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Observations of Bluegills Fed Selenium-Contaminated *Hexagenia* Nymphs Collected from Belews Lake, North Carolina

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Belews Lake, a 1563-ha reservoir in the Piedmont region of North Carolina, was impounded in the early 1970's to provide cooling water for a 2240 Mw coal-fired electric generating station. Investigations conducted by the North Carolina Wildlife Resources Commission and Duke Power Company (Harrell et al. 1978; Van Horn 1979) indicated that prior to 1976, Belews Lake supported a fishery which was typical of most reservoirs in the area. However, cove rotenone sampling in 1976 and 1977 yielded extremely low numbers of young-of-the-year fish, especially game species, at all sample locations except for a hydrologically remote uplake area fed by one of two principal tributaries to Belews Lake. Following elemental analyses of fish tissue samples and extensive water quality studies conducted by Weiss and Anderson (1978) and Duke Power Company, the large scale reproductive failure was attributed to elevated selenium concentrations resulting from discharges to the lake from a coal ash settling pond proximal to the power plant (Cumbie and Van Horn 1978).

Hatchery-raised bluegill (*Lepomis macrochirus*) fingerlings stocked in several net-isolated Belews Lake coves during 1979 experienced a rapid uptake of coal ash-associated trace elements, principally selenium, in skeletal muscle and visceral tissues (Duke Power Company, unpublished data). Generally, higher selenium concentrations and evidences of acute toxicity, including exophthalmos and extreme abdominal distention, were observed only among fish having unrestricted access to sediments and benthos. Among that group of bluegills, considerable mortality occurred within six days of stocking. Bluegills which were caged above the sediments in the same coves, and presumably had less opportunity to feed on benthic organisms, concentrated selenium to a lesser degree. Evidence of acute selenium toxicity was not observed in the latter group of fish.

Those observations, in addition to information provided by inspection of fish stomach contents and tissue elemental analyses of fish and benthic macroinvetebrates, strongly implicate predation on contaminated benthos as an important pathway for selenium uptake and resulting toxicity to fish in Belews Lake. However,

direct experimental evidence relating levels of dietary selenium to toxic effects among warm water fish species is sparse. The present study was intended to document the occurrence of selenium toxicity in bluegills fed to satiation a diet consisting of a common benthic food organism found in Belews Lake.

MATERIALS AND METHODS

Bluegills were electroshocked from Lake Norman, North Carolina, a nearby hydroelectric impoundment, on 2 September 1982 and acclimated to laboratory well water at 21 °C over a one-week period. During acclimation the bluegills were fed a combination of brine shrimp (*Artemia salina*) and mealworms *ad libitum*. At the end of acclimation, eight bluegills of similar length ($\bar{X}$=60.5 mm; S.D.=2.7 mm) were removed from the holding tank and anesthetized in a 290 mg/L solution of tricaine methanesulfonate (MS-222) at 21 °C for one minute. The length and wet weight of each bluegill were determined, and the fish were randomly distributed among eight test enclosures. The bluegills were allowed to recover from the anesthetic for one day before initiating the feeding experiment.

Burrowing mayfly nymphs (*Hexagenia limbata*) were collected during July 1982 from a shoreline area 0.4 km east of the ash settling pond sluice water return to Belews Lake. Sediment samples were obtained using sweep nets and washed on site with lake water. Mayfly nymphs were removed with forceps, placed in a container of lake water, and transported 145 km to our laboratory. The nymphs were then placed in 50-mL polystyrene vials, covered with 5 to 10 mL demineralized water, sealed, and frozen. Because an adequate source of uncontaminated mayfly nymphs was not readily available, a laboratory culture of mealworms (*Tenebrio molitor* larvae) was selected to provide a low-selenium diet for feeding control bluegills. Four replicate composite subsamples of both high- and low-selenium diets were analyzed for wet weight selenium concentrations by neutron activation analysis at North Carolina State University, Raleigh, North Carolina. Previous examination of tissue concentrations of several coal ash-related trace elements (antimony, arsenic, chromium, selenium, titanium, vanadium, and zinc) had revealed that only selenium was elevated among Belews Lake mayfly nymphs relative to mayfly nymphs collected from waters near our laboratory (Duke Power Company, unpublished data).

The eight bluegills were isolated among paired chambers, separated by polyvinyl chloride (PVC)-coated wire mesh, in four 56.8-L glass aquaria. The aquaria, which were fitted with PVC standpipes and drains, were immersed in a 21 °C water bath, and provided with a continuous flow (~30 L/h) of well water and continuous aeration.

