PALLID STURGEON PROPAGATION - 1999
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 the station had ever spawned a pallid sturgeon. Unfortunately a power short to the boiler resulted in a loss of all eggs. 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. Any progeny resulting from the 1999 spawn would have to be held on station at Garrison Dam NFH. Tanks and plumbing were temporarily installed to accommodate a last minute quarantine imposed on all FWS hatcheries holding sturgeon. Stocking or transfer of any hatchery reared fish was put on hold until further information was available on the status of the virus in the wild.

Objectives

This year our goal is to locate a pair of gravid females with the hopes of successfully spawning the fish. Our original intent was to ship any resulting fry to Gavins Point NFH for further culture. A subsample of fry was to be retained on station to determine our rearing capabilities and to serve as a backup for Gavins Point NFH. If we were able to have our facilities usable by the time the pallids spawn, we would maintain up to 10 family lots as backup source for broodstock development. The viral outbreak and quarantine that followed changed our goals somewhat. All fry resulting from the spawning effort would need to be held on station until the quarantine was lifted. We will be utilizing the catheterization method again this year for determining egg stage and plan a rigorous monitoring program of the egg development. A sample of unfertilized eggs will be taken to determine if initial cleavage will result from the unfertilized eggs. Testing will also be performed to determine the effects of hydrogen peroxide as a fungicide. This year we will be relying on palpation methods to collect eggs. We will also be providing holding tanks for fall captured fish to be used at Gavins Point NFH. The fish will be closely monitored for signs of fungal infections and abrasions. Malachite green will be used topically to control any fungal outbreak. Oxytetracycline will be administered as needed to combat any infections that develop and as standard protocol on all captured fish.

Fall Capture 1998

Results

September 22, 1998 the first of three pallids was brought to Garrison Dam NFH prior to transferring to the Gavins Point NFH. The fish was a 27 pound male. The second fish, a female, was captured on 9/30, a recapture from 1992. The fish was captured by NDGF and carried a white dangler tag. A second female was captured on October 6. This fish was captured during the spawn in 1993 and was running eggs. On November 3, the three pallids were transported to Gavins Point for spawning. The female captured by NDGF died of unknown causes during the winter at Gavins Point NFH.

Spring Capture 1999

Methods and Results

Between April 12 and April 15, pallids were captured near the confluence of the Missouri and Yellowstone Rivers using 6" X 10" mesh trammel nets. Many of the captured fish were concentrated on the north side of the Missouri River bank adjacent from the mouth of the Yellowstone River. They were apparently staging there waiting for an increase in flows to stimulate the spawning run. Ten of the captured fish were transported to the hatchery, four possible females and six suspected males. 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 50 degrees Fahrenheit. Salt at ½ % was applied routinely to the tanks. There were 3-4 crews floating trammel nets, two from Montana Game and Fish and two from the Missouri River FWMAO. A transport tank and truck were supplied by the Garrison Dam Fish Hatchery. This effort was timed well, river flows were low and more fish were captured in this four day period than in any other . A chronology of events:

4/12/99 - Two FWS crews arrive at Confluence at 3:00 pm and set nets. On the third drift a 31 lb male is captured at river mile 1583.5. A crew from Montana Game and Fish arrives to assist. We fished until 7:00 without capturing another pallid. The single male is transported to the hatchery.

4/13/99 - Four crews are netting at 9:30 am. The Montana crew captures two males at RM 1583.5 a FWS crew captures a third male in the same area - all before 11:30. After lunch a 51 lb female is captured by the FWS crew in the 'hot spot'. At 4:00 the nets are pulled and the four captured fish are transported to the hatchery. At the hatchery the female is catheterized and the eggs are staged. The GV position looks good - migration toward the pole is occurring. A biopsy is performed on the male captured on 4/12/99. The fish has a distended stomach and is either gravid or has eaten something fairly large. The biopsy reveals the later but we are unable to determine the sex since the body cavity is filled and probing to see the gonads wasn't possible. The testes were visible through a biopsy on a male captured today but the small incision size and location along with fluid in the cavity makes finding them difficult. The incision on the next male is done near the third scute just off midline. The muscle tissue here was too thick to allow for a visual of the testes and making the incision larger would probably create too much stress on

the fish. Fluid and blood loss with the existing incision was excessive. The fish was sutured and released. A biopsy was not performed on the last suspected male - the confirmation of sex is not worth the risk of infection.

4/14/99 - Four fish are captured in a single drift by the Montana crew. The truck is on the road midday back to the hatchery. At the hatchery a catheter was used to determine sex. The first fish tubed weighed 50 lbs and was thought to be a female. Nothing could be removed. The second, a 51 lb fish, was tubed and mature eggs were taken. The third fish had a distended stomach similar to the fish captured on the 12th, the catheter wasn't able to retrieve anything. Fat was removed from catheterizing the fourth, a 36 lb. fish.

4/15/99 - Two fish are taken back to the confluence, the fish with a distended stomach from the 14th and the fish that was not biopsied on the 13th. A few more pallids are captured, one presumed male pulled from the nets had a large goldeye in it's stomach. A large 51 lb fish is captured by the MTFWP and appears to be a female. The fish is taken back to the hatchery. At the hatchery the fish from today as well as the 50 lb unknown from yesterday are catheterized. The check revels mature eggs on today's fish - confirming a total of three mature females. The catheter removes a piece of testes from the 50 lb fish captured yesterday. The testes was placed on a slide using a 45X microscope and water was added - sperm was observed with motility.

