Evaluation of Risks to Fish Reproduction from the Kingston Steam Plant Coal Ash Spill

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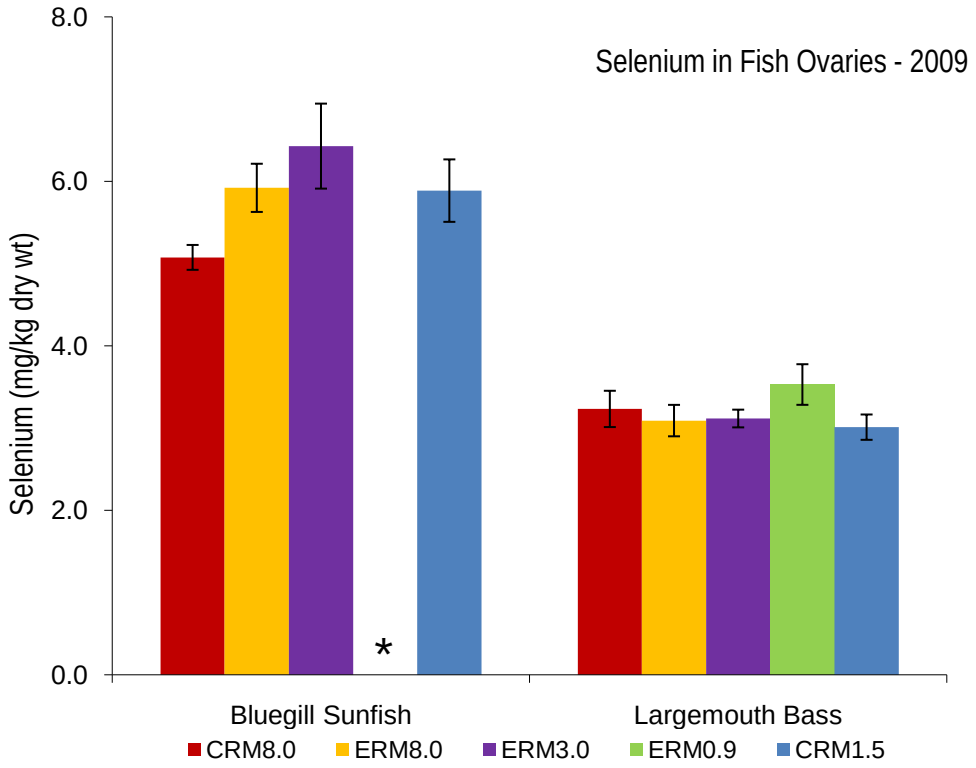
Contaminants in Fly Ash can Cause Reproductive Failure of Fish Populations

- Prolonged exposure to elevated selenium, a metal enriched in fly ash, has been shown to be capable of causing reproductive failure in effected fish populations
- Primary concern from selenium is maternal transfer from the adult females to their larvae
- Current selenium concentrations in fish ovaries from the Emory and Clinch Rivers are elevated in some species, but remain lower than a presumptive10 mg/kg threshold for reproductive impacts
- Other metals such as arsenic and mercury that could potentially effect fish reproduction are also enriched in fly ash



Note: Figure from poster by Fortner and Adams, this session; asterisk indicates insufficient ovary tissue from ERM 0.9 sunfish to conduct the analysis.

