512325	160	9520	56	40	5300	~200	2	163
3:45 pm 6/15	411D40360E	120	7140	15	65	1100	~350	5	173
5:15 pm 6/15	CRYOPRESERVATION	47.4	2750**	15	10 - 85	378	125	5	
5:15 pm 6/15	CONTROL # 350	6.2	360**	68	85	152	*	0	
6:15 pm 6/15	41476A0462	25	1488	34	35	2900	~1000	12	340
8:30 pm 6/15	41476A0462	50	2975	-	-	-	-	-	
10:15 pm 6/15	41476A0462	70	4165	-	-	-	-	-	
12:30 am 6/16	1F4849755B	43	2559	19	95	1200	~60	1	15
1:15 am 6/16	1F4849755B	61	3630	-	-	-	-	-	
1:45 am 6/16	1F4A143350	30	1785	19	90	1600	~20	<1	3
2:26 am 6/16	1F4A143350	13.5	803	-	-	-	-	-	
3:20 am 6/16	1F4A143350	73	4344	-	-	-	-	-	
3:50 am 6/16	1F4A143350	29	1726	-	-	-	-	-	
Total/Averages		728	43245	29		12478	1755	4	704

***** Control # 350 combined with production lots ******Includes eggs used in egg fertilization study (preserved in formalin)

Note: Very high mortality post hatch. Low survival probably a result of premature hormone injection. The Polarity Index indicated the egg stage was not advanced enough to be suitable for fertilization. Other possibilities include poor egg quality and elevated levels of toxic substances in the yolk sac. At hatch development for sturgeon is far from complete. Major differentiation of the anatomy takes place prior to the onset of feeding. During this time energy stores it the yolk sac are used up. Many harmful substances that have accumulated in the adult are passed on to the progeny in the fatty reserves of the yolk sac. The effects of these substances becomes apparent only after hatch when the developing larvae begin to utilize those energy store

Table 3. Female #411D235BOE Spawning Results (6/16-17/00)

FEMALE # 411D235BOE 'ALICE'									
TIME / DATE	MALE #	MLS EGGS	# EGGS	HATCHED FRY	% EGGS VIABLE	% MILT MOTILITY	# FRY @ 1 WEEK	% SURV TO 1 WEEK	# FISH 10/01/00
3:00 pm 6/16			3						
4:00 pm 6/16	ORIENTATION TEST		50						
5:30 pm 6/16		0	0						
7:30 pm 6/16	1F4A33194B	55	3273	2300	21	95	~1700	74	551
8:00 pm 6/16	7F7F054773	85	5058	2700	53	90	~1750	65	68
8:45 pm 6/16	CRYOPRESERVATION	39.5	2348	641	31	1 - 50	518	81	260
8:45 pm 6/16	CRYOPRESERVATION FERTILIZATION STUDY	28.1	1672	-		1-50	-		-
8:45 pm 6/16	CONTROL #94B	7.5	448*	172	67	95	**		-
8:45 pm 6/16	UNFERTILIZED CONTROL	2.2	132	0	0	-	-		-
8:45 pm 6/16	DEVELOPMENT SERIES #552	78	4611	?			250		-
9:40 pm 6/16	1F4A143350	85	5058	1400	28	90	~350	25	213
9:40 pm 6/16	115712453A	99	5891	1700	29	85	~950	56	648
10:50 pm 6/16	411D40360E	77	4582	1600	35	65	~60	4	34
10:50 pm 6/16	1F4849755B	77	4582	4000	21	85	~2400	60	2068
12:10 am 6/17	CRYOPRESERVATION	38.9	2316	426	18	0 - 35	~250	59	-
12:10 am 6/17	CRYOPRESERVATION FERTILIZATION STUDY	20.4	1215						-
12:10 am 6/17	CONTROL # 350	7.9	469*	97	31	90	**		-
12:10 am 6/17	1F4849755B	123	7319						-
12:10 am 6/17	1F4A004552	159	9461	2500	26	35	~1500	60	820
12:10 am 6/17	7F7B081579	167	9937	1200	12	85	~400	33	77
12:10 am 6/17	1F4849755B	118	7021						-
12:10 am 6/17	1F4A33194B	125	7438						-
2:05 am 6/17	1F4A4B5973	135	8033	175	2	0 / 5 / 15	0		0
2:05 am 6/17	41476A0462	165	9818	1350	14	35	~350		8
Total/Averages		1693	100735	20261	21	0 - 95	10478	52	4747