The pallids were checked visually for signs of stress and when we handled the fish to check for abrasions they were given a salt bath of ½ percent to relieve stress. The black rubberized finish on the holding tanks appears to be helping with stress and abrasions. The sutures from the biopsies on the suspected males are healing well and abrasions are not apparent on any fish. Water temperatures are increased periodically to mimic natural conditions. On June 2 the Bozeman FDC is at the station to check the pallids for a virus identified on shovelnose sturgeon at Gavins Point NFH this past winter and also found at Valley City NFH on pallids received from Gavins Point. For the tests fin and opercle punches are collected on four fish and the tissue is sent off for analysis. The three females are also staged to check maturation. Both progesterone and GV position tests were run. A picture of the GV position was taken and from that we were able to determine the classification index of 0.082 with an index of 0.07 representing a mature oocyte (as classified in the Hatchery Manual for the White Sturgeon). Results of the progesterone assay were positive for female #2204657963 (963) with 8 of 10 oocytes breaking down after the 24 hour incubation period. The results of the other two females were inconclusive. The egg shell was 'marbled' on these two making the position of the AV axis impossible to determine. No nucleus was observed on the eggs sampled. On June 7th the females were checked again. Female # 7F7F054855 (855) was exhibiting stress signs, capillaries red at the skin surface, and was given a shot of oxytetracycline. I have been trying to use the same entry site for the catheterization of the fish but was not able to find it on two of the fish. The new entry site on #115553544A (44A) and #963 was at the juncture of the two ducts. A sample of eggs from the three fish was collected and tests were run. Water temperature is at 62 F and flows were increased. The GV position from eggs of all three fish look good. The progesterone assay this time confirmed the receptiveness of the eggs to the hormone on female #855 and #44A , but this time was negative for #963. We are using saline rather than Leibovitz to incubate the eggs. I'm not sure why we are having conflicting results from the test- next year we'll stick with Leibovitz.

For reference the biopsy tube will slide through the duct until meeting a sphincter muscle between scute 8 and 9 . A mark on the catheter tube near the 4-5 abdominal scute would be about right, you should be in the bulk of the ovary/testes and past the juncture with the intestine. By not going past the mark for the 9th scute you should be able to avoid any damage to other internal organs, provided you don't allow the tube to rupture the kidney. Turn curvature of tube inward to facilitate membrane rupture and to avoid kidney puncture. The walls of the intestine are thick and would be difficult to rupture with the plastic tube.

On June 8th a warm-up dose of 0.01 mg/kg is given to two females, #855 and #44A at 11:00 pm. The following morning at 11:00 am they are administered the resolving dose of 0.09 mg/kg. The males are injected at 8:00 am with 0.05 mg/kg. Salt is added to the tank. Twenty-one hours later, June 10th, the male pallids are obviously reacting to the injection. The fish are active, swimming around the periphery of the tank. The female response is not as apparent. At 11:00 the males are checked for milt production. A full syringe is collected from four of the five suspected males. The fifth fish is now presumed to be a female with immature eggs. No eggs have been seen released in the tanks or in the screen over the tank outfall. At 11:15 the two females are palpated and eggs are expelled. Egg collection begins at 11:30 and continues about every hour until 9:00 pm. A total of eight collections for # 44A and six for # 855. Egg collections from #855, the fish that had received a shot of antibiotic three days earlier, are going well. The fish doesn't appear overly stressed and she is expelling very dark gray eggs in good quantity 200-500 mls/spawn. Fertility also appears to be very good with this fish on all four crosses made. On the other hand, female #44A doesn't appear to have responded as well to the hormone injection. Ovulation is slow and only small lots 5-190 mls of eggs per spawn attempt. The eggs have a more yellow tint to them and fertility doesn't appear to be as good. Egg samples from each take have been removed at about the three hour mark and put in formalin to determine % fertilized. Rebecca Baesler, a summer youth employee from the Bismark Fish and Wildlife Management Office will be analyzing the samples. The next morning eggs are collected from both females. In both cases the eggs are in poor condition, many are broken. They are fertilized with fresh milt but fertilization is almost non-existent. In approximately 40,000 eggs fertilized only one is found that is viable - this egg did eventually hatch. Eggs were incubated in a portable hatching battery in four and six inch jars. Each take was kept separate initially and after initial fertility checks were complete, the family lots were combined into a single jar when family lots were small enough. Green egg size was determined to be 58/ml. An accurate egg size on green eggs is difficult to determine since the eggs are still taking in water. A 30% difference in volume was noted on green eggs as compared to eggs after 3 hours of incubation. To confirm suspicions that unfertilized eggs undergo what appears to be initial cell divisions a small group of eggs from female #855 were incubated in a hatching jar for a day without being fertilized. The results after five hours indicated that there appears to be division, but by the following morning (16 hours) the eggs had turned white.

Greg Looney and Bill Wayman, both from the Warm Springs NFH/Technology Center in Georgia made the trip to assist us with spawning, particularly with milt collection. Bill is working on his Ph.D. which involves milt cryopreservation of North American sturgeon. Greg is currently working with the shortnose sturgeon restoration. The two have been collecting and freezing milt, analyzing ovarian fluid and taking blood samples as well as sharing their expertise with sturgeon propagation. We plan to use cryopreserved milt to fertilize eggs from the third female next week.

The male response to the hormone was good. Twenty-six hours after injections we were able to collect full syringes (60 ml) of milt at every attempt. The males were very active in the tanks for two days after the injections. Sperm production was good as was motility. Milt was collected in ziplock bags and stored on ice prior to using. Milt was viable in the bags for at least 24 hours without any care.

Eggs on the incubator began to develop fungus two days after spawning. Initially the fungused eggs were siphoned off. By June 14 the fungus was out of control - we initiated Malachite Green treatments at 65 ppm in a 2 minute standing bath. The eggs were poured from the incubation jars into a pan to facilitate the removal of fungused eggs. The treatment was administered in the pan. No additional treatments were necessary to control fungus. We did remove dead eggs periodically throughout the incubation. Incubation temperature was set at 59-60 degrees Fahrenheit.

