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May 4, 2012

Dr. Neil Carriker
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Tennessee Valley Authority Kingston Ash Recovery Project
Kingston, Tennessee

Subject: Preliminary Evaluation of 2011 ORNL Results: *Fish Reproduction*

Dr. Carriker,

I am pleased to submit to you a preliminary evaluation of 2011 *Fish Reproduction* Task results. The attached memorandum covers the spring 2011 fish collections in support of the TVA Kingston Ash Recovery Project from six locations in the Emory and Clinch Rivers. This personal communication is meant to provide a provisional assessment of the reproductive condition of fish collected at the beginning of the spring 2011 breeding season based on ongoing analyses of these samples. Data and tentative conclusions based on these data should be considered preliminary prior to the issuance of a draft report concerning these samples.

We hope this preliminary update is useful to you. If you have any questions or require additional information, please call me at (865) 574-9387.

Sincerely,

Mark S. Greeley, Jr.
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cc: M. J. Peterson, ORNL
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Preliminary Evaluation of 2011 ORNL Results:
Fish Reproduction Studies

Mark S. Greeley, Jr., ORNL

May 4, 2012

Introduction

On December 22, 2008, a dike containing fly ash and bottom ash at the Tennessee Valley Authority's (TVA) Kingston Fossil Plant in East Tennessee failed and released a large quantity of ash into the adjacent Emory River. A byproduct of coal combustion, coal ash contains a variety of metals and other elements, particularly selenium, with known effects on the reproduction and early development of exposed fish populations (Lemly 1993; Besser and others 1996). For instance, female fish in a coal ash-contaminated lake have been shown to have increased frequencies of atretic oocytes (dead or damaged immature eggs) and reductions in the overall numbers of developing oocytes (Sorensen 1988) associated with elevated body burdens of selenium. Similarly, a number of studies have reported increased incidences of developmental abnormalities in larval fish exposed to selenium through maternal transfer to the developing eggs either in the laboratory or in contaminated bodies of water (Woock and others 1987, Lemly 1999, Jezierska and others 2009), including sites where coal ash from power plants is the primary source of selenium contamination.

In order to address the potential risks of the fly ash spill to the reproduction of downstream fish populations in the Emory and Clinch Rivers, females from select sentinel fish species have been sampled from multiple locations in these river systems at the beginning of each spring breeding season from 2009 through 2012. Results of the 2009 and 2010 studies are reported in Greeley and others (2012). Preliminary results of the spring 2011 fish collections are presented in the current memorandum, while laboratory analyses of these samples are ongoing. Spring 2012 fish collections for fish reproduction, fish health, and fish bioaccumulation studies were recently completed and samples currently await analysis.

A significant finding of the 2009 – 2010 reproductive studies reported in Greeley and others (2012) was that the relative sizes of ovaries and developing oocytes in bluegill sunfish were significantly reduced at the Emory River fly ash spill site in the spring of 2010 as compared with other study sites, particularly reference sites located upstream of the spill. Since oocyte atresia was not increased in fish at this location in 2009, this apparent delay in ovarian development at the spill site was probably not the result of direct chemical toxicity of fly ash constituents to the developing oocytes but instead due to the substantial habitat alterations and food chain disruptions that significantly impacted this river reach during this first breeding season following the spill. Other than this apparent developmental delay at the spill site, there were no other significant reproductive abnormalities noted in female bluegill sunfish from any of the study sites sampled in 2009 or from sites sampled in the spring of 2010. Similarly, there was also no conclusive evidence of adverse reproductive effects in female largemouth bass sampled from sites downstream of the fly ash spill in either 2009 or 2010, or in redear sunfish sampled from these same sites in 2010.

Preliminary Results

Largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), and redear sunfish (*Lepomis microlophus*) were sampled in the spring of 2011 from six locations in the Emory and Clinch Rivers, including both upstream reference sites (ERM 8.0, LERM 2.0, and ERM 8.0) as well as downstream sites directly influenced by the spill (ERM 3.0, ERM 0.9, and CRM 1.5) (Fig. 1). All three fish species are multiple spawners, capable of producing several clutches of eggs over a period of a few days through a few weeks. To maximize comparability of reproductive condition across the study sites, fish were collected in April and May immediately prior to or during the first spawn of the season. For each fish sampled, the gonadsomatic index (GSI) − or relative size of the gonad to the fish body − was calculated, and weighed portions of the ovary were saved for later morphometric analysis of oocyte stages and condition and to estimate fecundity.

