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Effects of dietary methylmercury on growth performance and tissue burden in juvenile green (*Acipenser medirostris*) and white sturgeon (*A. transmontanus*)

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ABSTRACT

Triplicate groups of juvenile green and white sturgeon (30 ± 2 g) were exposed to one of the four nominal concentrations of dietary methylmercury (MeHg, 0 (control), 25, 50, and 100 mg MeHg/kg diet) for 8 weeks to determine and compare the effects on growth performance and mercury (Hg) tissue burden in the two sturgeon species. Mortality, growth performance as measured by percent body weight increase per day, hepatosomatic index, proximate composition of whole body, and Hg burden in the whole body, gill, heart, liver, kidney, and white muscle were determined to assess the adverse growth effects and bioaccumulation of dietary MeHg in sturgeon. Significantly higher mortality and lower growth rate ($p < 0.05$) were noted in green and white sturgeon fed the MeHg diets compared to the controls. Green sturgeon fed the MeHg diets exhibited earlier and more severe adverse effects compared to white sturgeon. Mercury accumulated in all tissues in a dose-dependent manner regardless of species, and the highest Hg concentrations were found in the kidneys of both species. Dietary MeHg had no significant effect ($p > 0.05$) on the whole body proximate compositions of either sturgeon species. In conclusion, green sturgeon was more susceptible to dietary MeHg toxicity than white sturgeon in our 8-week growth experiment based on the higher mortality and lower growth rate and body energy contents.

1. Introduction

Green (*Acipenser medirostris*) and white sturgeon (*A. transmontanus*) are two sturgeon species native to the San Francisco Bay-Delta (SFBD) (Moyle, 2002). The populations of the two sturgeon species have been in decline since the late nineteenth century; in particular, the southern population of green sturgeon in SFBD is currently listed as a threatened species (CNDDB, 2006). The population declines of green and white sturgeon have been attributed to a variety of factors, including habitat degradation and loss, overfishing, chemical contamination, and the presence of invasive species (Kohlhorst, 1980; Linville et al., 2002; National Marine Fisheries Service, 2006). Mercury (Hg) is one of several chemical contaminants of special concern in SFBD. Current Hg levels found in SFBD fish including white sturgeon were high enough to trigger the release of a human consumer advisory. The Hg levels found in SFBD white sturgeon are known to cause toxicity in northern pike (*Esox lucius*) (Drevnick et al., 2008) and fathead minnow (*Pimephales promelas*) (Drevnick and Sandheinrich, 2003). Thus, wild sturgeon in SFBD may already suffer chronic methylmercury (MeHg) toxicity. In addition, juvenile green and white sturgeon are known to spend 1–3 years in the SFBD water system before outmigration, suggesting that juvenile sturgeon may already be at risk of Hg contamination. However, there is no information on the effects of Hg on these sturgeon species.

As one of the three primary spawning areas for green sturgeon in California, the SFBD region is one of the most important areas for sturgeon populations (Moyle, 2002). Unfortunately, this area has a long history of Hg contamination dating back to the gold mining period between 1850 and 1960. During this period, an estimated 1200–3600 mton of inorganic Hg were released into the environment in California as a byproduct of hydraulic gold mining activities, and Hg levels in the SFBD still remain in excess of United States Environmental Protection Agency (USEPA) screening values (Fairey et al., 1997; Davis et al., 2002). Moreover, a portion of the introduced inorganic Hg can be converted by bacteria or fungi in the aquatic environment into to MeHg (Winfrey and Rudd, 1990; Morel et al., 1998), one of the most toxic forms of Hg (Matida et al., 1971; Boening, 2000) because of its high bioavailability and high affinity for thiol groups in biological systems (Morel et al., 1998;

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Pickhardt et al., 2006). Once introduced, MeHg is efficiently biomagnified through food chains (Mason et al., 1995; Hall et al., 1997) and has a tendency to bioaccumulate with increased age (Bache et al., 1971). Thus, fish in the upper trophic levels of food chains are likely to accumulate higher concentrations of MeHg than those in the lower trophic levels (Morel et al., 1998; Davis et al., 2002; Cizdziel et al., 2003). Long-lived sturgeon species in upper trophic levels in benthic food chains may accumulate significant levels of MeHg and may be susceptible to MeHg toxicity.

Mercury pollution in aquatic food webs has serious consequences, as can be observed in the tragedies in Minamata and Niigata, Japan, where MeHg accumulation in fish severely poisoned human consumers (Tsubaki and Irukayama, 1977). These incidents prompted many studies to monitor Hg and MeHg bioaccumulation in fish intended for human consumption. Until recently, limited information was available regarding MeHg toxicity in fish. Although substantial knowledge on Hg toxicity in fish species has been gathered, the majority of these studies were conducted using single, short-term (several days) or water-borne Hg exposure methods (Giblin and Massaro, 1973; McKim et al., 1976; Pentreath, 1976; Niimi and Kissoon, 1994; Elia et al., 2003; Leaner and Mason, 2004; Pickhardt et al., 2006; Monteiro et al., 2010), which are not relevant to the Hg exposure patterns that fish encounter in the environment. There are a limited number of studies investigating the chronic effects of dietary MeHg on fish, but almost no studies investigated its effect on sturgeon specifically.

Mainly due to white sturgeon's larger population size, most field sampling surveys in the SFBD were based on the sturgeon, so that there is a lack of information on the SFBD green sturgeon including chemical contaminations and stress responses. As a result of this, researchers are trying to use white sturgeon to build models of green sturgeon. Although green and white sturgeon are very closely related species, they may have different biological responses to environmental stressors such as MeHg. To test whether green and white sturgeon have a similar biological response to dietary MeHg, the current study identifies and compares sensitivity of the two sturgeon species by determining growth performances (mortality, growth rate, hepatosomatic index), whole body proximate composition, and Hg burdens in whole body and 5 tissues (gill, heart, liver, kidney, and white muscle).