At 10:00 pm on June 13, the initial dose of LH-RH was injected into the dorsal musculature of the third female (#963 or 55C). The following morning at 10:00 am she was administered the resolving dose. The four males were also checked at this time for milt production. Viable sperm was confirmed under scope in all four fish. Sperm count was low in two of the males (#A65 and # 16A) consequently they were given a shot of LH-RH at the normal rate. The other two were not injected. At 10:30 on June 15, the female was checked for ovulation, eggs were present. We promptly aspirated milt from two males and then palpated the female for eggs. In the process of removing her from the water a stream of eggs were expelled. We were able to collect 240 mls of eggs but lost about the same amount in the tank. An hour later we were unable to recover any eggs. At 1:30 a catheter was run up both mullerian ducts to check for eggs - nothing. There is a possibility that the sphincter muscle controlling egg passage into the ducts was being held shut by the female. To get around that problem the duct was ruptured near the vent and about 50 eggs were removed. The eggs were fertilized and were viable. At 3:05 pm an additional 50 eggs were recovered. Additional checks performed hourly until 7:30 pm didn't produce any eggs. At this point ovulation had been occurring for at least 9 hours but didn't appear to be progressing through the ovary as no further egg flow was noted. We hoped an additional shot might stimulate egg release. A booster shot of 2.4 mls LH-RH was given to the fish. Ovulation checks at 12:00 am and 3:00 am revealed nothing The following morning at 7:57 am we were able to express only 36 mls of eggs from the fish and they were not viable. Why we were able to express 3-400 mls of eggs on the first attempt and then only a few dozen after that remains unknown.
To assist with the post spawn recovery all of the pallids with the exception of female #885 were given an injection of oxytetracycline on 6/16. Stitches were removed from the fish that had been biopsied. Eggs were expressed from all three females. The females are expelling decomposing eggs without any assistance which should help to reduce infection. The fish are being monitored closely for any signs of stress.

Table 1 . Female # 115553544A Spawning Results

FEMALE # 115553544A					
TIME/DATE	**MALE #**	**TAG**	**MLS EGGS**	**# EGGS**	**% VIABLE**
11:48am 6/10	115552116A	S BLUE	6 mls	348	100
1:10 pm 6/10	115552116A	S BLUE	58 mls	3364	100
2:49 pm 6/10	1F477B3A65	S GREEN	125 mls	7250	100
3:50 pm 6/10	1F482F3F2B	S ORANGE	120 mls	6960	100
4:52 pm 6/10	1F4A27214F	N BLUE	190 mls	11020	100
5:52 pm 6/10	115552116A	S BLUE	112 mls	6496	97
7:43 pm 6/10	1F482F3F2B	S ORANGE	225 mls	13050	100
9:01 pm 6/10	1F477B3A65	S GREEN	89 mls	5162	99
8:24 am 6/11	115552116A	S BLUE	222 mls *	12876*	-
Total/Averages			925 mls	53650	100

‘ % viable’ from results of Missouri River FWMAO sampling at 3-4 hours postfertilization.

Table 2. Female # 7F7F054855 Spawning Results

FEMALE # 7F7F054855					
TIME/DATE	**MALE #**	**COLOR**	**MLS EGGS**	**# EGGS**	**% VIABLE**
11:33am 6/10	1F477B3A65	S GREEN	200 mls	11600	97
	1F482F3F2B	S ORANGE	250 mls	14500	98
12:51pm 6/10	115552116A	S BLUE	150 mls	8700	96
	1F4A27214F	N BLUE	140 mls	8120	96
2:52 pm 6/10	1F482F3F2B	S ORANGE	310 mls	17980	99
	1F477B3A65	S GREEN	210 mls	12180	99
3:54 pm 6/10	115552116A	S BLUE	150 mls	8700	44
4:49 pm 6/10	1F4A27214F	N BLUE	250 mls	14500	100
7:51 pm 6/10	1F477B3A65	S GREEN	450 mls **	26100	-
8:26 am 6/11	115552116A	S BLUE	430 mls *	24940*	-
Total/Averages			2110 mls	147,320	91

*eggs collected on 6/11 not included in totals ** 250 mls for fungicide test

Eggs were sampled to determine initial survival from the first spawn. Egg size ranged from

45.8/ml to 55.5/ml indicating a need for a more precise sampling method. Viability checks after six days were performed and appeared to be excellent for female # 855 with family lots ranging from 77-95% viable. Female # 44A was not nearly as impressive. Egg size for this fish ranged between 50-55.4/ml and viability was 16-40%. The viability ranges do not account for dead and fungused eggs removed prior to 6/16. See Table 3 for overall survival rates. The hatching jars were moved from the incubation battery to individual 3 foot circular tanks on June 16. There were a few larval fish hatching the following day with the bulk of the hatch occurring on June 18. The hatch was 95% complete June 19th. The remaining eggs were floated in a mesh frame in the tank. The tank's water supply was maintained through a ½ inch rubber hose. The flow into the tank was about 1 gpm through the incubation period but was increased significantly after the jars were emptied to keep the fry from #855 suspended. Fry from female 855 had an excellent hatch however they are not as active as those from 44A. Oxygen levels are normal. Without a strong current the fry from #855 settle out on the bottom. On June 21, approximately 50% of the fry from female #855 were removed in fungused mats from each of the four tanks. Losses with the other female are unnoticeable. The following morning live fry from three family lots of #855 along with a single cross, 44A X F2B, were sent to Bozeman for disease testing. Fry preserved in Davidson's Solution were also sent. Initial hatching occurred on June 22 from female #55C. June 23, fry from female # 55C are split into a second tank to reduce stress associated with overcrowding.

The mouths of the earlier family lots look developed but yolk plug is still apparent in intestine. The small mesh screen prevents natural zooplankton in the water supply to pass and a fair number of copepods have built up in the tanks. When the fry begin feeding there should be an ample supply of natural food in the tanks to get them started. June 24 we began introducing Biodiet #2 salmon starter into the water. The fish react to the food but I can't determine if any food is ingested. The hatch from female # 55C is nearly complete and we have only a handful of fry left from female # 855. June 25 cleaning of the tanks began to remove excess feed and unhatched eggs/shells and keep bacteria levels to a minimum. Copepods are still seen in the tanks and feeding is accomplished using a belt feeder as well as ad lib every hour or so during the day. June 29 - fish are actively feeding and mortality has been fairly low with losses about 1% daily. July 7-9 an actual count is performed on all tanks. A few fry are developing spinal curvature. A vitamin 'C' deficiency is suspected in the feed and new feed is ordered. Total mortality through July 12 averaged 13%.

