

PALLID STURGEON PROPAGATION -2001

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 and held for further growth in anticipation of an August stocking. 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 another year class of 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. The efforts and success of the 2000 spawn were clouded when that lot also tested positive for the virus and were destroyed. On the positive side, cryopreservation was determined to be an unqualified success with results comparable to that of fresh milt. The ability to cryopreserve milt will have a huge impact on increasing genetic diversity in the recovered population provided we put the effort necessary into capturing and collecting the gametes.

Objectives

Objectives for this year were focused on identifying the source of the Shovelnose Sturgeon Iridovirus (SSIV). The approach we had planned involved both increased research and isolation of spawning in the wild. Research into the advancement of a PCR diagnostic test was to be accomplished at the University of California, Davis campus under the direction of Dr. Ron Hedrick. Additional samples of the 2000 year class fish that had been clinical for the virus and now were considered carriers were sent this Spring for use in the development and testing process.

The second step, the isolation of spawning adults, meant a shift in spawning and rearing strategy. In an attempt to identify the presence of the virus in the wild we maintained spawning of the

adults and subsequent rearing of the progeny in water from the same source as the brood fish. Fish captured in the confluence area were transported to Miles City SFH for spawning. Fish captured in the Upper Missouri River (above Fort Peck) were spawned in a portable tank set up riverside and the eggs sent to the Bozeman FTC for rearing. The water source for Bozeman although not from the Upper Missouri River would be in the drainage for the upper Missouri River and for isolation purposes would achieve the same results.

The hatcheries of Garrison Dam and Gavins Point were placed under quarantine and would not be used as the source of restocking efforts this year unless quarantine restrictions were lifted. To accomplish recovery goals the Upper Basin Pallid Workgroup made the decision to proceed with measures necessary to get Garrison Dam NFH back on line with production. All pallids on station were destroyed and disinfection of the facilities was performed. All that remained to get the quarantine lifted at Garrison Dam NFH was to have sentinel fish cultured at the facility for a year without occurrence of the virus. It was decided that both pallid and shovelnose sturgeon in excess of the capacities at the Bozeman and Miles City hatcheries would be used as the sentinel fish. In the event that the virus was identified in the wild, the potential existed to utilize fish from Garrison Dam after the quarantine had been lifted. This allowed for a quasi-backup facility in the event of a catastrophic loss at either of the two Montana hatcheries and met the requirements of the one year culture of sentinel fish necessary to lift the quarantine.

Fall Capture 2000

Results

No Fall capture was accomplished due to the quarantine at Garrison Dam NFH and at Gavins Point NFH.

Spring Capture 2001

Methods and Results

Confluence Spawning

Through the efforts of the Montana Parks and Wildlife and US Fish and Wildlife personnel pallids were captured near the confluence for Spring spawning. Seventeen fish were captured with seven being recaptures. Two females and four males were held for spawning efforts. Fish considered suitable for spawning were transported to the Miles City SFH. A couple trips were made by the Garrison Dam NFH personnel to Miles City to transport potential brood fish and to stage the egg development of fish held there. On June 25th personnel from Gavins Point traveled to Miles City to perform the final staging and spawning. All pallids were given their initial injections at 5:00 pm on the 25th. The resolving dose was administered to the females the following morning at 7:45 am. Milt was also collected that morning from the males. Initial ovulation was detected at 4:00 pm on the afternoon of June 26th and spawning commenced at 6:00 pm. Egg collections were performed through the evening at 6:00, 8:00, 10:00 and 12:00 pm. The following morning eggs were taken at 6:30 and 9:00 am. Milt was collected at 3:30 on the 26th and again at 6:15 am on the 27th for cryopreservation (which was done upon arrival at Garrison Dam that afternoon and following morning). After spawning the fish were given an

injection of Nuflor and a 0.5% salt bath. Water temperatures were reduced to 55-60 degrees Fahrenheit. Egg size at Garrison Dam NFH was determined on June 28th counting a total of 691 eggs (3 family lots). There were significant differences in egg size determinations at the two facilities probably a result of equipment and techniques used. Based on previous year's data and comparing hatch results with egg volumes, it appears we are overestimating the egg size. The average egg size is probably closer to 45/ml.

Table 1 . Female #220E345E09 Spawning Results, Miles City SFH

FEMALE # 220E345E09				
MALE #	MLS EGGS	EGGS/ML	# EGGS	Hatch Location
1F4A111C6A	1715	47.0	80620	Miles City SFH
1F4A111C6A	150	48.7	7298	Garrison Dam NFH
1F4A27214F	1804	47.0	84790	Miles City SFH
1F4A27214F	142	48.7	6908	Garrison Dam NFH
Total/Averages	3811	47.1	179616	

Table 2 . Female #7F706672B Spawning Results, Miles City SFH

FEMALE # 7F706672B				
MALE #	MLS EGGS	EGGS/ML	# EGGS	Hatch Location
7F7D3C5708	858	41.4	35496	Miles City SFH
7F7D3C5708	175	54.5	9538	Garrison Dam NFH
115631222A	828	41.4	34272	Miles City SFH
115631222A	170	54.4	9265	Garrison Dam NFH
Total/Averages	2031	43.6	88571	

Upper Missouri River Spawning

Pallid spawning in the upper Missouri River was accomplished riverside near the James Kipp Recreation Area. The staff from the Bozeman FTC assembled a 16 foot circular fiberglass tank on the banks of the river. Water from the river was pumped into the tank continually to maintain temperature, flow, and oxygen levels. Pallid collections began the first week of June with two males collected. Milt from both males was taken at capture and the fish released. Milt was refrigerated and cryopreserved. On June 11th a female pallid was collected as well as the third male(E09). These two fish were placed in the tank in anticipation of the spawning event.. The staff from Garrison Dam NFH was requested to assist in the staging and spawning. On arrival at 12:30 the afternoon of the 12th, a sample of eggs was removed via catheter from the female. The polarity index was determined to be between 0.4 and 0.9 - within the range for initiation of

