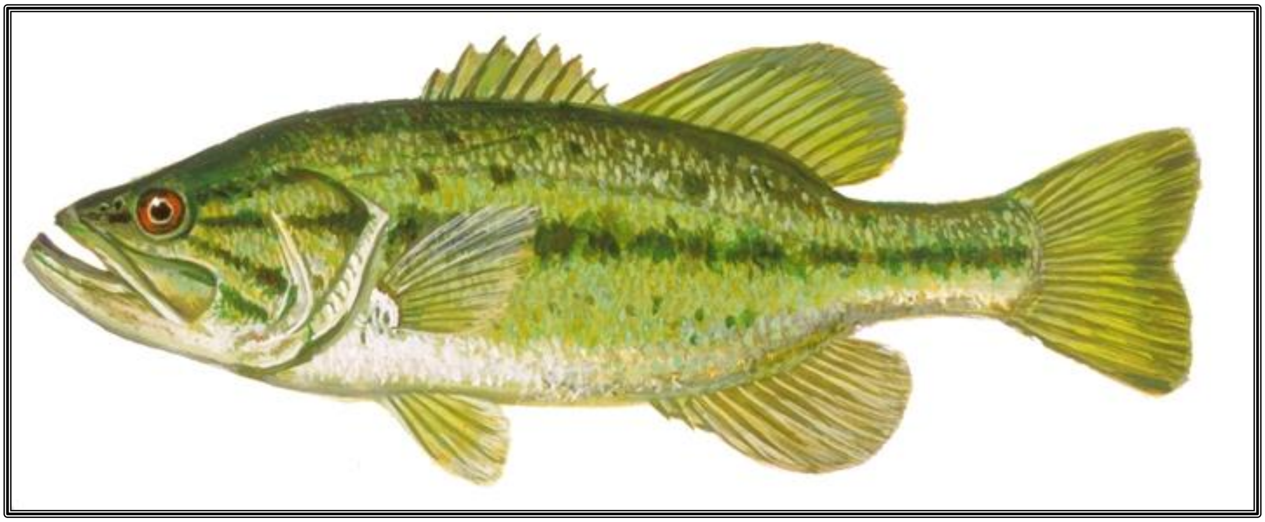
Selenium-Induced Developmental Effects Among Fishes in Select West Virginia Waters



West Virginia Department of Environmental Protection

January, 2010

Summary

In respect to the USEPA's draft whole fish tissue body burden criterion for selenium—7.91 mg/kg dry weight (USEPA, 2004), potentially revised to 11.1 mg/kg dry weight (USEPA, 2008)—the West Virginia Department of Environmental Protection (WVDEP) has studied selenium bioaccumulation among fishes residing in the State's lakes and streams since 2005. Additionally, due to concern regarding fish population health at locations subjected to elevated selenium inputs, particularly during the more sensitive developmental life stages of fishes (e.g. yolk-sac larvae), the WVDEP has collected and examined bluegill sunfish, *Lepomis macrochirus*, larvae (ichthyoplankton) from selected waterbodies since 2007. Also, in 2009, WVDEP began acquiring data about selenium concentrations within fish eggs, which is often used as a predictor of larval deformity rates (Lemly, 1997; Holm *et al*., 2005; Muscatello *et al*., 2006). Certain developmental deformities may also be observed among individuals surviving to later life stages (Nagano *et al*., 2007); consequently, WVDEP has conducted deformity surveys of adult fishes in selenium enriched waters as well as at reference locations since 2008.

Larval deformity rates were variable throughout the study duration but were nonetheless associated with waterborne selenium exposure. Reference locations produced age-based larval bluegill subsamples (24-168 hours) with deformity rates between 0% and 1.27%; whereas, locations with elevated seleniferous inputs exhibited bluegill ichthyoplankton deformity rates ranging from 0% to 47.56% in certain developmental stages (10-312 hours). However, these evaluations were not indicative of overall reproductive success or population sustainability, which must be determined via more detailed studies. Independent confirmation of selenium-induced larval deformities among bluegill populations sampled in 2008 was sought via collaboration with Dr. Diana Papoulias, Fish Research Biologist, United States Geological Survey (USGS), Columbia, MO, who verified the presence of developmental deformities. Maximum deformity rates among certain aged bluegill subsamples as determined through these evaluations were 19.28%, representing specimens collected from selenium-enriched waters. Concentrations of selenium within fish eggs also varied according to study location and ranged from <0.8 mg/kg dry weight among bluegill eggs at the control site to 64.62 mg/kg dry weight among largemouth bass, *Micropterus salmoides*, eggs collected from selenium-enriched waters. Searches for more mature, yet developmentally-deformed fishes revealed increased deformity rates (14%) among largemouth bass residing in the Upper Mud River Reservoir (UMRR), Lincoln County, West Virginia, as compared to deformity rates among largemouth bass found in the reference location (0%), Plum Orchard Lake (POL), Fayette County, West Virginia.

Background

Among oviparous vertebrates, the teleost fish egg and embryo contained within represents a life stage which is susceptible to the effects of bioaccumulative toxicants through the processes of maternal transfer (Russell *et al*., 1999). Specifically, potentially detrimental substances like selenium are accrued via maternal diet and stored in the liver. During oogenesis, or egg formation, these substances are transferred from the maternal liver, through the circulatory system, into the oocyte (egg cell) as yolk (Evans &Claiborne [eds.], 2006). Developmental deformities may then be incurred as a result of natural growth phenomena with the embryo utilizing contaminants (e.g. elevated selenium concentrations) instead of typical organic and inorganic constituents (Rolland, 2000; Strmac *et al*., 2002; Zambonino *et al*., 2005). Selenium, being a chemical analog of sulfur, is especially detrimental when substituting sulfur in protein synthesis as the structural confirmation is compromised. Selenium-induced developmental deformities can be manifest in several ways, but most often result in embryonic spinal curvatures, anomalous yolk-sac absorption, and malformed craniofacial features (Lemly, 1997; Holm *et al*., 2005). Such teratogenic conditions may be inherently lethal, or may cause a reduction in the overall fitness of the organism, resulting in delayed mortality (Gillespie & Baumann, 1986; Pyron & Beitinger, 1989).

The USEPA's draft whole fish tissue body burden criterion for selenium is based primarily on experimentally observed impacts to sunfish—specifically, bluegill sunfish (USEPA, 2004; USEPA, 2008). Therefore, the reproductive activities of bluegill sunfish were the focus of this study. More specifically, a comparison of reproductive success, measured through developmental deformities observed among yolk-sac larvae, of bluegill sunfish populations inhabiting selenium-enriched waters versus those residing in reference locations was the major objective of this investigation. However, important contributions from the scientific literature have recently been made via correlations between selenium concentrations in maternal eggs and subsequent deformity rates among developing larvae (Lemly, 1997; Holm *et al*., 2005; Muscatello *et al*., 2006). Accordingly, collections of fish eggs from gravid females of various species, inhabiting environs with diverse selenium inputs, were made by WVDEP personnel. Subsequent analyses of the selenium tissue concentrations within these eggs allowed for comparisons to egg tissue concentration thresholds for selenium suggested in the literature, albeit among different fish species, and were also an experimental objective. Furthermore, it was surmised that establishing such selenium egg tissue concentrations among certain species would provide valuable information regarding poorly understood bioaccumulation mechanisms (e.g. selenium accrual by stream fishes in fluvial environs). Lastly, developmental deformities persisting among more mature individuals are often observed at locales experiencing selenium-induced toxicity to the fish community (Lemly, 1993; Hamilton *et al*., $2005_{\mathbf{b}}$). In May, 2007, WVDEP and United States Fish and Wildlife Service (USFWS) biologists first observed a potentially developmentally-deformed largemouth bass at a selenium-enriched impoundment—the Upper Mud River Reservoir. Since that time, several deformity surveys have been conducted at this location and at reference sites in order to determine the persistence of such developmental deformities among the resident mature fish populations.

Study Locations

The primary study locations, which correspond to potential anthropogenic selenium inputs (Appendix A), were located within similar coal-bearing geologic strata and are warmwater fisheries managed by the West Virginia Division of Natural Resources—the Upper Mud River Reservoir and Plum Orchard Lake (Figure 1). These locations were sites of both fertilized bluegill egg collection and stripped (maternal) egg collection for selenium tissue concentration determination. The Upper Mud River Reservoir is a 306-acre impoundment located in Lincoln County, WV, which is formed by the dammed confluence of the main forks of the Mud River. It was first inundated in 1995. Upstream of this reservoir, significant surface mining activities have introduced elevated levels of selenium into the watershed. A previous WVDEP study identified Upper Mud River Reservoir as having the highest measured selenium bioaccumulation factor (BAF) among lentic waters evaluated in the State; higher BAFs are often more common in lentic environs. In contrast, Plum Orchard Lake is a 202-acre impoundment located in Fayette County, WV. Impounded in 1962, this reservoir has minimal upstream disturbance and low waterborne selenium concentrations. Subsequent to collection of fertilized bluegill eggs from these locations, specimens (eggs) were transported to WVDEP headquarters, Kanawha County, WV, for the ichthyoplankton rearing portion of the study.

Additional study locations were selected in regard to stream flow and selenium inputs, both of which may affect bioaccumulation in resident fluvial fish communities. At these locations, gravid (egg-bearing) females of certain fish species were stripped of their eggs, which were later analyzed for selenium content. Ash Fork, Nicholas County, WV, and Davis Creek, Kanawha County, WV, served as stream reference locations, containing fish communities exposed to low waterborne selenium levels. Seng Creek, Boone County, WV, and Mud River, Lincoln County, WV, were selected as stream fisheries with elevated waterborne selenium exposure due to upstream mining activities (Figure 1; Appendix A).

Figure 1. Study site locations in West Virginia.

Experimental Methods

ICHTHYOPLANKTON

The spawning activities of bluegill sunfish populations at the Upper Mud River Reservoir and Plum Orchard Lake were monitored throughout the spring seasons of the study years, 2007-2009. Observations of water temperature, male nest-building activities, and female staging were made while nest site locations were identified and geo-referenced. Once initiated, spawning phenomena were observed and photographed as well as restricted from extraneous human disturbance (e.g. at the Upper Mud River Reservoir swimming beach). Materials used by male bluegills to line nest depressions were inspected periodically until affixed eggs were found. Relict Asian clam, *Corbicula fluminea*, shells were primarily used by bluegills as nesting substrate in the Upper Mud River Reservoir; whereas, small cobblestones, twigs, and pine cones lined bluegill nests in Plum Orchard Lake.

Fertilized eggs, affixed to nesting substrate, were collected by hand and immediately transferred into 48-quart coolers filled with water from the spawning areas. The coolers were mechanically aerated by two battery-powered aerators and standard aquarium air stones. After securing the inundated eggs/nest substrate along the aerated cooler bottom, the specimens were transported to WVDEP headquarters, where 10-gallon aquaria were prearranged to accept the substrate-affixed eggs and collected water. Eggs and nest substrate were carefully and evenly distributed by hand along the aquarium floors, following the addition of the aerated water. Three 10-gallon aerated aquariums were used to contain the egg specimens from each study location, totaling six aquariums for entire ichthyoplankton rearing experiment (Figure 2). Eggs and nest materials not placed in the experimental aquaria were retained in the aerated coolers throughout the remainder of the study. Physicochemical parameters and other water quality constituents (e.g. dissolved metals and nutrients) were measured in the incubation medium (aquarium water) periodically throughout the study to assure habitat stability. Additionally, aquarium waters during the 2009 study year were treated with dilute (0.05%) bleach, sodium hypochlorite, and formaldehyde (1%) in order to curtail growth of the egg fungus, *Saprolegnia sp.*

Developing bluegill larvae were removed from the aquaria beginning 10-24 hours after reaching a post-hatch tail-free life stage. Larvae were extracted in fractions of the total number observed at 2-day intervals. Removal of the larvae was accomplished through a large diameter bulb pipette and larvae were transferred, along with a small volume of aquarium water, into sterile 250 ml plastic beakers. Photographs and video images of extant (living) larvae in the beakers were made via Bodelin ProScope HR at 50x magnification and an ocular lens microscope camera at various magnifications. Following photography, the larvae, in the beakers, were placed into a -80^{o}C freezer for approximately 20 minutes in order to induce a state of torpor. The beakers were then removed and the surficial chilled water was extracted with the bulb pipette, leaving the torpid larvae and a small volume of water at the bottom. Formaldehyde, buffered and diluted to 10%, was subsequently added to the beakers, totaling a volume of 200 ml, which instantly preserved the anesthetized larvae. The fixed larvae were retained in these vessels throughout the remainder of the study, being removed via bulb pipette only for deformity screening.