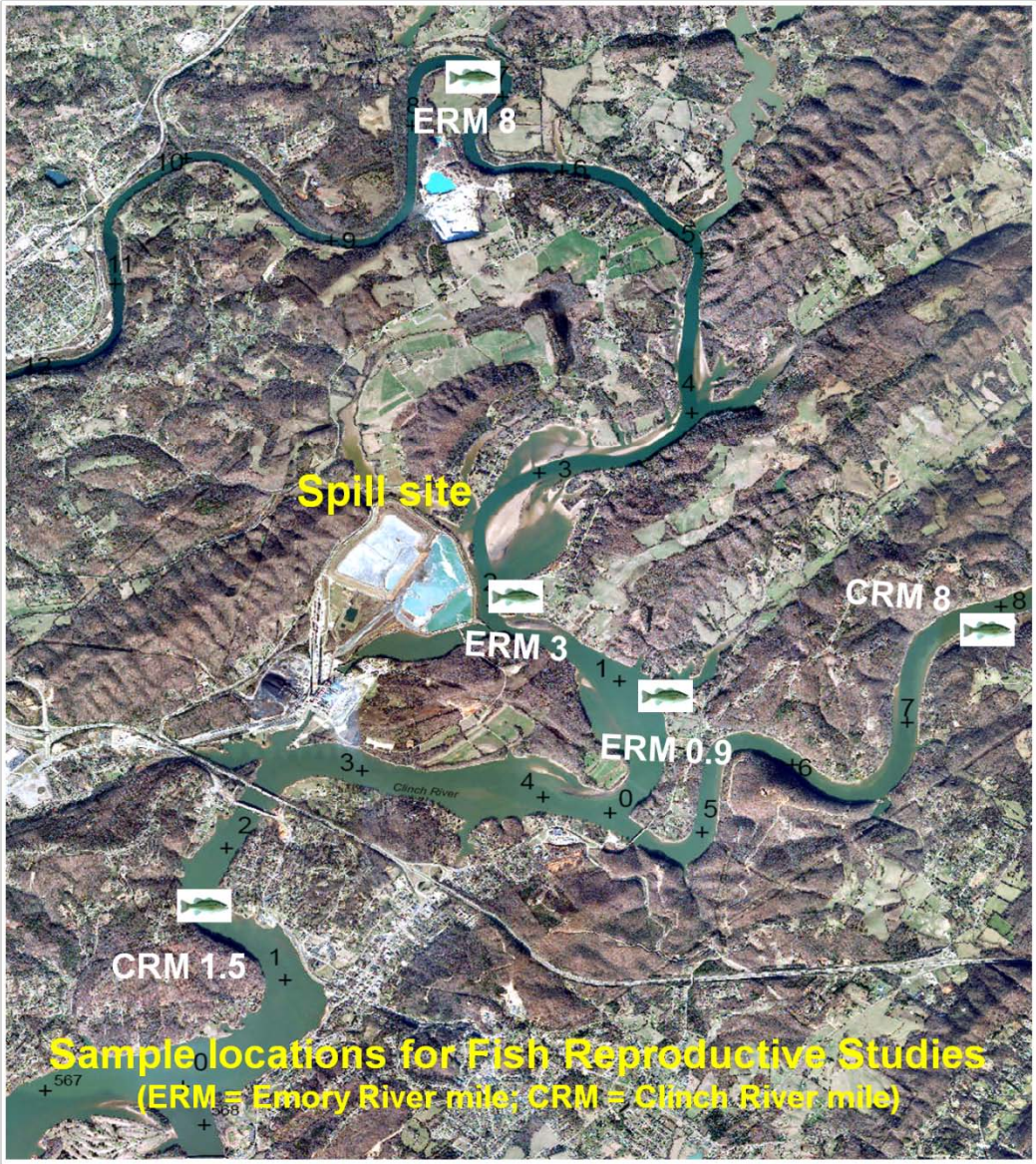
Outline: Evaluating Risks to Fish Reproduction from the Kingston Spill

- Field Evaluations of the Reproductive Condition of Fish in the Emory and Clinch Rivers following the Fly Ash Spill
  - Spring 2009
  - Spring 2010
- Short-term Laboratory Tests of the Effects of Direct Exposure to Fly Ash on Fish Early Development
- Long-Term Laboratory Studies of the Effects of Direct Exposure to Fly Ash on Fish Reproduction and Early Development
- Field Evaluation of Fish Larval Development in Emory and Clinch Rivers
- Potential Future Directions