ovulation. A fourth male(462) was collected that afternoon and milt from this fish was cryopreserved. Milt from the male captured the previous day was clear indicating that spermiation had not yet occurred in this fish. At 10:30 that evening the initial injections were given to the three fish on hand to initiate spawning. The female received 0.9 mls Ovaprim, the two males 0.2 mls LH-RH. The following morning at 11:00 the resolving dose was administered to the three pallids. We expressed milt from the two males at this time as well. Approximately 20 mls was taken from male E09. The milt was dilute but had a high motility-95%. Fifty mls. of milt was collected from male 462 of good quality and motility - 90%. That afternoon at 4:00 the female was checked for the presence of eggs - none seen. A full syringe of milt was taken from both males. At 8:00 that evening a 5th male(C5F) was captured and injected with 1.5 mls of LH-RH. At 5:15 the morning of June 14th, the female was checked for eggs. About 25 mls of eggs were recovered when palpated and were fertilized with fresh milt from male E09. At 7:30 the fish was again palpated to produce 40 mls of eggs. The eggs were fertilized with male 462. Egg collections at 10:30 provided the largest take at 300 mls. Eggs from this take were fertilized with milt from C5F. At 11:00 the fish was again palpated, this time only 20 mls were collected. Another hour passed and only 13 mls could be expressed. Concern was growing that ovulation was not continuing. We decided to wait a little longer between attempts to reduce the stress of handling. At 2:30 the collection increased to 125 mls. The next attempt at 3:45 was back down to 50 mls. Another 95 mls was recovered using a catheter at 4:07 and fertilized with 973. An additional 150 was taken using the catheter and fertilized with 139. At this point we had fertilized eggs from all family groups. Jim Peterson was present to collect disease samples, fin punches and fluids from the spawning fish at this time. The fish was spawned again at 6:00, 7:00, and 9:00 to increase representation in each of the family lots. At 9:00 we felt enough eggs had been collected to achieve our goals and there was no need to further stress the female. Eggs were incubated in a portable battery using four inch jars and water pumped from the spawning tank. Overnight water flows stopped in one of the jars (male 139 - 300 mls). Water temperatures during spawning fluctuated 12 degrees throughout the course of the day, and ranged from the mid 40's to the upper 60's. The temperature the day of spawning was 51-59°F.

On the morning of June 15th at 8:15 the female was palpated to assist in expelling eggs. In past years we have found that the females held in tanks are not very efficient at expelling ovulated eggs and that egg decomposition in the body cavity jeopardizes the fish's health. Eggs expelled the day following ovulation are typically clumped, broken, and flaccid. In this case the eggs still looked fairly good - a mixture of rigid and flaccid eggs. An astounding 640 mls were collected and fertilized with male C5F. Another group of 90 mls were expressed 15 minutes later and fertilized with 973. Her stomach at this point was fairly flaccid - apparently most of the eggs in her ovaries had released and been expelled. The female was then injected with Nuflor and released. Her strength had increased from the previous day and looked good upon release. The males were also given injections with the antibiotic, measurements and fin samples taken, and then released. Eggs were taken from the jars and transferred to small bags. Oxygen was used to fill the bags and they were sealed for transport to Bozeman and Garrison Dam. The eggs looked good upon arrival at Garrison Dam NFH. They were measured into the four inch incubation jars hung on individual tanks and allowed to incubate. Egg viability calculated using an average egg size of 50/ml and total recorded egg/prelarvae pickoff for those taken the first day ranged from a low of 82.6% (those in the jar where water flow had stopped overnight) to a high of 90.4%. Eggs taken the day following initial ovulation were 48.9 % for the large take and 69.5 % for the smaller group.

Table 3. Spawning Results, Upper Missouri River, MT

FEMALE # 411D262C1F						
TIME	DATE	MALE #	MLS EGG	# EGGS @ 50/ML	EGGS/ LOCATION	Hatch Location
5:15 a	6/14/01	411DOB4E09	25	1250	1250	Garrison Dam NFH
11:00 a	6/14/01	411DOB4E09	20	1000	7250	Bozeman FTC
2:30 p	6/14/01	411DOB4E09	125	6250		
10:30 a	6/14/01	411D0E2C5F	300	15000	3500	Garrison Dam NFH
					11500	Bozeman FTC
7:30 a	6/14/01	41476A0462	40	2000	2000	Garrison Dam NFH
12:10 p	6/14/01	41476A0462	13	650	650	Garrison Dam NFH
3 :45 p	6/14/01	41476A0462	50	2500	850	Garrison Dam NFH
					1650	Bozeman FTC
9:15 p	6/14/01	41476A0462	150	7500	7500	Bozeman FTC
4:15 p	6/14/01	17509415139	150	7500	2500	Garrison Dam NFH
					5000	Bozeman FTC
6:00 p	6/14/01	17509415139	100	5000	5000	Garrison Dam NFH
7:00 p	6/14/01	17509415139	150	7500	7500	Bozeman FTC
4:07 p	6/14/01	1F4A4B5973	95	4750	4750	Bozeman FTC
7:15 p	6/14/01	1F4A4B5973	35	1750	1750	Bozeman FTC
9:15 p	6/14/01	1F4A4B5973	75	3750	3750	Garrison Dam NFH
8:15 a	6/15/01	411D0E2C5F	640	32000	32000	Garrison Dam NFH
8:30 a	6/15/01	41476A0462	90	4500	4500	Garrison Dam NFH
TOTAL			2058	102900	102900	

Analysis

The backcalculated survival from egg to hatched fry revealed problems with early egg viability estimates (see Tables 4-6). These numbers show egg survival to hatch of 21% to 84% as compared to 49% to 98% (The exception of 125% survival which was likely a result of error in egg volume measurements). There are explanations for the differences. Egg size has been difficult to establish because of the nature of the eggs - after ovulation they slowly hydrate, consequently to get an accurate count on egg numbers, egg size and volume need to be taken at the same time. Egg viability is also difficult to establish. At the stage of egg development with four cell divisions you can differentiate between viable eggs and those undergoing cell division without fertilization. After that point you have cell divisions in non-viable eggs that cause an inflated estimate of percentage of 'good' eggs. Looking at the tables below you can get an understanding of the problem. It should be noted that the survival of eggs fertilized with refrigerated milt was considerably lower than that of fresh milt. These values were expected since the milt had been in storage for 7-8 days. Survival of the progeny of the confluence fish was significantly lower than that of the upper Missouri River progeny. A possible explanation for the lower survival could be poor egg quality brought about by the stress of capture and holding. There are apparently effects of related to either the genetics of the confluence fish or complications at spawning. Progeny from the family 72B X 22A are extremely variable in size and mortalities (primarily 'pinheads') from the four confluence families have extended out for longer than what is 'expected'.

