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Effect of dietary selenomethionine on growth performance, tissue burden, and histopathology in green and white sturgeon

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ABSTRACT

A comparative examination of potential differences in selenium (Se) sensitivity was conducted on two sturgeon species indigenous to the San Francisco Bay-Delta. Juvenile green (*Acipenser medirostris*), recently given a federally threatened status, and white sturgeon (*Acipenser transmontanus*) were exposed to one of four nominal concentrations of dietary L-selenomethionine (SeMet) (0 (control), 50, 100, or 200 mg SeMet/kg diet) for 8 weeks. Mortality, growth performance, whole body composition, histopathology, and Se burdens of the whole body, liver, kidneys, gills, heart, and white muscle were determined every 2 to 4 weeks. Significant ($p < 0.05$) mortality was observed in green sturgeon fed the highest SeMet diet after 2 weeks, whereas no mortality was observed in white sturgeon. Growth rates were significantly reduced in both species; however, green sturgeon was more adversely affected by the treatment. Dietary SeMet significantly affected whole body composition and most noticeably, in the decline of lipid contents in green sturgeon. Selenium accumulated significantly in all tissues relative to the control groups. After 4 and 8 weeks of exposure, marked abnormalities were observed in the kidneys and liver of both sturgeon species; however, green sturgeon was more susceptible to SeMet than white sturgeon at all dietary SeMet levels. Our results showed that a dietary Se concentration at 19.7 ± 0.6 mg Se/kg, which is in range with the reported Se concentrations of the benthic macro-vertebrate community of the San Francisco Bay, had adverse effects on both sturgeon species. However, the exposure had a more severe pathological effect on green sturgeon, suggesting that when implementing conservation measures, this federally listed threatened species should be monitored and managed independently from white sturgeon.

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1. Introduction

Green (*Acipenser medirostris*) and white sturgeon (*Acipenser transmontanus*) are two sturgeon species native to the San Francisco Bay Delta (SFBD) and both have exceptional biological, commercial, and ecological values (Moyle, 2002). Their populations, however, have been in steady decline since the nineteenth century (Billard and Lecointre, 2001). Recently, green sturgeon was listed as a species of special concern by the State of California and a threatened species by the United States Federal Government (California Natural Diversity Database (CNDDB), 2006). Elevated concentrations of chemical contaminants found in their diets are considered one of the primary causes of their decline (National Marine Fisheries Service, 2006).

Selenium (Se) is a major water contaminant in SFBD. It is an essential micronutrient for all vertebrates (NRC, 2005), as it is a major component of glutathione peroxidase, an enzyme that protects lipid membranes from oxidative damages at the cellular and subcellular levels (Arteel and Sies, 2001). However, at a slightly higher concentration, dietary Se is toxic to many aquatic animals (Lemly, 2002, 1985; Skorupa, 1998; Steward et al., 2004). In SFBD, major Se inputs include waste discharges originating from petrochemical and industrial manufacturing operations. The most significant source, however, is from irrigated agricultural practices on the seleniferous soils of the Central Valley (Lemly, 2004).

Most field surveys on SFBD sturgeon populations have been conducted on white sturgeon due to their larger natural population. Several such reports have noted elevated tissue Se concentrations [Se]s (up to 30 μg/g dry weight (dw) in the liver and 15 μg/g dw in the muscle; Urquhart and Regalado, 1991; Linville et al., 2002) in these animals. Similar tissue Se levels have been reported to cause toxic effects in freshwater and anadromous fish (Lemly, 2002).

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In contrast, very little is known about Se toxicity and tissue burden in green sturgeon. Although the two species are closely related, they exhibit different responses to environmental contaminants. Recent studies have demonstrated a higher sensitivity to dietary methylmercury (MeHg) in green sturgeon compared with white sturgeon (Lee et al., 2011, 2012). Therefore, information with regards to the physiological responses of green sturgeon to environmental contaminants, in general, should not be simply extrapolated from that of white sturgeon. The objective of our current study was to determine and compare the effects of elevated concentrations of dietary L-selenomethionine (SeMet) on the growth performance, tissue burden, and histopathology of juvenile green and white sturgeon.

2. Materials and methods

2.1. Diet preparation

Four isoenergetic and isonitrogenous purified diets were prepared according to Tashjian et al. (2006) and Lee et al. (2011). Different concentrations of L-selenomethionine (Fisher Scientific, Pittsburgh, PA) were added to a basal diet mixture to constitute the four nominal levels of 0 (control), 50, 100, and 200 mg SeMet/kg diet. These SeMet concentrations were chosen to span the range of projected dietary [Se]s in SFBD (Luoma and Presser, 2000) and the selenocompound was chosen as it is the predominant Se form found in natural diets of white sturgeon (Fan et al., 2002). Furthermore, previous studies have indicated that toxic responses in animals fed SeMet were similar to those fed diets containing naturally incorporated Se compounds (Hamilton, 2004).

2.2. Animal acquisition, experimental design, and animal maintenance

The acquisition, maintenance, handling, and sampling of animals were approved by the Campus Animal Care and Use Committee at the University of California, Davis and are as described by Lee et al. (2011). Due to the different spawning and hatching times of the two sturgeon species, the two experiments were conducted consecutively, 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. In brief, 300 juvenile sturgeon (mean weight of 30 ± 2 g) were used in each of the two experiments and they were randomly distributed into twelve 90-L tanks, resulting in 4 dietary groups with 3 replicate tanks per treatment. Daily rations of 3% body weight/day (BW/d) for the first 4 weeks and 2% BW/d for the second 4 weeks (Cui and Hung, 1995) were placed in an automatic feeder (Cui et al., 1996; Hung and Lutes, 1987) which dispensed feed continuously over 24 h. Water temperature, pH, and dissolved oxygen were maintained at 18–19 °C, 7–8, and 7–9 mg/L, respectively. The effluent water was sampled weekly and [Se] was determined by a certified laboratory (BSK Analytical Laboratory, Fresno, CA, using EPA 200.8 method) and ranged from undetectable to 4.2 μg/L.

