PALLID STURGEON PROPAGATION - 1998
Garrison Dam NFH

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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. 1996 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. Success was achieved at Gavins Point NFH marking a turning point in the recovery effort and a first for the Service. Two females were successfully spawned and this August the reintroduction of year old pallids will take place in the Yellowstone and Missouri Rivers.

Objectives

This year our goals remain the same - we will attempt to locate a pair of gravid females with the hopes of successfully spawning the fish. The eggs will be shipped to Gavins Point NFH for further culture. A subsample of eggs will be retained on station to determine our rearing capabilities and to serve as a backup for Gavins Point NFH. We currently do not have the facilities to maintain family lots separate and consequently our backup source could not be used for broodstock development. We will be utilizing the catheterization method for determining egg stage and plan on a rigorous monitoring program of the egg development. 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.

Fall Capture 1997

Methods and Results

September 16, 1997 the first of seven pallids was brought to Garrison Dam NFH prior to transferring to the Gavins Point NFH. The fish was a 46 pound female captured near Ryder Point at 11:00 am. The fish was injected with the antibiotic, Medamycin, and arrived at the hatchery at 6:30 pm. This fish had been previously captured and jaw tagged (098) in the early 1980's. The second fish, a male, was captured at 9:00 am on the 23rd at the Confluence. A third fish was picked up at 1:30 pm in the same location. The sex of this 60 pound fish has yet to be confirmed.

Both fish were transported back to the hatchery by hatchery personnel after Medamycin injections. The fourth fish, a 35 pound fish of unknown sex, was captured on the Missouri River, river mile 1576.5, one half mile below the landslide. This fish was captured at 4:00 pm, injected and arrived at the hatchery at 9:00 pm. The fifth fish was captured on the 25th at the confluence. This fish was a 53 pound female with a biopsy scar from a previous capture. (Another male was captured and released on this date). All fish were captured using trammel nets in 58-59 degree water. Flows in the Yellowstone and Missouri Rivers were low. The fish were caught over sandy substrate in 6-18 feet of water. All but the 35 pound fish were recaptures. On September 25, the right pectoral fin of the 46 pound fish was treated with Malachite for fungus on an injury received from the trammel net. October 1st, the 46 pound jaw tagged female was treated for a fungal invasion on her stomach and right pectoral fin using Malachite (Abrasions from transport and holding in the tank allow the fungus to attach and spread). The tanks were also treated with ½ percent salt. October 2, 1997 the Pallid Sturgeon Recovery Team was at the hatchery. The fish were removed from the tank and the tubing technique was demonstrated for the group as the preferred method for determining sex. Eggs were recovered from two of the five fish. The eggs appeared to be mature and should be suitable for 1998 progeny. October 15th another two pallids were captured, both recaptures. One fish was staged last year and predicted a year away from spawning, the second fish was unknown sex. On the 16th the two fish were examined. The 35 pound fish was determined to be a male by biopsy. The female was tubed but no eggs were recovered. On October 21, three of the pallids were transported to Gavins Point for spawning. The fish spawned in 1997 were returned to the Confluence. On the 28th the 60 pound fish, presumed an immature female was hauled back to the Confluence. November 5 the last three pallids held at Garrison were transferred to Gavins Point.

Spring capture 1998

Methods and Results

Between April 15 and April 20, four male pallids were captured and transported to the hatchery. April 22, a female pallid captured near Sicklefin Island just south of the confluence was brought to the hatchery for spawning. The fish were injected at the capture site with the antibiotic Medamycin prior to hauling and were held at the hatchery in a twenty foot tanks at 50 degrees Fahrenheit. Salt at ½ % was applied routinely to the tanks. On May 7th the temperature in the tanks was increased to 55 degrees F. On May19, water temperature was increased to 65 degrees and then to 70 degrees just prior to spawning.