*Fertilization study eggs included **Combined with production lot

Table 4. Female # 115713555A Spawning Results (6/22/00)

FEMALE # 115713555A						
TIME/DATE	**MALE #**	**MLS EGGS**	**# EGGS**	**HATCHED FRY**	**% VIABLE**	**% SURV TO A WEEK**
4:00 pm 6/22	orientation test	0.5	20			
5:00 pm 6/22	2202236E31	4	200	417	2.6	0
6:00 pm 6/22	2202236E31	3	150			
7:05 pm 6/22	2202236E31	0.5	23			
8:00 pm 6/22		0	0			
9:45 pm 6/22	2202236E31	54	3213			
9:45 pm 6/22	1F4A143350	~50	2975	222	1.4	0
11:05 pm 6/22	1F4A143350	6	350			
11:10 pm 6/22	1F4A143350	202	12019			
11:10 pm 6/22	2202236E31	210	12495			
Total/Averages		530	31427	639	2.0	0

Polarity Index of 0.18 on this female indicated it was not ready for induced ovulation. The Polarity Index had not changed between the April and June assays. It is assumed this fish if held longer would have 'ripened' and produced viable eggs. If we had the equipment and knowledge of the test in place this fish would not have been injected until a later date.

Year 2000 Pallid Production - December 1, 2000									
Tanks	♀	♂	**Hatch Number**	**6/27/00 Inventory**	**7/11/00 Inventory**	**8/1/00 Inventory**	**10/18/00 Inventory**	**12/01/00 Inventory**	**Viral Status**
FT 1, T 3	213	60E	1168	350	273	206	173	141	+
FT 6,9,S8	213	462	3219	1000	1133	959	340	339	+
FT 30, T 4,7	213	325	5583	200	386	264	163	157	
FT 29	213	350	1603	20	9	4	3	2	
FT 4	213	55B	1067	60	42	19	15	15	
FT 12	213	16A	164	81	62	32	29	26	
FT 19	213	A65	209	44	33	26	23	22	
FT 2,5, T 5, N8	BOE	94B	3975	1700	1115	1154	551	550	+
FT 11,18, 26	BOE	773	3139	1750	1500	1082	68	67	+
FT 3,7,10,14 T 2,9, S20	BOE	55B	6173	2400	3673	2587	2068	1608	+
FT 8,16*27,28 T 6, N20	BOE	552	3163	1750	1000	1774	820	792	+
FT 22	BOE	462	1395	350	300	306	8	8	
FT 15, 23	BOE	579	1187	400	200	86	77	42	
FT 25	BOE	60E	1593	60	67	37	34	34	
T 8	BOE	350	1480	350	450	297	213	213	
T 1, 10, N20	BOE	53A	1978	950	950	681	648	626	
FT 17	?	?	35	21	7	7	7	7	
FT 13	BOE	14F	132	106	92	75	70	58	+
FT 20	BOE	A65	268	178	146	115	83	83	
FT 21	BOE	F2B	223	159	126	97	1	1	
FT 24	BOE	16A	274	75	52	32	26	26	
TOTAL			38028	12004	11616	9840	5420	4817	