2.3. Growth performance, tissue sampling, proximate composition and selenium analysis

Fish were batch weighed on a weekly basis and feed rations were adjusted accordingly. Growth performance, tissue sampling, and diet and tissue [Se]s were determined as previously described by Lee et al. (2011) and Huang et al. (2012). For [Se] analysis, each sample was analyzed in triplicates with one replicate spiked with a sodium selenate standard to verify Se recovery. Dolt-4 (National Research Council Canada) was analyzed simultaneously with the experimental samples and the observed concentration (6.89 mg Se/kg dw) was within the certified standard range (7.06 ± 0.48 mg Se/kg dw). The [Se]s determined in the 0, 50, 100, and 200 mg SeMet/kg diet were 2.2 ± 0.2, 19.7 ± 0.6, 40.1 ± 1.5, and 77.7 ± 3.6 mg Se/kg dw, respectively. Whole body samples were lyophilized and pulverized prior to proximate composition and energy content determinations, which were determined according to AOAC, 1984, 1995, respectively.

2.4. Tissue processing and light microscopy procedures

After 4 and 8 weeks of exposure, three fish from each tank were randomly captured and euthanized with an over-dose of tricaine methanesulfonate solution (1 g/L, Argent Chemical Laboratories, Redmond, WA). Gills, heart, liver, trunk kidneys, and skeletal muscle were surgically removed, fixed, sectioned, stained, examined, and photographed according to Lee et al. (2012).

2.5. Statistical analysis

Statistical analyses were conducted using JMP 7.0 statistical software program (SAS Institute, Cary, NC). A two-way analysis of variance with interactions was used to test for significant differences among the four dietary SeMet concentrations and between the two sturgeon species. The Tukey's honestly significant difference test was used for multiple comparisons among dietary SeMet 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 the mean ± standard error unless noted otherwise.

3. Results

3.1. Mortality and growth performance

Significant mortality was observed in green sturgeon fed the 200 mg SeMet/kg diet from week 2 and by week 8, mortality was also seen in the 100 SeMet/kg diet group (Table 1). At the end of the study, green sturgeon exhibited a mortality of 7.7% and 23% at the 100 and 200 mg SeMet/kg diet treatments, respectively. In contrast, no mortality was observed in the white sturgeon.

Growth rates (% BWI/d) were reduced significantly in both species. After 8 weeks, green sturgeon showed depressed growth rates in all the treatment groups, when compared with the control. In contrast, white sturgeon showed depressed growth rates only at the 100 and 200 mg SeMet/kg diet treatment groups. Although growth rate was significantly higher in the control green sturgeon group, compared with that of the white sturgeon, green sturgeon was more sensitive to SeMet than white sturgeon, at all dietary SeMet levels.

Similarly, by week 8, hepatosomatic index (HSI) of green sturgeon exposed to the two upper SeMet treatments was significantly decreased compared with the control. In contrast, dietary SeMet had no significant effect on the HSI in white sturgeon.

3.2. Whole body proximate composition

Significant increases in moisture content were observed in green sturgeon fed the two highest SeMet diets. Similarly, whole body crude protein, lipid and energy contents were also significantly reduced in these treatment groups (Table 2). In white sturgeon, significant increase, compared with the control, was observed in whole body moisture content in the 200 mg SeMet/kg diet group. Significant decreases were observed in lipid contents at the 100 and 200 mg SeMet/kg diet groups. Similar decrease in energy content was also observed at the 200 mg SeMet/kg diet group.

Table 1
Growth performances of green and white sturgeon exposed to different levels of dietary selenomethionine (SeMet) for 2, 4, 6, and 8 wk.

Parameters	mg SeMet/kg diet	2 wk		4 wk		6 wk		8 wk	
		Green	White	Green	White	Green	White	Green	White
Mortality (%)	(0) Control	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
	50	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b
	100	0 b	0 b	0 b	0 b	0 b	0 b	7.7 ± 4.4 b	0 b
	200	5.3 ± 1.3 a	0 b	12.1 ± 1.5 a	0 b	16.7 ± 2.1 a	0 b	23.1 ± 4.4 a	0 b
% BWI/d[a]	(0) Control	4.5 ± 1.8 a	3.0 ± 2.1 cd	11.9 ± 6.1 a	7.1 ± 0.4 b	6.3 ± 15.9 a	3.7 ± 6.5 b	6.6 ± 14.9 a	4.2 ± 14.1 b
	50	3.8 ± 3.9 ab	3.6 ± 0.2 bc	6.8 ± 8.4 bc	7.8 ± 3.6 b	3.1 ± 14.8 bc	3.9 ± 10.5 b	2.6 ± 16.0 c	4.2 ± 22.5 b
	100	2.0 ± 3.2 ef	2.7 ± 1.2 de	3.2 ± 11.1 de	4.6 ± 4.4 cd	1.0 ± 8.7 d	2.5 ± 10.6 c	0.8 ± 4.1 de	2.8 ± 20.6 c
	200	0.7 ± 1.1 g	1.5 ± 3.2 fg	0.8 ± 7.6 f	1.9 ± 3.9 ef	-0.1 ± 3.7 d	0.9 ± 6.8 d	-0.1 ± 4.3 e	1.0 ± 11.0 d
HSI[b]	(0) Control	1.9 ± 0.1 c	3.2 ± 0.2 ab	2.0 ± 0.1 bc	3.5 ± 0.3 a	1.8 ± 0.3 c	3.0 ± 0.2 ab	2.0 ± 0.1 cd	2.6 ± 0.2 bc
	50	2.3 ± 0.2 bc	3.2 ± 0.2 ab	1.9 ± 0.2 bc	3.7 ± 0.2 a	1.4 ± 0.1 c	3.3 ± 0.3 a	1.3 ± 0.0 de	3.6 ± 0.2 a
	100	2.0 ± 0.2 c	3.4 ± 0.1 a	1.8 ± 0.3 bc	2.8 ± 0.2 ab	1.1 ± 0.2 c	3.2 ± 0.4 a	0.8 ± 0.2 e	3.0 ± 0.1 ab
	200	2.0 ± 0.4 c	3.3 ± 0.1 a	1.2 ± 0.1 c	2.7 ± 0.3 ab	0.8 ± 0.0 c	1.9 ± 0.1 bc	0.9 ± 0.1 e	2.2 ± 0.4 bc

Values represent the mean ± SE ($n = 3$), and different letters denote significant differences ($p < 0.05$) among treatments and between species within each exposure periods.
[a] Percent body weight increase per day (%BWI/d) = 100 × (final body weight − initial body weight)/(initial body weight)/number of days. Initial body weight of the sturgeon were 30 ± 2 g (mean ± SE).
[b] Hepatosomatic index (HSI) = 100 × liver weight/body weight.

