GROWTH INHIBITION OF SIBERIAN STURGEON (*ACIPENSER BAERII*) FROM DIETARY AND WATERBORNE FLUORIDE

Xiaotao Shi,[a,b] Ping Zhuang,[a,b] Longzhen Zhang,[b] Guangpeng Feng,[b] Liqiao Chen,[a] Jianyi Liu,[b] Ruifang Wang[a,c]

Shanghai, China

SUMMARY: As part of a recent study, growth trials were conducted to evaluate effects of dietary and waterborne fluoride ions (F) on juvenile Siberian sturgeon (*Acipenser baerii*). In 12-week dietary exposure experiments, growth was inhibited by diets with 710.2 or 1478.3 mg F/kg, but not with 162.6 or 360.8 mg F/kg compared to the control (75.2 mg F/kg). In 90-day waterborne exposure experiments, growth was inhibited at 7.8, 18.7, and 51.8 mg F/L but not at 3.1 mg F/L compared to the control at 0.26 mg F/L. These findings indicate that F can cause adverse effects on the growth of Siberian sturgeon.

Keywords: *Acipenser baerii*; Fish diet; Fluoride and growth; Siberian sturgeon.

INTRODUCTION

Widespread occurrence of ionic fluoride (F) is well known to cause harmful effects in both humans and animals. Aquatic life can take up F from both food and water.[1] Fish can accumulate F through the food chain, but few reports have determined the toxicity of dietary F to fish, although the study of krill meal as an alternative to fish meal as a source of F has been reported.[2,3] The amount of F present in water is highly variable, depending upon the individual geological environment and the severity of the F pollution.[1] Levels as low as 0.5 mg F/L in water may be toxic to certain species of fish, particularly in "soft" waters.[1] Adverse effects of F to fish include growth inhibition, behavioral changes, histopathological abnormalities, disturbances of metabolic enzymes, and bone disorders.[1] The natural F concentration of ground/surface water in parts of China is reported to be higher than 5 mg/L with some as high as 45 mg/L,[4] which greatly exceeds the 1.0-mg F/L China national water standard for fisheries.[5] However, the effects of waterborne F to fish are relatively less reported. [6,7]

Siberian sturgeon (*Acipenser baerii*) is one of the sturgeon species widely raised in fish farms in China. In a recent investigation, now extended here, we reported the effects of waterborne F on weight gain and hard and soft tissue accumulation of F in 400 juvenile Siberian sturgeon.[8] These same juvenile sturgeon, plus an additional 400, were used in the present study to evaluate effects of both dietary and waterborne F on their growth and related parameters.

MATERIALS AND METHODS

Fish, diet, and experimental design: Eight hundred juvenile Siberian sturgeons were obtained from Hangzhou Qiandaohu Xunlong Sci-tech Development Co. Ltd in Zhejiang Province. The fish were stocked in an indoor aquaculture facility

[a]School of Life Science, East China Normal University. [b]East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jungong Road 300, Shanghai 200090, China. [c]College of Aqua-life Science and Technology, Dalian Fisheries University.
For correspondence: Dr P Zhuang, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jungong Road 300, Shanghai 200090, China.
Email: pzhuang@online.sh.cn; sxtshanghai@163.com.

located at the East China Sea Fisheries Research Institute of the Chinese Academy of Fishery Sciences, Shanghai, and acclimated to ambient light and 23±1°C water for 15 days. Four hundred fish were used for the dietary F (DF) exposure trial while the other four hundred fish were used for the waterborne F (WF) exposure trial. Fish used for DF were fed in excess three times per day with a commercial diet (43.2% crude protein, 15.4% crude fat, 12% carbohydrate, 8.9% ash according to the manufacturer, Shandong Shengsuo Feed Company, Yantai City, China) during acclimation period and gradually weaned to an artificial meal based diet (43.2% crude protein, 12.7% crude fat, 12% carbohydrate, 12.1% ash). Fish used for the WF trial were fed in a similar way but only 2 times a day. The artificial fish-meal based diet contained 75.2 mg F/kg.

As described in part in our recent report,[8] juvenile Siberian sturgeon (12.30±0.05 cm body length, 11.93±0.05 g wet body weight for DF; 10.83±0.05 cm body length, 8.55±0.09 g wet body weight for WF) were chosen and assigned to 15 rectangular concrete (DF) or plastic blue (WF) tanks (100×78×55 cm) with 12 fish in each tank. The mean lengths and weights of fish did not differ among treatments (LSD; $p>0.05$). Then, for DF and WF, fish were designated randomly to one of the four F exposure treatments plus one control treatment, resulting in triplicate tanks for each treatment.