Year 2000 Pallid Production - Mortality															
Tanks	♀	♂	**~Hatch Number**	**Prelarvae Mortality**		**Siphon * Mortality**		**Amoeba** Mortality**		**Other** Mortality**		**Water** Mortality**		**Lot Totals**	
				Number	%	Number	%	Number	%	Number	%	Number	%	Total Mortality	INV # 12/1/00
FT 1, T 3	213	60E	1168	784	67	172	45	0	0	71	33	0	0	1027	141
FT 6,9,S8	213	462	3219	2005	62	286	24	554	60	35	4	0	0	2880	339
FT 30, T 4,7	213	325	5583	5133	92	176	39	88	32	26	9	3	1	5426	157
FT 29	213	350	1603	1584	99	16	84	0	0	1	33	0	0	1601	2
FT 4	213	55B	1067	1010	95	33	58	0	0	9	38	0	0	1052	15
FT 12	213	16A	164	87	53	40	52	0	0	11	30	0	0	138	26
FT 19	213	A65	209	173	83	9	25	0	0	5	19	0	0	187	22
FT 2,5, T 5, N8	BOE	94B	3975	2210	56	967	55	176	22	72	9	0	0	3425	550
FT 11,18, 26	BOE	773	3139	1144	36	570	29	1256	88	102	7	0	0	3072	67
FT 3,7,10,14 T 2,9, S20	BOE	55B	6173	1833	30	1156	27	911	29	187	6	478	15	4565	1608
FT 8,16,27,28 T 6, N20	BOE	552	3163	1200	38	876	45	141	13	154	14	0	0	2371	792
FT 22	BOE	462	1395	1014	73	61	16	295	92	17	5	0	0	1387	8
FT 15, 23	BOE	579	1187	907	76	190	68	0	0	48	53	0	0	1145	42
FT 25	BOE	60E	1593	1530	96	21	33	0	0	8	19	0	0	1559	34
T 8	BOE	350	1480	1046	71	150	35	46	16	25	9	0	0	1267	213
T 1, 10, N20	BOE	53A	1978	869	44	399	36	17	2	67	9	0	0	1352	626
FT 17	?	?	35	17	49	11	61	0	0	0	0	0	0	28	7
FT 13	BOE	14F	132	28	21	25	24	0	0	10	13	11	14	74	58
FT 20	BOE	A65	268	107	40	42	26	20	17	16	13	0	0	185	83
FT 21	BOE	F2B	223	72	32	44	29	95	89	11	10	0	0	222	1
FT 24	BOE	16A	274	214	78	24	40	0	0	10	28	0	0	248	26
TOTAL			38028	22967	60	5268	35	3599	37	885	9	492	5	33211	4817

* Siphon percent mortality calculated after total survival was adjusted from prelarvae mortality

** Percent mortality calculated from total survival after losses to prelarvae and siphon mortality

BREEDING PLAN Suggested by the Genetics Department, UC Davis		
Female #	**Male #**	
	Suggested	**Used**
1F47715752	1F4A143350	1F4A143350
	757B081579	1F48512325
	1F4A004552	411D440360E
	1F4849755B	1F4849755B
		41476A0462
220F0F6213	115525534A	1F48512325
	7F7B081579	411D440360E
	2202236E31	41476A0462
	1F4A143350	1F4A143350
		1F4849755B
411D235BOE		1F4A33194B
		115712453A
		7F7F054773
		1F4A143350
		411D440360E
		1F4849755B
		1F4A004552
		757B081579
		1F4A4B5973
		41476A0462
115713555A	1F4849755B	
	2202236E31	2202236E31
	115525534A	
	1F4A143350	1F4A143350
Males to the right are not included in the 1 X 4 crosses suggested	1F4A33194B	
	115712453A	
	7F7F054773	

	Fort Peck fish

Dr. Bernie May made the suggestions for mating analyzing eight microsatellite loci with 2-7 alleles per loci. The recommendations were based on mating the most dissimilar fish.