For the duration of the experiment, all fish were fed to satiation twice daily at intervals spaced at least six hours apart. Bluegills 1, 2, 3, and 4 served as controls, and were fed finely chopped mealworms, while bluegills 5, 6, 7, and 8 were fed freshly

thawed and chopped Belews Lake mayfly nymphs. Feeding during each session was individualized for each bluegill, and pre-weighed food portions were provided until repeatedly refused. Uneaten food was retrieved from the tank bottom, blotted to remove excess moisture, and weighed, thereby providing a determination of the total wet weight of food consumed. Any behavioral or physiological anomalies were recorded at each feeding session. Aquaria were siphoned clean and water temperatures were recorded daily.

Mortalities that occurred prior to the last experimental feeding session were removed and skeletal muscle and liver samples were dissected for neutron activation analysis of selenium at North Carolina State University. At the termination of the experiment, surviving fish were placed in polyethylene bags containing approximately 3 L of aquarium water topped with pure oxygen, and shipped live via air freight to the Department of Pharmacology and Toxicology, University of Texas, Austin, Texas, for histological examination.

The live bluegills were received at the University of Texas approximately five hours after being shipped. All fish were sacrificed by severing the spinal cord. Liver samples were dissected for subsequent neutron activation analysis of selenium at the University of Texas Nuclear Reactor Teaching Laboratory. Skeletal muscle samples were returned for analysis of selenium at North Carolina State University. Sections were prepared for optical microscopic examination of liver, kidneys, heart, spleen, stomach, intestines, gills, and skeletal muscle. Preparation consisted of fixing tissue sections in 10% neutral buffered formalin, parafin embedding, and staining with hematoxylin and eosin. Methanol-fixed, Giemsa-stained blood smears were also prepared for optical examination.

All selenium tissue concentrations were determined by neutron activation analysis, either at North Carolina State University, or the University of Texas. At North Carolina State University, samples and selenium standards obtained from the National Bureau of Standards and the International Atomic Energy Agency were irradiated for 12 h in a 1-Mw Pulstar Nuclear Reactor at a neutron flux of 1.5×10^{13} n/cm^2/s. Following a 10-d monitored decay, 1500-s counts were made on 24 and 25% large volume lithium drifted germanium detectors coupled to a computerized gamma acquisition and data processing system. Analytical methods used at the University of Texas for neutron activation analysis were described previously (Sorensen and Bauer 1983).

Linear regression lines were determined for ingestion of selenium and tissue concentrations among the eight bluegills. Correlation coefficients were determined at the $p \leq 0.05$ and $p \leq 0.01$ levels of significance. Also, skeletal muscle selenium concentrations were compared among mealworm and mayfly-fed fish using a t-test at a 0.05 level of significance.

RESULTS AND DISCUSSION

Three of the four bluegills provided the 13.6 µg Se/g mayfly diet died within the 44-d exposure period, while all control fish appeared healthy throughout the feeding experiment. Mortalities among the mayfly-fed fish occurred on the 17th, 35th, and 44th day of feeding (Table 1).

Total quantities of food consumed by bluegills fed low- and high-selenium diets were comparable at the end of the 44-d feeding regime, although dietary selenium exposures among the two groups differed by a considerable margin. Mean daily selenium ingestion for Fish 5 through 8 (mayfly-fed) ranged from 1.6 to 1.8 µg Se. Mean daily intake among Fish 1 through 4 (mealworm-fed) remained well below 0.1 µg Se.

Gross physiological, as well as behavioral, indications of selenosis became evident in all four bluegills provided the 13.6 µg Se/g mayfly diet between 13 and 20 d of dietary exposure. No abnormal conditions were observed among the four mealworm-fed bluegills throughout the entire 44-d feeding schedule. Directly observable physiological anomalies noted in each mayfly-fed fish, such as abdominal distention and exophthalmos, were generally associated with a rapid onset of edema. Similar observations have been described previously in bluegills exposed to toxic levels of selenium (Adams 1976; Duke Power Company, unpublished data). These effects were generally manifested over a period of two to four days following the first indications of toxicity. Associated with these physiological disruptions was a gradual loss of equilibrium. Fish stricken with severe edematous swelling exhibited a strong buoyant tendency. When approached for feeding or observation, those fish would expend considerable effort attempting, with limited success, to maintain a position in the lower part of the water column; the location most preferred by the control bluegills.