Table 4. Survival Success - Upper Missouri River

FEMALE # 411D262C1F											
SPAWN DATE	MALE #	MLS EGGS	# EGGS @ 50/ML	% EGGS VIABLE	# EGGS / DEAD FRY REMOVED	HATCH FRY 6/21/01	% SURV EGG TO HATCH	# FRY @ 10 DAYS	% SURV TO 10 DAYS	# FRY @ 30 DAYS	% SURV TO 30 DAYS
6/14/01	411DOB4E09	25	1250	83.9	201	1558	124.6	1528	98.1	1430	91.8
6/14/01	41476A0462	70	3500	86.5	474	2932	83.8	2782	94.9	2150	73.3
6/14/01	1F4A4B5973	75	3750	89.9	378	1997	*53.3	1932	96.7	1480	74.1
6/14/01	411D0E2C5F	70	3500	90.4	337	2936	83.9	2744	93.5	2071	70.5
6/14/01	17509415139	200	10000	82.6	1745	5817	*58.2	5349	92.0	4464	76.7
6/15/01	411D0E2C5F	640	32000	48.9	16358	6800	21.3	4269	62.8	2863	42.1
6/15/01	41476A0462	90	4500	69.5	1372	2947	65.5	2390	81.1	1922	65.2
TOTAL		1170	58500	64.3	20865	24993	42.7	21004	84.0	16410	65.7

* refrigerated milt

Table 5. Survival Success - Confluence

FEMALE # 7F7F06672B											
SPAWN DATE	MALE #	MLS EGGS	# EGGS @ 54.5/ml	% EGGS VIABLE	# EGGS / DEAD FRY REMOVED	HATCH FRY 7/1/01	% SURV EGG TO HATCH	# FRY @ 10 DAYS	% SURV TO 10 DAYS	# FRY @ 30 DAYS	% SURV TO 30 DAYS
6/26/01	115631222A	170	9265	97.2	260	5308	57.3	2701	50.9	1884	35.5
6/26/01	7F7D3C5708	175	9538	98.2	168	4913	51.5	3097	63.0	2070	42.1
TOTAL		345	18858	97.7	428	10221	54.2	5798	56.7	3954	38.7

Table 6. Survival Success - Confluence

FEMALE # 220E345E09											
SPAWN DATE	MALE #	MLS EGGS	# EGGS @ 48.7/ml	% EGGS VIABLE	# EGGS / DEAD FRY REMOVED	HATCH FRY 7/1/01	% SURV EGG TO HATCH	# FRY @ 10 DAYS	% SURV TO 10 DAYS	# FRY @ 30 DAYS	% SURV TO 30 DAYS
6/26/01	1F4A111C6A	150	7298	95.2	347	5008	68.6	2873	57.4	2342	46.8
6/26/01	1F4A27214F	142	6908	95.7	299	4550	65.9	2693	59.2	1540	33.8
TOTAL		292	14255	95.5	646	9558	67.1	5566	58.2	3882	40.6

Table 7. Milt Cryopreservation

TAG NUMBER	CAPTURE AREA	AMOUNT OF SOL'N MLS	NUMBER OF STRAWS 0.5 ML	NUMBER OF STRAWS 5 ML	DILUTION CRYOPRO TECTANT	STORAGE FACILITY	DATE OF FREEZING
3A65	CONFLUENCE	2.5 3	5 6	2		WSFTC GAD	2000
194B	CONFLUENCE	2.5 2.5	5 5	2		WSFTC GAD	2000
4773	CONFLUENCE	2.5 2.5	5 5	2		WSFTC GAD	2000
6E31	CONFLUENCE	2.5 2.5	5 5	0		WSFTC GAD	2000
4552	CONFLUENCE	2.5 2.5	5 5	0		WSFTC GAD	2000
453A	CONFLUENCE	2.5 2.5	5 5	0		WSFTC GAD	2000
3350	CONFLUENCE	2.5 2.5	5 6	0		WSFTC GAD	2000
2C5F	Upper Missouri	10 10 10	20 20 20	1 1 1		WSFTC GAD GAP	2001
0462	Upper Missouri	10 10 10	20 20 20	1 1 1		WSFTC GAD GAP	2001
5973*	Upper Missouri	2.5 2.5 2.5	5 5 5	0		WSFTC GAD GAP	2001
5139*	Upper Missouri	5 5 5	10 10 10	0		WSFTC GAD GAP	2001
4E09 (2265)	Upper Missouri	10 10 10	20 20 20	1 1 1		WSFTC GAD GAP	2001
1C6A	CONFLUENCE	10 5 5	20 10 10	2	1:1 AKOS	GAD WSFTC GAP	2001
214F	CONFLUENCE	12.5 5 5	25 10 10	6	1:1 AKOS	GAD WSFTC GAP	2001
5708	CONFLUENCE	10 5 5	20 10 10	4	1:1 AKOS	GAD WSFTC GAP	2001
222A	CONFLUENCE	10 5 5	20 10 10	4	1:1 AKOS	GAD WSFTC GAP	2001

