Ohio Lake Erie Commission

Lake Erie Protection Fund

Viability of the Progenies of Lake Sturgeon in Lake Erie- Most Endangered Acipenserid Fish

Project Duration:

October 4, 2001 – June 30, 2004

Final Report Submitted by

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1. Introduction

Until the end of the 20th century, Lake Erie had one of the most abundant sturgeon population in North America. In 1885, 5 million pounds of sturgeon were caught in the lake. During 1872, 13,880 sturgeon of average weight 50 lbs were smoked in Sandusky, Ohio. As a result of overfishing, pollution and loss of riverine spawning habitats, lake sturgeon in Lake Erie have become extremely rare.

It is believed that suitable riverine systems exist now for Lake Erie sturgeon spawning in Detroit River and other tributaries, a historical evidence also exists for lake spawning near Dunkirk, N.Y. Therefore, the only way to reestablish this fish in Lake Erie is to protect spawning ground, increase survival of juveniles and stock hatchery-produced fingerlings, which would return as adults and successfully reproduce. This project will set up an example of how to develop and implement techniques to restore vanishing species extremely important to the Lake Erie ecosystem and local economy.

Protection of breeding grounds of wild populations in the large ecosystems such as Lake St. Clair and Lake Erie will be a major means to the conservation and reestablishment of many sturgeon populations. However, without introduction of mass propagation methods, the species (population) may indeed become extinct. The breeding program of endangered sturgeon must be combined with evaluating gametes quality, possible survival in the wild and the formation of the cryopreserved sperm bank. Thus far (1999-2001) no progenies of lake sturgeon were observed in Detroit River. However, male lake sturgeons from Lake Erie can be caught and cryopreservation of sperm would allow preserving genetic diversity and prevent one more

population from disappearing. Development of the techniques for successful androgenesis and subsequently mass propagation of the species and maintaining its biological diversity are elements essential for saving genetic lines of lake sturgeon from Lake Erie. We will establish biochemical indicators of health status of lake sturgeon that will enable us to predict its reproduction potential and larval survival.

1.1. Project Focus

The specific strategic actions of the *Lake Erie Protection & Restoration Plan* that this proposal addresses are (i) "restore healthy and productive populations of historically important native sportfish and (ii) reestablish viable fisheries for lake sturgeon."

1.2. Objectives

The main objective of the project is to collect and analyze gametes from lake sturgeon in Lake Erie system (Detroit River). For comparison, gametes will be also collected from fish in the St. Clair system, Lake Winnebago (WI) and lake sturgeon raised in captivity (OSU). This objective will contribute towards establishing the biological principles that may contribute to the conservation of this threatened species and restore species diversity among different populations, and developing criteria to evaluate viability of lake sturgeon progenies and identify possible mechanisms (vitamin deficiency) affecting survival of the larval stages in the wild.

The specific objectives of the project are (i) analysis of vitamins in lake sturgeon gametes and carrying experiments of fertilization and progenies viability, (ii) determination of the embryonic stages survival, conditions during yolk absorption, and concentration of vitamins

possibly responsible for EMS in sturgeon, and (iii) development and propagation of lake sturgeon broodstock and maintenance of several generations in captivity (OSU) in order to gain experience on culturing techniques essential to the success of stocking programs.

2. Accomplishments

2.1. Study 1: Vitamins C and E concentrations in gametes of lake sturgeon from Detroit River

During May and June, 2002, the unseasonable weather conditions and a sewage spill in the Detroit River prevented the intended sampling of representative number of lake sturgeon for vitamin analyses of gametes. In 2002, only one lake sturgeon was caught and sampled from the Detroit River, south east of Fighting Island (gender undetermined, total length 150 cm, fork length 136 cm, weight 22 kg). In 2003, no spawning lake sturgeon was caught (Jim Boase has been using baited set lines in May 2003). We quote a portion of the report submitted by Jerry McClain and James Boase of the US Fish and Wildlife Service Alpena Fishery Resources Office.