2. Materials and methods

2.1. Animal acquisition

Green sturgeon larvae were obtained from artificially spawned captive F1 broodstocks (1 female and 3 males) originating from the Klamath River (Conte et al., 1988; Van Eenennaam et al., 2001) on 15 March 2007. They were reared and fed commercial feeds (Hung et al., 1987; Deng et al., 2003) for 90 days. They were then gradually weaned to a purified diet (Hung and Lutes, 1987; Hung et al., 1987) one week prior to the initiation of dietary MeHg exposure. White sturgeon larvae were obtained from a sturgeon farm (Sacramento, CA) after artificial spawning of a captive broodstock (Doroshov et al., 1983) on 17 May 2007. The larvae were reared and fed diets similar to the green sturgeon for 97 days. They were similarly weaned to the purified diet (Hung and Lutes, 1987; Hung et al., 1987) one week prior to the initiation of the experiment. The care, maintenance, handling, and sampling of sturgeon were performed according to protocols approved by the Campus Animal Care and Use Committee of UCD.

2.2. Diet preparation

Four isoenergetic and isonitrogenous purified diets similar to those used in previous studies (Hung and Lutes, 1987; Hung et al., 1987; Tashjian et al., 2006) were used in this experiment (Table 1). The diet contained sufficient nutrients to support robust growth in juvenile white sturgeon (Hung and Lutes, 1987; Hung et al., 1987) and has been shown to be suitable for testing the effects of dietary selenomethionine in juvenile white sturgeon (Tashjian et al., 2006). The ingredients of the diets were combined and thoroughly mixed, water was added, and the appropriate concentration of MeHg chloride (K&K Laboratories, Div. ICN Biomedicals, Inc., dissolved in 100% ethanol) was added to the mixture to constitute the nominal levels of 0 (control), 25, 50, and 100 mg MeHg/kg in the diets; at most 6 mL of ethanol was added to a kg of diet, and most ethanol evaporated during the processes of experimental diet preparation, including pelleting and fan drying overnight. The range of MeHg concentrations used in the current study was chosen because (1) the concentrations in this range affected the mortality and growth of green sturgeon in an unpublished study (R.C. Kaufman, unpublished data) and (2) this design allows us to compare our results with the results of two previous studies on other fish species (Rodgers and Beamish, 1982; Houck and Cech, 2004). The diets were stored in plastic bags at −20 °C before use. Daily rations (3% body weight/day for the first 4 weeks and 2% body weight/day for the second 4 weeks, determined based on maximum % body weight increase and minimum feed/gain ratio) (Cui and Hung, 1995) were placed in an automatic feeder, dispensing the feed into the 90-L circular tanks continuously through 24 h (Hung and Lutes, 1987). Every week, weight of fish in each tank was measured and feed ration was adjusted accordingly. Initially, 25 fish were introduced into each tank (8.3 g fish/L water). Three fish were removed every 2 weeks for tissue samplings and an additional 3 fish were removed every 4 weeks for whole body samplings.

Table 1
Sturgeon purified diet formulation and proximate composition.

Formulation	
Ingredients	g/kg diet
Vitamin-free casein	310
Wheat gluten	150
Egg albumin	40
Dextrin	295
Corn oil	60
Cod liver oil	60
Celufil hydrolyzed	40
FNG-2b vitamin premix[a]	10
BT-m mineral premix[b]	30
Choline chloride	5
Santoquin	0.2
Nutrients	*Proximate compositions*
Moisture	80
Crude protein	428
Crude lipid	126
Ash	33
Energy content (cal/g)	5011.9

All ingredients were purchased from the USB Biochemical Corp. (Cleveland, OH) except FNG-2b, which was purchased from ICN Nutritional Biochemicals (Aurora, OH).

[a] FNG-2b vitamin premix contained the following ingredients (g/kg premix): thiamine mononitrate (1.5), riboflavin (2.5), niacin (20), calcium pantothenate (5), pyridoxin HCl (2), vitamin B_{12} (0.02), folic acid (1), biotin (0.15), inositol (50), ascorbic acid (50), *para*-amino benzoic acid (30), butylylated hydroxyanisole (9), menadione sodium bisulfite (0.125), vitamin A acetate (500,000 units/g) (0.8), vitamin D (400,000 units/g) (0.6), vitamin E acetate (250 units/g) (48).

[b] BT-m mineral premix composition was prepared as described (Bernhart and Tomarelli, 1966).

2.3. Experimental design and animal maintenance

Both of the green and white sturgeon growth experiments were conducted for 8 weeks using the same batch of diets and the same tank system (Hung and Lutes, 1987). They were conducted consec-

utively, with the green sturgeon experiment conducted between June 20th and August 8th, 2007 and the white sturgeon experiment between August 29th and October 17th, 2007. The two experiments were not conducted simultaneously because of the different spawning and hatching times of the two sturgeon species. For each experiment, 300 juvenile sturgeon of similar size (average 30 ± 2 g) were randomly assigned to twelve circular fiberglass tanks. Each tank received flow-through aerated well water at a flow rate of 3 L/min, and the water temperature, pH, and dissolved oxygen were 18-19 °C, 7–8, and 7–9 mg/L in the tanks, respectively.