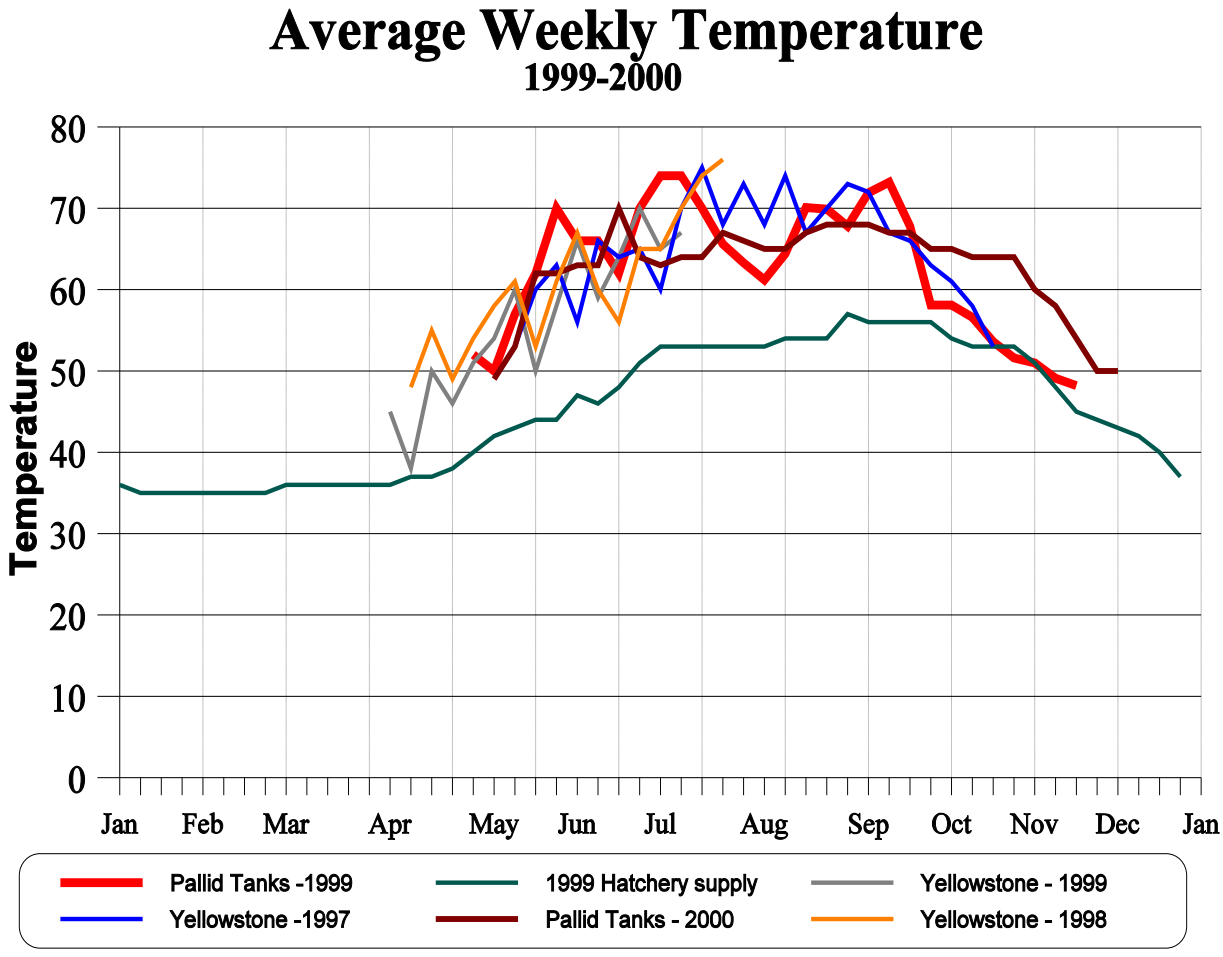
Average Weekly Temperature
1999-2000
Temperature
80
70
60
50
40
30
20
10
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Pallid Tanks -1999
1999 Hatchery supply
Yellowstone - 1999
Yellowstone -1997
Pallid Tanks - 2000
Yellowstone - 1998