Pallid # 1F4A363031, was biopsied on April 23 to determine sex. No eggs were observed and gonadal tissue was not apparent. The fish was thought to be a male**.** Checks were made weekly using a catheter to determine egg stage beginning on April 23 and continuing up until the injection. We used a 1/8 inch rigid plastic tube to catheterize the fish, rupturing the Muellerian duct on the right side to recover eggs from the ovary. Both the progesterone assay and the germinal vesicle position tests were used to determine the correct time to iniate ovulation. The GV position gradually moved closer to the periphery of the egg until the spawn time when it nearly touched the periphery. The eggs sampled on the nineth of June for the progesterone assay gave positive results. The nucleus was apparent in only 2 of the 20+ eggs sampled. We

immediately injected the four males with LH-RHa at the rate of 0.045mg/kg body weight. The injections were given at 3:30 pm on June 10. The female was injected at the standard warm-up dosage of 0.01 mg/kg body weight. She was injected again the following morning with the resolving dose at 8:00 am. The pallids were injected with LH-RHa intermuscular. The hormone was diluted with saline at 5 milligrams LH-RHa / 10 mls saline. On the morning of June 11 the male with the green ribbon, tag #'s 220D0EF0B and 220E5E551E, was observed swimming rapidly around the tank with the water flow. The behavior appears to be typical for fish that have responded to the hormone injections. That afternoon at 1:15 we collected milt from all four males and checked quality and viability under the scope. The milt looked good with nearly 100% activation. At this time only a small amount was extracted indicating that spermiation was not yet advanced. The female was palpated hourly beginning at 1:20 pm to check for ovulation. She ovulated between the check conducted at 4:00 and 6:00 pm. Eggs were observed on the floor of the tank and were flowing from her vent with any movement of her caudal peduncle. Milt was again taken from the four males prior to stripping the female and held in separate syringes on ice. The males were much more active in the tank swimming both with and against the flow of the water. Each of the four males easily filled a 55 ml syringe. In preparation for spawning, the female was lifted from the water with her upper body placed on a table in the tank. The person lifting the tail placed his hand over the vent to prevent the loss of eggs as the fish was lifted. A large bowl was placed under the vent and the hand covering the vent was removed. A small amount of eggs ranging from 30-335 mls were collected during each spawn attempt. Pressure was used to expel the eggs from the vent after the initial flow had stopped. The entire procedure was repeated seven times between 6:00 pm and 12:20 am. We used suction hose at the 6:15 attempt to collect eggs but did not penetrate the vent. This was done to determine what affect if any machanical damage from suction would have. The fish was laid on her back in a stretcher while the Muellerian duct was ruptured at the juncture. Eggs were being expelled at this time. A vacuum apparatus was used to suction the eggs as they flowed from the vent into a collection container. The amount of suction was regulated to minimize the damage to the eggs. The remainder of the egg collection was accomplished using palpation. The total number of eggs recovered was 2310 mls and egg size determined to be 62/ml giving a total of 143,220 eggs. Of this number only the first seven takes had viable eggs leaving us with 1710 mls (106,020 eggs). Eggs were incubated in hatching jars at 68-70 degrees Fahrenheit using separate jars for each take and family lot. Egg samples were taken approximately every two hours to monitor development. The first checks reviled a very high fertilization rate. Later results showed survival much less impressive. The eggs were rolled in the hatching jars to prevent the spread of fungus which began on the 13th of June. Eggs that were taken using the suction tube had lower survival rates than those expressed from the fish. The rates corresponded to the amount of suction used to recover the eggs. Eggs were also recovered from the tank to give an estimate of total eggs released. Milt was added to the eggs and they were incubated to determine if there would be any hatch. There was a small number (approximately 300) of these that did hatch, probably from eggs expelled immediately prior to collection.