For DF, NaF (analytical reagent grade, Sinopharm Chemical Reagent Co. Ltd, Shanghai, China) was added to the same fish meal based diet mentioned above (also used as the control diet) to make apparent F concentrations to be 150, 350, 700, and 1500 mg F/kg diet. The resulting dietary F concentrations were 75.2 (Control), 162.6, 360.8, 710.2, and 1478.3 mg F/kg. As noted above, DF fish were fed in excess three times a day with these diets containing different concentrations of F. For WF, fish were exposed to measured F concentrations of 0.26±0.07 (control), 3.1±0.5, 7.8±0.8, 18.7±1.8, and 51.8±3.5 mg/L for 90 days. Stock solutions of 1000 mg F/L were prepared from NaF in double distilled water. The control diet in DF was used for all treatments in WF. For both growth trials, water used was filtered tap water pre-aerated for more than 3 days. The remaining pellets and excreta were removed from the aquaria with a suction hose. There was a minimum of 80% medium renewal daily by pumping the target solution into the rearing tank.

The sturgeon were reared under ambient photoperiod which approximately was 12-hr light/12 hr dark. The water quality parameters during the study were temperature 23±1°C, pH between 7.3 and 7.9, dissolved oxygen >8.0 mg/L, hardness as $CaCO_3$ 22±4 mg/L, and total ammonia nitrogen <0.02 mg/L.

Sampling: All fish in each group were measured to get the body weights and body lengths under anesthesia by MS-222 (300 mg/L). The sampling time points for DF fish were 0, 4, 8, and 12 weeks, and for the WF fish were 0, 30, 60, and 90 days. The growth parameters were calculated as follows: SGR (Specific growth rate) = $100\times(\ln W_2-\ln W_1)/T$, FR (Feeding Ratio) = $200\times F/[(W_2+W_1)\times n\times T]$, FCR (Food conversion ratio) = $F/[n\times(W_2-W_1)]$, DWG (Daily weight gain) = $(W_2-W_1)/(n\times T)$, DFC (Daily food consumption) = $F/(n\times T)$, CF (Condition factor) = W/L^3, where W denotes body weight, W_1 and W_2 represent initial and final weights, respectively, L denotes body length, F is the total feed consumption, n is the number of individuals, and T is the time in days.

Data and statistical analysis: As reported earlier,[8] means and standard errors (SE) were calculated for each of the measured parameters. Data were analyzed with STATISTICA (Version 6.0) (StatSoft, Inc.). Statistical differences were evaluated using a one-way analysis of variances (ANOVA) followed by the Fisher's Least Significant Difference (LSD) test for multiple pair-wise comparisons. Statistically significant differences were accepted at $p<0.05$. Normality and homogeneity of variances were verified using the Kolmogorov–Smirnov test and the Levene test, respectively.

RESULTS

No mortality was found during the experimental period in either the DF or WF fish. As seen in Table 1, the growth of the juvenile Siberian sturgeon exposed to DF ≤360.8 mg/kg for 12 weeks was not adversely affected compared to the control, but negative growth effects were observed in fish fed a diet with 710.2 and 1478.3 mg F/kg. For SGR, DWG, and DFC, fish fed 710.2 and 1478.3 mg F/kg had a lower level than the control, whereas fish fed DF with F ≤360.8 mg/kg did not. FR and CFF of fish fed 1478.3 mg F/kg were significantly lower than those of the control; no significant differences among treatments were found in FCR.

Table 1. Growth parameters (mean±SE) of juvenile Siberian sturgeon exposed to different dietary F concentrations for 12 weeks

Item[a]	Measured dietary F concentration in different treatments (mg/kg)				
	Control (75.2)	162.6	360.8	710.2	1478.3
Initial BW (g)	11.99±0.04	12.02±0.03	11.82±0.25	11.87±0.11	11.95±0.11
Final BW (g)	200.24±4.47	197.77±3.60	192.51±4.78	178.94±3.73*	159.67±6.96*
SGR	3.13±0.02	3.11±0.02	3.10±0.01	3.01±0.03*	2.88±0.05*
DWG [g/d]	2.09±0.05	2.06±0.04	2.01±0.05	1.85±0.04*	1.64±0.08*
DFC(g)	2.48±0.07	2.47±0.02	2.29±0.05	2.14±0.04*	1.88±0.10*
FCR	1.19±0.03	1.2±0.02	1.14±0.02	1.16±0.02	1.15±0.03
FR (%)	2.34±0.06	2.36±0.03	2.25±0.05	2.25±0.03	2.19±0.05*
Initial CF	6.34±0.05	6.41±0.19	6.31±0.26	6.49±0.23	6.54±0.16
Final CF	5.94±0.05	5.92±0.08	5.89±0.04	5.8±0.04	5.77±0.05*