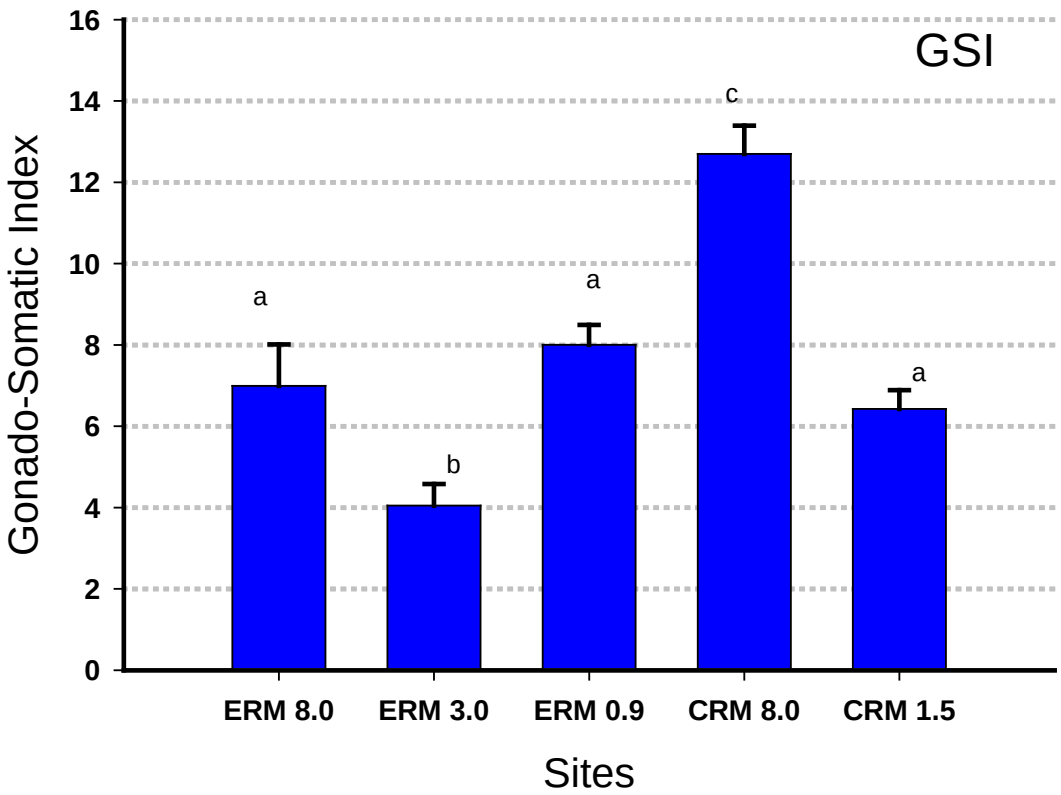
Field Studies of Fish Reproductive Condition: Study Design

- Conducted along with spring monitoring of fish health and bioaccumulation in 2009 and 2010 at five primary study sites (see following slide for locations)
- Female fish were sampled at the beginning of their respective breeding seasons
- Metal bioaccumulation was analyzed in ovaries and other tissues
- Batch fecundity and the quality of the developing eggs were also assessed
- Target sample sizes were 8 or more females of 3-5 fish species (bluegill sunfish, largemouth bass, white crappie, channel catfish, redear sunfish)

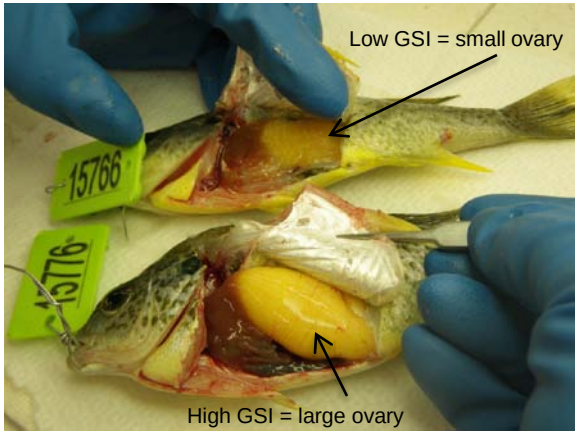
Study Sites for Field Evaluations of Fish Reproductive Condition



Site Comparisons of Bluegill Sunfish Reproductive Condition - Spring 2009: Ovary Size