Figure 2. Typical aquarium layout, note *Corbicula fluminea* relict shells along bottom.

Screening of the preserved bluegill larvae for developmental deformities was accomplished via light microscopy using VWR VistaVision dissecting microscopes. Anomalous larvae were grouped into four categories: craniofacial, spinal, yolk-sac, and other deformities. Reference literature regarding ichthyoplankton identification and selenium-induced developmental deformities included Lemly's (1997) "A Teratogenic Deformity Index for Evaluating Impacts of Selenium on Fish Populations," and Lippson and Moran's (1974) Manual for Identification of Early Developmental Stages of Fishes of the Potomac River Estuary, as well as other related publications (Holm, 2002; Janz & Muscatello, 2008). Confirmation of larval deformities among 2008-collected specimens was performed by Dr. Diana Papoulias, Fish Research Biologist, USGS, Columbia, MO. Dr. Papoulias evaluated 60.5% of 10-hour larvae, 89.2% of 120-hour larvae, and 40.6% of 192-hour larvae that WVDEP reared and examined in 2008. Histological assessments of these bluegill ichthyoplankton were made via standard light and specialized microscopy (Papoulias, pers. comm., 2009).

EGG TISSUE

Gravid female stream and lake fishes were collected by standard electrofishing techniques (USEPA, 2000). Individuals were live-kept in ambient water until dissection, when the gonads were removed from the body cavity. Ovaries were further dissected and the eggs within were removed via small laboratory spatula. Once extracted, eggs were placed in individual sterile beakers, or were composited with the eggs of other individuals of the same species to achieve a minimum analytical mass. Specimens were kept at <0ºC until chemical analyses were performed in accordance to USEPA's Laboratory Analysis Method 200.3, which is the basis for derivation of whole-body selenium concentrations in fish tissue (USEPA, 1991).

Results and Conclusions

2007 ICHTHYOPLANKTON

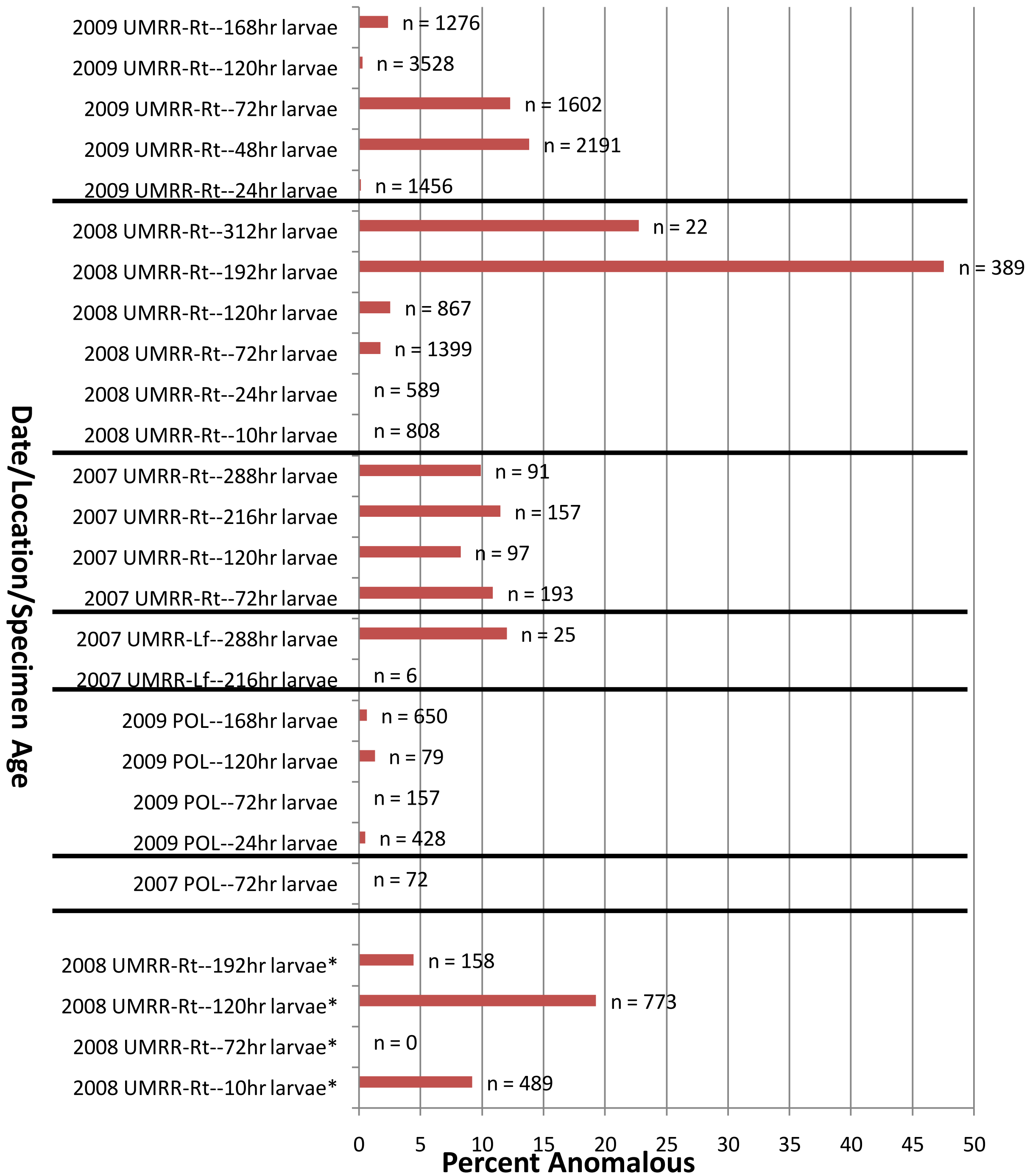
Deformity rates among 2007 bluegill ichthyoplankton were as high as 12.0% of the sampled population; although, the total number of specimens evaluated was relatively small at 569 individuals (Table 1; Figure 3). These deformity rates could not be compared to those from a reference population in the Left Fork/Mud River portion of the Upper Mud River Reservoir as it was later found to be influenced by the selenium-enriched part of the impoundment. However, a small number of bluegill larvae, 72 individuals, were collected from another reference location, Plum Orchard Lake. Upon evaluation of these reference specimens for anomalies, none were found to be deformed—0% (Table 1; Figure 3). Of the deformities among specimens from selenium-enriched locations, most were categorized as spinal anomalies; however, each of the deformity categories was represented in the population (Appendix C).

2008 ICHTHYOPLANKTON

During the 2008 study year, the location of a productive reference (non-impacted) spawning bluegill sunfish population was of paramount importance; a point of comparison to the presumably selenium-impacted ichthyoplankton was an experimental necessity. Consequently, Plum Orchard Lake, Fayette County, WV, was targeted as such a reference location due to its geography/geology as well as its low anthropogenic selenium input. However, attempts to locate bluegill nests within the impoundment were unsuccessful as the prolific growth of fragrant waterlilies, *Nymphaea odorata*, covered the surface water and shaded the lake's bottom from visible observation.

Bluegill ichthyoplankton were collected from the selenium-influenced population in the Upper Mud River Reservoir. The age-based deformity rate among these specimens ranged from 0 to 47.56% (Table 1). The total number of specimens evaluated by WVDEP in 2008 was 4,074. Dr. Diana Papoulias, USGS, Columbia, MO, confirmed the presence of selenium-induced developmental deformities among a subset (1420 individuals) of the 2008 Upper Mud River Reservoir bluegill ichthyoplankton. Dr. Papoulias' observed age-based deformity rate ranged from 2.54% to 19.28% (Table1; Figure 3; Appendix C) Dr. Papoulias' maximum observed deformity rate of 19.28% corresponds to WVDEP's larval deformity rate evaluation of 2.54%, considering 89.2% of the same specimens. WVDEP's evaluation of 192-hour larvae revealed a deformity rate of 47.56% as compared to 4.43% by Dr. Papoulias, who examined only 40.6% of the total specimens. Consideration must be given to Dr. Papoulias' use of higher resolution microscopy and that these confirmations are not based on evaluations of identical subsamples.

Figure 3. Larval bluegill sunfish, *Lepomis macrochirus*, deformity rates at study locations, 2007-2009.



***Observations made by Dr. Diana Papoulias, USGS (see Appendix C for details)**

During the 2008 study year, the prevalence of the egg fungus, *Saprolegnia sp.*, in all experimental aquaria necessitated the removal of entrapped larvae from the fungal mass, or mycelium. Since it was documented that developmentally deformed individuals were not able to swim normally (Gillespie & Baumann, 1986), these specimens were carefully extracted from fungal hyphae and were included in the deformity evaluations in the same manner as individuals freely captured. Many of larvae removed from the fungal mycelium were found to be developmentally deformed.

Table 1. Deformity evaluations of bluegill sunfish, *Lepomis macrochirus*, ichthyoplankton 2007-2009.

Site	Date Preserved	# Specimens Evaluated	Observed Anomalies	% Anomalous
UMRR-Rt	6/1/07	193	21	10.88
UMRR-Rt	6/4/07	97	8	8.25
POL	6/6/07	72	0	0.00
UMRR-Rt	6/8/07	157	18	11.46
UMRR-Lf	6/8/07	6	0	0.00
UMRR-Lf	6/11/07	25	3	12.00
UMRR-Rt	6/11/07	91	9	9.89
UMRR-Rt	6/4/2008	808	0	0.00
UMRR-Rt*	6/4/2008	180	6	3.33
UMRR-Rt*	6/4/2008	158	30	18.99
UMRR-Rt*	6/4/2008	151	9	5.96
UMRR-Rt	6/5/2008	589	0	0.00
UMRR-Rt	6/7/2008	1399	24	1.72
UMRR-Rt*	6/7/2008	698	damaged	damaged
UMRR-Rt*	6/7/2008	216	damaged	damaged
UMRR-Rt*	6/7/2008	147	damaged	damaged
UMRR-Rt	6/9/2008	867	22	2.54
UMRR-Rt*	6/9/2008	46	25	54.35
UMRR-Rt*	6/9/2008	585	112	19.15
UMRR-Rt*	6/9/2008	102	6	5.88
UMRR-Rt*	6/9/2008	40	6	15.00
UMRR-Rt	6/12/2008	389	185	47.56
UMRR-Rt*	6/12/2008	158	7	4.43
UMRR-Rt*	6/12/2008	150	damaged	damaged
UMRR-Rt	6/19/2008	22	5	22.73
UMRR-Rt	6/13/2009	1456	2	0.14
UMRR-Rt	6/16/2009	2191	303	13.83
UMRR-Rt	6/18/2009	1602	197	12.30
UMRR-Rt	6/20/2009	3528	9	0.26
UMRR-Rt	6/22/2009	1276	30	2.35
POL	6/16/2009	428	2	0.47
POL	6/18/2009	157	0	0.00
POL	6/20/2009	79	1	1.27
POL	6/22/2009	650	4	0.62

*Determinations made by Dr. Diana Papoulias, USGS.

2009 ICHTHYOPLANKTON

Attempts to collect a reference population of bluegill ichthyoplankton in 2009 were successful as 1,314 specimens were preserved from Plum Orchard Lake (Table 1; Figures 3 & 7). Among these individuals, a maximum deformity rate of 1.27% was observed, and most anomalies were considered slight. In contrast, larval deformity rates were as high as 13.83% among bluegill ichthyoplankton collected from the Upper Mud River Reservoir (Table 1), and many individuals were impacted by yolk-sac edema (Figures 4, 5, & 6). A total of 10,053 individuals were examined from the selenium-influenced population. Observations of larval viability in the aquaria also revealed the phenomenon of delayed mortality, where certain impacted individuals did not survive beyond defined life stages (e.g. 72-hour larvae afflicted by yolk-sac edema eventually died due to their deformities). Such delayed mortalities, particularly related to yolk-sac edema, were likely a result of congestive heart failure caused by increased fluid in the pericardial region (Figure 5) and/or feeding disruption caused by the ventral extension, or gape, of the mandible (Figure 6).