July 20 we received word from the Bozeman Disease Lab that there was no viral involvement relating to the loss of fry from female #855. Histology results indicated 'degenerative and necrotic changes in the GI epithelia.' I would assume that bacteria in the GI tract would cause the described changes fairly rapidly after death. Crystal Hudson, director of the fish health lab, diagnosed the mortality as relating to poor egg quality - premature hatch. I would disagree with the premature hatch diagnosis since it is not consistent with what we observed at the hatchery. The eggs from #855 hatched at the same rate as eggs from #44A and mortality in #44A is not acute. Poor egg quality is a possibility considering the varied response each of the females had to the hormone, but the response of female #855 was by far the best of the three. The eggs from this fish also appeared the best and had the highest hatch rate. There is no question that more is information is needed and the techniques developed to determine when egg development is optimum for hormone induced ovulation. The test procedures used, both GV position and GV breakdown appear to be inadequate or not as precise as desired.

Table 3. Egg Size and Survival Data

Egg Data by Family Lot									
Family cross	**green eggs**	**eggs (6/16)**	**% eggs remaining from green (6/16)**	**sample size**	**egg size (#/ml)**	**mls disp.**	**% viable Egg sample (6/16)**	**mls eggs (6/16)**	**mls eggs (6/10)**
855 X 16A	17,400	5,483	31.5	146	52.1	2.8	87.7	120	300
855 X A65	35,380*	14,705	41.6	112	46.7	2.4	76.8	410	610*
855 X F2B	32,480	19,697	60.6	122	55.5	2.2	91.0	390	560
855 X 14F	22,620	10,486	46.4	87	45.8	1.9	95.4	240	390
Subtotal	**107,880**	**50,371**	**46.7**	**467**	**50.2**	**9.3**	**87.7**	**1160**	**2110**
44A X 16A	10,150	1,024	10.1	160	50.0	3.2	25.6	80	175
44A X A65	12,470	872	7.0	171	55.2	3.1	15.8	100	214
44A X F2B	20,010	738	3.7	216	55.4	3.9	11.1	120	345
44A X 14F	11,020	1,450	13.2	144	51.4	2.8	40.3	70	190
Subtotal	**53,650**	**4,084**	**7.6**	**691**	**53.2**	**13.0**	**23.2**	**370**	**924**
55C X A65	**13,920**	**6,878**	**49.4**	**169**	**56.3**	**3.0**	**56.3**	**240**	**240**
Total/Ave	**175,450**	**61,333**	**35.0**	**1327**	**52.5**	**25.3**	**55.7**	**1770**	**3274**

*250 mls excluded for fungicide test

NOTE: A sample of eggs was taken from each family lot on June 16 to determine at that point what the viability was in the jar. The % viability was used to estimate the hatch numbers for each lot (Table 4.).

In an additional experiment, a sample of eggs that had been fertilized after 5 hours was compared to a sample which had not been fertilized at five hours post spawn to evaluate if the culturists could determine the difference. Of the 35 eggs that had milt added, 9 had obviously cleaved (8 divisions). From this we can predict 25.7% viability. Unfortunately the family lot was not recorded. If the eggs were from female 44A the % fertility would be consistent with the overall results of this family (23.2 % viability). The non-fertilized sample contained 31 eggs, none of which had true cleavages. There were some random furrows in a few eggs but most looked like the stage representing initial fertilization - dark animal pole with radiations from center. These eggs were coated with Fullers earth.

Table 4. Survival of eggs and fry

Survival of Pallid Eggs and Fry to One Month								
Family Lot	# Eggs Green	% Survival Green - Prehatch	% viable from fertility test	# Eggs Prethatch (includes nonviable eggs)	% viable of egg subsample 6/16	% Survival prehatch eggs - Fry	Fry count @ hatch*	% Survival Green-fry (7/7-7/9)
855 X 16A	17,400	32	70	5,483	88	78	4,256	24.5
855 X F2B	32,480	61	98	19,697	91	80	15,809	48.6
855 X A65	35,380	42	98	14,705	77	70	9,987	28.2
855 X 14F	22,620	46	98	10,486	95	84	8,786	38.8
Subtotal	**107,880**	**47**	**91**	**50,371**	**86**	**77**	**38,838**	**36.0**
44A X A65	12,470	7	100	872	16	23	202	1.6
44A X 14F	11,020	13	100	1,450	40	43	616	5.6
44A X F2B	20,010	4	100	738	11	18	135	0.7
44A X 16A	10,150	10	99	1,024	26	28	288	2.8
Subtotal	**53,650**	**8**	**100**	**4,084**	**23**	**30**	**1,241**	**2.3**
55C X A65	**13,920**	**49**	**-**	**6,878**	**56**	**49**	**3,400**	**24.4**
Total/Ave	175,450	35	95	61,333	81	71	43,479	24.7

* All fry from female cross 855 died within eight days post hatch and no fry inventory was performed. The numbers in the chart assume fry from this fish had post mortality losses (11.8 %) comparable to the other two female lots.

NOTE: 'Green egg to prehatch egg % survival'should approximate the ' % viability from fertility test' if the test gave accurate results (assuming little loss during incubation). The '% viability of egg subsample' taken on 6/16 should approximate the 'prehatch egg-fry' numbers. Prehatch egg count should be close to initial fry inventory when eggs have separated and been siphoned off.. That number is often used when the fry would be damaged by an actual inventory or when an inventory is two cumbersome. Typically by prehatch eggs are fairly clean. Most dead eggs have been discarded and what is left will hatch. In the case of the three pallids this year, female # 44A had poor eggs and dead eggs were not picked off to eliminate the possibility of discarding a live one. This is why the % viability of the egg subsample was low. Female # 855 had excellent eggs and nearly all dead eggs were removed prior to hatch consequently her subsample results were high. Female # 55C had good quality eggs but had not been incubating as long as the other two female crosses. The eggs from # 55C had not fungused or floated off as well at this stage and resulted in a viability of 56 %.