Table 9. Broodstock Data

PALLID STURGEON BROODSTOCK DATA					
Tag Number	**Date**	**Sex**	**Weight**	**Other Info**	**Capture Site**
1F47715752	4/11/00	F	55	mature eggs	confluence
1F4A436E66	4/11/00	F	46	immature eggs	confluence
1F477B3A65	4/11/00	M	27		confluence
7F7F054773	4/11/00	M	50		Missouri River
7F7F065A3D	4/11/00	F	55	atritic eggs - MFWP yellow tag	confluence
1F4A143350	4/11/00	M	28		Missouri River
1F4B225A1A	4/12/00	?	31	old fish	confluence
1F4A004552	4/12/00	M	25		confluence
7F7B081579	4/12/00	M	32		confluence
1F4A33194B	4/12/00	M	45		confluence
115712453A	4/12/00	M	27		confluence
1F4849755B	4/13/00	M	33		confluence
115713555A	4/13/00	F	57		confluence
115676690A	4/13/00	F		immature hybrid shovelnose??	confluence
115525534A	4/17/00	M	32		confluence
2200F0F6213	4/17/00	F	36	missing scute	confluence
2202236E31	4/17/00	M	61		confluence
1F4A4B5973	6/6/00	M	28	LEW	Ft Peck headwaters
41476A0462	6/8/00	M	35	PEROT	Ft Peck headwaters
1F48512325	6/8/00	M	28	HARVEY	Ft Peck headwaters
7F7D487531	6/12/00	M	32	LENNY	Ft Peck headwaters
7F7E6C1A60	6/13/00	M	33	CLARK	Ft Peck headwaters
411D40360E	6/13/00	M	17	POMP	Ft Peck headwaters
411D235BOE	6/15/00	F	45	ALICE	Ft Peck headwaters

Conclusions

2000 spawning effort was successful in producing 20 family lots of pallid sturgeon.

Domperidone appears effective as an antidopamine blocker and should be used on wild captured fish in stressful environments in conjunction with hormone injections.

Efforts above Fort Peck paid off in providing viable milt for the recovery effort as well as an additional female. Logistics of transporting and storing sperm from wild caught fish during the spawning run was accomplished.

Cryopreservation techniques were demonstrated successful. Methanol (MeOH) and dimethyl sulfoxide (DMSO) were the two cryoprotectants added to the milt prior to freezing. Three concentrations were used, 5%, 10%, and 15%. All trials had success, however the MeOH at the 5% and 10 % rate were by far the best. Straw sizes used were 0.5 and 5 ml. Both were successful.

Genetic mapping of adults held at the hatchery was accomplished. An opportunity exists to establish the identity of unmarked fish released from the hatchery and captured in the wild using genetic mapping. If we continue to collect fin samples from all broodstock, research geneticists at UC Davis are confident they can trace unmarked fish back to the parent cross. This technique could be used in cases where we want to release eggs or fry into the recovery areas but would like them 'marked' to differentiate from natural reproduction.

The Polarity Index information corresponds well to the overall performance of the eggs this year and in years past and should be used extensively in the future to track egg stage and predict appropriate injection time.

Using a siphon to remove excess feed and fecal material in tanks with high densities during the first few weeks post hatch can cause mortality. Apparently fry siphoned incidental to removing feed have a good chance of dying from stress or mechanical injury.

High nitrogen levels cause gill damage and allow for invasion by amoeba parasites.

Amoeba outbreaks caused rapid mortality in infected tanks ranging from 28-100% mortality in approximately 10 days. CASE: FT-6 (BOE X 773) had 416 fish on July 20. An amoeba outbreak on 7/29 occurred and 11 days later all fish had died. We had not determined a treatment for the amoeba at this point.

Formalin treatments using a 100 ppm concentration for a 1 hour static treatment was successful in amoeba control.

The 'Missouri River Iridovirus' has the potential to cause low level mortalities in compromised fish. CASE: FT-11 (BOE X 773) had 408 fish on July 20. On August 9 the tank was diagnosed positive for the iridovirus. During the 'virus phase', between July 20 and August 16, there were 10 mortalities, 2% of the total. On the 17th of August the tank was infected with the anmoeba, within 7 days mortalities were at 30% of the total. Two days after formalin treatments mortalities ceased.

Although viral outbreaks and pathogen problems were present, a considerable amount of information was gained which will hopefully advance the recovery effort.

Recommendations from 2000 and results:

Research other cues/tests that will enable us to determine receptiveness of egg to hormone injections. Consult other sturgeon producers. *The polarity index, the ratio of the distance between the GV and inner cell membrane and the diameter of the oocyte provides the cue to egg maturation level. An index of less than 0.1 indicates eggs that will likely respond to hormone injections. This stage is further confirmed using the progesterone assay (The assay has been revised to allow 16 hours of incubation at ambient temperatures rather than the 24 hours originally used and uses Ringers solution in place of Leibovitz). These methods are currently used by the white sturgeon producers (Stolt Seafarms and The Fishery and for the recovery efforts of the Kootenai white sturgeon). The Polarity Index results on pg. 28 illustrate how well spawning events have tracked the Polarity Index.*