Table 2
Whole body proximate composition (%) and selenium burden of green and white sturgeon exposed to different levels of dietary selenomethionine for 4 and 8 wk.

Parameters	mg SeMet/kg diet	4 wk		8 wk	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Moisture	(0) Control	82.9 ± 0.7 ab	78.4 ± 0.4 c	82.9 ± 0.5 b	76.7 ± 0.4 d
	50	82.4 ± 0.5 ab	77.1 ± 0.5 c	83.5 ± 0.6 b	77.5 ± 0.4 cd
	100	83.0 ± 0.7 ab	77.8 ± 0.3 c	86.5 ± 0.8 a	77.9 ± 0.1 cd
	200	85.3 ± 1.3 a	79.6 ± 1.0 bc	88.2 ± 0.2 a	79.5 ± 0.5 c
Crude Protein	(0) Control	10.2 ± 0.1 ab	11.5 ± 0.1 a	11.5 ± 0.3 a	11.6 ± 0.3 a
	50	10.6 ± 0.4 ab	11.4 ± 0.3 a	11.0 ± 0.3 a	11.4 ± 0.0 a
	100	10.5 ± 0.4 ab	11.6 ± 0.1 a	9.3 ± 0.5 b	11.7 ± 0.2 a
	200	9.4 ± 0.6 a	11.3 ± 0.4 a	7.8 ± 0.2 b	11.3 ± 0.5 a
Crude Lipid	(0) Control	2.9 ± 0.5 c	6.2 ± 0.3 ab	2.5 ± 0.4 d	7.9 ± 0.3 a
	50	2.1 ± 0.3 cd	7.7 ± 0.3 a	1.3 ± 0.1 de	6.8 ± 0.4 ab
	100	1.5 ± 0.3 cd	6.6 ± 0.3 ab	0.4 ± 0.1 e	6.1 ± 0.2 b
	200	0.7 ± 0.2 d	5.2 ± 0.9 b	0.2 ± 0.0 e	4.5 ± 0.3 c
Energy (kcal/g)	(0) Control	5.4 ± 0.1 b	6.4 ± 0.1 a	5.4 ± 0.1 c	6.6 ± 0.0 a
	50	5.1 ± 0.1 bc	6.7 ± 0.1 a	5.0 ± 0.0 d	6.5 ± 0.1 a
	100	4.9 ± 0.1 cd	6.5 ± 0.1 a	4.6 ± 0.0 e	6.4 ± 0.0 ab
	200	4.6 ± 0.1 d	6.3 ± 0.2 a	4.4 ± 0.1 e	6.1 ± 0.1 b
mg Se/kg dw	(0) Control	6.5 ± 0.9 e	7.3 ± 0.8 e	7.1 ± 0.9 d	5.6 ± 0.3 d
	50	21.7 ± 0.5 c	15.3 ± 1.6 d	22.8 ± 0.9 c	20.1 ± 0.5 c
	100	26.2 ± 1.2 bc	22.5 ± 0.9 c	27.8 ± 1.4 bc	31.8 ± 0.3 b
	200	30.6 ± 0.7 ab	34.3 ± 2.5 a	34.3 ± 0.3 b	47.1 ± 4.3 a

Values represent the mean ± SE ($n = 3$), and different letters denote significant differences ($p < 0.05$) among treatments and species within the exposure period. Initial body composition (%): Moisture 83.0 ± 0.6 and 80.2 ± 0.8, crude protein 10.5 ± 0.3 and 9.9 ± 0.4, lipid 1.8 ± 0.2 and 5.3 ± 0.2, energy (kcal/g) 5.1 ± 0.1 and 6.3 ± 0.1 in green sturgeon and white sturgeon, respectively. Initial whole body Se concentrations in green and white sturgeon were 7.2 ± 0.3 and 4.8 ± 0.5 mg Se/kg dry weight (dw), respectively.

Moisture, lipid, and energy contents of green sturgeon were significantly different from those of white sturgeon at all levels of dietary SeMet. Noticeably, crude protein contents of green sturgeon fed the 100 and 200 mg SeMet/kg diets were significantly lower than those of white sturgeon in the same treatment groups. However, the most significant differences were observed in crude lipid contents between the two species.

3.3. Se burden

Different patterns in whole body Se burden were also observed between the two species (Table 2). White sturgeon accumulated Se in a dose and duration-dependent manner. In contrast, whole body Se in green sturgeon did not increase much after week 4 and there was no obvious dose-dependent Se accumulation. Pattern of Se accumulation among tissues were also different between the two species (Tables 3a and 3b). Selenium levels in the gills and kidneys of green sturgeon showed little increase after week 2 and week 4, respectively. In the white muscle, however, [Se] was found to have increased in a dose dependent manner up to the 100 mg SeMet/kg diet level. Liver [Se] increased continuously throughout the 8 weeks, except in those fed the 200 mg SeMet/kg diet, where [Se] decreased after reaching a concentration asymptote at week 6. Similarly in the heart, [Se] plateaued after reaching a maximum concentration at week 4. In contrast, tissue Se burden of white sturgeon generally increased with increasing exposure duration. In the 200 mg SeMet/kg diet group, the highest Se levels were observed at week 6. The highest tissue Se levels in green sturgeon were observed in the liver, whereas the highest Se levels in white sturgeon were seen in the kidneys.

3.4. Histopathological alteration

Histological examination showed progressions of marked lesions in the kidneys and liver of both species after each sampling period (Tables 4 and 5 and Figs. 1 and 2). Mild histological changes

Table 3a
Selenium tissue burden (mg Se/kg dw) in green and white sturgeon exposed to different levels of dietary selenomethionine (SeMet) for 2 and 4 wk.