* milt collected and refrigerated at capture

Table 8. Broodstock Data

PALLID STURGEON BROODSTOCK DATA					
Tag Number	Date	Sex	Weight	Other Info	Capture Site
7F7F06672B	4/24/01	F	43	SPAWN recapture 10/08/92	confluence
220E345E09	4/24/01	F	59	SPAWN	confluence
115631222A	4/24/01	M	29	SPAWN	confluence
7F7D3C5708	4/26/01	M	50	SPAWN recapture 10/22/92	confluence
1F4A111C6A	4/24/01	M	30	SPAWN recapture 4/14/99	confluence
1F4A27214F	4/26/01	M	48	SPAWN recapture 4/99, 4/00	confluence
1F482F3F2B	4/25/01	M	29	recapture 4/12/99	confluence
1F4A363031	4/25/01	M	39	recapture 4/97, 4/98, 4/99	confluence
1F4B225A1A	4/24/01	U	31	recapture 4/14/98, 4/12/00	confluence
115679374A	4/25/01	U	39		confluence
115529097A	4/25/01	U	33		confluence
220E587576	4/25/01	U	12		confluence
220E5F4928	4/26/01	U	41		confluence
220F107A6F	4/25/01	M	37		confluence
115669540A	4/25/01	U	29		confluence
115676635A	4/25/01	F	55		confluence
115544332A	4/24/01	M	56	Staged @ MCSFH and released	confluence
411DOB4E09	6/11/01	M	-		Ft Peck headwaters
41476A0462	6/12/01	M	34.0	recapture 7/92, 7/99, 6/00 'Perot'	Ft Peck headwaters
1F4A4B5973	6/05/01	M	26.4	recap 6/00 'Lew' Refrigerated	Ft Peck headwaters
17509415139	6/07/01	M	31.3	Refrigerated milt	Ft Peck headwaters
411D0E2C5F	6/13/01	M	33.0		Ft Peck headwaters
411D262C1F	6/11/01	F	49.1		Ft Peck headwaters

Notes:

Seven of seventeen fish captured in the confluence area were recaptures - 41%.

Two of six fish captured in the upper Missouri River were recaptures - 33%.

Milt taken from two of the upper Missouri River fish was refrigerated 7-8 days prior to use and still had good fertilization results. Milt from all upper Missouri River fish was cryopreserved as well as from the four spawned confluence fish.

Table 9. Inventories, Transfers and Mortality records - Hatch through September 1.

Year 2001 Pallid Production												
Tanks	♀	♂	Hatch Number	Split Tank (Number)	Split Tank (Number)	Split Tank (Number)	June mort	July mort	Aug mort	7/1/01 Invent ory	8/1/01 Invent ory	9/1/01 Invent ory
FT-1	CIF	973	1997	(1805)			65	127	0	689	0	0
				FT-25(1243)	(987)		14	242	0	1229	0	0
4 fish to BFHC 8/25/01					T-67(409)		0	1	228	0	408	180
				T-51(272)	T-51(578)		0	203	13	0	647	309
					T-59(325)		0	0	18	0	0	307
10 fish to BFHC 8/14/01				T-70(290)			0	0	79	0	290	201
TOTALS							79	573	338	1925	1345	997
% Mortality by Month							4	30	25			
FT-2	CIF	E09	1558	(1388)			30	140	0	941	0	0
				FT-9(587)	(488)		4	95	0	583	0	0
					T-80(159)		0	0	0	0	159	159
*PUMP FAILURE T-58 HAD 383 MORTS THROUGH 7/18					T-58(329)		0	329	0	0	0	0
					T-58(485)		0	485	0	0	0	0
					T-58(316)		0	7	0	0	309	159
					T-60(150)		0	0	2	0	0	148
TOTALS							34	1056	2	1524	468	466
% Mortality by Month							2	69	0			
FT-3	CIF	139	5817	(5225)			468	124	0	980	0	0
				FT-13(3414)			45	130	0	1268	0	0
					FT-19(2101)	(1809)	2	290	0	2099	0	0
					T-66(809)		0	4	1	0	805	0
COMBINED WITH OTHER TANKS					N-20 (804)		0	0	0	0	0	0
					T-53(1000)		0	57	0	0	943	0
COMBINED WITH OTHER TANKS					N-20 (943)		0	0	0	0	0	0
					T-64(888)		0	5	15	0	883	0
COMBINED WITH OTHER TANKS					N-20(868)		0	0	0	0	0	0
					FT-8(250)		0	8	0	0	0	0
					T-79(242)		0	0	0	0	242	242
				FT-26(955)			21	242	0	934	0	0

Year 2001 Pallid Production

				T-68(363)		0	3	74	0	360	286	
COMBINED W/ FT-3 FISH		T-50(521)		T-50(329)		0	292	4	0	558	0	
COMBINED WITH OTHER TANKS					N-20(554)	0	0	1	0	0	3168	
		T-71(335)				0	0	25	0	335	310	
TOTALS						536	1155	120	5281	4126	4006	
% Mortality by Month						8	22	3				
Tanks	♀	♂	Hatch Number	Split Tank (Number)	Split Tank (Number)	Split Tank (Number)	June mort	July mort	Aug mort	7/1/01 Invent ory	8/1/01 Invent ory	9/1/01 Invent ory
FT-4	CIF	462	2932	(2537)			150	245	0	1474	0	0
				FT-27(1308)			8	222	0	1300	0	0
					T-69(740)		0	0	39	0	740	351
						T-66(350)	0	0	16	0	0	334
COMBINED W/ FT-4 FISH					T-59(338)		0	0	0	0	0	0
			T-59(512)			0	222	10	0	628	0	
COMBINED WITH OTHER TANKS					S-20(618)		0	0	23	0	0	1285
8 fish to BFHC 8/14/01				T-60(717)		0	6	13	0	711	0	
COMBINED WITH OTHER TANKS					S-20(690)		0	0	0	0	0	0
TOTALS						158	695	101	2774	2079	1970	
% Mortality by Month						5	25	5				
FT-5	CIF	462	2947	(2096)			562	289	0	1224	0	0
				FT-14(1161)	(964)		6	191	0	1155	0	0
					FT-10(250)		0	12	0	0	0	0
						T-81(238)	0	0	6	0	238	232
				T-65(714)		0	4	34	0	710	326	
						T-64(350)	0	0	47	0	0	303
			T-61(935)			0	7	8	0	928	0	
COMBINED WITH OTHER TANKS					S-20(870)		0	0	16	0	0	854
OTC MARKED 700PPM/8H			8/15		FT-1(50)		0	0	0	0	0	50
TOTALS						568	503	111	2379	1876	1765	
% Mortality by Month						19	21	6				