Mortality in the developing fry a nearly a month old appears limited to fish that have not accepted the artificial diet. Natural zooplankton coming through the supply line is not able to sustain the fish. If we had the tank space to isolate the fish not accepting the artificial diets we could move them out and start an labor intensive feeding program collecting natural feeds. This may be an issue we can address for next year. We are obviously weeding out some genetic traits by not accommodating these fish. On the other hand, man-hours and space will be an issue in the attempt to keep these few fish alive. This may fall in the realm of our Fish Tech Centers. Mortality other than emaciated fish may have been attributed in part to the cleaning method. A siphon was used to remove excess feed/fecal materials and in the process live fish were siphoned up. The fish were returned to the tanks but obviously underwent stress. We opted to use a fine mesh net to remove the waste in conjunction with draining the tanks. Cleaning tanks for the first month was very labor intensive. As the fish grew the screen size in the tank was increased and eventually the tanks became nearly self-cleaning.

The larval fish were held in the four foot circular tanks at the hatchery and were fed Biodiet's soft-moist salmon starter diet #2 and #3. The five lots of fish were kept in separate tanks and at varied densities. The fish were fed ad lib using belt feeders. No attempt was made to fed by body weight to reduce stress associated with handling. The fish were presumed to be fed in excess. The fish were held at temperatures near 70 degrees throughout the summer. An inventory was done monthly to check growth. Water temperature was lowered during the month of September from the low 70's to the upper 50's (Table 8.) to match reservoir temperatures and prepare the fingerlings for the transfer to Valley City NFH. Boilers were turned off and serviced to prepare for salmonid incubation. The temperature dorp over the last 20 days of September was 16 degrees (74.1-58.1). The fish continued to feed but rates were significantly lower. Feeding rates were cut in half to reduce the amount of wasted feed on the tank bottoms. By the end of October the temperature had dipped to 51 degrees and when the fish were transferred on November 18th it was 48 degrees. The Mortalities August-November were virtually nonexistant. The few fish that died were sent to the Bozeman FHC to check for the 'virus'- all ok. The fish had fungus on the gills and was most likely the cause of mortality. Water quality in Sept/Oct was poor due to the lake turnover. Bacterial gill problems were associated with salmonid mortalities during this time frame as well. The fingerlings were tagged with elastomer tags during the last of October and early November to allow them to be mixed at Valley City NFH. The tagging appeared to effect the fish very little. The elastomer tag color's black and orange caused some trouble. The material set up in the syringe over a short time period and had to be reordered to finish the tagging. Overall the fish looked good. The fish had essentially no growth during the last two months due most likely to the drop in temperature (Table 6.). Two family lots showed a decrease in body weight of 12-13%. The scale used to inventory the fish reads at 0.2 lb increments. The 'loss' of weight for the two lots was 0.2 - 0.4 lbs. That amount could easily be an error in weighing (water weight from the net). The results do show that these fish are a warm water species. I would have expected some growth since temperatures during the two month period averaged in the upper 50's. Body condition was good, a condition factor should be developed to assess the 'relative' condition of the fish to compare with either wild fish or other year classes of hatchery fish. There was a fair amount of variation in sizes within family lots. It appears that we have some genetic variability. It would be nice to have some baseline

work done on the fish.
The pallids, both larval and adults were held at Garrison Dam throughout the summer. The adults were maintained in the two twenty foot tanks and fed on rainbow trout at will. The pallids appeared to be effective predators and were able capture the trout without much trouble. They adapted well to their captivity and recovered fine from the stress of spawning. On September 28, after the quarantine had been lifted, the adults were released at the highway 85 bridge near Williston, ND. The Bismark FWMAO crew made two trips to the release site.

Genetic mapping should be on the list of priorities for the future of these fish, especially the future broodstock. The procedure is currently in use on the Atlantic Salmon at the Cronin National Salmon Station and they are pleased with the results (Mickey Novak, (413) 548-9010). Tim King of the USGS in Leetown, WV (304)724-4450 is currently working on genetic mapping. He suggested the use of this procedure to increase the heterozygousity of the population. The way I understand the process you can increase the occurrence of recessive genes expressing themselves by selecting for parents with those gene types. The technique allows you to actually increase the genetics of the current population. Typically you run the risk of losing these recessive genes in small populations. This may be one method of dealing with the limited gene pool. This past winter I requested help from him in getting cost estimates and details of the procedure. He reluctantly agreed to assist us (they are swamped) for the spring of 1999 in mapping the broodstock. The cost for the 10-20 fish would be approximately $500. To do the procedure a 1/4" clip is removed from the caudal fin, chilled overnight in 95% absolute ethanol, and shipped FedEx to the lab in Leetown. They would send back the results prior to the spawning event to be used for selecting the 'best' crosses to make using individuals with the most divergent genotypes. It never happened. I would encourage the Recovery Team to investigate this procedure further and possibly incorporate genetic mapping into the recovery plan. It would be prudent use of the best science available to us to assist in the recovery effort at a minimal cost. We could go a step further and complete mapping on future domestic broodstock as well. The investment here is in the $15,000 range for a lot of 300 fish. I have made the proposal (1999) to the FWS for funding but it ranked low (61) in the region's fishery needs. Possibly some external pressure from the Recovery Team and other outside agencies could elevate the need if it is something we desire. Funding from other agencies may be needed to get this program on line.

We had assistance from Warm Springs Fish Technology Center this past spring with cryopreservation research. Bill Wayman and Gregg Looney will be returning this spring to conduct further investigations. If we get a breakthrough in sperm cryopreservation it do a lot to increase the available gene pool for the future. Hopefully they will get satisfactory results on some of the earlier 'southern' sturgeon species this spring that can be of use to the pallids. It's unfortunate this technology isn't available for use today. This is another area of research that could use additional funding for more extensive work.