Have genetic mapping completed on broodstock prior to spawning and utilize information to select the best crosses (if we have enough broodstock where choices are necessary). This information can become part of the genetic baseline of information for this population of sturgeon. *Jeff Rodzen, UC Davis, suggested the method of genetic mapping had been used successfully to identify the parents of a random mix of progeny with a high degree of accuracy in white sturgeon. One test was 99% accurate using 450 fish looking at 3 markers and another with 100% success on 80 fish. He suggested that with a collection of unmarked fish recovered from a stocking event in the wild, he could assign the recovered fish to individual matings provided he had genetic makeup of the parents. The process has merits if we decide to stock fish at an earlier age. Indicates a need to collect fin samples of wild caught males that are used in the recovery process as well.*

If the opportunity exists, spawn females at extended intervals to allow for experimental opportunities as dictated by the polarity index and progesterone test. *Sturgeon apparently spawn over an extended period of time depending on egg maturation, temperature, diurnal cues, and flows. Condensing our spawning operations into a single event or even over the course of a couple weeks may not be the most prudent approach, in fact we may inadvertently be selecting for individual that spawn only during a short time frame. Spawning at the hatchery should coincide with a favorable Polarity Index. Premature hormone injections have shown to cause poor to no survival. In broodstock development we know it is advantageous to collect fish from the entire spawning run and we should try to adjust our spawning practices to accommodate for the early and late spawners as well. There are obvious disadvantages at the hatchery in this approach, on the other hand it will allow for extended time for research on cryopreservation, optimal water temperatures, flow rates, toxicity testing and so forth.*

Continue work on cryopreservation. *The success of the Warm Springs FTC in achieving fertilization of pallids was a major breakthrough for sturgeon cryopreservation. A major thrust of the recovery effort should center around the developing storage capabilities at Garrison Dam NFH and additional testing of the techniques employed this year at Garrison Dam NFH by Bill Wayman and Gegg Looney.*

Disinfect eggs with Betadine to reduce the chances of viral infection - GAP used 50,75,and 100 ppm for 30 and 60 minutes with success. It would be valuable to determine what concentration exists inside the egg at the different rates. Also, what level is necessary to destroy the virus. *Betadine at 50 ppm for 30 minutes was used during water hardening the eggs to disinfect. The*

measure is typically used to disinfect salmonid eggs received from other facilities. The treatment apparently was not effective in preventing the transfer of the virus for several reasons; 1) the virus is transmitted vertically and the treatment provides only topical disinfection, 2) the rate was to low or duration to short, or3) the virus is transmitted from the water source.

Test survival of sturgeon hatched at 70 degrees vs. 60 degrees. Faster hatch rates may preclude the need to treat fungus. Determine threshold and optimal levels.

Start with fresh Biodiet #2 salmon starter and progress to larger sizes - grower diets. The fish grew well on the #2 and #3 starter diets but could easily consume the 1mm Biodiet grower pellets. *The 1 mm Biodiet grower worked well. We tried to convert the 1999 progeny to our production trout and salmon diet from Rangen. The fish did not accept the diet and were switched back to a Biodiet diet. Later they were converted to a Silver Cup trout diet with a lower fat content.*

Maintain water temperatures on developing fingerlings near 70 degrees for optimum growth

Increase screen sizes to facilitate the self cleaning design of the circular tanks and maintain better water quality.
Screen sizes make a tremendous difference in our ability to keep good hygiene in the larval tanks. A slime develops on excess feed and fecal materials in the water which in turn causes gill problems and mortality in the developing larval fish. If we were able to graduate more quickly to larger screen sizes to allow passage of excess feed and fecal materials it would improve the health and survival of the sturgeon during that vital first month of life.