Year 2001 Pallid Production

Tanks	♀	♂	Hatch Number	Split Tank (Number)	Split Tank (Number)	Split Tank (Number)	June mort	July mort	Aug mort	7/1/01 Invent ory	8/1/01 Invent ory	9/1/01 Invent ory
FT-6	CIF	C5F	~2936	(~2406)			192	140	0	~1721	0	0
				FT-18(~1600)			0	62	0	~1600	0	0
COMBINED FT-28, 29 AND 18 FISH					N-8(~1538)	DISPOSED	0	380	0	0	0	0
350 FISH DISCARDED 8/21				T-52(778)			0	122	7	0	728	371
COMBINED WITH OTHER TANKS				S-8(226)			0	18	3	0	208	205
FROM S-8 8/21					N-8(600)		0	0	1	0	0	599*
TOTALS		* NOT INCLUDED IN TOTALS					192	722	10	3321	936	576
% Mortality by Month							7	22	1			
FT-7	CIF	C5F	1021	(419)			454	148	0	567	0	0
COMBINED WITH OTHER TANKS				S-8(419)			0	33	5	0	386	381
TOTALS							454	181	5	567	386	381
% Mortality by Month							44	32	1			
FT-8	CIF	C5F	709	(311)			279	119	0	430	0	0
COMBINED WITH OTHER TANKS				S-8(311)			0	24	4	0	287	283
TOTALS							279	143	4	430	287	283
% Mortality by Month							39	33	1			
FT-10	CIF	C5F	800	(358)			125	317	0	675	0	0
COMBINED WITH OTHER TANKS				S-8(358)			0	28	4	0	330	326
TOTALS							125	345	4	675	330	326
% Mortality by Month							16	51	1			
FT-11	CIF	C5F	896	(638)			123	135	0	773	0	0
				T-62(638)			0	3	19	0	635	416
COMBINED WITH T-63					T-61(200)		0	0	23	0	0	177
TOTALS							123	138	42	773	635	593
% Mortality by Month							14	18	7			
Tanks	♀	♂	Hatch Number	Split Tank (Number)	Split Tank (Number)	Split Tank (Number)	June mort	July mort	Aug mort	7/1/01 Invent ory	8/1/01 Invent ory	9/1/01 Invent ory

Year 2001 Pallid Production

FT-12	CIF	C5F	1066	(712)		154	200	0	912	0	0
COMBINED WITH FT-6 FISH				T-52(72)		0	0	0	0	0	0
COMBINED WITH T-63 FISH				T-63(640)		0	0	28	0	640	412
				T-61(200)		0	0	22	0	0	178
TOTALS						154	200	28	912	640	590
% Mortality by Month						14	22	4			
FT-21	E09	C6A	5008	(2677)	MILES CITY EGGS	0	2331	0	5008	0	0
				FT-17(1324)	(992)	0	332	0	0	0	0
				FT-2(378)		0	47	0	0	0	0
COMBINED WITH FT-17 FISH					T-78(331)	0	0	9	0	331	322
COMBINED WITH FT-2 FISH				T-78(614)		0	0	9	0	614	355
					T-50(250)	0	0	4	0	0	246
				T-54(1016)		0	419	55	0	597	292
					T-50(250)	0	0	3	0	0	247
				T-83(337)		0	0	1	0	337	336
TOTALS						0	3129	81	5008	1879	1798
% Mortality by Month						0	62	4			
FT-22	E09	14F	4550	(2511)	MILES CITY EGGS	0	2039	0	4550	0	0
				FT-20(1463)		0	380	0	0	0	0
COMBINED FT-22 AND FT-20 FISH				T-55(688)	T-55(689)	0	687	11	0	690	379
COMBINED T-55 AND T-76 FISH					T-53(300)	0	0	0	0	0	300
COMBINED FT-22 AND FT-20 FISH				T-76(395)	T-76(359)	0	0	10	0	754	544
COMBINED T-55 AND T-76 FISH					T-53(200)	0	0	0	0	0	200
TOTALS						0	3106	21	4550	1444	1423
% Mortality by Month						0	68	1			
FT-23	72B	22A	5308	(2282)	MILES CITY EGGS	0	3026	0	5308	0	0
				T-57(850)		0	365	82	0	485	403
				FT-29(1119)		0	290	0	0	0	0
				FT-6(500)		0	190	0	0	0	0
					T-82(310)	0	0	23	0	310	287
COMBINED FT-23 AND FT-29 FISH				T-75(329)		0	0	0	0	0	0

Year 2001 Pallid Production

COMBINED FT-23 AND FT-29 FISH		T-75(313)			0	0	51	0	642	591		
TOTALS					0	3871	156	5308	1437	1281		
% Mortality by Month					0	73	11					
Tanks	♀	♂	Hatch Number	Split Tank (Number)	Split Tank (Number)	Split Tank (Number)	June mort	July mort	Aug mort	7/1/01 Invent ory	8/1/01 Invent ory	9/1/01 Invent ory
FT-24	72B	708	4913	(2382)	MILES CITY EGGS		0	2531	0	4912	0	0
				FT-18(1020)			0	206	0	0	0	0
					T-56(783)		0	363	91	0	420	329
COMBINED FT-24 AND FT-18 FISH					T-77(237)		0	0	0	0	0	0
COMBINED FT-24 AND FT-18 FISH			T-77(320)				0	0	122	0	557	435
				FT-28(1042)			0	322	0	0	0	0
					FT-7(400)		0	67	0	0	0	0
COMBINED FT-28 AND FT-7 FISH						T-74(333)	0	0	0	0	0	0
COMBINED FT-28 AND FT-7 FISH					T-74(320)		0	0	49	0	653	604
TOTALS							0	3489	262	4912	1630	1368
% Mortality by Month							0	71	16			
FT-28	CIF	C5F	877	(204)			601	72	0	276	0	0
COMBINED FT-28, 29,30 AND 18				N-8(204)	DISPOSED 8/21		0	50	12	0	154	0
TOTALS							601	122	12	276	154	0
% Mortality by Month							69	44	8			
FT-29	CIF	C5F	848	(125)			641	82	0	207	0	0
COMBINED FT-28, 29 ,30 AND 18				N-8(125)	DISPOSED 8/21		0	31	7	0	94	0
TOTALS							641	113	7	207	94	0
% Mortality by Month							76	55	7			
FT-30	CIF	C5F	~583	(~200)			154	229	0	~282	0	0
COMBINED FT-28, 29,30 AND 18				N-8(~200)	DISPOSED 8/21		0	47	12	0	153	0
TOTALS							154	276	12	282	153	0
% Mortality by Month							26	98	8			