Tissues	mg SeMet/kg diet	2 wk		4 wk	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Kidney	(0) Control	ND	8.0 ± 1.5 a	10.7 ± 0.4 d	9.1 ± 1.6 d
	50	ND	18.1 ± 0.8 b	34.2 ± 0.3 bc	29.5 ± 1.0 cd
	100	ND	36.0 ± 0.5 c	53.1 ± 10.4 ab	50.7 ± 6.0 abc
	200	ND	54.3 ± 2.4 d	50.7 ± 1.8 abc	71.2 ± 2.2 a
Liver	(0) Control	6.1 ± 1.1 c	5.8 ± 1.4 c	4.2 ± 0.4 d	4.9 ± 0.7 d
	50	14.0 ± 1.3 bc	12.4 ± 1.2 bc	23.3 ± 3.2 bc	14.2 ± 1.1 cd
	100	25.6 ± 2.9 ab	16.1 ± 0.7 bc	31.4 ± 6.9 bc	20.9 ± 1.1 bcd
	200	39.5 ± 7.1 a	23.3 ± 0.8 b	65.6 ± 6.1 a	32.3 ± 1.2 b
Gill	(0) Control	6.6 ± 0.2 f	8.0 ± 1.6 ef	6.7 ± 0.2 e	7.0 ± 1.5 e
	50	23.2 ± 1.2 cde	17.5 ± 1.9 def	26.6 ± 0.2 d	25.3 ± 0.3 d
	100	32.5 ± 2.0 bcd	34.7 ± 2.6 bc	35.5 ± 0.6 cb	40.7 ± 3.6 c
	200	44.4 ± 4.4 ab	51.6 ± 6.5 a	48.1 ± 1.5 b	60.3 ± 2.7 a
Heart	(0) Control	9.1 ± 0.7 d	7.6 ± 1.0 d	7.6 ± 0.7 f	6.7 ± 1.1 f
	50	22.7 ± 1.3 bc	17.0 ± 0.4 cd	25.2 ± 0.8 e	26.8 ± 1.0 de
	100	28.8 ± 0.8 b	29.7 ± 1.5 b	34.9 ± 1.2 cd	42.0 ± 1.1 bc
	200	43.1 ± 3.8 a	42.0 ± 4.0 a	45.6 ± 1.2 ab	53.1 ± 4.2 a
White muscle	(0) Control	8.4 ± 0.6 e	11.7 ± 0.8 de	9.0 ± 0.2 d	9.5 ± 0.3 d
	50	20.4 ± 0.1 bc	17.6 ± 0.7 cd	25.6 ± 0.1 c	25.3 ± 0.3 c
	100	26.9 ± 0.3 ab	25.9 ± 1.3 a b	32.2 ± 1.2 b	29.5 ± 0.5 bc
	200	32.2 ± 3.6 a	33.2 ± 0.8 a	34.7 ± 2.6 ab	40.4 ± 2.3 a

Values represent mean ± SE ($n = 3$) and different letters denote significant differences ($p < 0.05$) among treatments and species within each exposure period and tissue type. Initial Se concentrations (mg Se/kg dw) in green and white sturgeon were as follows: gill 6.6 ± 0.1 and 4.8 ± 0.5; heart 6.3 ± 0.6 and 6.5 ± 1.3; liver 7.0 ± 1.0 and 3.1 ± 0.3; kidney ND and 6.3 ± 0.9; and white muscle 7.6 ± 0.2 and 8.94 ± 0.2, respectively. ND: not determined and dw: dry weight.

Table 3b
Selenium tissue burden (mg Se/kg dw) in green and white sturgeon exposed to different levels of dietary selenomethionine (SeMet) for 6 and 8 wk.

Tissue	mg SeMet/kg diet	6 wk		8 wk	
		Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Kidney	(0) Control	9.1 ± 0.7 e	8.2 ± 1.3 e	8.5 ± 0.6 d	9.3 ± 0.9 d
	50	35.1 ± 1.0 cd	28.1 ± 1.8 de	33.3 ± 0.6 c	33.5 ± 0.3 c
	100	60.1 ± 12.6 b	54.8 ± 1.2 bc	53.0 ± 9.8 bc	54.5 ± 3.6 bc
	200	44.4 ± 1.3 bcd	127.6 ± 8.1 a	58.1 ± 2.6 b	93.3 ± 5.6 a
Liver	(0) Control	5.1 ± 0.8 c	4.7 ± 0.5 c	6.1 ± 0.3 c	4.2 ± 0.1 c
	50	32.6 ± 1.1 bc	16.0 ± 1.1 bc	34.4 ± 3.5 bc	28.0 ± 10.4 bc
	100	78.4 ± 10.5 a	26.6 ± 1.5 bc	86.1 ± 9.7 a	30.1 ± 1.0 bc
	200	106.5 ± 14.5 a	46.8 ± 2.6 b	87.0 ± 11.2 a	56.3 ± 2.6 ab
Gill	(0) Control	6.0 ± 0.2 e	6.6 ± 1.0 e	5.4 ± 0.3 e	7.6 ± 0.7 e
	50	29.3 ± 1.4 cd	20.7 ± 5.3 d	29.5 ± 0.6 d	26.7 ± 3.3 d
	100	34.1 ± 3.5 bc	45.2 ± 2.1 b	39.3 ± 0.6 c	46.4 ± 0.7 bc
	200	45.1 ± 1.6 b	60.6 ± 0.3 a	51.6 ± 1.6 b	69.5 ± 2.4 a
Heart	(0) Control	5.5 ± 0.5 d	6.4 ± 0.3 cd	5.3 ± 0.3 f	8.8 ± 0.5 f
	50	23.6 ± 0.9 bcd	26.0 ± 1.1 bcd	24.4 ± 0.3 e	28.9 ± 0.4 de
	100	29.5 ± 1.6 bc	41.0 ± 4.2 ab	33.0 ± 1.4 cd	45.8 ± 1.7 b
	200	35.5 ± 3.3 ab	58.2 ± 12.4 a	35.6 ± 2.1 c	70.6 ± 2.1 a
White muscle	(0) Control	10.0 ± 0.5 e	9.5 ± 0.3 e	8.4 ± 0.4 e	9.2 ± 0.7 e
	50	29.7 ± 1.0 cd	25.2 ± 0.6 d	31.1 ± 0.3 cd	27.0 ± 1.1 d
	100	31.4 ± 0.7 bcd	37.4 ± 3.4 ab	37.0 ± 0.3 bc	41.3 ± 0.6 b
	200	35.7 ± 1.9 abc	42.6 ± 1.1 a	36.8 ± 1.2 bc	57.9 ± 1.2 a

Note: See Table 3a.

were noted in the skeletal and heart muscles (results not shown). However, no prominent histological changes were observed in the gills of either species at all times.