Figure 4. Multiple edematous bluegill larvae, *Lepomis macrochirus*, of similar age from UMRR.

Figure 5 (Left) & Figure 6 (Right). Bluegill larvae with pericardial yolk-sac edema and gaped mandibles.

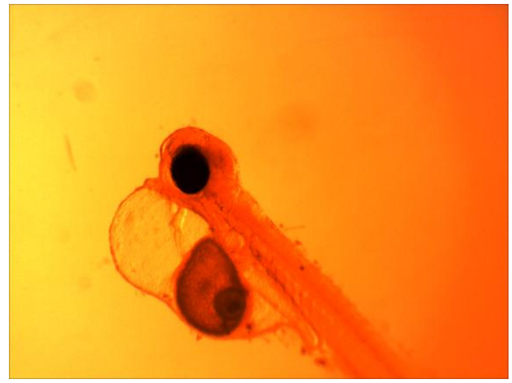
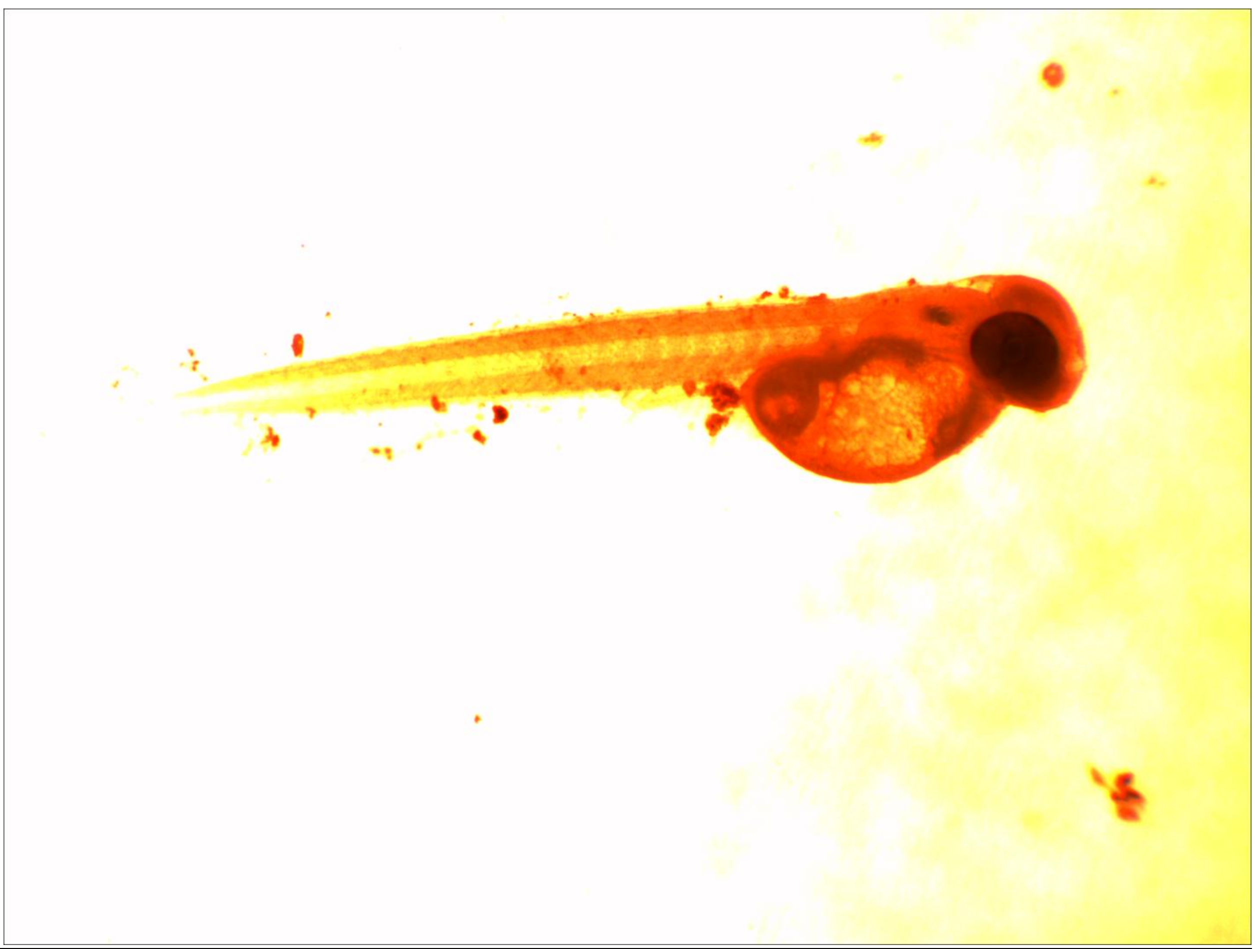


Figure 7. Developmentally typical POL bluegill larva in yolk-sac life stage.



EGG TISSUE SELENIUM CONCENTRATIONS

In comparison to published values (thresholds) associated with elevated larval deformity rates, fish eggs from various species were collected during the 2009 study year and analyzed for selenium concentration (mg/kg dry weight). At stream locations, minnow (family Cyprinidae) and sucker (family Catostomidae) females were collected and stripped of their eggs. Seng Creek, a selenium-enriched stream, contained creek chubs, *Semotilus atromaculatus*, with egg tissue selenium concentrations as high as 23.75 and 21.85 mg/kg dry weight, respectively (Figure 8; Appendix B). In contrast, a creek chub collected from a reference location, Ash Fork, exhibited an egg tissue selenium concentration of 6.5 mg/kg dry weight, and at Mud River, which is also selenium enriched, creek chub eggs had accrued 17.7 mg/kg dry weight selenium. Among the suckers, Davis Creek populations of white suckers, *Catostomus commersoni*, representing a reference location, had accrued egg tissue selenium concentrations of 5.1 and 3.9 mg/kg dry weight, respectively. These egg concentrations were less than those representing selenium-enriched locations (e.g. Mud River) where white suckers exhibited egg tissue selenium concentrations of 14.7 mg/kg dry weight.

The sunfishes (family Centrarchidae) are purported to be among the most sensitive fishes in regard to potential selenium-induced reproductive impacts (USEPA, 2004). Therefore, collection of sunfish eggs for selenium tissue concentration analyses was an important study objective. At impounded reference and selenium-enriched locations, both bluegill sunfish and largemouth bass gravid females were targeted for this purpose. Plum Orchard Lake, the reference location, contained bluegill and largemouth bass females with egg tissue selenium concentrations of <0.8 mg/kg dry weight and 8.7 mg/kg dry weight, respectively. Whereas, sunfish eggs collected from the Upper Mud River Reservoir, a selenium-enriched environ, contained comparatively more selenium: bluegill eggs had 9.8 mg/kg dry weight and largemouth bass eggs had accrued selenium concentrations as high as 64.6 mg/kg dry weight.

ADULT DEFORMITY SEARCH

Searches for developmentally-deformed individuals, which have survived to maturity, were conducted at Plum Orchard Lake and the Upper Mud River Reservoir in October, 2007 and 2008, focusing again on the sunfishes—bluegills and largemouth bass. These searches were initiated after observations of deformed adult fishes were made by WVDEP and USFWS personnel in May, 2007, (Figure 9) and following literature reviews revealing the occurrence of this phenomenon in other selenium-impacted fish populations (Lemly, 1993; Hamilton *et al*., $2005_{\mathbf{b}}$). In 2007, 50% of adult largemouth bass examined in the Upper Mud River Reservoir were considered to have some degree of craniofacial deformity, with approximately 50 individuals evaluated. In 2008, 12% of adult largemouth bass were craniofacially deformed from the Upper Mud River Reservoir population, with 117 individuals evaluated. In contrast, a 2008 survey of the Plum Orchard Lake adult largemouth bass population revealed 0 deformities, with 35 individuals evaluated (Appendix E).

Figure 8. Egg tissue selenium concentrations from fishes collected at study locations.

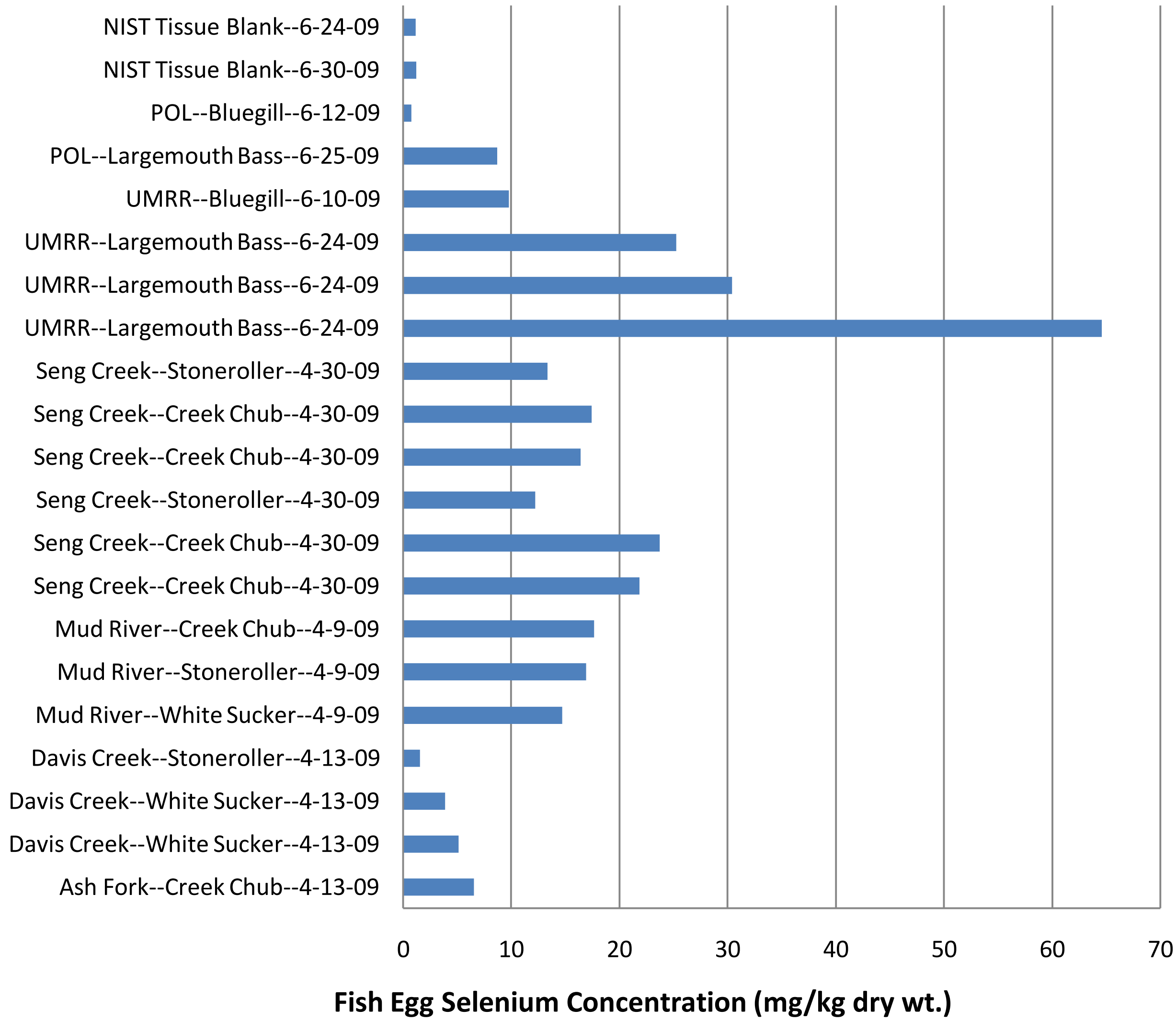


Figure 9. Adult largemouth bass, *Micropterus salmoides*, from UMRR with deformed facial features.