Another behavioral change was consistently observed within 96 h of the physiological manifestations mentioned above. Affected fish, all of which had previously demonstrated a voracious appetite for the selenium-laden mayfly nymphs, reduced their food intake, until all food was rejected. Food avoidance was previously observed among rainbow trout (*Salmo gairdneri*) fed a pelletized diet high in selenium (13 µg Se/g dry wt.) supplemented as sodium selenite (Hilton et al. 1980). This response is common to other organisms, including mammals, which are fed diets containing toxic concentrations of selenium (National Academy of Sciences 1976). Each bluegill that died did so following a period of food avoidance which ranged from 4 to 11 d. Two experimental fish fed the high-selenium diet experienced a distinct remission of observable signs of selenosis, both physiological and behavioral, following a prolonged period during which all food was refused. Among the other two mayfly-fed fish, food avoidance apparently

Table 1. Selenium ingestion and tissue selenium concentrations (wet wt.) in bluegills (*Lepomis macrochirus*) fed to satiation for 44 consecutive days. Percent analytical error is given for results of neutron activation analysis, when available

Bluegill no.	Initial wet wt. (g)	Total days fed	Total selenium ingested (µg)	Selenium ingested per g of initial bluegill wet wt. (µg)	Final tissue selenium concentration (µg Se/g wet wt.)	
					skeletal muscle	liver
1	3.3	44	3.2	0.96	1.972 ± 5%	-
2	2.3	44	2.7	1.2	1.813 ± 5%	-
3	3.2	44	1.7	0.52	2.057 ± 5%	5.[b]
4	2.7	44	2.6	0.98	1.811 ± 5%	7.[b]
5	3.1	44	70.7	22.8	7.503 ± 3%	69.[b]
6	2.1	44[a]	75.0	35.7	7.936 ± 3%	86.[b]
7	2.4	17[a]	27.2	11.3	5.097 ± 3%	8.466 ± 3%
8	3.1	35[a]	62.4	20.1	5.438 ± 3%	15.079 ± 1%

[a]Mortality occurred on the day indicated

[b]Values reported from neutron activation analysis at the University of Texas. All other tissue selenium concentrations were determined by neutron activation analysis at North Carolina State University

occurred too late or for too short a duration before resumption of feeding, so that detoxification or elimination processes did not produce any obvious remission of selenosis-related conditions before mortality occurred. Even in the instances of selenosis remission the bluegills eventually resumed eating the selenium-laden diet, and repeated the pattern of toxicosis.

Dietary selenium had a profound effect on the amount of selenium assimilated in both skeletal muscle and liver tissues of the bluegills. Selenium concentrations were significantly ($\alpha = 0.05$) greater in skeletal muscle of the mayfly-fed than in mealworm-fed bluegill skeletal muscle. A strong correlation ($r=0.96$; $p \leq 0.01$) was found between the amount of selenium ingested by the eight individual bluegills and their respective skeletal muscle selenium concentrations. Selenium concentrations of about 2 µg/g wet weight in skeletal muscle samples taken from the four control bluegill were slightly above concentrations reported previously for *Lepomis* spp. obtained from nearby uncontaminated waters (Cumbie and Van Horn 1978; Duke Power Company, unpublished data). The slightly elevated selenium concentrations among those fish could have resulted from either an indeterminable exposure to selenium prior to collection of the fish, or more likely, from direct bioconcentration from the 0.61 µg Se/g mealworm diet. These slightly increased selenium burdens among the control fish caused no adverse effects that could be determined.

Selenium concentrations in liver tissues were also significantly correlated ($r=0.87$; $p \leq 0.05$) with dietary intake of selenium. Analysis of liver samples of all mayfly-fed and two of the mealworm-fed bluegills indicated a propensity for selenium accumulation, as determined from the slopes of the regression lines, more than 12X that of skeletal muscle over the 44-d exposure.

Optical microscopic examination of the bluegills fed Belews Lake mayfly nymphs revealed several characteristic degenerative conditions observed in the tissues of other *Lepomis* specimens collected from selenium impacted environments (Sorensen and Bauer 1983; Sorensen et al. 1982; Sorensen et al. 1984). In general, histopathologic effects in the present study were closely associated with the vascular system, as evidenced by changes in the blood, as well as in tissues closely associated with detoxification and elimination processes.