3.4.1. Trunk kidney

After exposure to dietary SeMet, the kidneys of both sturgeon species exhibited marked histological changes, compared with the controls. These changes included increased tubular epithelium degeneration (TED), renal corpuscular disintegration (CD), and interstitial tissue degeneration (ITD) (Table 4 and Fig. 1c–h). Tubular epithelium degeneration was mainly characterized by hydropic degeneration, pyknosis, and cell necrosis (Fig. 1c, e, and h). Characterization of CD included the collapse of glomerular capillary loop, hypertrophy of mesangial cells, thickening of Bowman's capsule layers, and collapse or enlargement of Bowman's space (Fig. 1c, e, and h). Lastly, ITD was identified by necrotic area and loss of tissue (Fig. 1g and h). In general, pathological alterations of the kidneys were proportional to the dose and duration of SeMet exposure.

Compared with week 4, both species displayed a more severe and higher frequency of TED, CD, and ITD in the kidneys at week 8 (Table 4). The most serious damage occurred in the tubular epithelium as TED for both species (Table 4 and Fig. 1). Although some of the lesion scores were the same between the two species, green sturgeon exhibited more severe kidney pathology in all of the SeMet treatment groups (Table 4).

Table 4
Kidney histopathological alterations of green and white sturgeon exposed to a graded levels of dietary selenomethionine (SeMet) for 4 and 8 wk.

	mg SeMet/kg diet							
	Control		50		100		200	
	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Histopathology at 4 wk								
TED	0	0	++	+	+++	++	+++	+++
CD	0	0	0	0	+	++	++	++
ITD	0	0	0	0	+	+	+	+
Histopathology at 8 wk								
TED	0	0	+++	++	+++	+++	+++	+++
CD	0	0	++	+	++	++	++	+++
ITD	0	0	0	0	++	+	+++	++

Lesion severity scoring: 0 = absent or rarely observed, + = mild (affected less than 10%), ++ = moderate (affected greater than 10% but less than 50%), and +++ = severe (affected greater than 50%). TED, tubular epithelium degeneration; CD, renal corpuscular disintegration; ITD, interstitial tissue degeneration. *N* = 9.

Table 5
Liver histopathological alternations of green and white sturgeon exposed to a graded levels of dietary selenomethionine (SeMet) for 4 and 8 wk.

	mg SeMet/kg diet							
	Control		50		100		200	
	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon	Green sturgeon	White sturgeon
Histopathology at 4 wk								
GD	0	0	+	0	++	+	+++	+
VD	0	0	++	0	++	+	+++	+++
Histopathology at 8 wk								
GD	0	0	++	0	+++	+	+++	++
VD	0	0	++	+	++	++	+++	++

Lesion severity scoring: 0 = absent or rarely observed, + = mild (affected less than 10%), ++ = moderate (affected greater than 10% but less than 50%), +++ = severe (affected greater than 50%). GD, glycogen depletion; VD, vacuolar degeneration including single cell necrosis. *N* = 9.

3.4.2. Liver

After 4 weeks, the livers of both species showed marked histological alterations, including glycogen depletion (GD) and vacuolar degeneration (VD) (Table 5 and Fig. 2). In both species, the progression of the aforementioned alterations was generally proportional to the dose and duration of exposure. However, between the two species, the green sturgeon livers exhibited more severe GD and VD (Table 5 and Fig. 2c–h).

4. Discussion

4.1. Mortality and growth depression

In the current study, green sturgeon exhibited significant higher mortalities at the highest SeMet treatment, which is equivalent to a 78 mg Se/kg diet. However, similar to Tashjian et al. (2006), who reported a mean survival rate of 99 ± 4% in white sturgeon exposed to diets containing up to 191 mg Se/kg for an 8 week period, no significant mortalities were observed among white sturgeon in the current study. Although green sturgeon appeared to be more sensitive to dietary Se, the mortality rate was still lower than that of other fish species. A mean mortality of 37.5% was observed in Chinook salmon parr (*Oncorhynchus tshawytscha*) after an 8.6-week exposure to a 35.4 mg Se/kg diet (Hamilton et al., 1990). Arshad et al. (2011) reported a mean mortality of 25% in juveniles of beluga sturgeon (*Huso huso*) exposed to dietary Se at levels between 1.26 and 20.26 mg/kg for 8 weeks.

Compared with white sturgeon, the significantly higher mortality in the green sturgeon may be a consequence of their higher initial growth. Deng et al. (2002) reported faster growth rates in juvenile green sturgeon when compared with white sturgeon of similar age. As faster growth rate reflects a higher energy demand, the green sturgeon may have been in an overall lower energy state, especially since the diets were provided in a fixed daily ration and adjusted on a weekly basis. The low HSI, whole body lipid and energy content, and glycogen storage in the hepatocytes are all indicative of the low energy reserves in the green sturgeon.

Compared with other fish species from similar studies, green sturgeon exhibited a more severe growth rate depression. At 8 weeks, green sturgeon fed the 50 and 100 mg SeMet/kg diets (equivalent to 19.7 and 40.1 mg Se/kg diet, respectively) had their average growth rates reduced to 39% and 12% of that of the controls, respectively. In contrast, growth rates of Chinook salmon parr were only reduced to 77.9% and 37.3%, when given an 18.2 and 35.4 mg Se/kg diet in the form of SeMet for 60 days (Hamilton et al., 1990). Interestingly, juvenile beluga sturgeon fed a 20.26 mg Se/kg diet, in the form of SeMet, for 8 weeks, exhibited increased growth rates (Arshad et al., 2011). The observed reduction in growth among the green sturgeon may be a combined physiological response to: (1) the higher energy demand during the rapid initial growth phase and (2) energy relocation/adaptation to chronic Se toxicity. Thus, reduced growth is likely a physiological tradeoff for achieving a comparatively lower Se-induced mortality, as to what were seen in the aforementioned studies.