Table 5. Pallid survival and growth

Family lot	Tank #	Mort	July 7-9	Mort	Aug 16	#/lb	Mort	Sept 16	#/lb	% overall surv	% survival w/o lost fish
44A X A65	1	23	178	29	53	317	1	52	28.9	26	50
44A X F2B	2	27	105	30	78	236	2	77	22.6	58	57
44A X 14F	5	90	477	97	191	423	1	185	29.8	33	50
44A X 16A	6	23	271	23	93	313	1	93	24.5	32	66
55C X A65	3	19	719	53	115	337	0	115	25.0	16	62
55C X A65	4	20	811	58	163	477	0	162	31.2	20	68
55C X A65	7				48	473	0	48*	24.0	100	100
55C X A65	8				90	473	0	90*	28.1	100	100
55C X A65	9	114	723	103	302	473	0	163*	29.1	20	43
55C X A65	10	184	810	143	307	404	1	307	37.4	31	48
TOTAL		483	4094	536	1302	391	6	1292	29.4	28	51

* Tank 9 split into T-7 & T-8

Table 6. Final Inventory (11/18/99)

Family lot	Tank #	Number	Weight	Number / Lb.	Number / Lb. (9/21)	% Weight change	Tag Color	Mortality (9/16-11/18)
44A X A65	1	52	1.6	32.5	28.9	-13	Green	0
44A X 16A	2	93	3.4	27.4	24.5	-12	Yellow	0
44A X F2B	3	77	3.6	21.4	22.6	6	Pink	0
44A X 14F	4	81	3.4	23.8	29.8	18	Black	1
44A X 14F	5	103	4.2	24.5	29.8	18	Black	0
55C X A65	11	459	20.0	23.0	28.9	20	Orange	3
55C X A65	12	411	15.2	27.0	33.0	5	Orange	1*
TOTALS		1276	51.4	24.8	29.4			5

* 10 fish sent to Bozeman FHC for viral testing - all results negative

Table 7. Pallid Progeny Survival

Survival by Lot								
Family Lot	Color	Inventory 7/9	Inventory 8/16	Inventory 9/21	Inventory 11/18	% survival*	Total Mortality	Lost ?
44A X A65	Green	178	53	52	52	62	30	94
44A X F2B	Pink	105	78	77	77	71	32	-4
44A X 16A	Yellow	271	93	93	93	80	24	154
44A X 14F	Black	477	191	185	184	65	99	194
55C X A65	Orange	3063	887	885	870	28	362	1831
TOTALS/AVE		4101	1302	1292	1276	31	570	2259

* Does not include fish not accounted for (lost column)

Table 8.

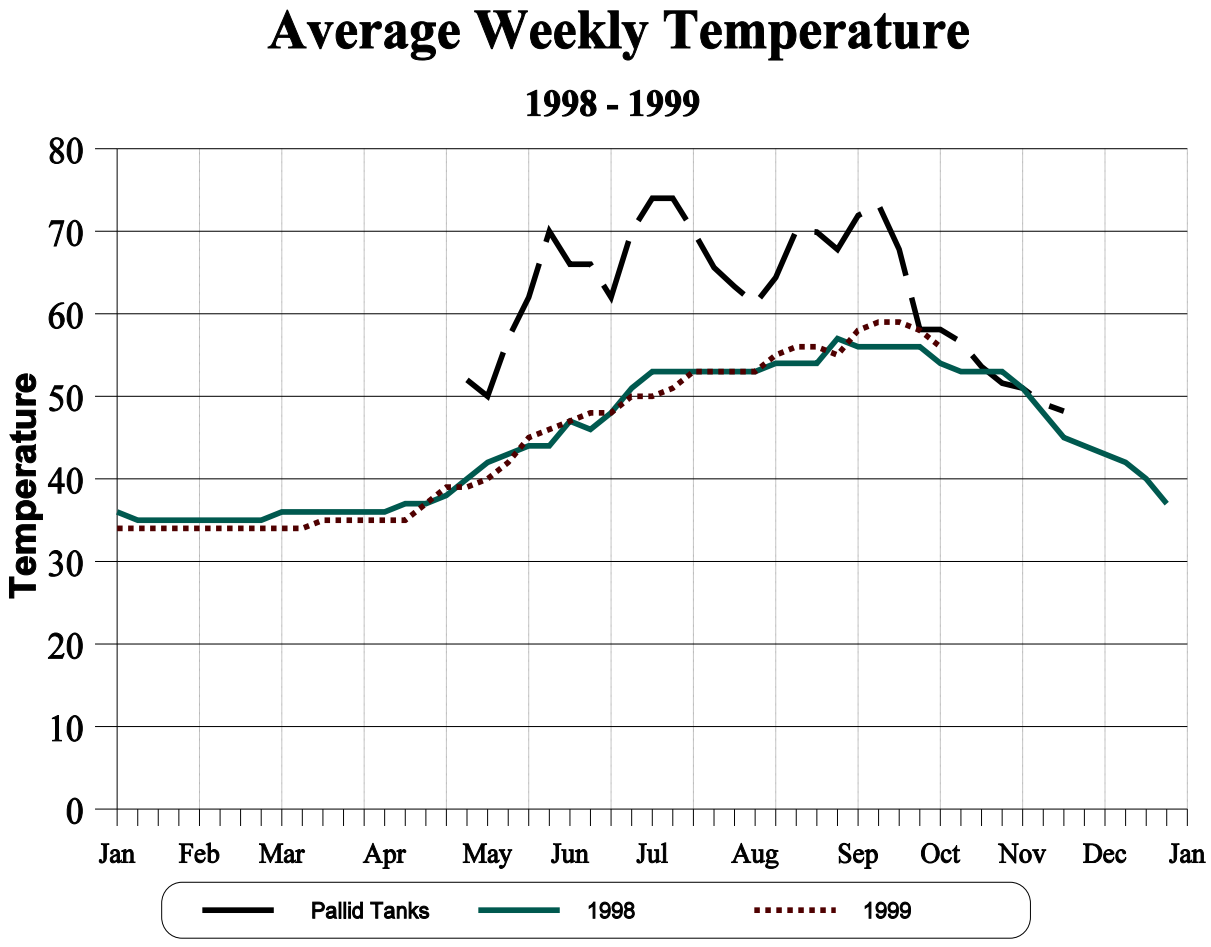


Table 9. Broodstock Data

PALLID STURGEON BROODSTOCK DATA					
capture site RM	**date**	**sex**	**weight**	**other info**	**tag number**
Missouri River 1583.5	4/12/99	male	31 lbs		1F482F3F2B
Missouri River 1583.5	4/13/99	male	31 lbs	monel 097	115552116A
Missouri River 1583.5	4/13/99	female	51 lbs		7F7F054855
Missouri River 1583.5	4/13/99	unknown	35 lbs		7F7D7C2E4B
Missouri River 1583.5	4/13/99	unknown	30 lbs	220C7D0429	2204523542
Missouri River 1583.5	4/14/99	male	50 lbs		1F4A27214F
Missouri River 1583.5	4/14/99	male	36 lbs	biopsy scar	1F477B3A65
Missouri River 1583.5	4/14/99	female	51 lbs		115553544A
Missouri River 1583.5	4/15/99	female	51 lbs	2204657963	220F01755C