Asterisks (*) indicate significant differences relative to control ($p<0.05$). [a]Abbreviations: BW Body Weight; SGR Specific Growth Rate; FR Feeding Ratio; FCR Food conversion ratio; DWG Daily Weight Gain; DFC Daily Food Consumption; CF Condition Factor.

As seen in Table 2, growth, DWG, and DFC were significantly lower after 90 days in fish exposed to WF at 7.8, 18.7, 51.8 mg/L compared to the control. With 51.8 mg F/L, SGR was significantly decreased compared to control. FCR, FR, and CFF in groups exposed to WF did not differ significantly from the control.

Table 2. Growth parameters (mean±SE) of juvenile Siberian sturgeon exposed to different measured waterborne F concentrations for 90 days

Item[a]	Measured waterborne F concentration in different treatments (mg/L)				
	Control (0.26)	3.1	7.8	18.7	51.8
Initial BW (g)[b]	8.55±0.18	8.57±0.22	8.57±0.37	8.43±0.15	8.61±0.18
Final BW (g)[b]	175.88±4.43	170.67±4.15	157.91±4.50*	155.30±3.22*	150.14±4.44*
SGR[b]	3.36±0.05	3.32±0.04	3.24±0.07	3.24±0.04	3.18±0.05*
DWG [g/d]	1.86±0.02	1.80±0.03	1.66±0.04*	1.63±0.02*	1.57±0.03*
DFC(g)	1.94±0.02	1.90±0.02	1.84±0.04*	1.74±0.02*	1.57±0.03*
FCR	1.05±0.04	1.06±0.03	1.11±0.05	1.07±0.03	1.06±0.01
FR (%)	2.11±0.07	2.13±0.05	2.22±0.10	2.13±0.05	2.10±0.03
Initial CF	6.73±0.09	6.80±0.08	6.63±0.19	6.75±0.14	6.70±0.10
Final CF	6.01±0.11	6.00±0.08	5.98±0.06	5.99±0.09	5.95±0.08

Asterisks (*) indicate significant differences relative to control ($p<0.05$). [a]Abbreviations: BW Body Weight; SGR Specific Growth Rate; FR Feeding Ratio; FCR Food conversion ratio; DWG Daily Weight Gain; DFC Daily Food Consumption; CF Condition Factor. [b]Data are those reported earlier for the 0.26 mg F/L control and for "nominal" water F concentrations of 4, 10, 25, and 62.5 mg/L.[8]

DISCUSSION

Most reports about effects of dietary F on fish are related to krill meal, which contains a high F concentration.[3,9,10] Moren et al.[3] reported that NaF added at 110–190 mg F/kg did not inhibit growth of Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), Rainbow trout (*Onchorhyncus mykiss*), and Atlantic halibut (*Hippoglossus hippoglossus*), similar to the present study where growth was not affected by a diet containing 162.6 mg F/kg. The difference in FR between fish fed 1478.3 mg F/kg and the control group showed that fish might avoid the toxicity of F by decreasing feeding quantity, which was common in other species when exposed to toxicants. CFF denotes the body condition of fish. In the present study, fish fed 1478.3 mg F/kg were slenderer (lighter for a given length) than control fish as showed by CFF. F from water caused adverse effects to fish in the present study, similar results were found in *Anabas testudineus*, *Channa punctatus*, *Clarias batrachus*, *Heteropneustes fossilis*, and *Chitala ornata*, when exposed to waterborne F over a range of 6.9 to 52.5 mg/L in a captive pond.[11] As mentioned in the Introduction, we recently reported that waterborne F caused F accumulation in bone, cartilage, and various soft tissues of these juvenile Siberian sturgeon,[8] but here we have reported further details of the growth parameters and growth inhibition of the higher level DF as well in the same WF sturgeon.

The diminished growth in the high-F exposure group may be attributed to several reasons. First, F ions act as enzymatic poisons, interrupting protein synthesis, increasing glycolysis, activating antioxidative pathways, and causing haematological abnormalities.[1,7,12] Second, behavioral changes such as respiratory alterations and violent, erratic movement, which might influence the metabolism of fish,[13] were observed in fish exposed to 18.7 and 51.8 mg F/L. Third, histopathological changes might be caused by F, because the toxicity of F to fish has been reported to be related to histopathological changes in gill, kidney, or intestine.[6] Fourth, the accumulation of F in tissues especially in bone might influence growth.[8,9] The reason for the growth inhibition in the present study should be further investigated.