4.2. Whole body proximate composition

Proximate analysis is a good indicator of the overall physiological condition of a fish (Ali et al., 2005). In the present study, changes in proximate composition, most notably the significant decreases in protein, lipid, and energy contents, indicated that both species were experiencing physiological stress induced by dietary SeMet exposure. However, the treatment effect was more severe on green sturgeon, as the white sturgeon seemed to be in an overall better

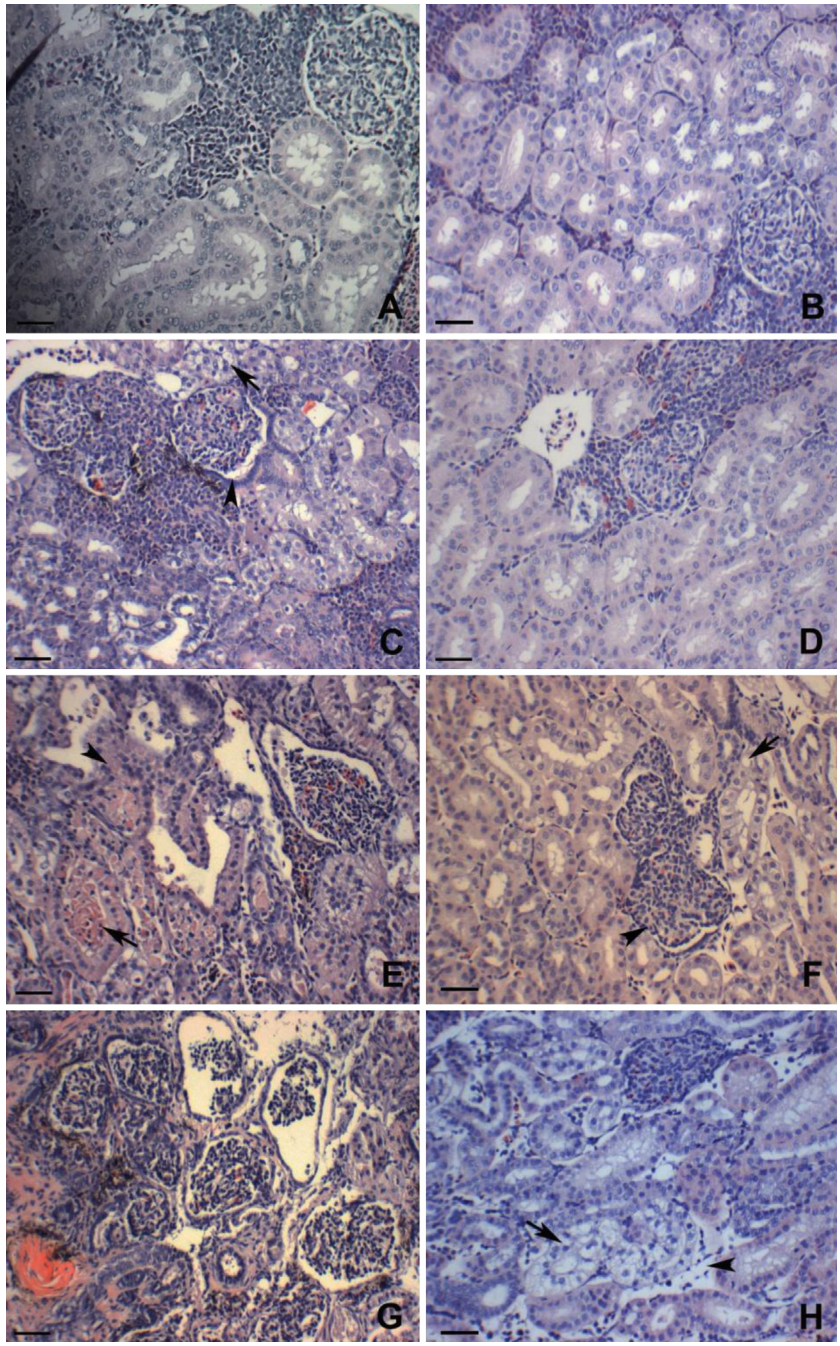

Fig. 1. The trunk kidney of *Acipenser medirostris* (left) and *A. transmontanus* (right) stained with hematoxylin/eosin: (A) and (B) kidneys of individuals from the control groups. (C) Kidney of *A. medirostris* exposed to 50 mg SeMet/kg diet for 8 weeks showing hydropic degeneration (arrow) and renal corpuscular disorganization (arrow head). (D) Kidney of *A. transmontanus* exposed to 50 mg SeMet/kg diet for 8 weeks showing slightly enlarged tubular cells. (E) Kidney of *A. medirostris* exposed to 100 mg SeMet/kg diet for 8 weeks showing severe tubular cell death (arrow head) and tubular inclusion (arrow), and renal corpuscular disintegration. (F) Kidney of *A. transmontanus* exposed to 100 mg SeMet/kg diet for 8 weeks showing moderate tubular hydropic degeneration (arrow) and collapse of glomerular capillary (arrow head). (G) Kidneys of *A. medirostris* exposed to 200 mg SeMet/kg diet for 8 weeks showing necrotic areas. (H) Kidney of *A. transmontanus* exposed to 200 mg SeMet/kg diet for 8 weeks showing severe tubular epithelium degeneration including hydropic degeneration (arrow) and loss of interstitial tissues (arrow head). All scale bars = 50 μm.

physiological condition, given the higher lipid and energy contents of their control group.

Chemical contaminants have been shown to induce physiological stress in teleosts. Beyers et al. (1999) reported that largemouth bass (*Micropterus salmoides*) utilize energy relocation to compensate for the additional energetic costs associated with toxic exposures. As described in Selye's general adaption syndrome (Selye, 1955), the authors observed a two stage energy relocation in the largemouth bass: first, an allocation of resources from somatic and reproductive growth, which have little effect on the overall energy status of the animal; and second, the allocation of body reserves such as somatic lipid and protein, which can put the animal in an energy-deficient state. Furthermore, when the stressor persists for sufficient length of time and magnitude, the animal would inevitably enter exhaustion, the third and final stage of stress adaption (Selye, 1955).