2.4. Sampling protocol

Fish were fasted for 24 h prior to sampling to allow the evacuation of food in the gastrointestinal tract. At 0 week, 9 fish were sampled and randomly pooled into 3 groups of 3 fish each to be used as controls for whole body proximate composition and Hg burden. At 4 and 8 weeks, three fish were removed from each tank and euthanized in a tricaine methanesulfonate solution (MS 222, 0.5 g/L, Argent Chemical Laboratories, Redmount, WA). The euthanized fish were blotted dry on paper towels, weighed and length-measured individually. The three fish from the same tank were wrapped together in aluminum foil, and stored at −20 °C until they were freeze-dried and pulverized for later determination of whole body proximate composition and Hg burden. Three additional fish from each tank were randomly captured and euthanized with MS 222 at 2, 4, 6, and 8 weeks, and pooled within tank to determine Hg tissue burden (at 0 week, additional 9 fish were sampled and randomly pooled into 3 groups for Hg tissue burden). The gill (gill filaments only), heart, liver, kidney, and white muscle were dissected. For sampling the posterior kidney, the entire urinary ducts were first removed, and then the posterior part of the kidney tissues was taken surgically by slitting the boundaries of the kidney. A cubical section (~2 cm) of white muscle at the midpoint of the body was removed from each fish. The sampled tissues were wrapped in aluminum foil, instantly frozen in liquid nitrogen, stored at −80 °C, freeze-dried, and hand ground to a homogenous powder using a mortar and pestle for Hg analysis.

2.5. Mercury analysis

The total concentration of Hg, rather than MeHg, was determined in the fish. This was because MeHg is known to constitute over 90% of Hg present in fish (Bloom, 1992; Amlund et al., 2007), and the USEPA recommends that because of the high cost of MeHg analysis total Hg be determined with the assumption that the majority of Hg is present in the form of MeHg. Total Hg concentrations were determined using a USEPA-approved Method 7473, a direct Hg analyzer (DMA-80, Milestone, Inc., Shelton, CT). The detection limit of this method is 0.01 ng of total Hg. Each sample was analyzed in duplicate. All samples were analyzed simultaneously with a certified reference material (DORM-2 dogfish liver, National Research Council, Canada) and blanks to ensure that suitable recovery was maintained throughout the analyses. The measured reference material values (mean 4.62 μg Hg/g dry weight) were within the certified standard range (4.64 ± 0.26 μg Hg/g dry weight). All results are presented on a dry weight basis.

2.6. Statistical analysis

Statistical analyses were conducted using the JMP 7.0 statistical software program (SAS Institute, Cary, NC). A two-way analysis of variance with interaction was used to test for significant differences among the four dietary MeHg concentrations and between the two species of sturgeon. The Tukey Honestly Significant Difference test was used for multiple comparisons among dietary MeHg concentrations and between the two species at each time point. Statistical significance was tested at the 0.05 probability level, and all values are presented as mean ± standard error unless noted otherwise.

3. Results

3.1. Mortality and growth performances

The mortality of green and white sturgeon exposed to diets with different concentrations of MeHg is presented in Tables 2a and 2b. After 8 weeks of exposure, significantly higher mortality was observed in green sturgeon fed diets with 50 mg or higher MeHg/kg compared to the controls, and in white sturgeon fed a diet with 100 mg MeHg/kg (Table 2b). Significantly higher mortality was first observed at 4 weeks in the green sturgeon fed the 100 mg MeHg/kg diet and at 6 weeks in the white sturgeon fed the same diet (Tables 2a and 2b). After 8 weeks of exposure, mortality reached 71.8% and 100% in the green sturgeon fed the 50 and 100 mg MeHg/kg diets, respectively, while mortality increased to 2.6% and 38.5%, respectively, in the white sturgeon fed the same diets for the same duration. No significant mortality was observed in the white sturgeon fed the 50 mg MeHg/kg diet in this experiment. After 8 weeks of exposure, the mortality in green sturgeon fed the 50 and 100 mg MeHg/kg diets was significantly higher than that in white sturgeon fed the same diets.

A significantly lower growth rate (%BWI/day) was observed in green sturgeon fed the 100 mg MeHg/kg diet for 4 weeks and those fed the 50 mg MeHg/kg diet for 8 weeks when compared to the controls. After 8 weeks of exposure, a significantly lower growth rate was observed in white sturgeon fed the 100 mg Hg/kg diet but not in those fed the 25 or 50 mg MeHg/kg diets. The growth rates in white sturgeon fed 25 or 50 mg MeHg/kg diets were not significantly different. While the growth rate of green sturgeon fed the control diet was higher than that of white sturgeon fed the same diet during these experiments, green sturgeon fed diets with 50 or higher mg MeHg/kg after 4 weeks exhibited more severe growth rate reductions than did white sturgeon at and after 4 weeks (Tables 2a and 2b).

There was no significant effect of dietary MeHg on the hepatosomatic index (HSI) in white sturgeon throughout the 8 week dietary exposure (Tables 2a and 2b). Green sturgeon fed the 100 mg MeHg/kg diet showed a significant decrease in HSI compared to the control at 6 weeks (Tables 2a and 2b). There was a significantly lower HSI in green sturgeon fed diets with 25 or higher mg MeHg/kg after 4 weeks and 50 or higher mg MeHg/kg after 6 weeks than those in white sturgeon fed the same diets (Tables 2a and 2b).

3.2. Whole body proximate composition

Dietary MeHg had no significant effect on whole body moisture, crude protein, lipid, or energy content in either species (Table 3). However, the whole body moisture content in green sturgeon was significantly higher than that in white sturgeon in each treatment group, whereas whole body crude protein, lipid, and energy contents in white sturgeon were significantly higher than those in green sturgeon in each treatment group (Table 3).