As the laboratory analyses of 2011 ovary samples are still ongoing, this preliminary evaluation of the relative reproductive condition of female fish at the various study sites in the spring of 2011 is based primarily on the gross morphological appearance of fish and ovary samples and on the GSI as an indicator of reproductive condition. It should be noted that all female fish collected from the Emory and Clinch Rivers in the spring of 2011, regardless of species or location, were reproductively mature based on ovarian size and appearance and overall degree of oocyte development. However, the specific reproductive status of sampled fish within the breeding population – whether pre-spawning, in the midst of a spawn, or immediately post-spawn − did vary substantially from site to site (Table 1). Fish collected prior to the first spawn of the season were characterized by relatively large ovaries and lacked obvious signs of recent spawning activity such as reddened and enlarged gonadal vents, while fish collected during or just after this first spawn still had relatively large but somewhat flaccid ovaries and roughened and red gonadal vents.

Percentages of fish considered to be pre-spawning in the spring of 2011 differed considerably from site to site and from species to species. In the case of bluegill sunfish, percentages of pre-spawning females ranged from 0% in samples from CRM 1.5 to 100% at ERM 8.0 (Table 1). Similar variation in specific reproductive status was observed in the largemouth bass samples, with no pre-spawning fish seen in samples from two study sites (LERM 2.0 and ERM 8.0) as compared with 75-88% pre-spawning fish in samples from the other four study sites. The reproductive status of redear sunfish was by contrast similar across all study sites (Table 1).

Average GSIs of fish sampled in the spring of 2011 differed significantly between some study sites. For instance, the GSIs of bluegill sunfish at CRM 1.5 were significantly lower than at the other study sites (Table 2), apparently due to all fish sampled from this site having spawned prior to sample collection. Similarly, the average GSIs of largemouth bass at two study locations (LERM 2.0 and ERM 8.0) were also significantly lower than at the other four sampling locations, again due to fish spawning at these sites prior to sample collection. In contrast, the GSIs of redear sunfish demonstrated little site to site variation in these spring 2011 samples.

Although the GSI is a widely-used and easily measured indicator of fish reproductive status and environmental stress (Nikolsky 1963), it is evident from these results and elsewhere (Greeley 2002) that it is best suited for use as part of a larger suite of reproductive indicators since it provides only limited data on the specific reproductive condition of the gonads.