At the two highest dietary SeMet levels, physiological assessments indicated that green sturgeon were in the exhaustion stage. Characteristics such as glycogen depletion of hepatocytes, increased histopathology in the liver and kidneys, depressed

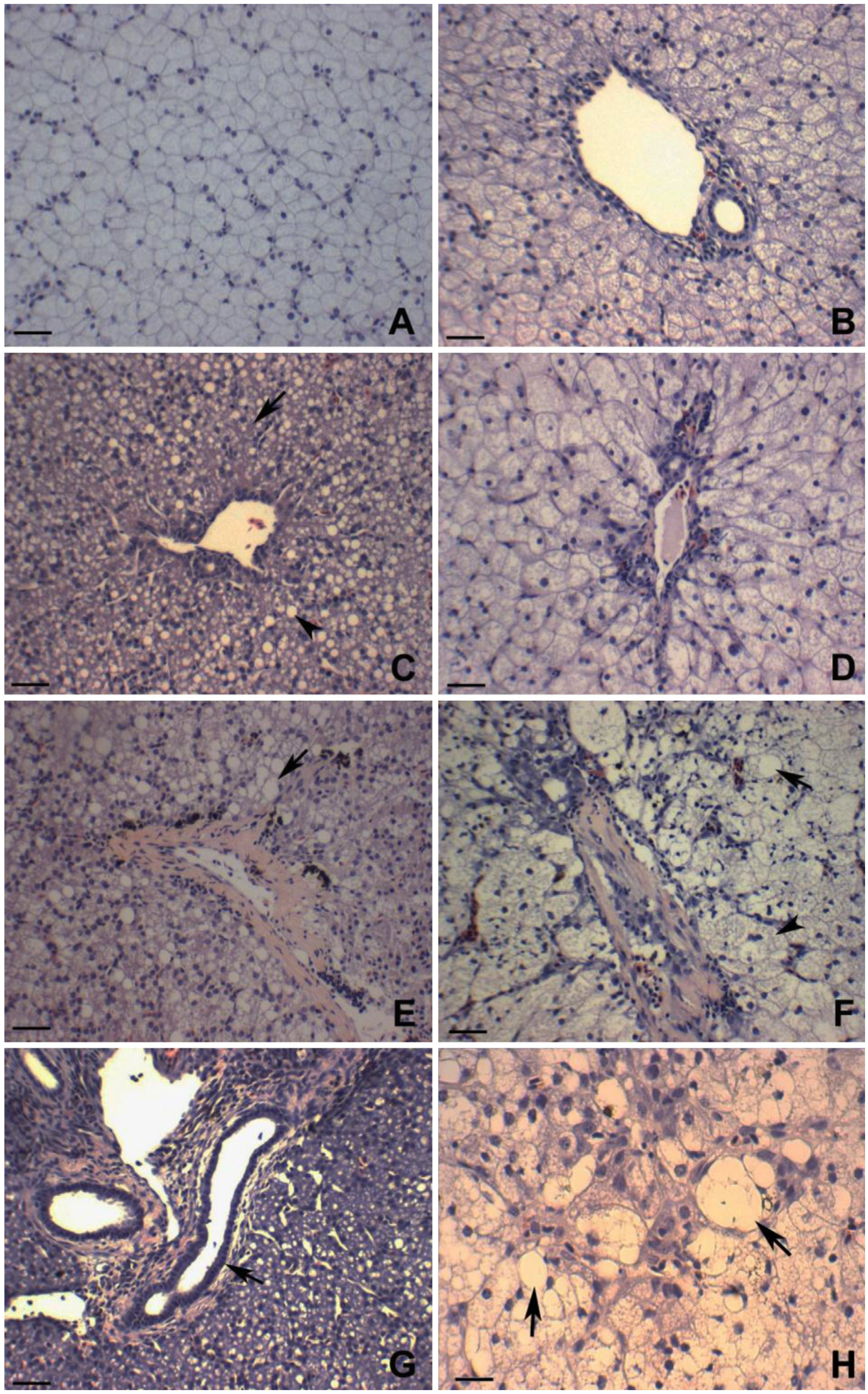

Fig. 2. The liver of *Acipenser medirostris* (left) and *A. transmontanus* (right) stained with hematoxylin/eosin: (A) and (B): Livers of individuals from control groups. (C) Liver of *A. medirostris* exposed to 50 mg SeMet/kg diet for 8 weeks showing moderate glycogen depletion (GD) (arrow) and vacuolar degeneration (VD) (arrow head). (D) Liver of *A. transmontanus* exposed to 50 mg SeMet/kg diet for 8 weeks showing slightly enlarged hepatocytes with unclear cell membranes. (E) Liver of *A. medirostris* exposed to 100 mg SeMet/kg diet for 8 weeks showing severe VD (arrow). (F) Liver of *A. transmontanus* exposed to 100 mg SeMet/kg diet for 8 weeks showing VD (arrow) and necrotic cells (arrow head). (G) Liver of *A. medirostris* exposed to 200 mg SeMet/kg diet for 8 weeks showing severe GD,VD, and dilation of bile duct (arrow). (H) Liver of *A. transmontanus* exposed to 200 mg SeMet/kg diet for 8 weeks showing VD (arrows). All scale bars = 50 μm, except the scale bar at (*H*) = 25 μm.

growth rates, and increased mortality were observed in these animals. By week 4, the animals have entered the second stage of energy mobilization, as seen in the largemouth bass (Beyers et al., 1999), in which more body constituents, such as lipid and protein, were utilized to meet the additional energy cost associated with Se toxicity. In comparison, the white sturgeon seemed to remain in the resistance state, given that their protein levels remained unaffected by SeMet. Furthermore, their body lipid contents were also significantly higher. The species difference, again, may be due to the rapid initial growth phase of juvenile green sturgeon, in which the associated high metabolic cost led to a comparatively more energetically vulnerable status. The exact cause of the observed reduction in body lipid is unknown, however, as multiple factors such as reduced food intake due to unpalatability of SeMet enriched feed and increased energy demand for Se detoxification may be involved.

4.3. Se burden

In general, whole body Se burden increased with dietary Se level and exposure duration; however, by week 4, the extent of Se bioaccumulation have slowed down in green sturgeon (Table 2). Avoidance to Se-contaminated food has been reported in waterfowl (Heinz and Sanderson, 1990) and teleost species (Hilton et al., 1980). Unpalatability of foods containing high concentrations of Se was suggested as a factor leading to food avoidances observed in birds and fish species (Ogle and Knight, 1989). In the current study, decreased feeding was noted in green sturgeon, from week 4 onwards, in the two highest SeMet groups. However, similar observation was not made during the first 4 weeks of exposure. Other Se toxicity mechanisms, such as musculature dysfunction may have also contributed to decreased food consumption in this study. Substitution of methionine (Met) by SeMet, in the disulfide bond of muscle actin filament, can generate radical oxygen species (ROS) leading to mechanical malfunction of the organ (Dalle-Donne et al., 2001; Palace et al., 2004). Histological changes observed in the white muscle of both sturgeon species (results not shown) in this study support possible musculature malfunctioning. Similarly, SeMet substitution may have also occurred in the heart muscle, as indicated by mild histological changes in the heart tissues (results not shown), and may have compromised the cardiovascular function of these animals. Thus, it is more likely that the decrease in feeding observed in the latter 4 weeks, the starvation effect, was a secondary effect of Se toxicity, such as locomotor dysfunction, rather than unpalability relating to the high SeMet content.