LITERATURE COMPARISONS & IMPLICATIONS

In regard to published studies which have similarly examined selenium-induced deformity rates among developing ichthyoplankton and suggested threshold rates for the sustenance of population health, the available information is concise—20% deformed larvae, above background or reference conditions, compromises the population (Lemly, 1997; USEPA, 2004; Muscatello *et al*., 2006). In this study, 2009 reference population deformity rates among bluegill larvae collected from Plum Orchard Lake were documented maximally at 1.27%. At the selenium-enriched location, the Upper Mud River Reservoir, maximum bluegill larval deformity rates were also below 20% in 2007 and 2009 sample populations (12.3% and 13.83%, respectively); however, in 2008, deformity rates were nearly 50% of the sampled population. Although this information suggests that the bluegill sunfish ichthyoplankton in the Upper Mud River Reservoir is potentially impacted due to the elevated occurrences of selenium-induced larval deformities documented in this study, more detailed evaluations of larval hatchability and survivability are needed to verify population-level effects.

Unlike the literature regarding selenium-induced larval deformity rates and subsequent fishery health, many published studies provide information regarding egg tissue concentrations of selenium which are predictive of toxicity. Muscatello *et al*. (2006) found that when concentrations of selenium reached 33.55 mg/kg dry weight among the eggs of northern pike, *Esox lucius*, 20% of the developing larvae exhibited deformities above background populations. Lemly (2002) suggests a selenium egg tissue concentration of 10 mg/kg dry weight as a threshold, above which selenium egg concentrations are predictive of a rapid increase in teratogenic deformities among developing larvae. Additionally, increased selenium egg tissue concentrations were correlated to an increase in deformities among razorback suckers, *Xyrauchen texanus*, as well as were indicative of smaller egg diameters and hatchability (Hamilton *et al*., 2005_a). In comparison, most of the selenium egg tissue concentrations of the fishes examined in this study were less than 30 mg/kg dry weight; however, all except one sample of fish eggs collected from selenium enriched locations exceeded 10 mg/kg dry weight (Figure 8; Appendix B). Of particular interest was selenium concentrations in the eggs of fishes collected from fluvial environs, to which fewer comparable published studies exist, and the amount of selenium accrual by the eggs of largemouth bass inhabiting the Upper Mud River Reservoir. In regard to the latter, a single female containing eggs with a selenium concentration of 64.62 mg/kg dry weight was documented; this value is considerably high in comparison to selenium egg concentrations of other fishes described in the literature (Gillespie & Baumann, 1986; Holm *et al*., 2005).

Since some developmental deformities among ichthyoplankton may persist into more mature fishes, particularly in regard to less severe anomalies, extensive population surveys of fishes residing in waters experiencing larval teratogenesis were necessary to determine the extent of surviving deformed adults. In general, craniofacial deformities, particularly those related to the lower jaw (mandibular) malformations, are among the most common anomalies observed in pollutant-impacted fish populations (Rolland, 2000; Al-Harbi, 2001; Strmac *et al*., 2002; Nagano *et al*., 2007). Such anomalous adult fishes have been observed in this study (Figure 9) among individuals collected at selenium-enriched locations (e.g. the Upper Mud River Reservoir). Although there are no clear thresholds suggested in the literature regarding percentages of impacted populations that are predictive of impending deleterious conditions, a few published studies have documented population collapses of fish communities when observed anomalous adults have exceeded 20-50% of the sampled individuals (Lemly, 1997; Hamilton, 2005_b). Therefore, according to these guidelines, the number (percentage) of deformed adults observed among largemouth bass populations at the Upper Mud River Reservoir is within the range considered to be impacted by selenium toxicity.

Acknowledgements

The information in the following report, prepared by the West Virginia Department of Environmental Protection, was gathered primarily by biologists Jason Morgan, Robert Row, Ben Lowman, and Jeff Ginger, with assistance from Mike McDaniel, Shawn Huffman, Ryan Pack, and Chris Barry. Several other staff scientists and water quality professionals also collaborated in the completion of the project: Jeff Bailey, Patrick Campbell, Dave Montali, Janice Smithson, and John Wirts. The cooperation of the West Virginia Division of Natural Resources was also greatly appreciated.

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Appendix A

Water Quality

STREAM_NAME	ANCODE	MILE	DATE	ANALYTE	Q	Se (ppm)
Davis Creek	WVK-39	2.6	05-Sep-02	Se Total	<	0.005
Davis Creek	WVK-39	3.3	20-Sep-07	Se Total	<	0.001
Davis Creek	WVK-39	13.7	04-Jun-02	Se Total	<	0.005
Plum Orchard Lake	WVK-65-Z-(L1)		23-Jul-07	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)		29-May-08	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	25-Apr-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	25-Apr-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	25-Apr-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	24-May-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	24-May-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	24-May-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	22-Jun-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	22-Jun-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	22-Jun-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	15-Aug-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	15-Aug-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	27-Sep-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	27-Sep-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	27-Sep-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	11-Dec-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	11-Dec-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	11-Dec-06	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	09-May-07	Se Total	<	0.001
Plum Orchard Lake	WVK-65-Z-(L1)	0.04	29-Oct-08	Se Total	<	0.001
Seng Creek	WVKC-42	0	16-Jul-02	Se Total		0.007
Seng Creek	WVKC-42	0	16-Jul-02	Se Total		0.008
Seng Creek	WVKC-42	0	05-Sep-02	Se Total		0.006
Seng Creek	WVKC-42	0	05-Sep-02	Se Total		0.006
Seng Creek	WVKC-42	0	16-Oct-02	Se Total	<	0.005
Seng Creek	WVKC-42	0	29-Oct-02	Se Total	<	0.005
Seng Creek	WVKC-42	0	14-Nov-02	Se Total		0.006
Seng Creek	WVKC-42	0	14-Nov-02	Se Total		0.006
Seng Creek	WVKC-42	0	09-Dec-02	Se Total		0.009
Seng Creek	WVKC-42	0	28-Jan-03	Se Total		0.009
Seng Creek	WVKC-42	0	24-Feb-03	Se Total	<	0.005
Seng Creek	WVKC-42	0	20-Mar-03	Se Total		0.009
Seng Creek	WVKC-42	0	14-Apr-03	Se Total	<	0.005
Seng Creek	WVKC-42	0	14-May-03	Se Total		0.006
Seng Creek	WVKC-42	2.5	15-Nov-05	Se Total		0.04
Seng Creek	WVKC-42	2.5	13-Dec-05	Se Total		0.042
Seng Creek	WVKC-42	2.5	04-Jan-06	Se Total		0.023
Seng Creek	WVKC-42	2.5	08-Feb-06	Se Total		0.031
Seng Creek	WVKC-42	2.5	13-Mar-06	Se Total		0.026

STREAM_NAME	ANCODE	MILE	DATE	ANALYTE	Q	Se (ppm)
Seng Creek	WVKC-42	2.5	13-Apr-06	Se Total		0.015
Seng Creek	WVKC-42	2.5	13-Apr-06	Se Total		0.015
Seng Creek	WVKC-42	2.5	02-May-06	Se Total		0.023
Seng Creek	WVKC-42	2.5	13-Jun-06	Se Total		0.025
Seng Creek	WVKC-42	2.5	12-Jul-06	Se Total		0.041
Seng Creek	WVKC-42	2.5	24-Aug-06	Se Total		0.03
Seng Creek	WVKC-42	2.5	17-Oct-06	Se Total		0.023
Seng Creek	WVKC-42	2.5	19-Apr-07	Se Total		0.024
Seng Creek	WVKC-42	3.9	16-Jul-02	Se Total		0.018
Seng Creek	WVKC-42	3.9	05-Sep-02	Se Total		0.0089
Seng Creek	WVKC-42	3.9	16-Oct-02	Se Total		0.008
Seng Creek	WVKC-42	3.9	29-Oct-02	Se Total		0.01
Seng Creek	WVKC-42	3.9	14-Nov-02	Se Total		0.012
Seng Creek	WVKC-42	3.9	09-Dec-02	Se Total		0.014
Seng Creek	WVKC-42	3.9	28-Jan-03	Se Total		0.01
Seng Creek	WVKC-42	3.9	24-Feb-03	Se Total		0.009
Seng Creek	WVKC-42	3.9	20-Mar-03	Se Total		0.045
Seng Creek	WVKC-42	3.9	14-Apr-03	Se Total		0.03
Seng Creek	WVKC-42	3.9	14-May-03	Se Total		0.009
Ash Fork	WVKG-5-H	0.1	09-Nov-05	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	19-Dec-05	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	04-Jan-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	09-Feb-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	13-Mar-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	13-Mar-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	03-Apr-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	02-May-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	12-Jun-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	11-Jul-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	21-Aug-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	05-Oct-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	05-Oct-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.1	16-Oct-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.25	04-Jan-06	Se Total	<	0.001
Ash Fork	WVKG-5-H	0.3	05-Jun-08	Se Total	<	0.001
Mud River	WVOGM	2.5	24-Sep-08	Se Total	<	0.001
Mud River	WVOGM	25.4	22-Sep-08	Se Total	<	0.001
Mud River	WVOGM	52.8	18-Sep-08	Se Total		0.003
Mud River	WVOGM	52.8	18-Sep-08	Se Total		0.004
Mud River	WVOGM	57.4	17-Sep-08	Se Total		0.004
Mud River	WVOGM	61.7	18-Sep-08	Se Total		0.006
Mud River	WVOGM	61.7	10-Nov-08	Se Total		0.004
Mud River	WVOGM	67.4	21-Dec-06	Se Total		0.008
Mud River	WVOGM	74.1	05-Mar-09	Se Total		0.0096
Mud River	WVOGM	74.1	07-Apr-09	Se Total		0.0071
Mud River	WVOGM	74.1	12-May-09	Se Total		0.0121

STREAM_NAME	ANCODE	MILE	DATE	ANALYTE	Q	Se (ppm)
Mud River	WVOGM	74.1	22-Jun-09	Se Total		0.0216
Mud River	WVOGM	74.3	21-Dec-06	Se Total		0.01
Mud River	WVOGM	75.6	30-Nov-05	Se Total		0.0084
Mud River	WVOGM	75.6	13-Dec-05	Se Total		0.007
Mud River	WVOGM	75.6	05-Jan-06	Se Total		0.005
Mud River	WVOGM	75.6	05-Jan-06	Se Total		0.005
Mud River	WVOGM	75.6	08-Feb-06	Se Total		0.0087
Mud River	WVOGM	75.6	15-Mar-06	Se Total		0.004
Mud River	WVOGM	75.6	05-Apr-06	Se Total		0.005
Mud River	WVOGM	75.6	03-May-06	Se Total		0.007
Mud River	WVOGM	75.6	13-Jun-06	Se Total		0.005
Mud River	WVOGM	75.6	21-Aug-06	Se Total		0.008
Mud River	WVOGM	75.6	20-Sep-06	Se Total		0.014
Mud River	WVOGM	75.6	03-Nov-06	Se Total		0.008
Mud River	WVOGM	75.6	06-Jun-07	Se Total		0.022
Mud River	WVOGM	75.6	10-Nov-08	Se Total		0.015
Mud River	WVOGM	77.1	27-Jul-05	Se Total		0.007
Mud River	WVOGM	77.1	16-Sep-08	Se Total		0.009
Upper Mud River Reservoir	WVOGM-(L1)		16-May-07	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)		24-Jul-07	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)		25-Jul-07	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)		04-Oct-07	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)		04-Oct-07	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)		19-Mar-08	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)		20-Mar-08	Se Total	<	0.001
Upper Mud River Reservoir	WVOGM-(L1)		05-Nov-08	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)	0.05	04-Oct-07	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)	1.4	03-Nov-05	Se Total		0.006
Upper Mud River Reservoir	WVOGM-(L1)	1.4	12-Jan-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	12-Jan-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	12-Jan-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	12-Jan-06	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)	1.4	12-Jan-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	12-Jan-06	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)	1.4	27-Feb-06	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)	1.4	27-Feb-06	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)	1.4	27-Feb-06	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)	1.4	04-Apr-06	Se Total		0.0036
Upper Mud River Reservoir	WVOGM-(L1)	1.4	04-Apr-06	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)	1.4	04-Apr-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	17-Apr-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	17-Apr-06	Se Total		0.001
Upper Mud River Reservoir	WVOGM-(L1)	1.4	17-Apr-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	24-May-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	24-May-06	Se Total		0.001
Upper Mud River Reservoir	WVOGM-(L1)	1.4	24-May-06	Se Total		0.004