Table 1. Spawning Results

Female # 7F7F056171								
Time/Date	Hrs. Post Ovulation	Pit Tag #	Eggs mls (6/13) 2 day old	Quality on 6/13	% Eye-up (6/15)	Suction / Palpation	% Hatch	# fry @ 78/ml
6:10 pm 6/11	1:10	1F47760123	30 mls	excellent	38 %	S	19% *	5304
6:15 pm 6/11	1:15	220E5E551E	180 mls	good	15%	S	12% *	3120
6:20 pm 6/11	1:20	1F4A363031	120 mls	poor	0.02%	S		
6:25 pm 6/11	1:25	1F4A4E5772	95 mls	fair	34%	S	11% *	2964
7:45 pm 6/11	2:45	1F4A363031	285 mls	excellent	51%	P	20% *	4914
8:05 pm 6/11	3:05	1F47760123	80 mls	excellent	38%	S	19% *	
9:30 pm 6/11	4:30	1F47760123	335 mls	excellent	37%	P	19% *	
9:50 pm 6/11	4:50	220E5E551E	240 mls	good	32%	P	12% *	
10:25 pm6/11	5:25	1F4A4E5772	70 mls	excellent	13%	P	11% *	
12:20 am6/12	7:20	1F4A4E5772	275 mls	excellent	13%	P	11% *	
8:00 am6/12	15:00	1F4A363031	200 mls		0%			
9:25 am6/12	16:25	220E5E551E	100 mls		0%			
10:45 am6/12	17:45	1F4A4E5772	150 mls		0%			
3:00 pm 6/12	22:00	220E5E551E	150 mls		0%			
total/averages			2310 mls		29% **		16%**	16302

* indicates overall hatch for each male - eggs were combined just prior to hatch
** doesn't include eggs taken with 0% eye-up

Eggs were incubated in four inch jars with a minimal roll. Fungused eggs were siphoned off daily throughout incubation. No chemicals were used to treat fungus. The eggs were incubated in separate jars for each take until the morning of 6/15 when hatching first began. At that point four trough were set up and all eggs from each family lot were combined and hatched into separate troughs. The hatch was earlier than anticipated. Water temperature on the eggs was maintained at 68-70 degrees F. through egg development and hatch. The total temperature units for hatch was approximately 130 T.U.'s. Last year's T.U.'s to hatch at Gavins Point was in the range of 250-350 at 56-57 degrees F. In spite of our unpreparedness the hatch went off fine and the fry appeared to be in good shape. The following morning, June 16, the hatch was complete. Fry were sampled to determine size using a 5 ml buret. 63 fry displaced 0.81 mls of water giving a sample size of 78/ml. (At Gavins Point they were sampled again using a finer tube and

averaged 83.5/ml. The counts were made by family lot as follows: 1F47760123 (blue)-81/ml; 220E5E551E (green)-84/ml; 1F4A363031 (pink)-83/ml; 1F4A4E5772 (orange) -86/ml.) Using the average of 83.5/ml we boxed up 17,450 fry in four boxes for air shipment to Gavins Point NFH. The NDGF plane flew into the CORPS landing strip at 8:00 to transport the fry. They were in transport about 3 hours and arrived at the hatchery in Yankton in excellent shape. (At Gavins Point all fry from the family 7F7F056171 X 220E5E551E and 7F7F056171 X 1F4A4E5772 died over the first weekend. Approximately 2000 fry remained from the other two family groups. All six family crosses from two females spawned at Gavins Point also died at that same time. Personnel at Gavins Point suspected poor water quality to be the cause of mortality.) Approximately 200 fry left in the jars were removed and were placed in a trough at Garrison Dam NFH to experiment with. By the next morning most of the fry had died. Two days post hatch we were down to about 30 fry. The remaining fry were put on feed but all eventually died. Cause of death unknown. The fry were in a trough beside a tank of 150,000 smallmouth bass fry. The water supply was identical, trough identical, and the bass did just fine. Water temperature was maintained in the upper 60's. The mortality pattern was very similar to what was experienced last year. We have a lot yet to learn.