Examination of erythrocytes of Fish 5 revealed that a considerable number of cells were abnormally shaped (poikilocytic), and had larger than normal nuclei, compared to those of a control bluegill (Figure 1). Additionally, increased numbers of nuclear shadows (structures associated with parts of old or fragile cells) were observed in the blood of Fish 5. Similar observations have been reported for redear sunfish (*L. microlophus*) collected from selenium-contaminated areas (Sorensen and Bauer 1983).

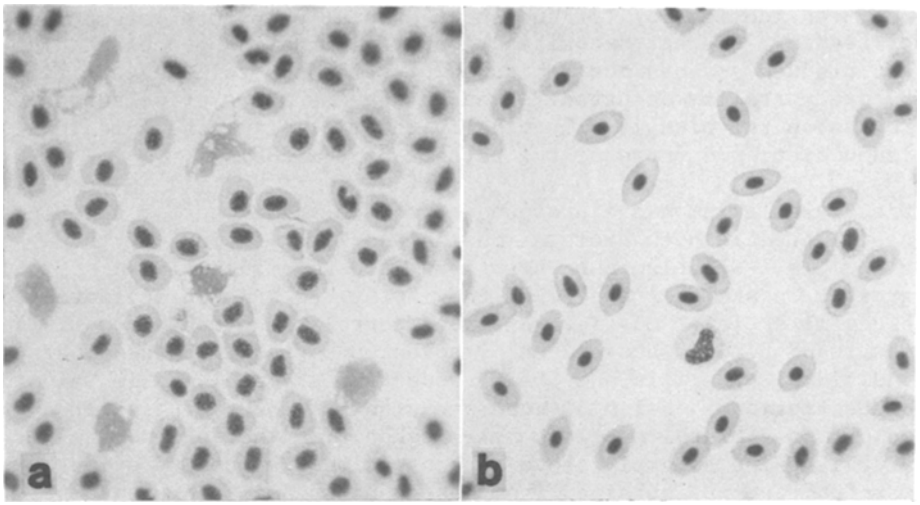

Figure 1. (a) Erythrocytes from Fish 5. Nuclear shadows (irregular contours) and abnormally shaped erythrocytes with larger than normal nuclei are numerous. Giemsa, 695X (b) Normal elliptical erythrocytes with oval nuclei from Fish 4. A neutrophil occurs slightly below the center of the field. Giemsa, 695X

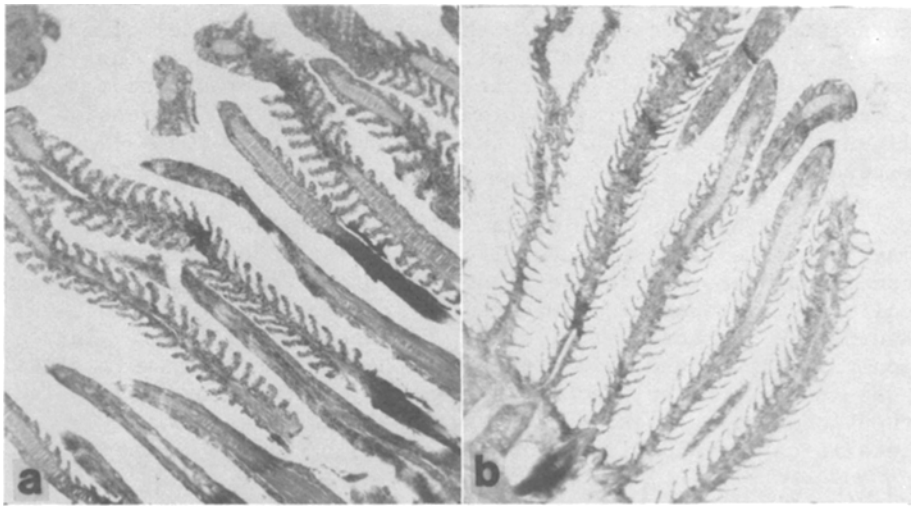

Figure 2. (a) Portion of a gill arch from Fish 5 showing several filaments with lamellae of considerably greater diameter than normally observed in fish of this genus. Hematoxylin and eosin, 70X (b) Portion of a gill arch from Fish 2 showing the normal, somewhat spiney appearance of typical lamellae. Hematoxylin and eosin, 70X