Growout Phase

The growout phase of the pallid and shovelnose sturgeon showed a pattern similar to what we had observed in previous years. During the first couple weeks post hatch there is a spike in mortalities even prior to initiation of feeding which is apparently due to complications in prolarvae development. My assumptions are that either egg quality or ovulation timing was not suitable and the losses are a result. The results of poor egg quality are apparent when you look at initial survival in the two family lots produced the second day of spawning (C5F and 462). There are also losses that occur related to acceptance of larval diets but not to the extent I previously thought. By the end of the first month mortalities had stabilized and the usual 1-2 % mortality/month was observed. Midway through August we once again saw spikes in mortality in a few tanks similar to what we observed last year. Assuming the mortalities were a result of parasites we began formalin treatments (100 ppm for 1 hour). Samples of pallids from tanks experiencing the outbreaks were sent off to Bozeman for testing. Results of the testing confirmed gill amoeba and Costia. Formalin treatments were successful in treating the parasites. Results were later received from a histological perspective indicating a two-fold problem: 1) osmoregulatory problems related to the treatment and 2) nutritional problem (possibly rancid feed). Feed samples were held in conditions representative of our routine and submitted for rancidity testing. The result of the test indicated that rancidity was not a problem. Formalin treatments were administered as in previous years and were necessary to control disease outbreaks so no change in treatments were made. On the positive side, no iridoviral agents were detected.

The first of September we began the process of inventorying and disposing of surplus pallids to reduce densities. Shortly after the inventory process we again saw mortality spikes in a few tanks. Treatments of formalin were initiated and samples submitted. Results of the disease work indicated similar problems histologically to what was seen earlier this year. However, parasites were not detected on this occasion. Incidentally the UV dosage requirements needed to treat the parasite Costia is 318 mWs/cm² - about ten times that of most fish pathogens and 4 1/2 times more UV than our unit will emit. No information is available on the dosage requirements of amoebas but, because of their size, the dosage is likely fairly high.

September through November mortality was practically nonexistent in the progeny of the Confluence fish. The fish from the upper Missouri River had some trouble in September but then recovered. Nine tanks representing four of the five upper Missouri River fish had monthly mortalities of 25% or greater. The mortalities occurred over a two week period and then ceased. The diagnosis was the same - no viral agents detected. Water temperatures through the year have been maintained using boilers at about 67° F. Growth has been good this year. The average size of the upper Missouri River fish on November 29th was 17 fish per pound (7.6" FL). The average size of the confluence fish was 20 per pound (6.8" FL). The fish appear in excellent condition going into the winter months. With the exception of families CIF X E09 with a 5% mortality, all other pallid family lots have had mortality rates of 3% or less.

Table 10. Mortality records																		
Year 2001 Pallid Production - Mortality by Lot - August Inventory through December 1																		
Tank #	♀	♂	START #	Aug mort	% mortality	9/1/01 Inventory	Sept mort	% mortality	10/1/01 Inventory	Oct mort	% mortality	11/1/01 Inventory	Nov mort	% mortality	Month/Number added disposed	total % mort	12/01/01 inventory	
Upper MO River Progeny																		
51	CIF	973	322	13	4%	309	91	29%	218	4	2%	214	2	1%		0	34%	212
59	CIF	973	325	18	6%	307	76	25%	231	5	2%	226	0	0%		0	30%	226
67	CIF	973	408	228	56%	180	114	63%	66	3	5%	63	0	0%		0	85%	63
70	CIF	973	280	79	28%	201	156	78%	45	0	0%	45	0	0%	8	-10	81%	45
LOT TOTAL			1335	338	25%	997	437	44%	560	12	2%	548	2	0%		-10	59%	546
Upper MO River Progeny																		
52	CIF	C5F	378	7	2%	371	14	4%	286	14	5%	272	1	0%	8	-71	8%	271
61	CIF	C5F	400	45	11%	355	33	9%	299	2	1%	297	0	0%	9	+32	22%	297
62	CIF	C5F	435	19	4%	416	5	1%	284	1	0%	283	2	1%	9	-127	5%	281
63	CIF	C5F	440	28	6%	412	6	1%	294	3	1%	291	0	0%	9	-112	7%	291
N8	CIF	C5F	600	1	0%	599	7	1%	592	2	0%	590	0	0%		0	2%	590
S8	CIF	C5F	611	15	2%	596	12	2%	510	1	0%	509	1	0%	9	-74	4%	508
LOT TOTAL			2864	115	4%	2749	77	3%	2265	23	1%	2242	4	0%		-352	7%	2238
Upper MO River Progeny																		
64	CIF	462	350	47	13%	303	89	29%	220	12	5%	208	7	3%	9	+6	45%	201
65	CIF	462	360	34	9%	326	43	13%	296	9	3%	287	9	3%	9	+13	27%	278
66	CIF	462	350	16	5%	334	131	39%	299	8	3%	291	10	3%	9	+96	65%	281
69	CIF	462	390	39	10%	351	156	44%	0	0	0%	48	0	0%	9	-263	30%	48