The pallid sturgeon broodstock were held in the 20 foot tanks following spawning to monitor for infection/stress from handling. The fish appeared to be recovering fine. Then another setback occurred on June 21. The female we had spawned started showing signs of stress on the afternoon of June 19, swimming high in the water column occasionally breaching the surface with her head or tail. Saturday the 20th her behavior was the same. Sunday morning she was found floating in the center of the tank. When handled she would swim away but was obviously weak. She was given her third shot of antibiotic and sprayed topically with Betadine as a last effort to save her life but died a few hours later. The fish had signs of infection on the anterior ventral portion of her body and some minor open sores from tank abrasion. The signs were not apparent earlier in the week. The fish was cut open to determine if there were any indications to the cause of death internally. The ovaries were enlarged with fatty tissue and speckled with eggs. The margins of the organs had a white perimeter. The Muellerian duct was punctured in two places from egg collection, neither showed any signs of inflammation or infection. The antibiotic used had an expiration date of 12/97. It was initially thought it may not be effective. A sample was sent off to Bozeman FHC for analysis. They confirmed that it was effective against bacteria and cause of death was unknown. We are dealing with older spawning females, predisposed to stress, which may likely be the cause for mortality.

Table 2. Pallid Broodstock Data

Tag Number	Date	Sex	Wt. lbs	Milt	Other Info	capture site
1F47760123	4/15/98	M	33	Y		Yellowstone confluence
1F4A363031	4/16/98	M	42	Y		Yellowstone River mi 0.5
1F4A24076C	4/20/98	M	26	Y	1F4A4E5772	Missouri River Sicklefin I
220E5E551E	4/20/98	M	33	Y	220D0E1F0B	Missouri River Sicklefin I
7F7F056171	4/22/98	F	66	-		Missouri River Sicklefin I

Conclusions

Success was achieved in spawning and larval pallids were produced. Despite all our attempts to alleviate stress and infection on the female pallid she died. The loss of a single wild fish is not the outcome we would like, however the genetics from this older fish will be replaced in the wild through stockings next fall. The tradeoff is justified. Hopefully we will be able to overcome the problems we have had in the recovery of the females.

Obviously the temperature units (TU's) used to calculate hatch are quite variable depending on the actual temperature used in incubation. Further experimenting with varying temperatures will allow us to better predict hatch times. Survival didn't appear to be effected by the warmer incubation temperature.

Air transport of larval pallids was successful. Unfortunately we were not able to perform and tests on the shipment of eggs.

The tubing technique used to determine egg stage was very effective. A series of eggs has been preserved to record development. It would be nice and more practical to have a series of photos to document development. We are still holding out on the photographic equipment.

We did experiment with egg viability at varying times post ovulation. Our results did not confirm what Gavins Point NFH had reported last year. We had viable eggs up to 7 hrs. 20 min. Post ovulation. The next take at 15 hours and beyond did not yield any viable eggs. Our results` are similar to other published findings with the white sturgeon.

Egg survival was disappointingly low with an overall hatch of only 16%. I feel that we had an extremely high fertilization rate and that are losses were the result of excessive roll on the eggs. Next year the flows during the incubation process will be very low. Hopefully we will see a dramatic improvement in the hatch.

Once again the female brood fish had excessive amounts of fat in the ovaries. The fish appeared to be a clone of the one spawned last year. Same weight and body dimensions. It makes you wonder if we don't have high percentage of siblings in our wild population. Genetic analysis of the wild fish should be high on the list of priorities.

We maintained approximately 200 larval fry at Garrison Dam NFH for experimental rearing purposes. The fry consisted of eggs that had not hatched prior to shipping the larvae to Gavins Point NFH and the crippled fish that were not able to swim out of the jars. Even though we didn't have the best lot of fish for the test, we were not able to keep a single fish alive for longer than a couple weeks. There needs to be additional research on the rearing problems here at Garrison Dam NFH.

Recommendations

- Patience- inject after confirmation of egg maturity
- Photograph results of egg maturation tests and polarity tests (Bozeman Equipment?)
- Transport fry, not eggs (If practical experiment with shipping times on eggs)
- Hold young pallids at 2-3 locations
- Experiment with growing pallids at Garrison to determine why we have had problems with survival
- Investigate possibilities to save female brood fish