Experiment with different densities to determine an optimum or a threshold

Compare production in circular tanks vs. rectangular troughs and dark vs. light tanks

Are there any negative results using aluminum troughs? No luck in 1997 & 1998 *The presence of a brass mesh screen in incubator jars caused Acipenser gueldenstaedti prelarvae to become almost immobile and lie on the bottom for long periods without any movement (Sturgeon Fishes, Dettlaff et al., pp192-194). We had fry that responded similarly in aluminum troughs. Worth additional research?? We utilize aluminum screens in our fiberglass tanks. May indicate a need to switch to slots cut in pvc instead.*

Compile a database of spawning events from all pallid spawns at the hatcheries and attempt to pattern spawning times taking into account spawning temperatures, stage of egg development (PI), injection regimes, and survival.

Recommendations for 2001

Establish a Pallid Sturgeon Health Policy that is acceptable to State and Federal Agencies involved in the recovery effort using the 'best science' available with the assurance that the policy is dynamic and will be revised as information becomes available. Risk management should play into the developing of the policy.

Based on recommendations from the Fish Health Policy, reevaluate stocking rates and timing to accommodate the risks associated with long term culture.

Develop cryopreservation capabilities at Garrison Dam NFH and storage capabilities at Gavins Point and Valley City.

Use polarity index to determine time for inducing spawn

Check for GV breakdown post injection on females past 24 hour time frame.

Collect milt from males captured at large and overnight ship to hatchery for cryopreservation

Collect fin samples from all wild captured pallid sturgeon to establish a genetic profile for the population(s)

Apply cryopreservation techniques to the 2001 spawning effort (Will also work with cryopreservation techniques on shovelnose sturgeon)

Monitor and maintain safe nitrogen levels in the water.

Monitor with periodic inspections for iridovirus presence

Treat with formalin when mortality levels show a rise - even if amoeba can't be identified.

Maintain records of everything

Increase screen sizes to facilitate the self cleaning design of the circular tanks and maintain better water quality. Use a siphon in tanks only when the density levels allow safe removal of excess materials without risk of siphoning larval fish.

Test survival of sturgeon hatched at 70 degrees vs. 60 degrees. Faster hatch rates may preclude the need to treat fungus. Determine threshold and optimal levels. Determine optimal 'roll' rates.

Determine the effectiveness of formalin, hydrogen peroxide, and ultraviolet disinfection in the control of fungus on eggs to determine a safe alternative to malachite green.

Determine the effects of UV disinfection on amoeba, viruses, and other potential pathogens

Compile a database of spawning events from all pallid spawns at the hatcheries and attempt to pattern spawning times taking into account spawning temperatures, stage of egg development (PI), injection regimes, and survival.

Side Notes

A male pallid (1F477B3A65) used in 1999 to make the 'green' and 'orange' families was recaptured this spring and injected with LH-RH to induce spermiation. The male did produce viable sperm which indicates that it is possible for males to spawn annually.

A female shovelnose sturgeon (hybrid suspect) with pit tag # 115676690A was captured in April and catheterized on 4/18/00. Small (~ 1mm) yellow eggs were removed from the ovaries. The fish was held in the 20 foot circular tanks until September 27 when it was moved to pond 24 to overwinter. The fish was catheterized the same day and a sample of 2.5 mm black eggs were recovered. Apparently this fish will be spawning in the spring of 2001. This suggests that it is possible for female shovelnose sturgeon to spawn every two years.

The information from these two wild fish is consistent with what the white sturgeon growers have found. Female white sturgeon in captivity will produce viable eggs every two years and the males produce sperm annually.

May be possible to determine if ovulation has occurred in fish injected with hormones by collecting a sample of eggs in a catheter, boiling, and bisecting. The eggs should have undergone GV breakdown with the oocyte in the metaphase II stage of meiosis. According to the book Sturgeon Fishes by Dettlaff, most oocytes reach this stage 2-3 hours prior to ovulation. It also suggests that at ovulation all eggs have reached this prefertilization stage. The presence of a nucleus would indicate that meiosis has not advanced far enough yet.