STREAM_NAME	ANCODE	MILE	DATE	ANALYTE	Q	Se (ppm)
Upper Mud River Reservoir	WVOGM-(L1)	1.4	22-Jun-06	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)	1.4	22-Jun-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	22-Jun-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	17-Aug-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	17-Aug-06	Se Total		0.006
Upper Mud River Reservoir	WVOGM-(L1)	1.4	27-Sep-06	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)	1.4	27-Sep-06	Se Total		0.005
Upper Mud River Reservoir	WVOGM-(L1)	1.4	27-Sep-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	28-Nov-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	28-Nov-06	Se Total		0.002
Upper Mud River Reservoir	WVOGM-(L1)	1.4	28-Nov-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	21-Dec-06	Se Total		0.004
Upper Mud River Reservoir	WVOGM-(L1)	1.4	11-May-07	Se Total		0.007
Upper Mud River Reservoir	WVOGM-(L1)	1.4	11-May-07	Se Total		0.006
Upper Mud River Reservoir	WVOGM-(L1)	1.4	30-May-08	Se Total		0.003
Upper Mud River Reservoir	WVOGM-(L1)	4.19	04-Oct-07	Se Total		0.005

Appendix B

Fish Egg Selenium Concentrations

Stream Name	Mile	Date	Species	Portion	Q	Se (ppm dry wt.)
Ash Fork	0.4	13-Apr-09	Creek Chub	Fish Eggs		6.549
Davis Creek	10.6	13-Apr-09	White Sucker	Fish Eggs		5.13
Davis Creek	10.6	13-Apr-09	White Sucker	Fish Eggs		3.92
Davis Creek	10.6	13-Apr-09	Stoneroller	Fish Eggs		1.56
Mud River	74.6	09-Apr-09	White Sucker	Eggs-Composite		14.735
Mud River	74.6	09-Apr-09	Stoneroller	Eggs-Composite		16.923
Mud River	74.6	09-Apr-09	Creek Chub	Eggs-Composite		17.674
Seng Creek	3.6	30-Apr-09	Creek Chub	Fish Eggs		21.851
Seng Creek	3.6	30-Apr-09	Creek Chub	Fish Eggs		23.75
Seng Creek	3.6	30-Apr-09	Stoneroller	Eggs-Composite		12.269
Seng Creek	3.6	30-Apr-09	Creek Chub	Fish Eggs		16.416
Seng Creek	3.6	30-Apr-09	Creek Chub	Fish Eggs		17.478
Seng Creek	3.6	30-Apr-09	Stoneroller	Eggs-Composite		13.4
Upper Mud River Reservoir		24-Jun-09	Largemouth Bass	Fish Eggs		64.615
Upper Mud River Reservoir		29-Jun-09	Largemouth Bass	Fish Eggs		30.462
Upper Mud River Reservoir		29-Jun-09	Largemouth Bass	Fish Eggs		25.261
Upper Mud River Reservoir		10-Jun-09	Bluegill	Fish Eggs		9.783
Plum Orchard Lake		25-Jun-09	Largemouth Bass	Fish Eggs		8.722
Plum Orchard Lake		12-Jun-09	Bluegill	Fish Eggs	<	0.8
None-Standard Reference Material		30-Jun-09	NIST-1947 Lake Michigan Fish Tissue	Tissue Blank		1.25
None-Standard Reference Material		24-Jun-09	NIST-1947 Lake Michigan Fish Tissue	Tissue Blank		1.199

Appendix C

Larval Fish Evaluations

Sample Location	Aquarium	Date	Vial	# larvae	% deformed
UMRR	6	6/9/2008	10	46	54
UMRR	cooler	6/9/2008	11	585	19
UMRR	4	6/9/2008	13	102	6
UMRR	5	6/9/2008	14	40	15
UMRR	4	6/12/2008	19	158	4
UMRR	6A	6/4/2008	21	180	3
UMRR	4B	6/4/2008	24	158	19
UMRR	6B	6/4/2008	25	151	6
UMRR	cooler	6/12/2008	17	150	not examined
UMRR	6	6/7/2008	31	698	not examined
UMRR	5	6/7/2008	33	216	not examined
UMRR	cooler	6/7/2008	34	147	not examined

Dr. Diana Papoulias' 2008 bluegill evaluations

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
Left Fork/UMRR	6/11/07	08/09/07	*Lepomis macrochirus*	25	N/A	12 days post-hatch	0	3	0	0	0
Right Fork/UMRR	6/11/07	08/09/07	*Lepomis macrochirus*	67	N/A	12 days post-hatch	2	3	0	0	1
Right Fork/UMRR	6/8/07	08/09/07	*Lepomis macrochirus*	116	N/A	9 days post-hatch	1	0	0	0	0
Left Fork/UMRR	6/8/07	08/09/07	*Lepomis macrochirus*	6	N/A	9 days post-hatch	0	0	0	0	0
Right Fork/UMRR	6/8/07	08/09/07	*Lepomis macrochirus*	41	N/A	9 days post-hatch	17	0	0	0	0
Right Fork/UMRR	6/4/07	08/09/07	*Lepomis macrochirus*	97	N/A	5 days post-hatch	5	3	0	0	0
Right Fork/UMRR	6/1/07	08/09/07	*Lepomis macrochirus*	193	N/A	3 days post-hatch	7	7	0	0	7
Right Fork/UMRR	6/11/07	08/09/07	*Lepomis macrochirus*	24	N/A	12 days post-hatch	1	1	0	0	1
Right Fork/UMRR	5/31/07	08/09/07	*Lepomis macrochirus*	274	N/A	1 day post-hatch	—	—	—	—	—
UMRR	6/7/08	7/23/08	*Lepomis macrochirus*	12	#1=11 #3=1	~ 72 hour YSL	0	0	0	0	0
UMRR	6/7/08	7/23/08	*Lepomis macrochirus*	80	#3	~48 hour YSL	0	0	0	0	0
UMRR	6/7/08	7/23/08	*Lepomis macrochirus*	133	#1* (#2)	~72 hour YSL	0	0	0	0	0
UMRR	6/7/08	7/23/08	*Lepomis macrochirus*	3	1	~72 hour YSL	2*	0	0	1^	0
UMRR	6/7/08	7/24/08	*Lepomis macrochirus*	231	Mostly 1/some 2*	variable ~72 hour YSL	0	0	0	0	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/7/08	7/24/08	*Lepomis macrochirus*	393	2	~72 hour YSL Some 24 hour larvae	0	0	0	0	0
UMRR	6/7/08	7/24/08	*Lepomis macrochirus*	57	3 & 4 (~10)	~24 hour YSL, variable	0	0	0	0	0
UMRR	6/7/08	7/24/08	*Lepomis macrochirus*	15	1	~72 hour YSL	13[A]	0	0	2[B]	1[C]
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	21	1	72 hour YSL	0	0	0	0	0
UMRR	6/4/08	8/4/08	*Lepomis macrochirus*	56	1	Tail-bud stage (in egg capsule)	0	0	0	0	0
UMRR	6/4/08	8/4/08	*Lepomis macrochirus*	78	1	Tail-free stage (out of capsule)	0	0	0	0	0
UMRR	6/4/08	8/4/08	*Lepomis macrochirus*	13	3	Early Embryo with decay	0	0	0	0	0
UMRR	6/4/08	8/6/08	*Lepomis macrochirus*	180	1	Tail-free stage (out of capsule)	0	0	0	0	0
UMRR	6/4/08	8/6/08	*Lepomis macrochirus*	38	Mostly 1 & 2 some 3	Tail-free, ~10 hour post hatch	0	0	0	0	0
UMRR	6/4/08	8/6/08	*Lepomis macrochirus*	151	1	Tail-free, ~10 hour post hatch	0	0	0	0	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/4/08	8/6/08	*Lepomis macrochirus*	158	1	Tail-free, ~10 hour post hatch	0	0	0	0	0
UMRR	6/4/08	8/6/08	*Lepomis macrochirus*	41	1	Tail-free, ~10 hour post hatch	0	0	0	0	0
UMRR	6/4/08	8/6/08	*Lepomis macrochirus*	27	1	Tail-free, ~10 hour post hatch	0	0	0	0	0
UMRR	6/4/08	8/4/08	*Lepomis macrochirus*	51	1	Tail-free, ~10 hour post hatch	0	0	0	0	0
UMRR	6/4/08	8/4/08	*Lepomis macrochirus*	15	1	Tail-free, ~10 hour post hatch	0	0	0	0	0
UMRR	6/9/08	8/4/08	*Lepomis macrochirus*	5	1	<96 hour, YSL	0	5	0	0	0
UMRR	6/9/08	8/4/08	*Lepomis macrochirus*	3	1,3	1<96 hour, YSL 1>24 hour, YSL 1<24 hour,YSL	0	1[A]	0	0	0
UMRR	6/9/08	8/4/08	*Lepomis macrochirus*	3	1	48 hour, YSL	0	1[A]	0	1[B]	0
UMRR	6/9/08	8/4/08	*Lepomis macrochirus*	8	1	>72 hour, YSL	1[A]	0	0	6[B]	1[C]