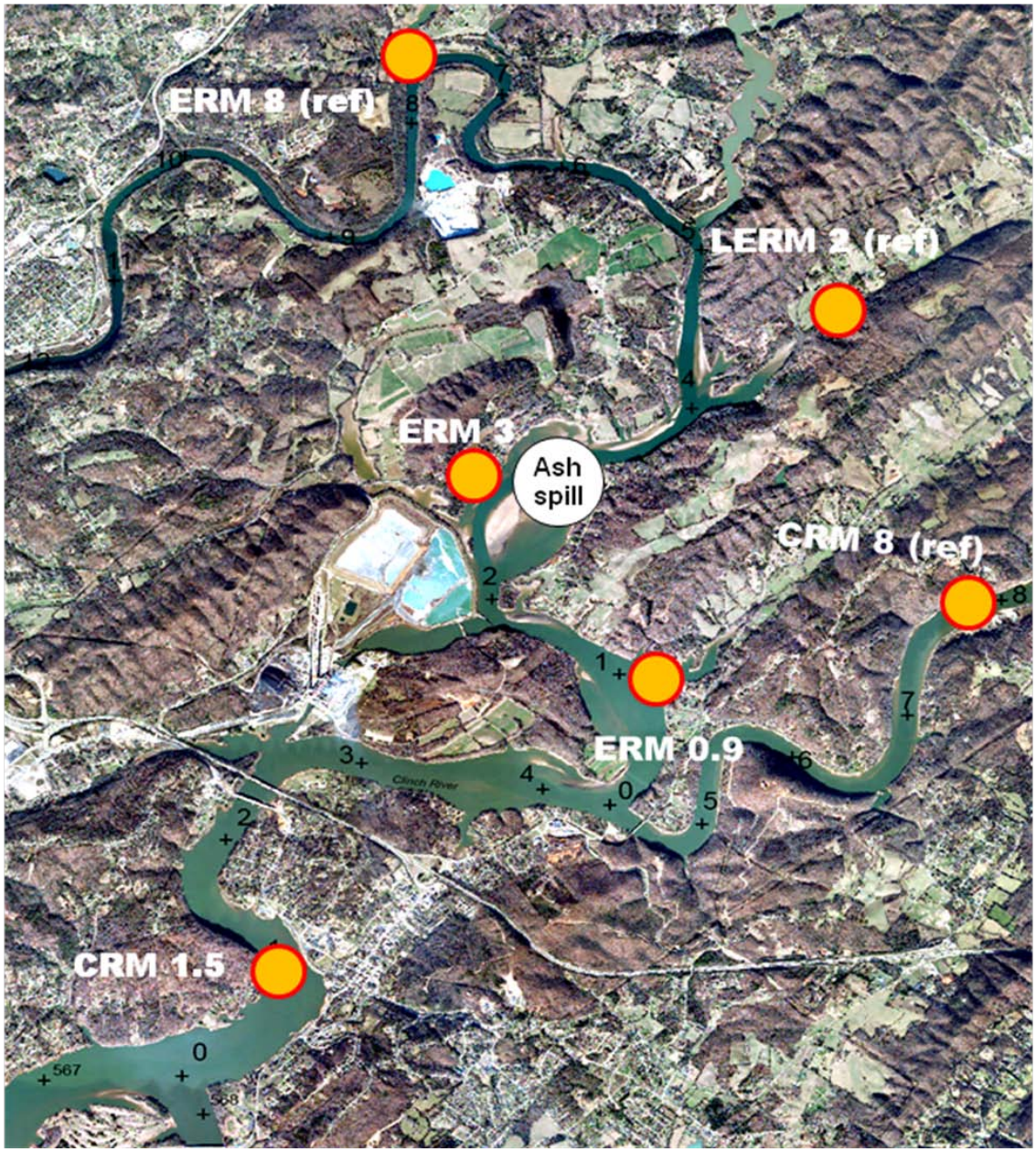

Figure 1. Map showing fish sampling locations in the Emory and Clinch Rivers for the Fish Reproduction Task. ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile (sampled only in 2010).

Table 1. Spawning status[a] of fish collected at the beginning of the spring 2011 breeding season from study locations in the Emory and Clinch Rivers. Includes fish from river reaches affected by fly ash (ERM 3.0, ERM 0.9, and CRM 1.5) as well as upstream reference sites (ERM 8.0 and CRM 8.0)[b]

	Spawning Status (% of sampled fish considered to be pre-spawn)		
Location	*Bluegill sunfish*	*Largemouth bass*	*Redear sunfish*
LERM 2.0	67	0	80
ERM 8.0	100	0	75
ERM 3.0	60	88	63
ERM 0.9	55	75	38
CRM 8.0	80	75	89
CRM 1.5	0	88	50

[a] Pre-spawning fish do not have the enlarged and reddened papillae of fish that have begun spawning nor ovaries with shed follicles
[b] ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile

Table 2. Gonadosomatic indices (GSIs) of fish collected at the beginning of the spring 2011 breeding season from study locations in the Emory and Clinch Rivers. Includes fish from fly ash-affected river reaches (ERM 3.0, ERM 0.9, and CRM 1.5) as well as upstream reference sites (ERM 8.0 and CRM 8.0)[a]

Location	2011 GSIs (means ± SEM)		
	Bluegill sunfish (n =7-11)	*Largemouth bass (n = 8)*	*Redear sunfish (n=8-10)*
LERM 2.0	7.8 ± 0.5	5.2 ± 0.3[c]	9.2 ± 0.7
ERM 8.0	9.2 ± 0.7	4.3 ± 0.3[c]	8.7 ± 1.0
ERM 3.0	7.5 ± 0.6	9.1 ± 0.9	8.6 ± 1.2
ERM 0.9	7.1 ± 0.6	8.7 ± 0.7	6.0 ± 0.8
CRM 8.0	8.3 ± 0.6	8.8 ± 0.9	7.7 ± 0.5
CRM 1.5	4.5± 0.3[b]	9.3 ± 0.7	8.2 ± 1.4

[a] ERM = Emory River Mile; CRM = Clinch River Mile; LERM = Little Emory River Mile

[b] Significantly lower at $\alpha = 0.05$ from GSIs at all other study sites

[c] Significantly lower at $\alpha = 0.05$ from GSIs at ERM 3.0, ERM 0.9, CRM 8.0, and CRM 1.5

Preliminary Conclusions

- All female fish collected during the spring 2011 fish reproduction, health, and bioaccumulation sampling event were reproductively mature based on the size and appearance of their ovaries.
- However, the reproductive condition of the sampled fish did vary from immediately pre-spawning through post-spawning, although all fish appeared capable of producing additional clutches of eggs during the remainder of the breeding season.
- The few statistically significant differences noted in the GSIs of sampled fish between study sites were apparently due primarily to differences in the pre- versus post-spawning status of bluegill sunfish and largemouth bass at these locations.
- There is no evidence from this preliminary evaluation of adverse reproductive effects or abnormalities in fish at sites influenced by the fly ash spill during the spring of 2011.

Additional Considerations and/or Uncertainties

The GSI provides only limited data as to the actual reproductive condition of the gonads. Completion of the morphometric analyses currently being conducted on ovarian samples from these fish, including the enumeration and staging of the developing oocytes and/or follicles and subsequent estimation of fecundity, is needed to accurately evaluate the reproductive condition of these spring 2011 fish samples.

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