3.3. Tissue Hg burden

Whole body Hg burden increased significantly in a dose-dependent manner in both green and white sturgeon, and no significant difference in whole body Hg burden between green and white sturgeon was observed (Fig. 1(A) and (B)). All treatment groups showed continuous increases of whole body Hg burden in both species without reaching a plateau (Fig. 1(A) and (B)).

Table 2a
Growth performances of green and white sturgeon exposed to dietary methylmercury for 2 and 4 wks.

	mg MeHg/kg diet	2 wks		4 wks	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Mortality (%)	0 (control)	0	0	0.0 b	0
	25	0	0	0.0 b	0
	50	0	0	0.0 b	0
	100	0	0	7.6 ± 3.0 a	0
%BWI/day[a]	0 (control)	4.8 ± 0.2 ab	3.3 ± 0.0 c	6.8 ± 0.2 a	4.0 ± 0.1 cd
	25	5.1 ± 0.2 ab	3.8 ± 0.1 c	6.6 ± 0.2 ab	4.0 ± 0.2 c
	50	5.3 ± 0.1 a	3.6 ± 0.1 c	6.1 ± 0.1 b	4.4 ± 0.1 c
	100	4.6 ± 0.0 b	3.3 ± 0.1 c	3.6 ± 0.2 d	3.3 ± 0.0 d
HSI[b]	0 (control)	2.3 ± 0.5 a	3.4 ± 0.3 a	3.2 ± 0.1 abc	3.5 ± 0.3 ab
	25	2.6 ± 0.1 a	3.2 ± 0.2 a	2.8 ± 0.2 bc	3.7 ± 0.1 a
	50	3.0 ± 0.1 a	3.3 ± 0.3 a	2.8 ± 0.1 bc	3.7 ± 0.2 a
	100	2.8 ± 0.2 a	3.2 ± 0.1 a	2.7 ± 0.1 c	3.8 ± 0.1 a

Values represent mean ± SE ($n = 3$) and letters denote statistical groupings among treatments in exposure period ($p < 0.05$).
[a] Percent body weight increase per day (%BWI/day) = 100 × ((final body weight − initial body weight)/(initial body weight))/number of days.
[b] Hepatosomatic index (HSI) = 100 × liver weight/body weight.

Table 2b
Growth performances of green and white sturgeon exposed to dietary methylmercury for 6 and 8 wks.

	mg MeHg/kg diet	6 wks		8 wks	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Mortality (%)	0 (control)	2.1 ± 2.1 c	0.0 c	7.7 ± 4.4 cd	0.0 d
	25	2.1 ± 2.1 c	0.0 c	7.7 ± 0.0 cd	0.0 d
	50	20.8 ± 5.5 bc	0.0 c	71.8 ± 17.5 ab	2.6 ± 2.6 d
	100	85.4 ± 5.5 a	35.4 ± 9.1 b	100 ± 0.0 a	38.5 ± 13.3 bc
%BWI/day[a]	0 (control)	7.5 ± 0.4 ab	4.1 ± 0.2 cd	8.2 ± 0.6 a	4.7 ± 0.2 cd
	25	7.7 ± 1.1 a	5.3 ± 0.2 bc	7.0 ± 0.3 ab	5.7 ± 0.1 bc
	50	5.7 ± 0.2 abc	4.8 ± 0.2 cd	3.3 ± 0.2 de	4.2 ± 0.2 cd
	100	1.4 ± 0.6 e	2.4 ± 0.1 de	NA[c]	1.5 ± 0.2 e
HSI[b]	0 (control)	2.7 ± 0.2 bc	3.1 ± 0.2 ab	2.4 ± 0.2 ab	3.0 ± 0.1 a
	25	2.2 ± 0.1 cd	3.0 ± 0.1 abc	2.0 ± 0.2 b	3.1 ± 0.2 a
	50	2.6 ± 0.2 bc	3.6 ± 0.2 a	2.8 ± 0.3 ab	3.3 ± 0.1 a
	100	1.5 ± 0.2 d	2.9 ± 0.2 abc	NA	2.6 ± 0.3 ab

Values represent mean ± SE ($n = 3$) and letters denote statistical groupings among treatments in exposure period ($p < 0.05$).
[a] See footnote in Table 2a.
[b] See footnote in Table 2a.
[c] NA indicates that data are not available due to mortality.

Table 3
Whole body proximate composition (%) of green and white sturgeon exposed to dietary methylmercury for 4 and 8 wks.