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/9/08	8/4/08	*Lepomis macrochirus*	24	3	<72 hour, YSL	0	0	0	0	0
UMRR	6/9/08	8/4/08	*Lepomis macrochirus*	70	1	>72 hour, YSL	0	0	0	0	0
UMRR	6/9/08	7/29/08	*Lepomis macrochirus*	52	1	>72 hour,YSL	0	0	0	0	0
UMRR	6/9/08	7/29/08	*Lepomis macrochirus*	16	3	72 hour , YSL	0	0	0	0	0
UMRR	6/9/08	7/29/08	*Lepomis macrochirus*	1	1*	72 hour , YSL	1	0	0	0	0
UMRR	6/9/08	7/29/08	*Lepomis macrochirus*	3	1	>72 hour, YSL	0	0	0	0	0
UMRR	6/9/08	7/29/08	*Lepomis macrochirus*	563	3*	>72 hour, YSL	0	0	0	0	0
UMRR	6/9/08	7/29/08	*Lepomis macrochirus*	79	3	72 hour, YSL	0	0	0	0	0
UMRR	6/9/08	7/29/08	*Lepomis macrochirus*	5	1 &3*	72 hour, YSL	4[A]	0	0	2[B]	2[C]
UMRR	6/9/08	8/14/08	*Lepomis macrochirus*	32	1	<96 hour larvae	0	0	0	0	0
UMRR	6/9/08	8/14/08	*Lepomis macrochirus*	3	3	72 hour larvae	0	0	0	0	0
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	147	1-98% 2-2%	~72 hour larvae	5[A]	0	0	0	0
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	12	1	~72 hour*, YSL	0	0	0	0	0
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	58	1	~20% 72 hour YSL ~80%< 72 hour YSL	0	0	0	0	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	96	1	72 hour, YSL	0	0	0	0	0
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	121	3	<72 hout, YSL	0	0	0	0	0
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	18	1 and 2	72 hour YSL	0	0	0	0	0
UMRR	6/7/08	7/28/08	*Lepomis macrochirus*	2	3	72 hour YSL	0	0	0	0	0
UMRR	6/5/08	8/12/08	*Lepomis macrochirus*	52	3	>24 hour YSL	0	0	0	0	0
UMRR	6/5/08	8/12/08	*No larvae observed*	0	0	0	0	0	0	0	0
UMRR	6/5/08	8/12/08	*Lepomis macrochirus*	13	3	~10 hour, YSL	0	0	0	0	0
UMRR	6/5/08	8/12/08	*No larvae observed*	0	0	0	0	0	0	0	0
UMRR	6/5/08	8/12/08	*Lepomis macrochirus*	49	3	>24 hour YSL	0	0	0	0	0
UMRR	6/5/08	8/12/08	*Lepomis macrochirus*	138	3 and 4	<24 hour, YSL	0	0	0	0	0
UMRR	6/5/08	8/12/08	*Lepomis macrochirus*	5	3	<24 hour, YSL	0	0	0	0	0
UMRR	6/5/08	8/12/08	*Lepomis macrochirus*	227	3	~24 hour YSL,	0	0	0	0	0
UMRR	6/5/08	8/12/08	*Lepomis macrochirus*	105	3	~24 hour YSL,	0	0	0	0	0
UMRR	6/19/08	12/8/08	*Lepomis macrochirus*	22	3	+120 hour	3[A]	2	0	0	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/12/08	12/8/08	*Lepomis macrochirus*	88	3	+96 hour	0	0	0	0	0
UMRR	6/12/08	12/8/08	*Lepomis macrochirus*	62	3	+96 hour	62	0	0	0	0
UMRR	6/12/08	12/11/08	*Lepomis macrochirus*	17	3	+96 hour	0	0	0	0	0
UMRR	6/12/08	12/11/08	*Lepomis macrochirus*	36	3	+96 hour	36[A]	0	0	1	0
UMRR	6/12/08	12/11/08	*Lepomis macrochirus*	82	3	+96 hour	82	0	0	0	0
UMRR	6/12/08	12/11/08	*Lepomis macrochirus*	76	3	+96 hour	0	0	0	0	0
UMRR	6/12/08	12/12/08	*Lepomis macrochirus*	24	3	+96 hour	0	0	0	0	0
UMRR	6/12/08	12/12/08	*Lepomis macrochirus*	4	3	+96 hour	4	0	0	0	0
UMRR	6/13/09	7/20/09	*Lepomis macrochirus*	583	1	24hr	0	0	0	0*	0
UMRR	6/13/09	7/20/09	*Lepomis macrochirus*	4	3	24hr	0	0	0	0*	0
UMRR	6/13/09	7/20/09	*Lepomis macrochirus*	1	1	≈ 12hr	0	0	0	0*	1
UMRR	6/13/09	7/20/09	*Lepomis macrochirus*	1	1	24hr	0	0	0	1	0
UMRR	6/13/09	7/22/09	*Lepomis macrochirus*	262	1	24hr tail-free	0	0	0	0	0
UMRR	6/13/09	7/22/09	*Lepomis macrochirus*	225	1*	≈ 18hr	0	0	0	0	0
UMRR	6/13/09	7/22/09	*Lepomis macrochirus*	3	2	≈ 12hr	0	0	0	0	0
UMRR	6/13/09	7/22/09	*Lepomis macrochirus*	377	1	≈ 18hr	0	0	0	0	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/16/09	7/23/09	*Lepomis macrochirus*	543	1	≈ 48hr	0	0	0	0	0
UMRR	6/16/09	7/23/09	*Lepomis macrochirus*	65	1	≈ 48hr	0	0	0	65	0
UMRR	6/16/09	7/23/09	*Lepomis macrochirus*	1	3	≈ 30hr	0	0	0	0	0
UMRR	6/16/09	8/27/09	*Lepomis macrochirus*	150	1	≈ 48hr	0	0	0	22	0
UMRR	6/16/09	8/27/09	*Lepomis macrochirus*	417	1	≈ 48hr	0	0	0	0	0
UMRR	6/16/09	9/8/09	*Lepomis macrochirus*	4	1	≈ 48hr	0	0	0	0	4
UMRR	6/16/09	9/10/09	*Lepomis macrochirus*	314	1	≈ 48hr	0	0	0	210	2*
UMRR	6/16/09	9/10/09	*Lepomis macrochirus*	697	1	≈ 48hr	0	0	0	0	0
POL	6/16/09	9/10/09	*Lepomis macrochirus*	146	1	≈ 24hr	0	0	0	0	0
POL	6/16/09	9/10/09	*Lepomis macrochirus*	1	1	≈ 24hr	0	0	0	1	0
POL	6/16/09	9/10/09	*Lepomis macrochirus*	2	2	≈ 24hr	0	0	0	0	0
POL	6/16/09	9/10/09	*Lepomis macrochirus*	94	2	≈ 24hr	0	0	0	0	0
POL	6/16/09	9/10/09	*Lepomis macrochirus*	1	1	≈ 24hr	1	0	0	0	0
POL	6/16/09	9/10/09	*Lepomis macrochirus*	2	2	≈ 24hr	0	0	0	0	0
POL	6/16/09	9/11/09	*Lepomis macrochirus*	182	1	≈ 24hr	0	0	0	0	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
POL	6/18/09	9/11/09	*Lepomis macrochirus*	86	1	≈ 48hr	0	0	0	0	0
POL	6/18/09	9/14/09	*Lepomis macrochirus*	26	1	≈ 48hr	0	0	0	0	0
POL	6/18/09	9/14/09	*Lepomis macrochirus*	1	2	≈ 48hr	0	0	0	0	0
POL	6/18/09	9/15/09	*Lepomis macrochirus*	41	1	≈ 48hr	0	0	0	0	0
POL	6/18/09	9/15/09	*Lepomis macrochirus*	3	2	≈ 48hr	0	0	0	0	0
POL	6/20/09	9/15/09	*Lepomis macrochirus*	4	1	≈ 72hr	0	0	0	0	0
POL	6/20/09	9/15/09	*Lepomis macrochirus*	1	1	≈ 72hr	0	1	0	0	0
POL	6/20/09	9/15/09	*Lepomis macrochirus*	15	1	≈ 72hr	0	0	0	0	0
POL	6/20/09	9/16/09	*Lepomis macrochirus*	58	1	≈ 72hr	0	0	0	0	0
POL	6/20/09	9/16/09	*Lepomis macrochirus*	1	2	≈ 72hr	0	0	0	0	0
UMRR	6/18/09	9/14/09	*Lepomis macrochirus*	108	1*	≈ 72hr	0	0	0	108	0
UMRR	6/18/09	9/14/09	*Lepomis macrochirus*	573	1*	≈ 72hr	0	0	0	0	0
UMRR	6/18/09	9/18/09	*Lepomis macrochirus*	291	1	≈ 72hr	0	0	0	0	0
UMRR	6/18/09	9/18/09	*Lepomis macrochirus*	89	1	≈ 72hr	0	0	0	89	0
UMRR	6/20/09	9/18/09	*Lepomis macrochirus*	889	1	≈ 96hr	0	0	0	0	0
UMRR	6/20/09	9/18/09	*Lepomis macrochirus*	6	1/3	≈ 96hr	6	0	0	0	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/22/09	9/30/09	*Lepomis macrochirus*	431	1	≈ 120hr	0	0	0	0	0
UMRR	6/22/09	9/30/09	*Lepomis macrochirus*	12	1	≈ 120hr	2*	10	0	0	0
POL	6/22/09	9/17/09	*Lepomis macrochirus*	312	1	≈ 96hr	0	0	0	0	0
POL	6/22/09	9/17/09	*Lepomis macrochirus*	3	1	≈ 96hr	2	1	0	0	0
POL	6/22/09	9/17/09	*Lepomis macrochirus*	181	1	≈ 96hr	0	0	0	0	0
POL	6/22/09	9/17/09	*Lepomis macrochirus*	152	1	≈ 96hr	0	0	0	0	0
POL	6/22/09	9/17/09	*Lepomis macrochirus*	1	2	≈ 96hr	0	0	0	0	0
POL	6/22/09	9/17/09	*Lepomis macrochirus*	1	1	≈ 96hr	1	0	0	0	0
UMRR	6/18/09	9/18/09	*Lepomis macrochirus*	481	1	≈ 48hr	0	0	0	0	0
UMRR	6/18/09	9/18/09	*Lepomis macrochirus*	2	1	≈ 48hr	0	0	0	0	0
UMRR	6/18/09	9/18/09	*Lepomis macrochirus*	58	1	≈ 48hr	0	0	0	0	0
UMRR	6/20/09	9/22/09	*Lepomis macrochirus*	122 7	1	≈ 72hr	0	0	0	0	0
UMRR	6/20/09	9/22/09	*Lepomis macrochirus*	1	2	≈ 72hr	0	0	0	0	0
UMRR	6/20/09	9/22/09	*Lepomis macrochirus*	2	1	≈ 72hr	1	0	0	1	0
UMRR	6/20/09	9/29/09	*Lepomis macrochirus*	140 2	1	≈ 72hr	0	0	0	0	0
UMRR	6/20/09	9/29/09	*Lepomis macrochirus*	1	1	≈ 72hr	0	0	0	1	0

Larvae Origin	Date Preserved	Evaluation Date	Species Identified	# Specimens Evaluated	Specimen Quality[b]	Developmental Stage (≈age)[c]	# Anomalous Spinal/Skeletal Features	# Anomalous Craniofacial Features	# Anomalous Fins	# Anomalous Yolk Sac Sorption	# All Other Anomalies
UMRR	6/22/09	10/1/09	*Lepomis macrochirus*	522	1	≈ 120hr	0	0	0	0	0
UMRR	6/22/09	10/1/09	*Lepomis macrochirus*	8	1	≈ 120hr	0	8	0	0	0
UMRR	6/22/09	10/1/09	*Lepomis macrochirus*	291	1	≈ 96hr	0	0	0	0	0
UMRR	6/22/09	10/1/09	*Lepomis macrochirus*	2	2	≈ 96hr	0	0	0	0	0
UMRR	6/22/09	10/1/09	*Lepomis macrochirus*	10	1	≈ 96hr	1	8	0	0	1

[a] **Quick Cooled, H_20 decanted, Innundated in Preservative**

[b] **1. Typical, Normal Development**

2. Mechanically Damaged by Handling

3. Evidence of Fungal Infestation

4. Evidence of Parasitism

[c] **YSL = Yolk Sac Larva**

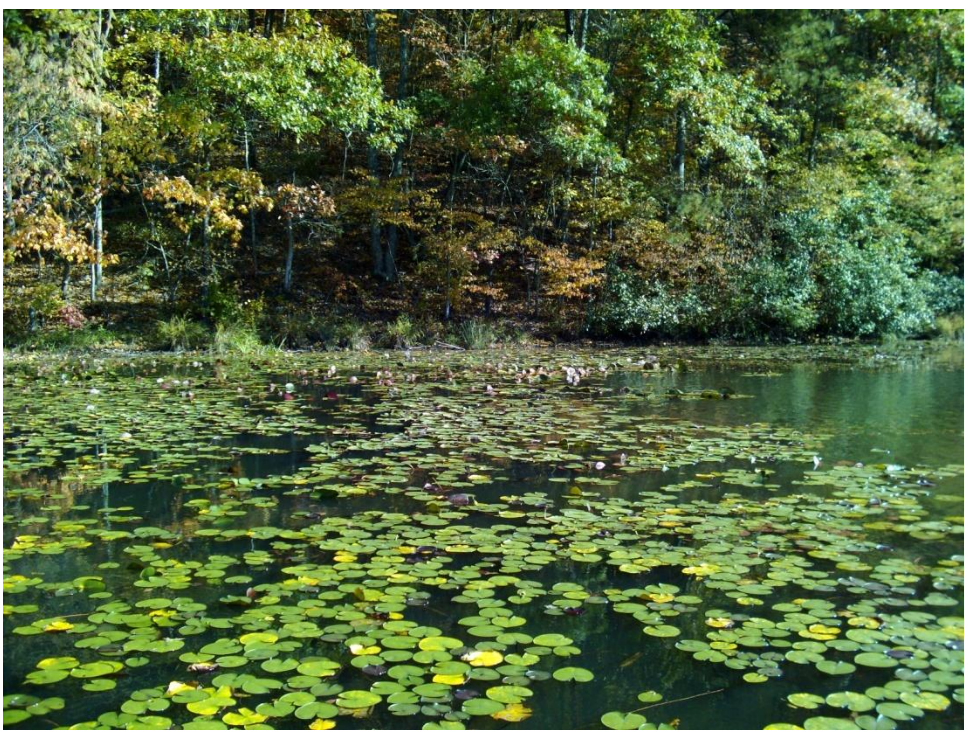
*Determination of edemic yolk sac not practical due to early stage of larvae (i.e. very little sorption has taken place)