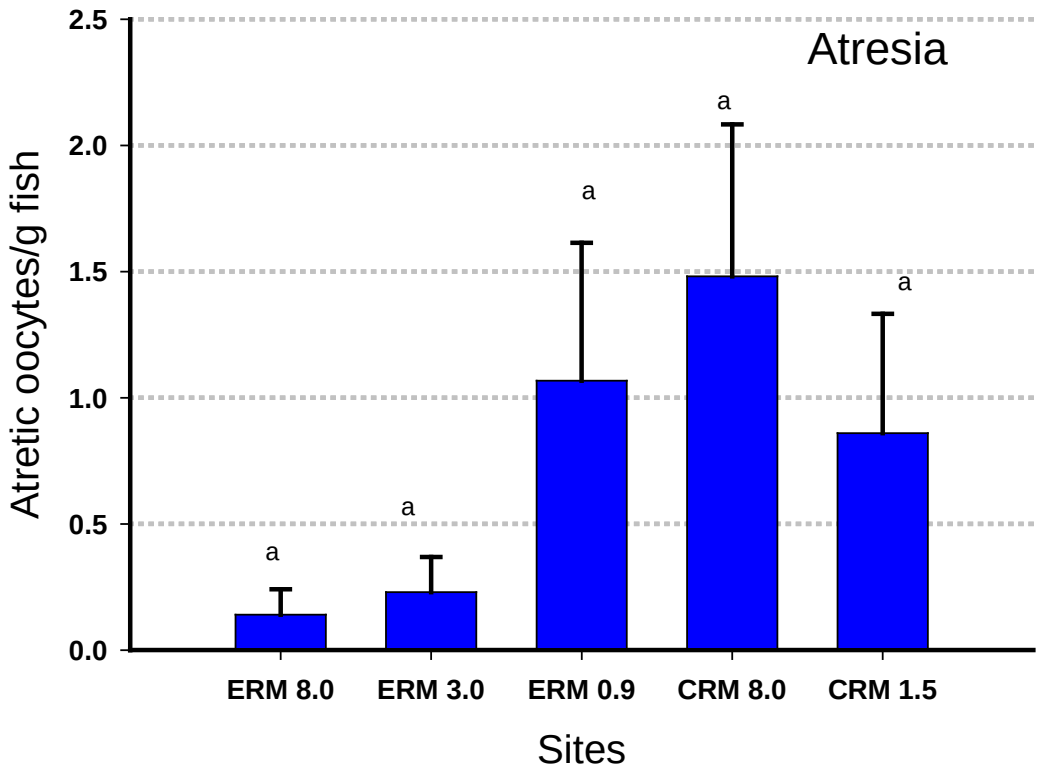
Bluegill Sunfish Photo by Richard T. Bryant

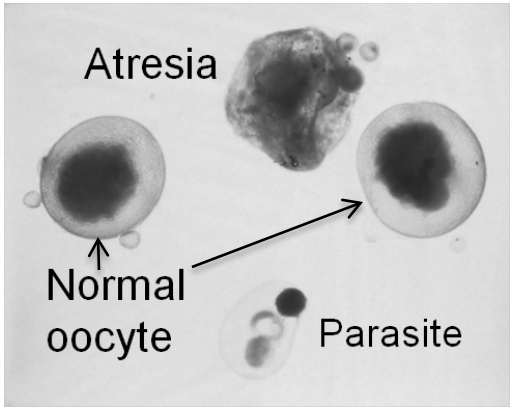


Examples above of sunfish with a high GSI (prespawning ovary) or a low GSI (immature ovary) are of redear sunfish, but bluegill sunfish ovaries are similar

Note: Letters above bars indicate statistical differences in site responses (for example, “a” is statistically different from “b”);
GSI = (ovary wt/ body weight) x 100;
ERM = Emory River Mile, CRM = Clinch River Mile.

Site Comparisons of Bluegill Sunfish Reproductive Condition - Spring 2009: Oocyte Viability