Conclusions

More questions than conclusions. Is there a procedure that determines the receptiveness of the fish to the hormone? The GV breakdown test should be it. Once the egg has been bisected and position of the GV is good, final confirmation using the GV breakdown should seal it. But, the difference in response of the three fish is baffling. So we look at other factors. We assume temperature plays a major role. Other fishes use photoperiod as well. The literature suggests water flows. These three factors no doubt play a major role in getting the egg ready but are not what determines the final release - at least not in the hatchery. The physiology says that once the nucleus has migrated to the animal pole and is positioned in the cortical ooplasm the hormone should override all other factors. Survival of the eggs should be dependant on how developed the egg is. Release of the egg from the ovary is controlled by hormone secretion (or injection). Are the dosages not adequate - or is it the egg not quite ripe? In the case of the third female, # 55C (49.4 % survival) there is from the flow of eggs initially, there was obvious release. Literature suggests that once the ovulation process has started there is no stopping. The release progresses through the ovary - but at what pace. In nature is it over several hours or days? The hormone is supposed to condense the whole process. That is why we are able to collect as many eggs in as short a time. It is also why we have overripe eggs overnight. It may also be why survival is less than optimum. The speed at which the hormone works may cause some damage to the developing egg. Could that be why female # 855 ovulated well and had good survival (46.7 %) but then all the fry died post hatch? What about the second fish # 44A. The fish released eggs on several attempts, obviously ovulation had occurred, but look at the quality - 7.6 % survival. Are other factors at play beyond our control? What about the age of these fish. The fish in the 60 lb class could possibly be 50-60 years old. The quality of eggs in older fish of other species declines with age. Maybe that is what we are seeing here. What about bioaccumulation? A predatory species with such a long life span exposed to agricultural runoff could have elevated levels heavy metals which might possibly cause defects in the offspring? I would think it unlikely that you would see 100% mortality but it is certainly possible. In the min 1980's we spawned shovelnose at Miles City and moved them as eggs to Gavins Point NFH. 100% of the offspring from these fish had ‘stub noses’. Gavins Point NFH has had very good success in the spawning attempts with most of their sturgeon*. We have tried to replicate conditions at Gavins Point to increase our overall success at Garrison Dam. Attempts so far have not paid off. One of the factors which may account for our poorer ovulation success is holding time. Gavins Point gets their fish in the fall. The fish have a chance to acclimate to the facility prior to spawning events. At Garrison Dam the fish are captured in mid-April, two months prior to spawning. The capture and transfer would create additional stress at a critical time. It would be interesting to monitor the stress levels in the fish at both hatcheries to see if there are any major differences. The *White Sturgeon Manual* suggests the hormone Thyroxine be used to improve ovulatory success in females that have undergone undue stress prior to spawn. It is suggested that this hormone makes the follicle more responsive to the LH-RH by secreting a maturation steroid hormone. It would be worth a try to see if we can increase our success rates. It would be interesting to test the hypothesis that spring captured fish respond better as well. If we are able to get funding which would allow for improvements in the Trout Building we could overwinter broodstock there and check their response against the spring captured fish.

* The fry from a female in 1998 all died of undiagnosed causes. The behavior of the fry was

described as being similar to those of the fry we lost this year. The assumed cause was suffocation from a silt load in the water. At the stage they died, the fry should have been suspended in the water column and silting over could not have occurred.
We need to break apart the different phases in larval development and try to determine what steps can be taken from initial spawning techniques through the incubation procedure to increase survival. There obviously is a huge difference in egg quality between fish. Is this due differences in the fish, differences in the stage of egg development prior to injection, reaction to the hormone, or timing of egg removal? The survival of eggs may also be influenced by many factors; timing and volume of added milt, de-adhesion, flow rate, fungus, fungicide, mechanical shock, and temperature to name a few. Any testing that we can accomplish to isolate the variables will help us progress towards the recovery of this species.

From the data on Table 5 some general assumptions can be made relating to growth and survival. Obviously survival increases with size. Survival doesn't appear to be effected by density (at least at the densities we had). Survival doesn't appear to be effected by family lot. Growth is effected by density either directly or indirectly (food availability). A huge discrepancy exists this year between recorded mortality and actual inventory numbers. Differences may be attributed to: 1) tank design 2) cannibalism 3) inaccurate inventory. Due to a manufacturing error, the fry tanks were shipped without a sump to accommodate the drain screen. Without the sump there was a high probability of fry being lost from the tanks during cleaning. The problem was not acknowledged until mid July. Cannibalism has been documented on larval shovelnose and could have occurred without being noticed due to water clarity and benthic behavior of the larvae, however is improbable that it wouldn't have been noticed on even a single occasion. An inaccurate inventory of mortality is unlikely to account for losses from ~100-600 fry. It is interesting to note that the fish in tank 2 are accounted for (plus a few). This indicates to me that the problem is most likely attributed to fry being flushed down the drain inadvertently during cleaning in all but tank 2. The tanks will be modified this winter to correct the problem and should be reflected in next year's survival.

It was interesting to note there was no real differences related to density, growth or survival. Generally it is recognized that stress, disease, and reduced growth rates are associated with higher densities. It would be interesting to experiment with raising sturgeon next year using controlled feeding and varied densities. Due to the sturgeon's nature of orienting itself with a surface, preferably the bottom but also the tank sides, raising the fish in circular tanks is not a very efficient way to utilize space. Taking this into account, density, as it related to sturgeon propagation is really measured in square surface area and not cubic. The density of the fish this year in the tanks with the excess fish appeared high - but evidently not at the threshold. For the 2000 production year rectangular troughs should be tried as an alternative to the circulars to see how they perform.