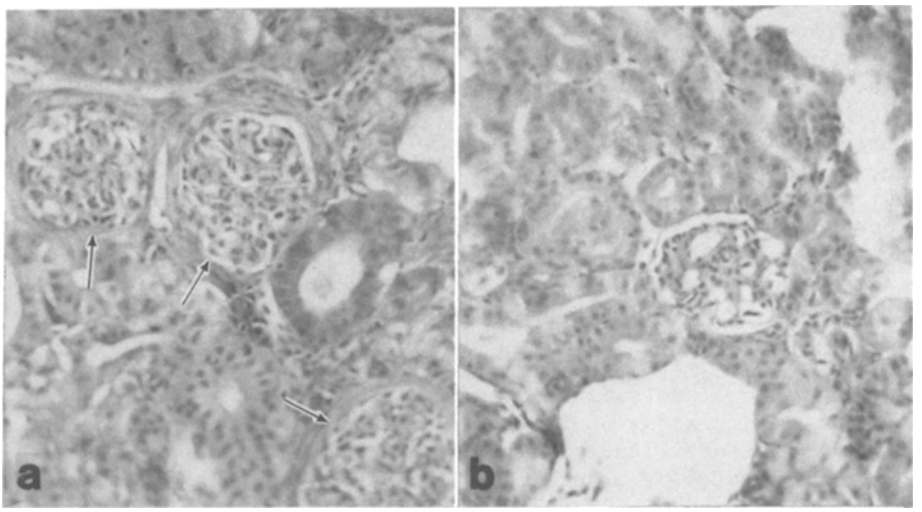

Figure 3. (a) Renal tissue from Fish 5 showing proliferative glomerulonephritis and periglomerular fibrosis in each of the three glomeruli indicated (arrows). Hematoxylin and eosin, 280X (b) Normal renal tissue from Fish 3 showing the absence of proliferative glomerulonephritis and of periglomerular fibrosis in the glomerulus at the center. Hematoxylin and eosin, 280X

Compared to the corresponding structures in the control fish, individual lamellae on the gill filaments of a bluegill fed Belews Lake mayfly larvae appeared considerably more swollen (Figure 2). Similar changes were observed in other *Lepomis* spp. collected from selenium contaminated lakes (Sorensen et al. 1982), although it was not determined if the condition was due to water-borne or dietary exposure. Results of this feeding experiment indicated that the swelling can be elicited entirely as a result of dietary selenium, as opposed to water-borne selenium (which was less than 2 µg/L in the well water supply). However, the relative contribution of dietary or water-borne selenium concentrations in producing this lesion in selenium-impacted habitats is unknown.

Central necrosis and extensive hepatocyte degeneration were observed in the parenchymal cells of the liver from Fish 5. Similar conditions were observed (Sorensen et al. 1982) in both green sunfish (*L. cyanellus*) and redear sunfish obtained from selenium-contaminated lakes. Degeneration of parenchymal hepatoexcytes was possibly a result of the high concentrations of selenium found in liver tissues, as well as cytotoxicity induced by kidney failure, and resulting uremia. Parenchymal hepatocytes appeared normal in the livers of the mealworm-fed bluegills.

Kidney tissues were markedly affected in bluegills exposed to a high level of dietary selenium (Figure 3). Proliferative

glomerulonephritis, a condition characterized by increased numbers of mesangial cells and matrix within the glomeruli, was evident in Fish 5. Among vertebrates, this condition is believed to result in permeability changes within the renal corpuscle, possibly leading to loss of blood (uremia) and amino acids (amino-aciduria) to the urine (Sorensen et al. 1982). Additional damage to the nephrons of the selenium-exposed bluegills was documented in the form of periglomerular fibrosis, and tubular vacuolation. Similar observations have been reported in other selenium-exposed *Lepomis* specimens (Sorensen et al. 1982). Nephrons of the control bluegills appeared normal.

Concentrations of selenium commonly found in benthic macro-invertebrates in areas of Belews Lake impacted by coal ash pond effluents are sufficiently high to cause selenosis when ingested at satiation rates by bluegills. Symptoms previously documented during Belews Lake field studies, including exophthalmia, edema, and abdominal distention were observed in laboratory-fed fish after 13 to 20 d of feeding at 21 ^{o}C. Additional observations consistent with selenium toxicity included food avoidance and loss of equilibrium. Selenium concentrations in both liver and skeletal muscle tissues were correlated with the degree of dietary exposure. Histopathological evidence indicated that the primary focus of the toxicant was on tissues commonly associated with detoxification (liver) and elimination (kidney, gills, and blood) of toxic substances. These effects were observed in lieu of any other significant source of selenium, either water- or sediment-borne, although the possibility that other undetermined contaminants may have contributed to the observed toxic effects cannot be discounted. The findings support the contention that a benthic dietary pathway contributes significantly to the present failure of the fishery of Belews Lake.

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