"A total of four lake sturgeon were captured with setlines, tagged and released in the Detroit River (DR) from 26 March – 16 May 2002 (Table 1). Although no lake sturgeon from the Detroit River was implanted in 2002, fish implanted in 2000 and 2001 were tracked in the river from April 23 to May 15, 2003. Six lake sturgeons were located in 2002 shortly after ice out and remained there until mid-May when a substantial oil spill on one of the tributaries (Rouge River) moved into the Detroit River. During repeated attempts following the oil spill, no implanted lake sturgeon could be found in the Detroit River. Due to the oil spill it was decided that setline

fishing should be abandoned for the season even though water temperatures were still below optimum (for spawning) (11-13°C)."

The lack of sexually mature lake sturgeons sampled from the Detroit River in 2002 and 2003 limited us from analyzing vitamins in their gametes and relate this with larval survival and viability. The negative effects of habitat destruction and further developments in the Detroit River area on lake sturgeon spawning are alarming. We are aware of efforts by Mr. Bruce Manny of the US Geological Survey (Ann Arbor, Michigan), US Fish and Wildlife Service (Alpena, Michigan), and private cooperators to raise funds to build artificial spawning reefs. There is evidence that lake sturgeon will utilize man-made habitats (Caswell et al. 2004).

2.2. Study 2: Vitamins C and E analyses of lake sturgeon gametes from St Clair River (Michigan) and St. Lawrence River (New York)

2.2.1. Vitamins C and E analyses of gametes from lake sturgeon broodstock from St. Clair River

Male and female fish were caught and sperm and eggs were collected for biochemical analysis. Samples of milt were cryopreserved for future evaluations. Morphological data, blood, and tissue samples from sturgeon caught by commercial fisherman in St. Clair River/Lake Huron waters in Canada were also obtained with the assistance of Alpena FRO and the Ontario Ministry of Natural Resources. Blood, tissues, egg, and seminal plasma samples collected in summer 2002 and 2003 were analyzed for vitamin C (ascorbic acid) and vitamin E (tocopherol) concentrations (Tables 2-4). Vitamins C and E have been shown to have significant effect on reproductive efficiency in fish (Dabrowski and Ciereszko 2001).

Vitamin E analysis. High performance liquid chromatography (HPLC) was used to detect three biologically significant isomers of vitamin E, α-tocopherol (αT), γ-tocopherol (γT) and δ-tocopherol (δT). The HPLC system comprised of a Beckman 101B pump (Beckman Insturments, Inc., Palo Alto, CA) a 5µm, 150 x 4.6 mm, C18 column (Phenomenex, USA), a column heater and temperature controller (Bioanalytical Systems, Inc., West Lafayette, IN) set at 40°C, and a programmable Jasco FP-920 fluorescence detector (Jasco Corporation , Tokyo, Japan). The mobile phase was 93% methanol (MeOH), 6.5% water, and 0.5% phosphoric acid (H_3PO_4) delivered at a flow rate of 1.0mL/min. Vitamin E was extracted from frozen samples (-80°C) lake sturgeon blood plasma, eggs and sperm pellets. Extracts were prepared by homogenizing the sample in methanol containing H_3PO_4 and pyrogallol followed by centrifuging at 5,000 RCF, then retaining the supernatant. Extracts were filtered with 0.45µm nylon encased syringe filters before injection. All three isomers were separated and detected. Concentrations of αT, γT, and δT were determined by comparison with a standard curve prepared simultaneously. Standards were purchased from Sigma Chemical Company (St. Louis, MO).

2.2.2. Collection of eggs and sperm from St. Clair River (Michigan) and St. Lawrence River (New York) and development of standardized fertilization techniques to evaluate gamete viability.

We conducted evaluation trials on maternal and paternal effects on gamete viability using the embryonic development (three days after gamete activation), hatching, and first-feeding stages as quality criteria. One-way ANOVA (SigmaStat Statistical Software) showed no significant differences ($\alpha = 0.05$) in fertilization rates among individual males and females from both locations (60.3% to 88.9% males from St. Lawrence River, 64.1% to 67.5% males from St.