Appendix D

Selected Study Photos

Upper Mud River Reservoir, Lincoln County, WV, 2008

Plum Orchard Lake, Fayette County, WV, 2008



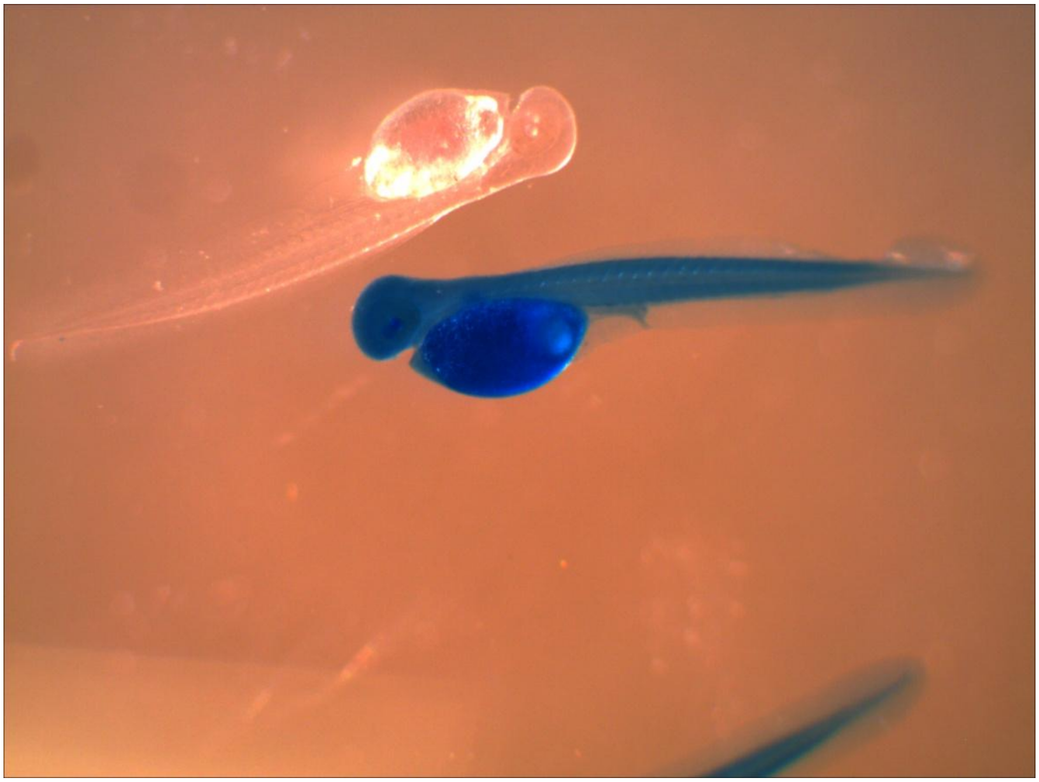
Largemouth Bass, *Micropterus salmoides*, from UMRR with slight facial deformities, 2008

Typical Largemouth Bass, *Micropterus salmoides*, from Plum Orchard Lake, 2008

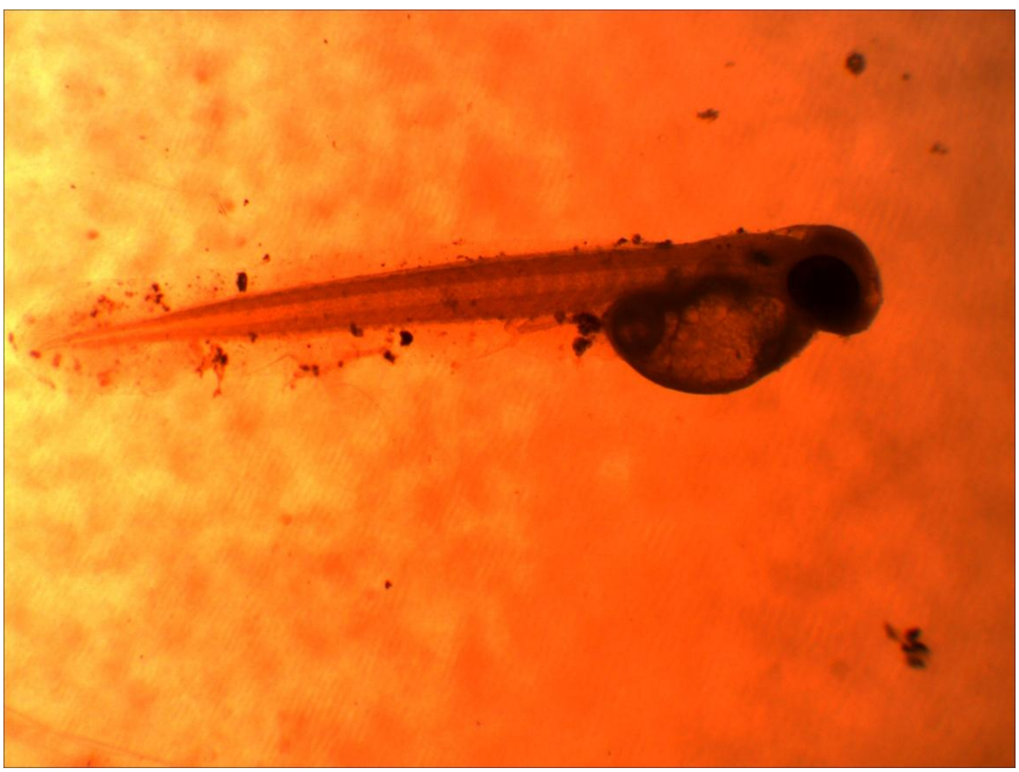
Fish Egg Collection, UMRR, 2008

Ichthyoplankton Rearing Laboratory Design, 2008

Developmentally-Deformed Bluegill, *Lepomis macrochirus*, Larvae from UMRR, 2009



Typical Bluegill, *Lepomis macrochirus*, Larvae from POL, 2009 (top specimens color-enhanced by stain) ↕



Appendix E

Related Documents Regarding Selenium and Fisheries Health

e-mail correspondence from Ben Lowman to Pat Campbell, 2-18-09

Observations on Deformed Fishes in the Upper Mud River Reservoir

In May, 2007, West Virginia Department of Environmental Protection biologists Ben Lowman, Jason Morgan, and Robert Row were conducting a birds' eggs collection survey at the Upper Mud River Reservoir, Lincoln County, West Virginia. Assisted by US Fish and Wildlife ornithologist Joe Skorupa, the group located a spawning beach being used by bluegill sunfishes, *Lepomis macrochirus*. The shallow water area included nest depressions lined with relict shells from Asian clams, *Corbicula fluminea*, as well as nest guarding males and schooling prenuptial females (Figures 1 and 2). Occasionally, a mature largemouth bass, *Micropterus salmoides*, would raid the nesting area in apparent attempt to predate bluegill eggs; however, the determined male bluegills successfully defended their nests, often driving the largemouth bass into the extreme shallows. It was on such an escape run that the biologists, photographing the spawning event, noticed a facial deformity on the nest-raiding largemouth bass. The bass appeared to have a protrusive lower jaw, one incapable of occlusion with the upper maxillae; yet, careful observation revealed a truncation of the maxillae, or upper jaw. The biologists discussed the rarity of such a deformation given the number of fishes that each has closely inspected during their respective careers.

Two days later, Jason Morgan and Robert Row, electroshocking fish in the same impoundment, collected another largemouth bass that exhibited severe facial deformity, also having an extremely truncated maxillae (Figure 3). This individual was of similar size to the nest-raiding bass. Again, biologists noted the scarcity of such individuals occurring in natural populations as such mal-fitness is usually a detriment to survival. Later, a discussion ensued regarding the likelihood of angling injuries or attempted predation leading to the observed deformities. It was agreed by all that uniqueness of the abnormalities combined with an apparent manifestation in certain aged fishes was evidence of mutation. Since selenium, the chemical analog of sulfur, is known to cause developmental deformities among vertebrates, and the inhabited environment of the fish was exposed to elevated selenium loads, the potential of selenium-induced deformities was raised.

Later in the summer, on August 3, 2007, the wildlife manager at the Upper Mud River Reservoir, Karen McClure, called WVDEP headquarters to discuss a deformed bluegill sunfish that had been turned in by an angler at the impoundment. The angler noted that the specimen was the second bluegill that he had caught that day with such deformities; consequently, he saved this second specimen for scientific study. DEP biologists collected the individual (Figure 4) and examined it for potential spinal kyphosis, a ventral bending of the cervical spine. Upon inspection, the biologists observed complete and symmetrical scaling overlying the spinal bend as well as a well pigmented integument (Figure 5). It was not conclusively determined if the anomaly was of developmental origin or injury related; however, the fish was retained for further analysis. Since then, biologists have continued electroshocking surveys of the fishes inhabiting the Upper Mud River Reservoir; and, in November, 2008, collected several individuals (largemouth bass) exhibiting various degrees of maxillae truncation (Figures 6). Anomalous fishes have not been observed at other, reference, impoundments (i.e. Plum Orchard Lake and Elk Fork Lake). Furthermore, developmental deformities among larval bluegill sunfishes are believed to be present, some having crainiofacial anomalies similar to those exhibited by the deformed adult largemouth basses (Figure 7). Independent verification of the larval fish assessments is currently being pursued.

It is now believed that a selenium-induced teratogenic developmental event has led to the deformities among largemouth basses in the Upper Mud River Reservoir. In support, a 12% (14 of 117 individuals examined) deformity rate among adult largemouth basses was revealed in recent (October 29, 2008, and November 5, 2008) electroshocking surveys of the impoundment. Contrastingly, a 0% (0 of 35 individuals examined) deformity rate of adult largemouth basses was observed in fishes collected from Plum Orchard Lake (October 29, 2008).

In summary, the Upper Mud River Reservoir continues to be the focus of selenium bioaccumulation studies undertaken by WVDEP biologists. Although the extent to which the wildlife populations inhabiting and residing around the impoundment can accrue bodily selenium and remain unaffected is still not fully known, it is likely that the first observations of selenium toxicity to the fish community have been made.

Figure 1. Bluegill sunfishes, *Lepomis macrochirus*, during spawming activities (photo taken 6/1/08).

Figure 2. Nest guarding male bluegill sunfish (photo taken 6/1/08).

Figure 3. Crainofacially deformed largemouth bass, *Micropterus salmoides* (photo taken 5/24/07).

Figure 4. Bluegill sunfish, *Lepomis macrochirus*, with spinal anomaly (photo taken 8/6/07).

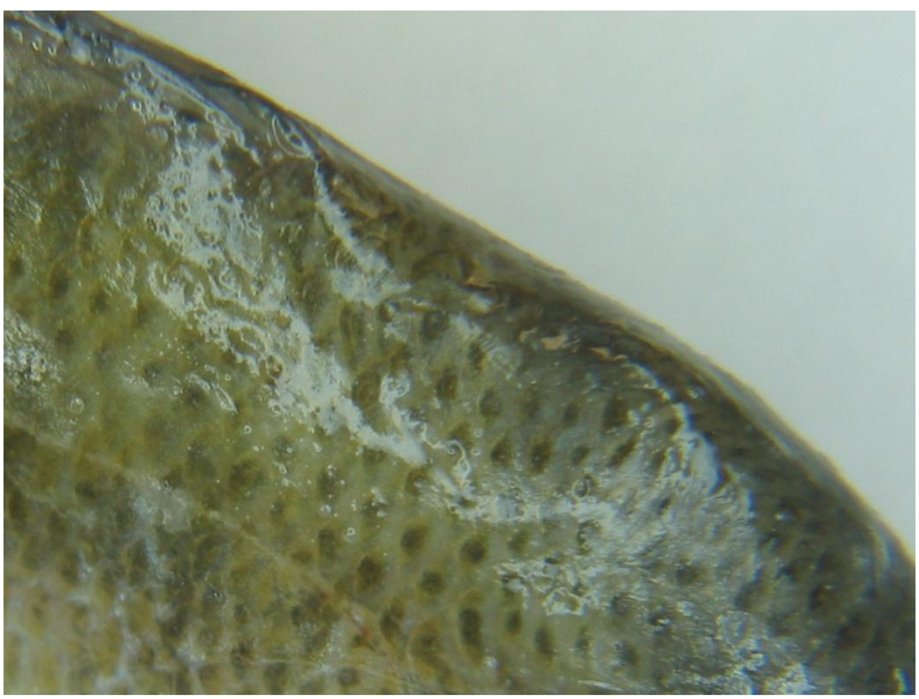


Figure 5. Scales overlying spinal bend in bluegill sunfish, *Lepomis macrochirus* (photo taken 8/6/07).