	mg MeHg/kg diet	4 wks		8 wks	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Moisture	0 (control)	82.3 ± 0.4 a	77.7 ± 0.4 b	82.7 ± 0.5 a	77.0 ± 0.3 b
	25	82.8 ± 0.7 a	78.3 ± 0.9 b	81.7 ± 0.2 a	76.5 ± 0.3 b
	50	82.5 ± 0.1 a	77.9 ± 0.3 b	82.3 ± 0.8 a	75.7 ± 0.4 b
	100	83.0 ± 0.2 a	78.7 ± 0.5 b	NA[a]	NA
Crude protein	0 (control)	9.8 ± 0.1 c	11.4 ± 0.1 a	10.5 ± 0.1 bc	12.0 ± 0.1 a
	25	9.6 ± 0.3 c	10.8 ± 0.0 ab	10.7 ± 0.2 b	11.9 ± 0.1 a
	50	9.8 ± 0.2 c	11.3 ± 0.2 a	9.9 ± 0.1 c	12.4 ± 0.2 a
	100	10.1 ± 0.1 bc	11.0 ± 0.3 ab	NA	NA
Crude lipid	0 (control)	3.3 ± 0.6 b	6.6 ± 0.3 a	2.5 ± 0.6 b	7.1 ± 0.2 a
	25	3.3 ± 0.4 b	6.2 ± 0.9 a	3.1 ± 0.2 b	7.7 ± 0.4 a
	50	3.0 ± 0.2 b	6.7 ± 0.2 a	2.1 ± 0.8 b	7.8 ± 0.3 a
	100	2.1 ± 0.4 b	5.8 ± 0.4 a	NA	NA
Crude ash	0 (control)	1.7 ± 0.0 bcd	2.1 ± 0.1 a	1.9 ± 0.1 a	2.1 ± 0.1 a
	25	1.6 ± 0.1 d	1.8 ± 0.1 abcd	1.7 ± 0.1 a	1.9 ± 0.1 a
	50	1.6 ± 0.1 cd	2.0 ± 0.1 ab	2.2 ± 0.0 a	2.1 ± 0.1 a
	100	1.8 ± 0.0 abcd	1.9 ± 0.1 abc	NA	NA
Energy (cal/g)	0 (control)	5424 ± 119 b	6450 ± 27 a	5272 ± 149 b	6535 ± 62 a
	25	5518 ± 107 b	6353 ± 116 a	5437 ± 52 b	6605 ± 87 a
	50	5382 ± 93 b	6489 ± 46 a	4964 ± 215 b	6591 ± 53 a
	100	5135 ± 121 b	6297 ± 85 a	NA	NA

Values represent mean ± SE ($n = 3$) and letters denote statistical groupings among treatments in exposure period ($p < 0.05$). Initial whole body proximate composition (%) of green sturgeon: moisture 83.2 ± 0.6, crude protein 10.5 ± 0.3, lipid 1.8 ± 0.2, ash 1.8 ± 0.0, and energy 5050 ± 36 cal/g and white sturgeon: moisture 80.2 ± 0.8, crude protein 9.9 ± 0.4, lipid 5.3 ± 0.2, ash 1.9 ± 0.1, and energy 6246 ± 27 cal/g.
[a] NA indicates that data are not available due to mortality.

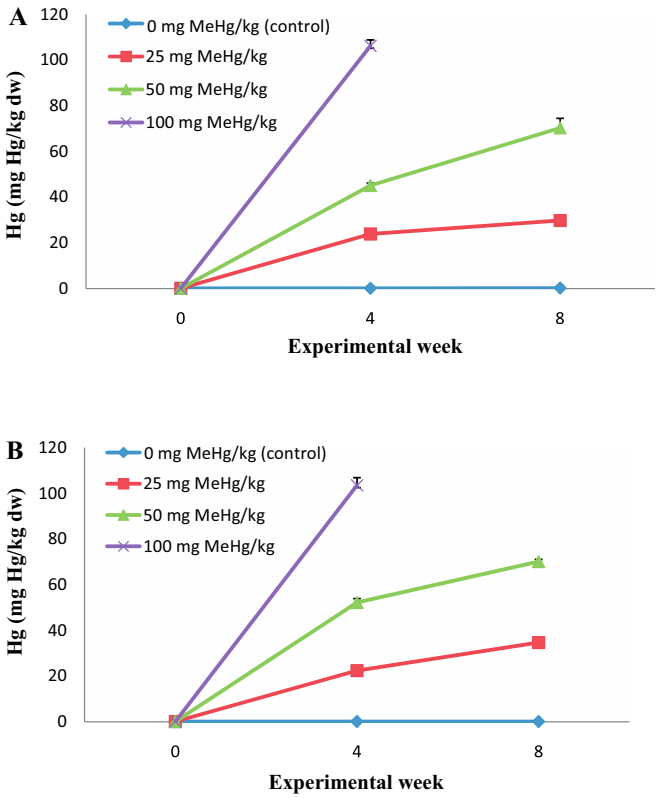

Fig. 1. Whole body Hg burden over time: (A) Whole body Hg burden (mg Hg/kg dry weight) of juvenile green sturgeon (n = 3 tanks, 3 fish per tank) exposed to four dietary MeHg levels (0, 25, 50, and 100 mg MeHg/kg). (B) Whole body Hg burden of juvenile white sturgeon (n = 3 tanks, 3 fish per tank) exposed to four dietary MeHg levels (0, 25, 50, and 100 mg MeHg/kg).

Significant levels of Hg accumulated in all five tissue types of both sturgeon species in a dose-dependent manner. The highest Hg burdens in both sturgeon species fed diets with added MeHg were found in the kidney throughout the experimental periods, followed by liver, white muscle, and gill in green sturgeon and white muscle, gill, and heart in white sturgeon. (Tables 4a and 4b). The differences in tissue Hg distribution patterns between green and white sturgeon were not always significant (Tables 4a and 4b). The Hg burdens of kidneys and gills in white sturgeon fed the 100 mg MeHg/kg diets seemed to reach a plateau after 4 and 6 weeks, respectively (Table 4b). There was a tendency that Hg burdens in the kidney of white sturgeon were higher than that in green sturgeon in the same treatment group (Tables 4a and 4b) and white muscle, gill, and heart tissue followed a similar trend as kidney. However, opposite trend was observed in liver tissue, in which green sturgeon showed higher Hg burden than white sturgeon (Tables 4a and 4b).