Clair River; 74.6% female from St. Lawrence River and 54.9% to 72.0% females from the St. Clair River). There was no difference in hatching rates among individual males and females from the St. Clair River (54.1% to 61.9% males; 54.3% to 65.2% females). Our results showed that there was a significant difference in the hatching rates between progenies obtained from individual males from New York when paired with the same female (32.3% to 75.5%).

2.3. Study 3: Analysis of plasma sex steroid hormone levels of lake sturgeon broodstock raised in captivity at The Ohio State University South Centers (Piketon, Ohio)

2.3.1. Methods

Lake sturgeons were sampled at the Ohio State University South Centers (Piketon, OH) on April 26, 2004. Fish were 100 ± 7 cm total length (range 87 – 108 cm) and 7.2 ± 1.5 kg in weight (range 4.2 – 10 kg). Blood samples were taken from 20 fish into 5 ml syringes using ½ inch 18 gauge needles. Syringes were rinsed with heparin before drawing blood to prevent coagulation. Blood samples were transported in 2 ml Eppendorf tubes to the Aquaculture Laboratory, Columbus, Ohio

Plasma levels of sex steroids including testosterone (T), 11-ketotestosterone (11-kT), estradiol-17β (E2) were measured by radioimmunoassay methods similar to those used previously (Ottobre *et al.* 1989; Rinchard *et al*., 2001).

2.3.2. Results

Concentrations of plasma sex steroids in captive lake sturgeon sampled on April 26, 2004 are reported in Table 5. The concentrations of E_2 were low, averaging 107 ± 56 pg/ml. The levels

of both androgens (T and 11-kT) were significantly higher than the ones of E_2 (2.9 ± 1.1 and 3.4 ± 1.7 ng/ml, respectively). A positive correlation was found between the concentrations of T and 11-kT (r = 0.84, n = 20, $P<0.01$).

2.3.3. Discussion

The concentrations of plasma sex steroids in captive lake sturgeon reported in this study were low in comparison to those reported in other sturgeon species during their gametogenesis (Barannikova *et al*., 2004). Thus, it appears most likely that the fish sampled are still in an early stage of their gonad development. In males, increases of androgens are usually associated with the gonial proliferation (Feist *et al*., 2004).

Levels of plasma sex steroid hormones have been used to sex cultured white sturgeon *Acipenser transmontanus* (Feist *et al.*, 2004). These authors reported that by 21 months of age male sturgeon raised at 18°C (California) could be separated from females based on the levels of T and 11-kT (>2 ng/ml). Wild immature white sturgeons with androgen levels greater than 3 ng/ml were also identified as males (Webb *et al*., 2002). Based on these data, we may suspect that a few of the lake sturgeon sampled on April 16, 2004 are males (see Table 5). A positive correlation was found between the weight of sturgeon identified as males based on plasma levels of 11-kT and T and the concentration of 11-ketotestosterone (Figure 1). However, further samplings are required to confirm these results.

2.4. Study 4: Development of techniques to cryopreserve lake sturgeon sperm

The manuscript entitled "Cryopreservation of semen from lake sturgeon (*Acipenser fulvescens*)" by Ciereszko et al. will be submitted to Transactions of the American Fisheries Society. In summary, the authors successfully cryopreserved sperm from lake sturgeon from St. Lawrence River that resulted in obtaining viable progenies. In addition, sperm used for cryopreservation was stored on ice for approximately 10 hours, and this procedure frequently diminish sperm quality.

Conclusions and Recommendations

Our results show potential paternal differences in hatching rates between individual males from New York when paired with the same female. Consequently, further studies should identify possible origin of variation in viability. This can be completed by standardization of egg/sperm ratio, but this task cannot be accomplished in the field.

The experimental diet composed of casein and casein hydrolysate was found inadequate for lake sturgeon (Froschauer 2004, in preparation). Our data have shown that a marine diet containing hydrolysed fish protein concentrate (Aglonorse, Nutreco, Norway) show promise for weaning lake sturgeon larvae to dry feed and achieving good growth. Variable feed acceptance and cannibalism were some of the major problems encountered during the feeding study. Future studies should evaluate techniques of preventing these problems during feeding experiments.