Figure 6. Largemouth bass, *Micropterus salmoides*, with truncated maxillae (photo taken 11/5/08).

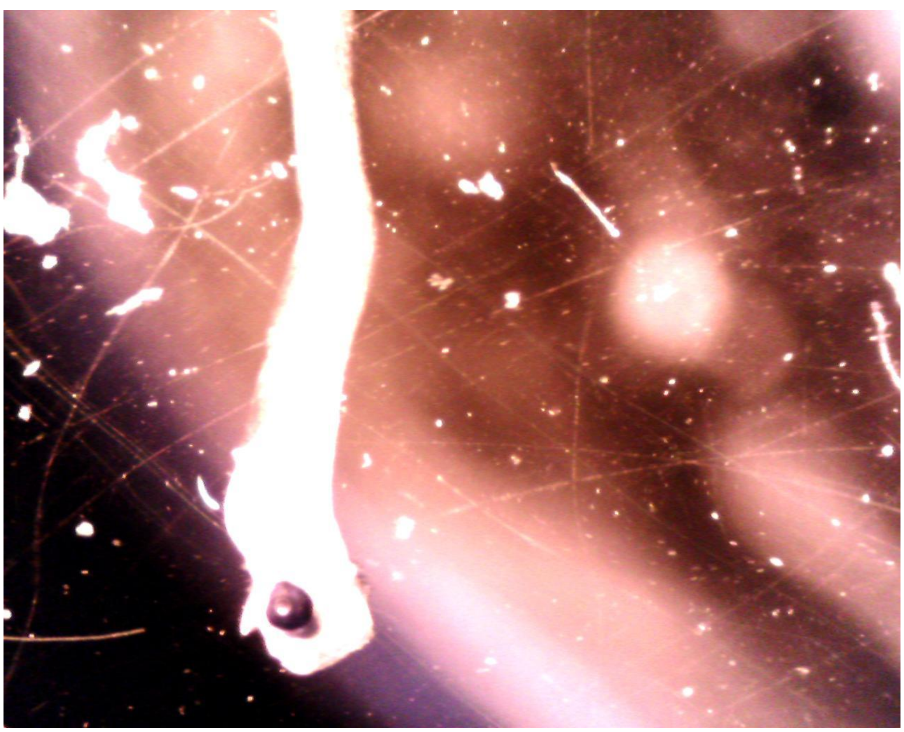


Figure 7. Larval bluegill sunfish, *Lepomis macrochirus*, with gaping jaw (photo taken 8/4/08).

MEMORANDUM

To: Patrick Campbell

From: Jeffrey Ginger

Date: December 10, 2009

Subject: Juvenile Black Bass Survey

Recent observations at the Upper Mud River Reservoir regarding selenium-induced deformities in adult black bass (*Micropterus spp.*) prompted a visual assessment survey throughout the impoundment. Typically, the right fork of Upper Mud River Reservoir has exhibited higher concentrations of selenium and poorer habitat. Selenium toxicity in the water body could potentially threaten fecundity and recruitment in fishes. The study concentrated on documenting the presence/absence of juveniles and multiple size classes of the bass to assess recruitment success. Anomalous and diseased fish were also documented as a measure of overall health. The study focused on game fish; therefore, other species were excluded from collection.

On November 12 and 16, 2009, DEP personnel conducted fish surveys at 10 sites in the Upper Mud River Reservoir in Lincoln County, West Virginia. Five sites from each fork, having similar habitats were sampled. Boat electro-fishing for 1,000 seconds at each site was employed to collect the fish, which were evaluated, measured, and returned to the reservoir, with very few retained as vouchers.

In general, habitat differed in each fork of the reservoir. The left fork contained more submerged vegetation; whereas, the right fork had more visible large woody debris. Overall, more fish were collected from the right fork (151 individuals) versus the left fork (137 individuals) and a larger number of juvenile black bass were collected in the right fork as well (Table 1). The majority of juveniles collected were spotted bass (*Micropterus punctalatus*) while the majority of the adult fish collected were largemouth bass (*Micropterus salmoides*). Hybridization between the two species was also evident. Most size/age classes of bass were represented in Upper Mud River Reservoir with more younger fish collected in the right fork. No young-of-the-year fish were collected. Approximately 5.2% of the fish collected were anomalous. The maxillary and spinoskeletal anomalies observed during the last sampling event were observed once again. Approximately 2.8% of the fish collected were diseased in some way.

At the present time, the population of black bass in the Upper Mud River Reservoir seems to exhibit stability based on the presence of juvenile fish and multiple size/age classes. Black bass anomalies occurring in this reservoir do, however, warrant further study with concentration on effects of selenium on the egg, larvae, and fry stages.

Respectfully,

Jeff Ginger

Table 1. Results of a Black Bass Survey on Selected Sites on the Upper Mud River Reservoir 11/12/09 & 11/16/09.

Site ID	Site Description	Latitude	Longitude	Fish Species	Size Class (mm)	# of Fish Collected	# Anomalous	# Diseased
#1	Left Fork	38 09 13.9	82 02 20.2	Largemouth Bass (LMB)	100-149	2	0	0
#1	Left Fork	38 09 13.9	82 02 20.2	LMB	150-199	4	0	1
#1	Left Fork	38 09 13.9	82 02 20.2	LMB	200-249	4	0	1
#1	Left Fork	38 09 13.9	82 02 20.2	LMB	300-349	3	0	2
#1	Left Fork	38 09 13.9	82 02 20.2	LMB	350-400	1	0	0
#1	Left Fork	38 09 13.9	82 02 20.2	Spotted Bass (SB)	200-249	1	0	0
#1	Left Fork	38 09 13.9	82 02 20.2	*Lepomis sp.*	100-149	8	0	0
#2	Left Fork	38 09 21.4	82 02 26.2	LMB	150-199	3	1	0
#2	Left Fork	38 09 21.4	82 02 26.2	LMB	200-249	1	0	0
#2	Left Fork	38 09 21.4	82 02 26.2	LMB	250-299	1	0	0
#2	Left Fork	38 09 21.4	82 02 26.2	LMB	350-400	3	0	0
#2	Left Fork	38 09 21.4	82 02 26.2	SB	250-299	1	0	0
#2	Left Fork	38 09 21.4	82 02 26.2	*Lepomis sp.*	100-149	5	0	0
#3	Left Fork	38 09 29.7	82 02 26.9	LMB	150-199	9	0	0
#3	Left Fork	38 09 29.7	82 02 26.9	LMB	200-249	3	0	0
#3	Left Fork	38 09 29.7	82 02 26.9	LMB	250-299	4	0	0
#3	Left Fork	38 09 29.7	82 02 26.9	LMB	300-349	3	0	0
#3	Left Fork	38 09 29.7	82 02 26.9	LMB	350-399	1	0	0
#3	Left Fork	38 09 29.7	82 02 26.9	LMB	450-499	1	0	0
#3	Left Fork	38 09 29.7	82 02 26.9	*Lepomis sp.*	100-149	3	0	0
#4	Left Fork	38 09 30.9	82 02 39.5	LMB	50-99	1	0	0
#4	Left Fork	38 09 30.9	82 02 39.5	LMB	100-149	1	0	0
#4	Left Fork	38 09 30.9	82 02 39.5	LMB	150-199	17	0	1
#4	Left Fork	38 09 30.9	82 02 39.5	LMB	200-	12	0	1

Site ID	Site Description	Latitude	Longitude	Fish Species	Size Class (mm)	# of Fish Collected	# Anomalous	# Diseased
					249			
#4	Left Fork	38 09 30.9	82 02 39.5	LMB	250-299	4	0	0
#4	Left Fork	38 09 30.9	82 02 39.5	LMB	300-349	4	1	0
#4	Left Fork	38 09 30.9	82 02 39.5	LMB	350-400	4	0	2
#4	Left Fork	38 09 30.9	82 02 39.5	*Lepomis sp.*	150-199	6	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	LMB	50-99	1	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	LMB	150-199	3	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	LMB	200-249	6	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	LMB	250-299	4	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	LMB	300-349	4	1	0
#5	Left Fork	38 09 36.7	82 02 46.6	LMB	350-400	2	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	LMB	400-449	1	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	*Lepomis sp.*	150-199	5	0	0
#5	Left Fork	38 09 36.7	82 02 46.6	SB	50-99	1	0	0
#6	Right Fork	38 08 26.6	82 02 42.7	LMB	300-349	3	1	0
#6	Right Fork	38 08 26.6	82 02 42.7	LMB	350-400	1	1	0
#6	Right Fork	38 08 26.6	82 02 42.7	LMB	450-499	2	0	0
#6	Right Fork	38 08 26.6	82 02 42.7	*Lepomis sp.*	150-199	5	0	0
#7	Right Fork	38 08 31.8	82 02 54.0	LMB	100-149	1	0	0
#7	Right Fork	38 08 31.8	82 02 54.0	LMB	150-199	4	0	0
#7	Right Fork	38 08 31.8	82 02 54.0	LMB	200-249	1	0	0
#7	Right Fork	38 08 31.8	82 02 54.0	LMB	300-349	1	0	0
#7	Right Fork	38 08 31.8	82 02 54.0	*Lepomis sp.*	100-149	1	0	0
#7	Right Fork	38 08 31.8	82 02 54.0	SB	50-99	9	0	0
#8	Right Fork	38 08 39.4	82 03 2.0	LMB	100-149	1	0	0
#8	Right Fork	38 08 39.4	82 03 2.0	LMB	150-199	4	1	0
#8	Right Fork	38 08 39.4	82 03 2.0	LMB	200-	5	0	0

Site ID	Site Description	Latitude	Longitude	Fish Species	Size Class (mm)	# of Fish Collected	# Anomalous	# Diseased
					249			
#8	Right Fork	38 08 39.4	82 03 2.0	LMB	250-299	1	0	0
#8	Right Fork	38 08 39.4	82 03 2.0	LMB	300-349	2	1	0
#8	Right Fork	38 08 39.4	82 03 2.0	LMB	450-499	1	0	0
#8	Right Fork	38 08 39.4	82 03 2.0	*Lepomis sp.*	150-199	3	0	0
#8	Right Fork	38 08 39.4	82 03 2.0	*Micropterus spp*. (juveniles)	50-99	4	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	50-99	9	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	100-149	3	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	150-199	1	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	200-249	8	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	250-299	15	3	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	300-349	8	1	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	350-399	4	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	LMB	400-449	3	2	0
#9	Right Fork	38 08 42.9	82 03 22.5	*Lepomis sp.*	≈150	3	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	*SB*(juveniles)	0-49	1	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	*SB*(juveniles)	50-99	21	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	*Micropterus spp*. (juveniles)	100-149	2	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	*Micropterus spp*.	150-199	1	0	0
#9	Right Fork	38 08 42.9	82 03 22.5	*Micropterus spp*.	300-349	1	0	0
#10	Right Fork	38 08 54.3	82 03 28.8	LMB	100-149	3	0	0
#10	Right Fork	38 08 54.3	82 03 28.8	LMB	150-199	1	0	0
#10	Right Fork	38 08 54.3	82 03 28.8	LMB	200-249	4	0	0
#10	Right Fork	38 08 54.3	82 03 28.8	LMB	250-299	2	0	0
#10	Right Fork	38 08 54.3	82 03 28.8	LMB	300-349	1	2	0
#10	Right Fork	38 08 54.3	82 03 28.8	LMB	350-399	5	0	0
#10	Right Fork	38 08 54.3	82 03 28.8	*Lepomis sp.*	170	1	0	0
#10	Right Fork	38 08 54.3	82 03 28.8	SB	184	1	0	0

Site ID	Site Description	Latitude	Longitude	Fish Species	Size Class (mm)	# of Fish Collected	# Anomalous	# Diseased
#10	Right Fork	38 08 54.3	82 03 28.8	SB(juveniles)	50-99	4	0	0