A technical problem exists for documenting egg fertilization rates which has led to misconceptions in overall survival rates of fry/eggs from the hatchery. There are several factors that influence accuracy when determining the survival of the developing eggs. When trying to determine the % fertility in the early hours of development you can misidentify fertilized eggs for non-fertile ones due to cleavages occurring in both. A study was conducted this year by the

MRFWMAO. Samples were taken about 4 hours post fertilization to determine fertility rates. Fertility rates are indicated on Table 4. as ‘ % Viable Fertility Test’ and show that the female with the poorest % survival had the highest % fertility with 100%. Another problem exists when enumerating a small volume of eggs - even small errors are magnified. It is difficult to get an accurate read on egg volume without compromising the well being of the eggs. As eggs develop and take in water during the first few hours the size also changes. Egg survival is constantly changing. The best method I have found for determining overall posthatch fry numbers is taking egg volume measurements and sample sizes immediately prior to hatch. Eggs left at this stage are usually viable since most of the dead eggs have fungused or floated to the top and have been picked off. The number may not represent the true fertility rates since some mortality occurs during the developing process, but it gets you in the ballpark - and it doesn’t cause any stress on the eggs or fry.

Recommendations

Research other cues/tests that will enable us to determine receptiveness of egg to hormone injections. Consult other sturgeon producers.

Try the hormone Thyroxine (triiodothyronine or T-3, Sigma) at 10 mg/kg body weight in 1 ml p. saline solution (*Hatchery Manual for the White Sturgeon*, p.48)

Try an underwater injection to reduce stress on the females

Run more precise tests using the progesterone assay (and Leibovitz) and verify test results with additional test lots.

Continue to administer oxytetracycline shots post spawn to reduce the risk of infection on broodstock

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.

If the opportunity exists, spawn females at weekly intervals to allow for experimental opportunities, ie., cryopreservation testing, egg maturation, ???

Continue work on cryopreservation

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

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

Modify tanks to include a sump and decrease the likelihood of escapement

Maintain shallower water levels (24" vs. 36") to facilitate cleaning and allow for visual inspection of the fish

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.

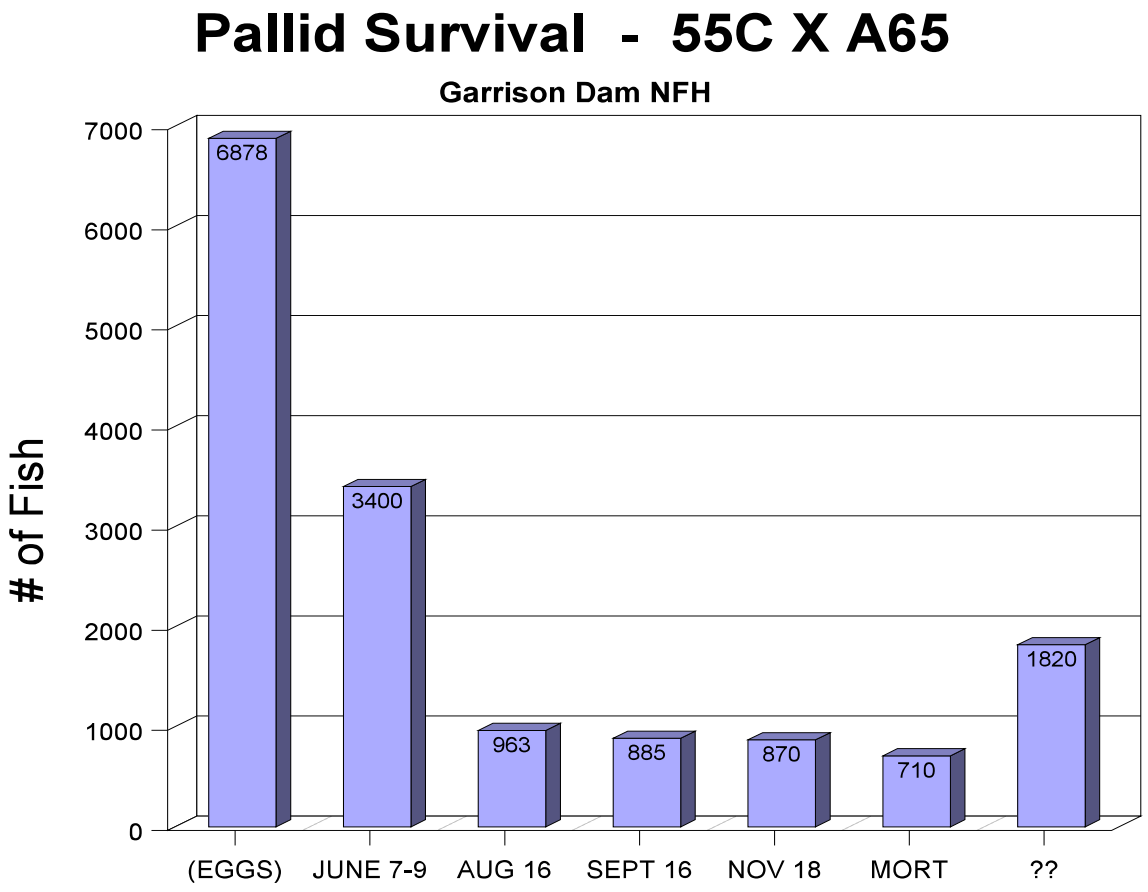
Maintain water temperatures on developing fingerlings near 70 degrees for optimum growth

Increase screen sizes to facilitate the self cleaning design of the circular tanks and maintain better water quality.

Experiment with different densities to determine an optimum or a threshold

Compare production in circular tanks vs. rectangular troughs

Are there any negative results using aluminum troughs? No luck in 1997 & 1998


Pallid Survival - 55C X A65
Garrison Dam NFH
# of Fish
7000
6000
5000
4000
3000
2000
1000
0
6878
3400
963
885
870
710
1820
(EGGS)
JUNE 7-9
AUG 16
SEPT 16
NOV 18
MORT
??

Pallid Survival - 44A

Garrison Dam NFH