4. Discussion

4.1. Mortality and growth performances

Mortality rates in the current study indicate that green sturgeon are more sensitive to dietary MeHg than white sturgeon, rainbow trout (*Onchorhynchus mykiss*) or Sacramento blackfish (*Orthodon microlepidotus*) fed diets with similar MeHg concentrations (Matida et al., 1971; Rodgers and Beamish, 1982; Houck and Cech, 2004). In previous studies with teleosts, Rodgers and Beamish (1982) found no mortality in rainbow trout exposed to 25, 45, or 95 mg MeHg/kg diets for 12 weeks. Similarly, Houck and Cech (2004) reported no significant mortality in Sacramento blackfish fed 22.2 or 55.5 mg MeHg/kg diets over 10 weeks. Significant mortality was observed in Sacramento blackfish fed diets with 55.5 mg MeHg/kg only after 35 weeks. However, juvenile beluga sturgeon (*Huso huso*) have been shown to be more sensitive than green sturgeon to dietary MeHg, in beluga sturgeon 100% mortality was observed when they were fed a 16.22 mg MeHg/kg diet for 6 weeks (Gharaei et al., 2008).

The significant depression in the growth rates of green and white sturgeon fed MeHg-added diets indicated the presence of adverse effects of dietary MeHg in both sturgeon species. The growth rate reduction in green sturgeon occurred earlier, and was more severe than in white sturgeon. Although a comprehensive mechanistic understanding of how MeHg exposure caused the observed decreases in green and white sturgeon growth rate remains unclear, a few hypotheses can be developed based on the current study and knowledge about MeHg toxicology. It is widely accepted that the high affinity of MeHg for the thiol or sulfhydryl groups of proteins underlies the mechanisms of MeHg toxicity (NRC, 2005). Binding of MeHg with sulfhydryl groups results in decreased enzyme activities, altered structural functionality, and problems in transport processes (Zalups and Lash, 1994). Furthermore, methylmercury is known to produce oxidative stress in fish, resulting in damage to various vital components of the biological system, from mitochondrial damages to altered behavior (Berntssen et al., 2003; Alves Costa et al., 2007; Berg et al., 2010).

When fish attempt to repair damage from MeHg toxicity, they confront additional energy demands, reallocating energy expenditures toward repairing the damage. In this experiment, fish were supplied with a fixed ration based on their body weight. Because the amount of available energy was fixed, the reallocation of energy toward metabolic maintenance for damage repair and adaptation most likely resulted in reduced somatic and reproductive growth (Beyers et al., 1999; Houck and Cech, 2004). In addition, this reallocation of energy may have led to behavioral modifications such as reduced swimming activity, which could in turn have led to depressed growth rates (Berntssen et al., 2003; Tashjian et al., 2006).

Depression of growth rates might be further exacerbated by damage to the nervous system, which is the most sensitive endpoint of oral exposure to MeHg (NRC, 2005). Both central and peripheral nervous systems can be damaged. Ataxia, muscle spasms, paralysis, impaired vision, loss of coordination, and hind limb crossing are common neurological signs of MeHg exposure in animals (NRC, 2005). In fish, dietary MeHg can cause structural injury in sensory, inner ear, and brain, suggesting adverse sensory, equilibrium behavior, and motor control (Baatrup and Doving, 1990; Skak and Baatrup, 1993; Berntssen et al., 2003), thus affecting the ability of active feeding. Matida et al. (1971) reported abnormal behavioral and neurological changes in rainbow trout fed dietary MeHg (25 ppm or above), and attributed the disability to feed to neurological impediment including the decline of visual power. Also, zebrafish (*Danio rerio*) after embryonic exposure to dietary MeHg showed reduced feed consumption of live prey (Samson et al., 2001), whereas consumption of pellets was not affected (Fjeld et al., 1998). Previous studies have reported decreased food consumption and aversion to MeHg-added diets (Matida et al., 1971; Rodgers and Beamish, 1982; Gharaei et al., 2008). However, Houck and Cech (2004) did not find any differences in food consumption rates among dietary MeHg treatment groups of Sacramento blackfish. In the current study, we did not notice any apparent abnormal feeding behavior in the first 4 weeks, but sluggish or no reaction to food introduction was observed after 4 weeks in green and white sturgeon fed diets of 50 or 100 mg MeHg/kg. Since we did not measure the feed consumption rate in the present study, a conclusion cannot be made, but decreased food consumption or aversion of food was likely to be a secondary factor in our experimental settings.

Table 4a
Mercury tissue burden (mg Hg/kg dw) in green and white sturgeon exposed to dietary methylmercury for 2 and 4 wks.