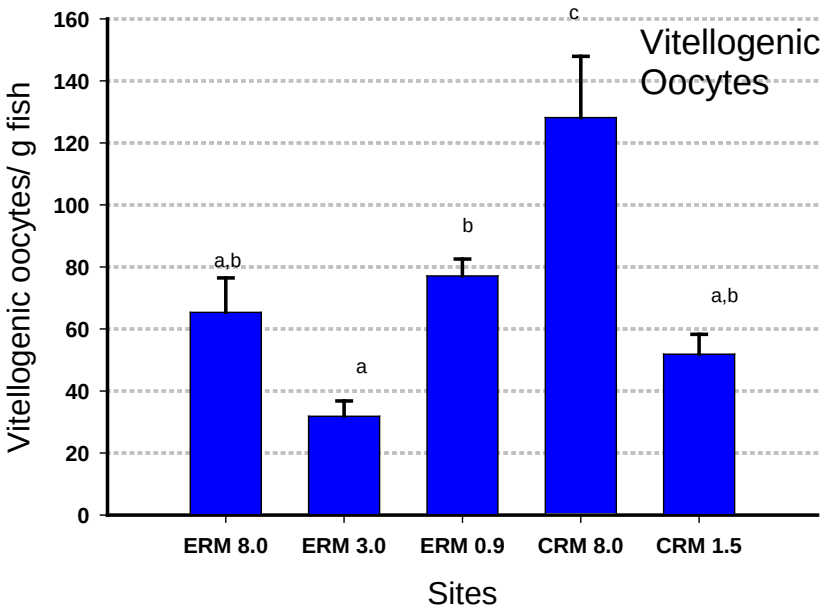


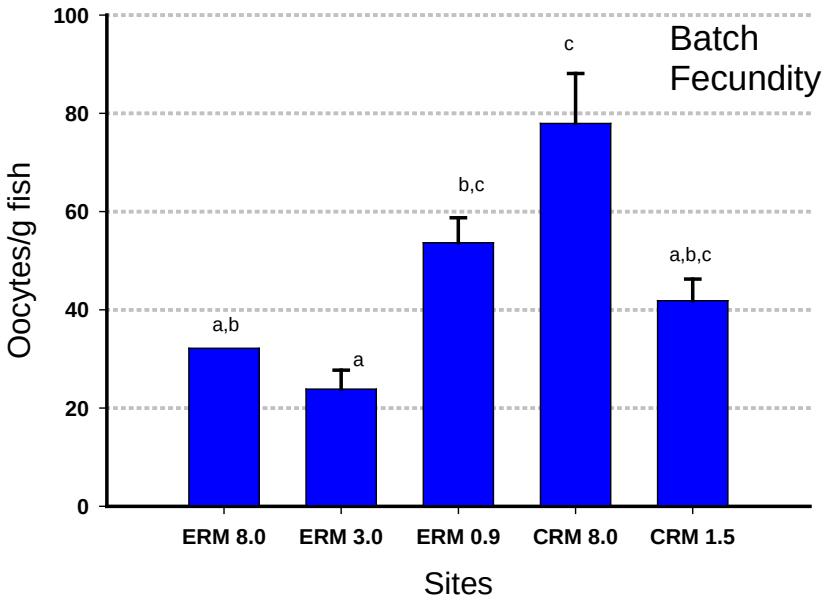


Picture of oocytes from a sunfish ovary illustrating the appearance of normal vitellogenic oocytes, an atretic oocyte, and a parasitic cyst present along with the oocytes within the ovarian tissue

Note: Letters above bars indicate statistical differences in site responses (for example, "a" is statistically different from "b"); atretic oocytes are dead or dying immature eggs (oocytes); ERM = Emory River Mile, CRM = Clinch River Mile.