According to the book Sturgeon Fishes by Dettlaff:

Excessive dosages of LH-RH do not appear to affect egg quality
Effectiveness of LH-RH increased when given in two injections 12 hours apart
Follicle enclosed oocytes can be incubated in coelomic fluid (or Ringers) and induced to ovulate using progesterone or pituitary in vitro
Sturgeon will not respond to hormone injections beyond their spawning range temperatures
Long term keeping of females at the spawning temperature causes egg quality to deteriorate
The longer wild captured females are kept the slower they are to respond to hormone injections and the lower the egg quality
Fertilizability of eggs decreases *gradually* over time. Eggs placed in water can be fertilized an hour or more after removal. If kept in coelomic fluid or saline the time is extended to 4-6 hours
One ml of milt contains ~ 1 X 10 to the tenth power of spermatozoa
Sperm life usually less than 5 minutes but has been documented up to 40 minutes
Milt can be stored up to 5-6 days on ice
Coelomic fluid should be decanted prior to fertilization
Both fertile and non-fertile eggs are sticky, but stickiness is more pronounced in fertile eggs
parthenogenetic development has been documented
Semi-dry method of fertilization gives best results
Add a mixture of 10 mls milt to 2000 mls (~2 quarts) of water for each kilogram of eggs

Virus particles can survive a week or so without a host (personnel communication w/ Ron Hedrick)

It takes an imaginary drop of water 1.4 years to travel 200 miles downstream through Lake Sakakawea but less than a day to travel the Missouri River 80 miles from Garrison Dam to Lake Oahe. (From the North Dakota Fishing Guide)

From what we know of the habitat requirements of sturgeon, although there are undoubtably sturgeon in the reservoir, the incidences of the fish in the lower end are probably minimal. Given the drop of water statement above, and the need of a host to keep the virus replicating, I would feel comfortable in stating the chance of fish getting infected from our water supply is pretty minimal.

The Kootenai River White Sturgeon of Montana, Idaho, and British Columbia is an endangered fish with a 1990 population of 880 fish. Recovery efforts for the two species (pallids and white) are similar as are the assumed reasons for the decline. A conservation hatchery operated by the Kootenai Tribe of Idaho provides 20 month old white sturgeon for restocking. The stocked fish have been WSIV positive.

No white sturgeon stocking is being carried out on the west coast since the occurrence of the three viruses. Prior to viral outbreaks commercial sturgeon producers were required to release a number of larval sturgeon. The reason for the release was more political than biological. The Game and Fish Agencies felt that public perception would favor the release from the commercial producers since the broodstock were taken from the wild. Natural recruitment is sufficient in most stretches to maintain the fishery. In other cases they use a 'trawl and haul' technique to move adult fish from areas of high concentration to those were recruitment is lacking. There was no reason to stock 'virus positive' fish.

Polarity Index

The Polarity Index is a tool used by sturgeon producers on the west coast to determine potential female spawners and predict ovulation time. The index has been revised slightly from what was published in The Hatchery Manual for the White Sturgeon. The index assumes a female is a candidate for ovulation when it has a Polarity Index of less than 0.1 (was 0.07). Using that information and newly purchased scope with a scale in the eyepiece I reviewed preserved eggs from past sturgeon spawned at Garrison Dam NFH.

Polarity Index Results			
Date	**Female**	**PI**	**Results**
6/15/00	**BOE**	**0.07**	**Successful spawn, fair egg flow, good survival**
6/6/00	**213**	**0.12**	**Ovulated well initially but egg flow was poor, poor fry survival**
6/6/00	**752**	**0.08**	**Ovulated but eggs were not expelled - went over ripe**
6/19/00	**55A**	**0.18**	**Ovulated very slowly and late, some hatch, no survival***
6/15/00	**HSN**	**0.65**	**Shovelnose spawned but no milt to fertilize with**
4/17/00	**213**	**0.18**	**Early spring**
4/13/00	**55A**	**0.18**	**Early spring**
4/12/00	**752**	**0.20**	**Early spring**
6/7/99	**44A**	**0.09**	**Slow spawn, poor survival**
6/7/99	**855**	**0.08**	**Ovulated well, dark eggs, good fertilization , no fry survival**
6/7/99	**963 (55C)**	**0.15**	**Ovulated once then quit, good survival**
4/14/99	**44A**	**0.15**	**Early spring**
4/13/99	**855**	**0.11**	**Early spring - fairly advanced egg stage**
10/6/98	**757F065E12**	**0.42**	**Fall capture**

***No change in the PI from 4/13 to 6/19**