In conclusion, dietary F ≤360.8 mg/kg or waterborne F ≤7.8 mg/L did not cause adverse effects on growth performance or survival to Siberian sturgeon. The maximum level of F in fish feeds is 350 mg/kg in EU,[14] and results from the present study support this guideline. However, as F specifically deposits in bone or exoskeleton,[15] considering the long life span of sturgeon to reach sexual maturity, F might have adverse effect on growth or other physiological function at a lower concentration. Longer term and low dosage growth trials with determination of F concentration in fish tissues should be conducted to ensure safe criteria of F to fish.

ACKNOWLEDGEMENTS

The project was supported by the National High Technology Research and Development Program of China (2008AA10Z227), Open Fund of Key Laboratory of Ecological Environment and Resources of Inland Fisheries (YM2007-06), and PhD Program Scholarship Fund of ECNU (20080036).

REFERENCES

1 Camargo JA. Fluoride toxicity to aquatic organisms: a review. Chemosphere 2003;50:251-64.
2 Yoshitomi B, Aoki M, Oshima S. Effect of total replacement of dietary fish meal by low fluoride krill (*Euphausia superba*) meal on growth performance of rainbow trout (*Oncorhynchus mykiss*) in fresh water. Aquaculture 2007;266:219-25.
3 Moren M, Malde MK, Olsen RE, Hemre GI, Dahl L, Karlsen Ø, et al. Fluorine accumulation in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), rainbow trout (*Onchorhyncus mykiss*) and Atlantic halibut (*Hippoglossus hippoglossus*) fed diets with krill or amphipod meals and fish meal based diets with sodium fluoride (NaF) inclusion. Aquaculture 2007;269:525-31.
4 Fuhong R, Shuquin J. Distribution and formation of high-fluorine ground water in China. Environ Geol Water Sci 1988;12:3-10.
5 Ministry of environmental protection of China. GB11607–Water quality standard for fisheries. 1989. [in Chinese]
6 Bhatnagar C, Bhatnagar M, Regar BC. Fluoride-induced histopathological changes in gill, kidney, and intestine of fresh water teleost, *Labeo Rohita*. Fluoride 2007;40:55-61.
7 Kumar A, Tripathi N, Tripathi M. Fluoride-induced biochemical changes in fresh water catfish (*Clarias Batrachus*, Linn.). Fluoride 2007;40:37-41.
8 Shi X, Zhuang P, Zhang L, Feng G, Chen L, Liu J, et al. The bioaccumulation of fluorine ion (F^-) in Siberian sturgeon (*Acipenser baerii*) under laboratory conditions. Chemosphere 2009;75:376-80.
9 Yoshitomi B, Aoki M, Oshima S, Hata K. Evaluation of krill (*Euphausia superba*) meal as a partial replacement for fish meal in rainbow trout (*Oncorhynchus mykiss*) diets. Aquaculture 2006;261:440-46.
10 Suontama J, Kiessling A, Melle W, WaagbØ R, Olsen RE. Protein from northern krill (*Thysanoessa inermis*), Antarctic krill (*Euphausia superba*) and the Arctic amphipod (*Themisto libellula*) can partially replace fish meal in diets to Atlantic salmon (*Salmo salar*) without affecting product quality. Aquac Nutr 2007;13:50-8.
11 Samal UN. Effect of fluoride on growth of certain freshwater fishes. Environ Ecol 1994;12:218-20.
12 Chlubek D. Fluoride and oxidative stress. Fluoride 2003;36:217-28.
13 Tarazona JV, Mufioz MJ, Ortiz JA, Nufiez MO, Camargo JA. Fish mortality due to ammonia exposure. Aquacult Fish Manage 1987;18:167-72.
14 EU [European Union]. Commission directive 2008/76/EC of 25 July 2008 amending Annex I to Directive 2002/32/EC of the European Parliament and the Council of 7 March 2002 on undesirable substances in animal feed as regards to lead, fluorine, and cadmium. Official J Eur Union 2008;198:37-40.
15 Julshamn K, Malde M, Bjorvatn K, Krogedal P. Fluoride retention of Atlantic salmon (*Salmo salar*) fed krill meal. Aquac Nutr 2004;10:9-13.