Table 10. Mortality records																		
Year 2001 Pallid Production - Mortality by Lot - August Inventory through December 1																		
81	CIF	462	238	6	3%	232	8	3%	224	0	0%	224	1	0%		0	6%	223
S20	CIF	462	2178	39	2%	2139	50	2%	1638	67	4%	1571	16	1%	9	-148	7%	1555
LOT TOTAL			3866	181	5%	3685	477	13%	2677	96	4%	2629	43	2%		-296	19%	2586
Tank #	♀	♂	START #	Aug mort	% mortality	9/1/01 Inventory	Sept mort	% mortality	10/1/01 Inventory	Oct mort	% mortality	11/1/01 Inventory	Nov mort	% mortality	Month/Number disposed	total % mort	12/01/01 inventory	
Upper MO River Progeny																		
58	CIF	E09	159	0	0%	159	10	6%	149	13	9%	136	13	10%		0	23%	123
60	CIF	E09	150	2	1%	148	42	28%	106	31	29%	75	3	4%		0	52%	72
80	CIF	E09	159	0	0%	159	7	4%	152	4	3%	148	1	1%		0	8%	147
LOT TOTAL			468	2	0%	466	59	13%	407	48	12%	359	17	5%		0	27%	342
Pallid Sturgeon (mixed lot)																		
N20	mix	mix	2779	1	0%	2778	4	0%	2047	21	1%	2026	13	1%		0	1%	2013
Upper MO River Progeny																		
68	CIF	139	360	74	21%	286	69	24%	165	0	0%	165	0	0%	9	-52	35%	165
71	CIF	139	335	25	7%	310	12	4%	238	3	1%	235	1	0%	9	-60	10%	234
79	CIF	139	242	0	0%	242	6	2%	236	8	3%	228	3	1%		0	7%	225
LOT TOTAL			937	99	11%	838	87	10%	639	11	2%	628	4	1%		-112	19%	624
Confluence Progeny																		
50	E09	C6A	500	7	1%	493	3	1%	300	0	0%	300	0	0%	9	-190	1%	300
54	E09	C6A	347	55	16%	292	4	1%	288	1	0%	287	1	0%		0	18%	286
78	E09	C6A	695	18	3%	677	4	1%	300	0	0%	300	0	0%	9	-373	2%	300
83	E09	C6A	337	1	0%	336	0	0%	250	0	0%	250	0	0%	9	-86	0%	250

Table 10. Mortality records																		
Year 2001 Pallid Production - Mortality by Lot - August Inventory through December 1																		
LOT TOTAL			1879	81	4%	1798	11	1%	1138	1	0%	1137	1	0%		-649	4%	1136
Tank #	♀	♂	START #	Aug mort	% mortality	9/1/01 Inventory	Sept mort	% mortality	10/1/01 Inventory	Oct mort	% mortality	11/1/01 Inventory	Nov mort	% mortality	Month/Number disposed	total % mort	12/01/01 inventory	
Confluence Progeny																		
53	E09	14F	500	0	0%	500	2	0%	300	0	0%	300	1	0%	9	-198	0%	299
55	E09	14F	390	11	3%	379	1	0%	300	1	0%	299	0	0%	9	-78	3%	299
76	E09	14F	554	10	2%	544	5	1%	298	0	0%	298	2	1%	9	-241	2%	296
LOT TOTAL			1444	21	1%	1423	8	1%	898	1	0%	897	3	0%		-517	2%	894
Confluence Progeny																		
74	72B	708	653	49	8%	604	2	0%	300	1	0%	299	3	1%	9	-302	6%	296
56	72B	708	420	91	22%	329	2	1%	299	0	0%	299	3	1%	9	-28	21%	296
77	72B	708	557	122	22%	435	7	2%	300	5	2%	295	1	0%	9	-128	20%	294
LOT TOTAL			1630	262	16%	1368	11	1%	899	6	1%	893	7	1%		-458	14%	886
Confluence Progeny																		
57	72B	22A	485	82	17%	403	3	1%	400	1	0%	399	1	0%		0	18%	398
75	72B	22A	642	51	8%	591	3	1%	299	1	0%	298	0	0%	9	-289	6%	298
82	72B	22A	310	23	7%	287	2	1%	250	0	0%	250	1	0%	9	-35	8%	249
LOT TOTAL			1437	156	11%	1281	8	1%	949	2	0%	947	2	0%		-324	10%	945
Shovelnose Sturgeon (cryopreservation)																		
72	HSN	CRY	509	11	2%	498	11	2%	487	0	0%	187	2	1%	10	-300	3%	185
73	HSN	CRY	274	4	1%	270	11	4%	259	2	1%	257	0	0%		0	6%	257
LOT TOTAL			783	15	2%	768	22	3%	746	2	0%	444	2	0%		-300	4%	442

Year 2001 Pallid Production - Monthly % Mortality																	
Tanks	♀	♂	Hatch Number	June% Mort	7/01/00 Inventory	July % Mort	8/1/00 Inventory	Aug % Mort	9/1/00 Inventory	Sept % Mort	10/1/00 Inventory	Oct % Mort	11/01/00 Inventory	Nov % Mort	12/01/00 Inventory	Disposed	Cumulative % mortality
51,59,67,70	CIF	973	1997	4%	1925	31%	1335	25%	997	44%	560	2%	548	0%	546	10	72%
52 (S8,N8)	CIF	C5F	2936	18%	3321	16%	728	2%	300	4%	286	5%	272	0%	271	421	46%
61,62,63,(52,S8,N8)	CIF	C5F	5196	68%	1645	22%	1275	7%	1183	4%	877	1%	871	0%	869	336	
S8	CIF	C5F	0		1314	8%	1211	2%	596	2%	510	0%	509	0%	503	5	
N8	CIF	C5F	0		1867	25%	1405	0%	599	1%	592	0%	590	0%	590	1405	
66,69,S20	CIF	462	2932	5%	2774	25%	3866	5%	3685	13%	2677	2%	2629	2%	2586	296	51%
81,64,65,S20,FT1	CIF	462	2947	19%	2379	21%											
58,60,80	CIF	E09	1558	2%	1323	18%	468	0%	466	13%	407	12%	359	5%	342	800*	27%
68,71,79	CIF	139	5817	8%	5281	22%	4126	3%	838	12%	639	2%	628	1%	624	112	87%
50,54,78,83	E09	C6A	5008		5008	62%	1879	4%	1798	1%	1138	0%	1137	0%	1136	649	64%
53,55,76	E09	14F	4550		4550	68%	1444	1%	1423	1%	898	0%	897	0%	894	517	69%
56,74,77	72B	708	4913		4912	67%	1630	16%	1368	1%	899	1%	893	1%	886	458	73%
57,75,82	72B	22A	5308		5308	73%	1437	11%	1281	1%	949	0%	947	0%	945	324	76%
Totals			43162		41607	50%	20804	19%	14534	11%	10432	1%	10280	1%	10192	2860	70%
	HSN	HSN					783	2%	768	3%	746	0%	444	0%	442	300	

* 800 died as a result of pump failure

Families CIF X 139 and CIF X 973 used refrigerated, week old milt.