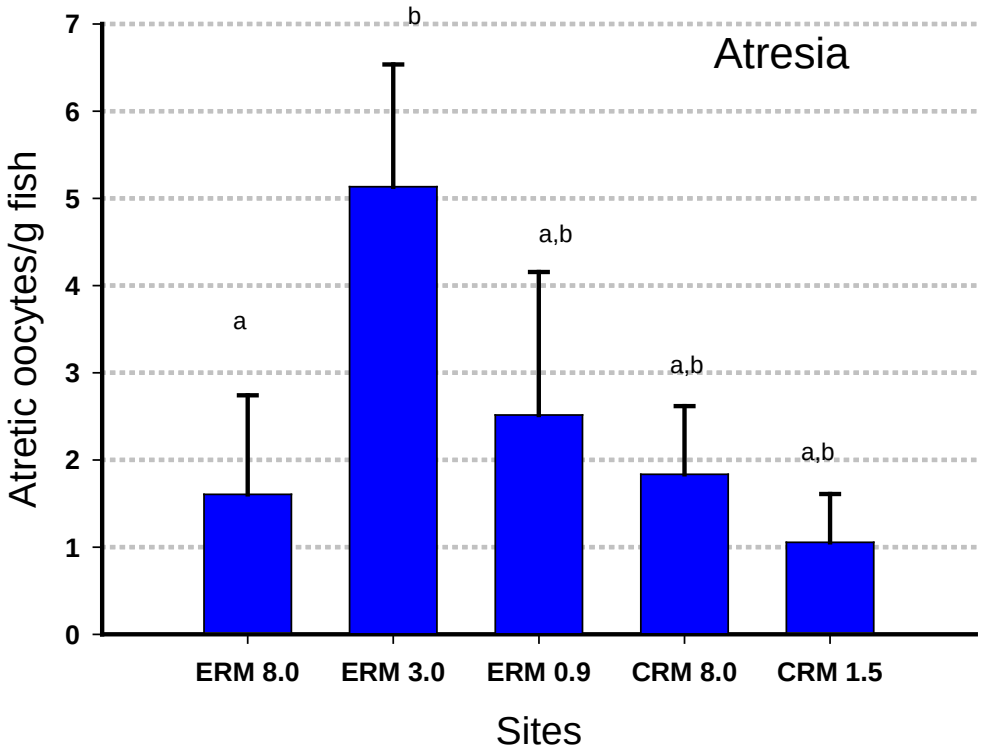
Site Comparisons of Bluegill Sunfish Reproductive Condition - Spring 2009: Fecundity





Note: Letters above bars indicate statistical differences in site responses (for example, “a” is statistically different from “b”);
vitellogenic oocytes = larger immature eggs in the process of accumulating yolk;
batch fecundity = number of vitellogenic oocytes in a leading clutch of developing oocytes destined to be spawned, or released from the ovary, at the same time, or alternatively the number of post-ovulatory follicles (the tissue layers wrapping the immature oocytes) left behind in a post-spawn ovary;
ERM = Emory River Mile, CRM = Clinch River Mile

Site Comparisons of Largemouth Bass Reproductive Condition - Spring 2009: Oocyte Viability



Largemouth Bass Photo by Richard T. Bryant

Note: Letters above bars indicate statistical differences in site responses (for example, “a” is statistically different from “b”);
atretic oocytes are dead or dying immature eggs;
ERM = Emory River Mile, CRM = Clinch River Mile

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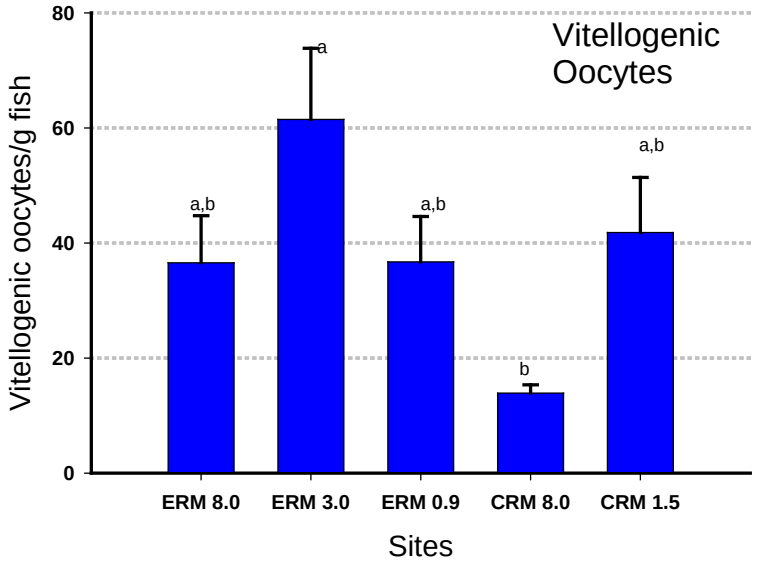
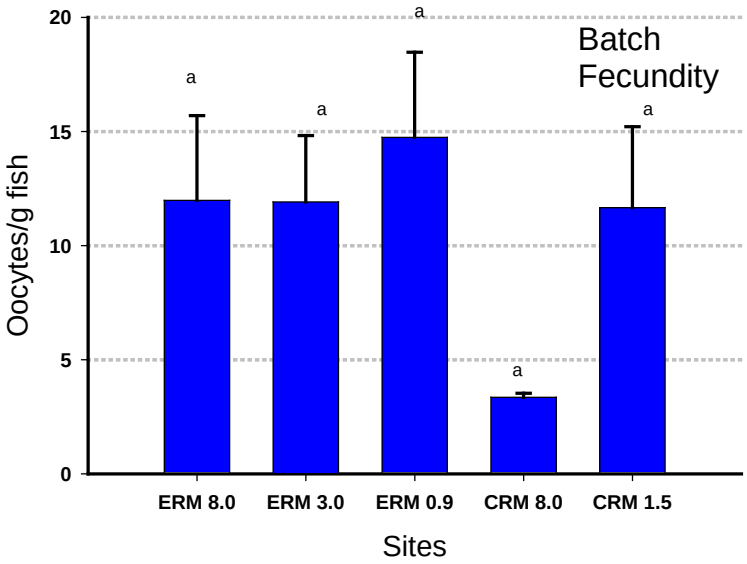
Site Comparisons of Largemouth Bass Reproductive Condition - Spring 2009: Fecundity