The highest Se burden was observed in the green sturgeon livers, at 6 weeks. However, the high liver [Se] may be a combined effect of decreased HSI (half the size of that of the controls), negative growth rates (%BWI/d), and decreased food consumption. Lee et al. (2011) reported similar findings in juvenile green sturgeon fed various levels of dietary MeHg for 8 weeks. Regardless of the mechanisms leading to the high organ Se accumulation, extensive liver damages were observed and likely were important factors contributing to the significant growth rate decline observed in green sturgeon and their subsequent high mortality.

Urine is the primary excretion route for Se. Although mammals can also excrete excess Se via feces and exhalation, the urine plays a quantitatively greater role in whole body Se homeostasis (Ellis et al., 1997; Ivancic and Weiss, 2001). Similarly, urine is also the primary Se excretory pathway in white sturgeon (Huang et al., 2012). In the current study, the significantly higher Se burden observed in white sturgeon kidneys suggests a more active depuration of Se (compounds) relatively to that of green sturgeon. However, study on both species using oral intubation and intravenous injection methods demonstrated similar SeMet assimilation and metabolism among the sturgeon (Silas S.O. Hung, University of California at Davis, unpublished date). Thus, the Se concentration plateau observed in the green sturgeon kidneys at post week 4 was likely due to decreased feed consumption rather than decreased urinary Se.

4.4. The trunk kidney

Histological changes in the kidneys in fish have been previously studied and are reliable and sensitive biomarkers for a wide variety of chemical exposures, including SeMet (Sorensen et al., 1984; Handy and Penrice, 1993; Thophon et al., 2003). In this study, the kidneys of sturgeon exposed to SeMet showed marked abnormalities, including TED, CD, and ITD. Collapsed glomerular capillaries, mesangial cell hypertrophy, abnormally abundant matrixes, thickened Bowman's capsule layers, and collapsed or enlarged Bowman's space were also observed in the renal corpuscles of SeMet exposed sturgeon. Similar damages were reported in green sunfish (*Lepomis cyanellus*) from Se-contaminated lakes (Sorensen et al., 1982, 1984) and in striped bass (*Morone saxatilis*) fed Se-contaminated live feed (Coughlan and Velte, 1989).

The extensive kidney lesions seen in both sturgeon species can be attributed to the primary excretory role of Se compounds (Suzuki, 2005) of the organ. The significant increase in green sturgeon whole body moisture content may be indicative of a compromised osmoregulation, given the extensive damages seen in the tubular epithelium. Other factors such as deprivation of energy and higher damages in the livers may also have contributed to the severe kidney lesions observed in green sturgeon, despite having a comparatively lower kidney Se burden compared to the white sturgeon.

4.5. Liver

The livers of both sturgeon species exposed to SeMet treatments exhibited adverse histological changes such as GD and VD, and are consistent with the histopathological lesions reported by Tashjian et al. (2006). Swollen hepatocytes and vacuolation were also reported in livers of green sunfish exposed to Se-elevated water (Sorensen et al., 1982, 1984). Reproductive failure was noted in the study and marked population decline followed suit. In the current study, the extent of the liver lesions may have also affected organ function, as seen in the decreased hepatocyte glycogen storage. Such will have an effect on glycogenesis and glycolysis, leading to an interruption of energy metabolism, as supported by the decrease in whole body energy content, growth, and the higher mortality in green sturgeon.

In addition, GD and single cell necrosis were also reported in Sacramento splittail (*Pogonichthys macrolepidotus*) fed SeMet-supplemented diets (Teh et al., 2004). Significant glycogen depletion was suggested as a result of increased liver glycogenolysis due to the excessive energy demand for repairing SeMet-induced damage and/or reduced food intake (Teh et al., 2004). Significant GD seen in the current study is thought to be an adaptation by the sturgeon to meet the high energy demand when exposed to high levels of dietary SeMet.

Laboratory studies reported hepatic oxidative stress in mallard ducks (*Anas platyrhynchos*) exposed to dietary SeMet (Hoffman, 2002). Increased dietary Se elevated plasma and hepatic GSH peroxidase activities, followed by an increased ratio of oxidized to reduced glutathione (GSSG:GSH) and hepatic lipid peroxidation. The oxidative effects were associated with teratogenesis, reduced growth, diminished immune function, and histopathological lesions. Similarly, oxidative stress is believed to have induced the histological changes observed in the current study. Deposition of dark pigments, which is thought as indicators of oxidative stress in northern pike (*Esox Lucius*; Drevnick et al., 2008), were also observed in the livers of sturgeon in the highest SeMet treatment groups and were found to be especially numerous in green sturgeon. Thus, liver damage, likely a result of Se-induced oxidative stress, may be a major factor contributing the higher susceptibility to Se toxicity by the green sturgeon in this study.

It is possible that the comparatively faster initial growth rates of juvenile green sturgeon have resulted in their energetically vulnerable states. As growth requires an increase in protein synthesis, green sturgeon may have experienced a higher frequency of Met substitution by SeMet in their functional proteins. Consequently, normal physiological functions may have been compromised by an increase in non-functional proteins, as well as the associated oxidative stress. The high energetic demands of their initial growth phase may have also compromised the species' ability to repair damages induced by Se Toxicity, leading to the stunted growth and higher mortality observed during the latter part of exposure trial.

5. Summary

The objective of this study was to compare the effects of high Se diets in the juvenile stage of two sturgeon species native to SFBD. Effects on growth parameters and histopathological alterations clearly indicated that green sturgeon is more sensitive to Se-laden diets compared with white sturgeon. Furthermore, the low SeMet diet (19.7 ± 0.6 mg Se/kg dw), which caused severe adverse effects in green sturgeon, is similarly to that of the levels found in SFBD benthic macro-invertebrates, which are a major dietary component of young sturgeon. As such, our results suggest that juvenile green sturgeon is more sensitive to Se toxicity and should be monitored and managed separately from white sturgeon when developing conservation measures to protect this threatened SFBD population segment from Se exposure.

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