Automatic feeders show promise for routine and large-scale feeding of lake sturgeon with dry feeds. The use of these feeders reduces manpower needs to feed the fish 24 h a day at ½ h intervals.

The elevated levels of T and 11-kT in some of the sampled lake sturgeon broodstock suggested the presence of sexually maturing males in the lake sturgeon raised in captivity at The Ohio State University, Piketon, Ohio. Further samplings are necessary in the following year to confirm this preliminary observation.

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Table 1. Mean length, weight, girth, and age for sturgeon collected from the Detroit and St. Clair Rivers in 2002 (McClain and Boase, 2004, unpublished report, National Fish and Wildlife Foundation)

Measure	Detroit River	St. Clair River
Total number caught	4	47
Mean length	1,583 mm	1,330 mm
Length range	1470 mm – 1,770 mm	799 mm – 1,900 mm
Mean weight	23.9 kg	15.0 kg
Weight range	18.5 kg – 34.0 kg	1.4 kg – 43.0 kg
Mean age	Na	20.0
Age range	Na	3-48

Table 2. Vitamin C and vitamin E concentrations in lake sturgeon biological samples collected in 2002 from the St. Clair River.

	Vitamin C			Vitamin E		
	Total AA (μg/g)	**Dehydro AA (μg/g)**	**Reduced AA (μg/g)**	**α-tocopherol (μg/g)**	**β+γ-tocopherol (μg/g)**	**δ-tocopherol (μg/g)**
Females						
Blood plasma (n=5)	3.0±0.5	1.9±.4	1.1±0.4	2.0±0.4	0.01±0.01	0.004±0.003
Liver (n=5)	101.6±50	36.5±20	65.1±32	27.8±11.8	0.28±0.14	0.002±0.001
Kidney (n=5)	85.7±24.8	47.5±6.9	68.0±10.6			
Immature ovary (n= 4)	87.0±24.8	8.2±6.6	8.3±4.0	9.9±2.7	0.1±0.07	0.003±0.002
Eggs (n=6)	98.8±17	19.1±6.4	79.7±16	11.0±2.5	0.1±0.06	0.005±0.005
Males						
Blood plasma (n=9)	2.8±0.8	1.9±0.4	0.9±0.6	1.7±0.8	0.01±0.004	0.005±0.003
Liver (n=9)	103.5±12	28.0±8.3	75.4±8.4	53.1±24.2	0.23±0.21	0.02±0.02
Kidney (n=9)	101.0±14.3	32.9±11.1	68.0±10.6			
Testis (n=9)	47.2±15.4	8.6±1.7	38.7±14.0			
Spermiating Males						
Seminal plasma (n=3)	1.1±0.9	1.1±0.9	0.0±0.0	1.3±0.9	0.01±0.008	0.07±0.06

Table 3. Vitamin C and vitamin E concentrations in lake sturgeon biological samples collected in 2003 from lake sturgeon at the St. Clair River in Michigan and the St. Lawrence River in New York.

	Vitamin C			Vitamin E		
	Total AA (μg/g)	**Dehydro AA (μg/g)**	**Reduced AA (μg/g)**	**α-tocopherol (μg/g)**	**β+γ-tocopherol (μg/g)**	**δ-tocopherol (μg/g)**
Michigan						
Eggs (n=3)	84.2±8.8	34.0±14.4	51.1±22.4	5.8±1.7	0.008±0.004	0.04±0.03
				0.94±0.5	0.0006±0.0003	0.0005±0.0004
Seminal pellets (n=4)	6.6±2.5	4.5±3.1	2.1±0.73	1.4±0.02	0.002+0.0009	0.011±0.0001
				0.74+0.17	0.0003±0.0002	0.003±0.002
Immature fish (n=15)				1.1±0.5	0.001±0.0008	0.006±0.004
				21.4±8.5	0.09±0.05	0.4±0.3
New York						
Eggs (n=2)	100.2±6.9	36.8±6.0	63.4±0.9	5.3±1.7	0.01±0.001	0.02±0.01
Seminal Pellets (n=9)	4.7±0.8	3.0±0.6	1.7±0.5	1.1±0.3	0.007±0.006	0.004±0.001

Table 4. Tocopherol (vitamin E) concentrations in lake sturgeon tissues and blood plasma collected in 2002 and 2003.