Tissue type	mg MeHg/kg diet	2 wks		4 wks	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Gill	0 (control)	0.1 ± 0.0 e	0.2 ± 0.0 e	0.1 ± 0.0 f	0.2 ± 0.0 f
	25	23.8 ± 1.2 d	23.5 ± 2.3 d	34.5 ± 1.8 e	41.5 ± 3.0 e
	50	54.2 ± 1.8 c	47.5 ± 0.8 c	63.6 ± 1.6 d	83.8 ± 2.9 c
	100	111.0 ± 5.3 b	136.9 ± 6.5 a	135.3 ± 4.8 b	155.3 ± 7.1 a
Heart	0 (control)	0.1 ± 0.0 e	0.2 ± 0.0 e	0.1 ± 0.0 f	0.1 ± 0.0 f
	25	17.1 ± 1.7 d	19.8 ± 2.7 d	23.2 ± 0.9 e	33.0 ± 1.8 de
	50	42.6 ± 1.4 c	40.3 ± 0.2 c	47.4 ± 3.1 d	73.5 ± 5.1 c
	100	71.7 ± 6.9 b	101.2 ± 4.4 a	105.0 ± 9.1 b	144.8 ± 4.7 a
Liver	0 (control)	0.1 ± 0.0 e	0.1 ± 0.0 e	0.1 ± 0.0 e	0.1 ± 0.0 e
	25	23.3 ± 2.4 cd	14.9 ± 2.2 d	32.0 ± 1.9 cd	25.1 ± 1.3 de
	50	42.2 ± 1.5 b	32.5 ± 3.1 bc	54.1 ± 0.6 c	53.1 ± 2.0 c
	100	79.5 ± 4.9 a	83.8 ± 1.8 a	125.5 ± 12.4 a	93.7 ± 7.1 b
Kidney	0 (control)	NA[a]	0.3 ± 0.0	0.2 ± 0.0 f	0.2 ± 0.0 f
	25	NA	43.6 ± 4.8	54.0 ± 0.9 de	41.0 ± 4.6 e
	50	NA	83.5 ± 1.4	88.4 ± 2.7 d	141.5 ± 5.5 c
	100	NA	215.2 ± 19.2	206.7 ± 12.6 b	298.0 ± 16.7 a
White muscle	0 (control)	0.1 ± 0.0 e	0.2 ± 0.0 e	0.1 ± 0.0 f	0.2 ± 0.0 f
	25	22.5 ± 0.7 d	17.6 ± 3.8 d	34.6 ± 0.8 e	40.7 ± 0.2 e
	50	45.9 ± 1.7 c	41.0 ± 2.5 c	62.6 ± 0.7 d	80.5 ± 2.5 c
	100	79.2 ± 4.7 b	112.9 ± 4.6 a	135.1 ± 4.6 b	177.5 ± 8.1 a

Values represent mean ± SE ($n = 3$) and letters denote statistical groupings among treatments in exposure period ($p < 0.05$). Initial Hg concentrations in green and white sturgeon were: gill 0.1 ± 0.0 and 0.2 ± 0.0, heart 0.13 ± 0.0 and 0.15 ± 0.0, liver 0.1 ± 0.0 and 0.1 ± 0.0, kidney NA and 0.2 ± 0.0, and white muscle 0.1 ± 0.0 and 0.2 ± 0.0 mg Hg/kg dw, respectively.
[a] NA indicates that data are not available due to mortality.

Further energetic deficiency from inhibition of nutrient absorption may have resulted in the depression of growth rate. Previous studies have shown that MeHg can interfere with gastrointestinal function in fish (Farmanfarmaian and Socci, 1985; Houck and Cech, 2004; Gharaei et al., 2008). Gharaei et al. (2008) reported severe pathological developments in the intestine walls of beluga sturgeon exposed to dietary MeHg. Farmanfarmaian and Socci (1985) also described that MeHg inhibited the absorption of amino acid (L-leucine) in the intestine of toadfish (*Opsanus tau*), resulting from the interactions of the compound with carrier proteins. Houck and Cech (2004) observed more fecal matter in Sacramento blackfish exposed to high-dose of MeHg compared to the control, and they attributed significantly reduced growth to the inhibition of nutrient absorption in the gut by MeHg. In the current study, we similarly observed an increased accumulation of mucous-like fecal matter at the bottom of the tank near the drainage in the highest treatment groups compared to the other groups. Therefore, irritation or inflammation in the gut may have occurred, and the subsequent inhibition of nutrient absorption may have led to a decrease in the growth rate of the sturgeon.

Table 4b
Mercury tissue burden (mg Hg/kg dw) in green and white sturgeon exposed to dietary methylmercury for 6 and 8 wks.

Tissue type	mg MeHg/kg diet	6 wks		8 wks	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Gill	0 (control)	0.2 ± 0.0 f	0.2 ± 0.0 f	0.2 ± 0.0 d	0.2 ± 0.0 d
	25	41.0 ± 2.7 e	57.1 ± 3.0 de	49.2 ± 0.9 c	50.0 ± 2.1 c
	50	74.2 ± 0.6 d	103.5 ± 6.6 c	103.3 ± 5.7 b	115.7 ± 8.6 b
	100	142.1 ± 11.9 b	175.6 ± 1.9 a	NA[a]	165.9 ± 16.1 a
Heart	0 (control)	0.1 ± 0.0 d	0.2 ± 0.1 d	0.1 ± 0.0 d	0.1 ± 0.0 d
	25	25.0 ± 1.2 cd	41.3 ± 1.7 c	27.0 ± 2.4 cd	43.6 ± 2.1 bcd
	50	42.9 ± 2.3 c	84.7 ± 0.1 b	81.1 ± 12.0 bc	96.7 ± 4.4 b
	100	107.6 ± 12.6 b	157.0 ± 11.3 a	NA	185.9 ± 29.8 a
Liver	0 (control)	0.1 ± 0.0 d	0.1 ± 0.0 d	0.1 ± 0.0 d	0.1 ± 0.0 d
	25	42.5 ± 3.5 cd	26.7 ± 2.6 cd	39.5 ± 2.4 bcd	27.9 ± 0.5 cd
	50	70.8 ± 3.1 bc	59.3 ± 4.3 cd	101.8 ± 11.9 b	62.8 ± 5.8 bc
	100	161.7 ± 39.2 a	132.8 ± 5.8 ab	NA	181.6 ± 30.2 a
Kidney	0 (control)	0.2 ± 0.0 d	0.2 ± 0.0 d	0.2 ± 0.0 d	0.3 ± 0.0 d
	25	51.6 ± 5.7 d	39.8 ± 9.1 d	66.4 ± 2.1 cd	111.2 ± 4.5 bc
	50	116.2 ± 2.8 c	125.5 ± 9.1 c	134.6 ± 1.8 bc	162.4 ± 40.0 b
	100	221.8 ± 17.3 b	285.5 ± 22.1 a	NA	267.4 ± 3.7 a
White muscle	0 (control)	0.2 ± 0.0 e	0.2 ± 0.0 e	0.2 ± 0.0 d	0.2 ± 0.0 d
	25	42.5 ± 0.7 de	51.4 ± 1.1 cde	50.8 ± 2.8 c	56.5 ± 0.9 c
	50	83.0 ± 3.6 bcd	103.3 ± 4.3 bc	115.2 ± 0.4 b	104.4 ± 6.8 b
	100	142.6 ± 33.2 b	212.1 ± 8.4 a	NA	231.8 ± 3.9 a

Values represent mean ± SE ($n = 3$) and letters denote statistical groupings among treatments in exposure period ($p < 0.05$).
[a] NA indicates that data are not available due to mortality.