Conclusions

To date no viral agents have been detected this year at any of the *hatcheries* culturing pallids or shovelnose. However, a preliminary positive finding was diagnosed in five of eight shovelnose samples sent on June 4th captured in the Yellowstone River near the mouth of the Powder River, MT . The diagnosis was made at UC Davis using a nested PCR test on October 22, 2001. Once the test is validated using histology we will have succeeded in accomplishing our primary objective for the year, *identifying the iridovirus in the wild*. It should also be noted that at this time few tests have been made histologically at the hatcheries and no PCR work has been done on the hatchery progeny. Upon completion of the tests on the progeny stocking recommendations can be made for 2002.

We were successful with our second goal as well, propagation of pallid sturgeon at the two Montana hatcheries. If the fish in the Miles City and Bozeman hatcheries remain disease free until the time of stocking next summer, we will have made an important contribution to the future of the pallid population, releasing nine additional family lots into the wild.

We also were successful at spawning in the wild. Keep in mind however that river stage was extremely low this year which made for ideal capture conditions during the peak of the spawn. Also note that in doing so we loose the security and controlled conditions of the hatchery environment. Murphy's Law will eventually take it's toll. We had some near misses at the site on CM Russell but in the end we achieved all our spawning goals.

At this hatchery we were able to do a better job of managing the fish with the additional tanks, UV disinfection and pressurized water. No mortality was attributed to poor water quality. Culture conditions were optimal for nearly the entire culture period. During periods of crisis the backup systems worked well. There still exist apparent problems related to parasites and feeds that need to be resolved but overall, growth and survival was better than past years.

Cryopreservation capabilities were advanced at the station. We are fully on line with state of the art cryopreservation capabilities compliments of the USACOE and WAPA. Nine additional milt samples were collected and froze this Spring. From a genetics standpoint, this was an important step in the recovery process.

Cryopreservation of shovelnose sturgeon milt and subsequent fertilization, hatch and survival of shovelnose progeny was accomplished by the Warm Springs FTC folks under the direction of Bill Wayman. The process validated his work last year on the other *Scaphirhynchus* species, the pallid sturgeon. Hopefully this success will be achieved with the *Acipenser* species as well.

Hopefully within the next few months and prior to the next spawning season we will have gained additional information to assist us in our strategy in achieving recovery.

Future Recommendations

As a result of incomplete testing on this year's progeny any future recommendations regarding the iridovirus are speculative. We will assume stockings of fish exposed to the virus but not clinical for the disease will be carried out wherever the virus has already been identified. Additionally if the fish held at Garrison also test negative for the virus we will have fish that may be released in select areas if so decided by the recovery team. A negative finding will allow the hatchery at Garrison to drop it's virus suspect status and will be allowed to fully utilize the facilities this Spring. What still remains in question is the use of rearing space at Gavins Point and Valley City NFH's for this coming Spring. In order to remove the quarantine in place changes will need to be made regarding the exotic status of the virus and it's pathogenicity. Without their contribution the recovery efforts won't be able to operate at capacity.

A scheduled pathogen monitoring program should be in place to test all pallid rearing facilities on a set time schedule to evaluate the presence and pathogenicity of the virus as well as to detect other parasites or feed problems. Since we have been unable to replicate the virus under laboratory conditions to test virulence, the next best evaluation technique would be monitoring of mortalities at the hatcheries. Virulence of the virus needs to be known to determine the classification of the iridovirus, it will be used to determine whether or not quarantines are necessary.

Evaluate the effect of UV disinfection on the iridovirus.

With or without a positive finding in the upper Missouri River, spawning on the river should be continued as long as conditions allow in order to incorporate as much of the genetic material as possible from that isolated population. In spite of the drawbacks of spawning at this site, the availability of pallids in spawning condition are a positive influence on the overall recovery of the species.

We should actively pursue collection of males in spawning condition and collect milt at capture for cryopreservation. We collect far more males annually than gravid females. The genetic makeup of future populations will be advanced significantly by these collections.

If space can be found to culture pallids at other facilities which allows for the expansion of pallid family lots produced, the option should be given consideration. The recovery of the genetic material from as many wild fish as possible will help reduce the founder effect which is inherent in any population as restrictive as this one.

Efforts this year should be directed at a review of stocking goals and population genetics.

The draft fish health policy for the pallid sturgeon should be finalized using the results of this years tests.

Develop feeds formulated for sturgeon. The Bozeman FTC is in the process of determining feeds suitable for the sturgeon. If there are feed related problems that are compromising the health of the cultured fish they should be explored

Continue with the genetic mapping of the wild population. A repository of the genetic information of the current population will allow us to analyze the genetic makeup of future populations to determine what effect if any hatchery propagation had on the genetics of the fish. The information may also have implications if we choose to begin larval stockings. With the information gained from genetic mapping, it is possible to identify unmarked hatchery progeny in future years.

Continue monitoring the polarity index of potential spawning females. Collect tagged females to determine frequency of spawn. We have demonstrated the males can spawn annually. Is it possible for the females to spawn every other year? It would also be interesting to evaluate the 1992 year class held at Gavins Point to determine stage of maturity.

The broodstock program at Gavins Point should be continued and the nine additional families propagated this year should be added to the current broodstock program. Once it has been determined that we have recruitment to spawning age in the wild population then the need for captive broodstock should be reevaluated.

Capture radio tagged females to determine stage of egg development. The MRFWMA is tracking females previously spawned at the hatchery. There are tagged females that have been at large 2 and 3 years since spawning. Recapturing and staging these fish will provide information on the spawning frequency of pallids in the wild. We have already determined that males can spawn annually.

Evaluate the OTC marked fingerlings to determine if this marking strategy can be used. Immerse larval fish this year at 7-800 ppm to see if it is effective as well.