2002 Sturgeon			
	α-tocopherol	**β+γ-tocopherol**	**δ-tocopherol**
Liver (n=14)	44.30±23.55	0.25±0.18	0.012±0.003
Eggs (n=11)	10.46±2.56	0.10±0.05	0.004±0.002
Seminal pellets (n=3)	1.25±0.9	0.013±0.008	0.065±0.05
Blood plasma (n=15)	1.11±0.47	0.001±0.0009	0.006±0.004
2003 Sturgeon			
	α-tocopherol	**β+γ-tocopherol**	**δ-tocopherol**
Liver (n=15)	21.4±8.5	0.09±0.05	0.35±0.30
Eggs (n=6)	5.56±1.89	0.009±0.003	0.035±0.028
Seminal pellets (n=4)	1.13±0.28	0.007±0.006	0.005±0.003
Blood plasma (n=23)	1.02±0.44	0.008±0.007	0.005±0.004

Table 5. Weight and concentrations of plasma sex steroid hormone in captive lake sturgeon sampled on April 26, 2004. * indicated sturgeon suspected to be male based on androgen levels.

Fish	Weight	Plasma sex steroids		
	(kg)	Estradiol (pg/ml)	Testosterone (ng/ml)	11-ketotestosterone (ng/ml)
1*	7.40	63	3.1	4.9
2	6.58	39	2.3	1.8
3*	7.50	158	4.6	5.8
4	7.15	72	2.2	2.6
5	10.00	72	1.7	2.2
6*	9.15	86	4.6	6.3
7	8.15	68	2.0	1.5
8	7.45	102	3.0	2.7
9*	7.60	53	5.6	4.8
10*	6.80	205	3.3	4.4
11	7.70	161	2.2	2.7
12*	9.45	216	4.2	6.5
13	4.20	204	2.5	2.1
14	9.20	72	1.9	2.0
15	7.05	119	2.5	1.4
16	5.15	78	2.0	2.0
17*	5.80	84	3.0	3.6
18*	6.75	146	3.6	5.5
19	5.70	38	2.1	2.7
20	5.95	95	2.1	2.5

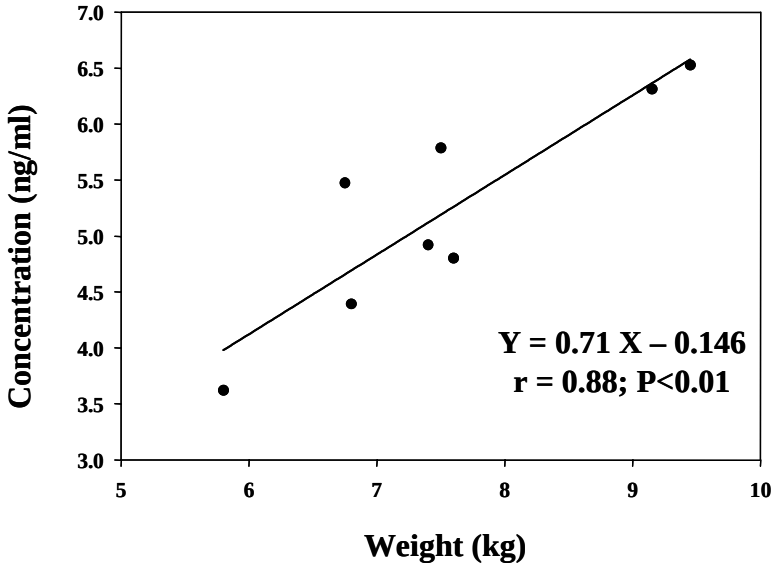

Figure 1. Relationship between weight and plasma 11-ketotestosterone levels in sturgeon identified as males based on plasma androgen levels.