4.2. Proximate composition

Dietary MeHg had no significant effect on whole body proximate composition in either green or white sturgeon. This finding confirmed that the decreases in the growth rate of green and white sturgeon were not due to changes in body composition. However, green sturgeon had lower whole body crude protein, lipid, and energy contents, but higher moisture content, than white sturgeon (Table 3). These findings indicated that green sturgeon have lower energy reserves compared to white sturgeon, which could make the green sturgeon more vulnerable than white sturgeon to MeHg toxicity.

4.3. Tissue Hg burden

Whole body Hg burdens increased in a dose-dependent manner in green and white sturgeon fed diets with added MeHg, and they were increased in a time-dependent manner. Similar levels of whole body Hg accumulated at each MeHg treatment groups for green and white sturgeon (Fig. 1(A) and (B)). The whole body Hg concentrations (5–22 mg Hg/kg wet weight) in green and white sturgeon fed MeHg-added diets for 8 weeks were comparable to ranges of Hg concentrations reported in previous studies (Matida et al., 1971, Rodgers and Beamish, 1982). Our results suggest that whole body Hg burden is a good bioindicator of dietary MeHg levels for both green and white sturgeon.

Similar to McKim et al. (1976) and Niimi and Kissoon (1994), we observed a relatively high muscle Hg burden compared to kidney and liver. Regine et al. (2006) concluded that the relatively high Hg concentration in the muscle tissue is a result of redistribution among internal organs, because muscle tissue ends up receiving most of MeHg assimilated (Ribeyre and Boudou, 1984; Wiener and Spry, 1996; Wiener et al., 2003). The different Hg distribution patterns between green and white sturgeon suggest differences in their biological characteristics to process MeHg.

Several factors may explain the differences in the tissue distribution of Hg between green and white sturgeon. Functional and structural characteristics of each sturgeon species may result in differences in the ability to modulate uptake through the intestine, transport processes in the circulatory systems, depuration mechanisms, and sequestration or storage in tissues for similar exposure conditions (Regine et al., 2006). For example, it was reported that the differences in amino acid concentrations of the digestive fluids between two species of fish could cause differences in the bioavailability of MeHg (Leaner and Mason, 2002). Those characteristics were reflected by the differences in total protein, lipid, moisture contents of whole body (Table 3), growth rates of the control treatment group (Tables 2a and 2b), and different Hg distributions between the two species. Also, green sturgeon are known as a more ocean-dwelling anadromous species compared to the more estuary-dwelling white sturgeon, an ecological characteristic that may have made green sturgeon evolve functionally and structurally differently from white sturgeon. Additionally, histopathological examination of both sturgeon species from this study revealed that kidney and liver were most affected by dietary MeHg, and these two tissues of green sturgeon were more severely affected than those of white sturgeon (J.-W. Lee, unpublished data). These different histological responses suggest that the two sturgeon species may be functionally or structurally different.

In addition to potential molecular or biochemical differences, there was evidence in the current study that green sturgeon have lower energy reserves than white sturgeon. Such evidence included lower whole body protein, lipid, and energy contents and lower HSI in green sturgeon than white sturgeon of similar sizes. When exposed to MeHg toxicity, the lower energy status, reallocation of energy toward metabolic maintenance for Hg-damage repair, and inhibition of nutrient absorption may have forced green sturgeon into a more unfavorable energy state, which resulted in higher growth rate depression. Such an energy shortage and subsequent decrease in locomotive activity may have put the green sturgeon into energy deprivation. The sturgeon in this stage could have passed over the stage of resistance and progressed to death, which is the stage three of the general adaptation syndrome (Selye, 1955).

In summary, mortality and growth rate of green and white sturgeon were adversely affected compared to controls when exposed to 50–100 mg MeHg/kg diets for 8 weeks. The lowest observed effect concentration in both sturgeon species was 50 mg MeHg/kg diet, while no observed effect concentration was 25 mg MeHg/kg considering all endpoints. Therefore, the dietary MeHg threshold at which no toxicity is exerted on juvenile green and white sturgeon is estimated to be between 25 and 50 mg MeHg/kg diet, based on the mortality, and growth rate. From extrapolation of the data in the current study, it is not inconceivable that dietary concentrations of 25 or less mg MeHg/kg can have chronic adverse effects on green and white sturgeon if an extended exposure duration or more sensitive experimental endpoints such as histopathology are applied. Our results also demonstrated that green and white sturgeon accumulated Hg in a dose-dependent manner. However, differences in Hg distributions in the tissues between the two sturgeon species suggested that they have different physiological mechanisms to process ingested MeHg. The increased mortality, decreased growth rate, and lower energy content indicated that juvenile green sturgeon were more susceptible to dietary Hg toxicity than juvenile white sturgeon. Thus, white sturgeon is not an appropriate reference for assessing the stress responses of green sturgeon from exposure to MeHg.

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