Note: Letters above bars indicate statistical differences in site responses (for example, “a” is statistically different from “b”);
vitellogenic oocytes = larger immature eggs in the process of accumulating yolk;
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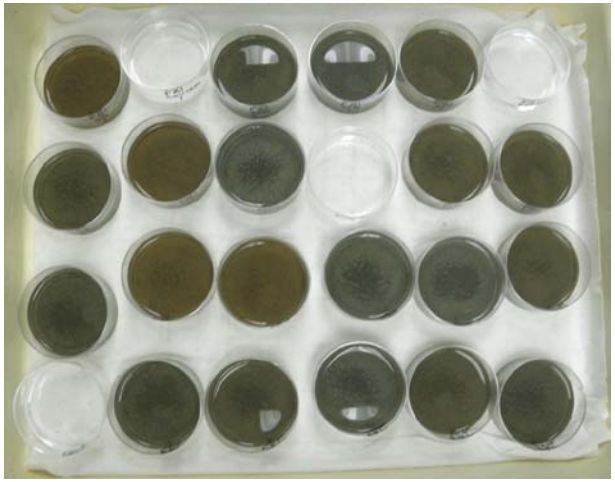
Field Studies of Reproductive Condition: Summary

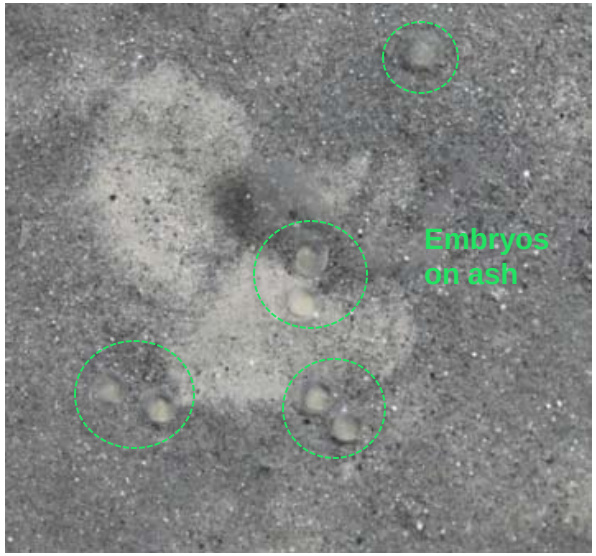
- Primary difference in the reproductive status of female bluegill at the spill site as compared to other study sites at the beginning of the 2009 breeding season was a slight delay in ovarian development as evidenced by smaller ovaries and fewer and less-developed vitellogenic oocytes
- Absence of a significant increase in oocyte atresia (one indicator of direct toxicity to the immature eggs) in bluegill sunfish at the spill site, along with the relative size-distributions of developing oocytes at the various study sites, suggests a probable bioenergetic rather than direct toxicity explanation for the observed reproductive delay at the spill site during the spring 2009 breeding season
- Although atresia was statistically higher in female largemouth bass at the spill site as compared to the upstream reference site in spring 2009, it was not elevated compared to other study sites
- Largemouth bass fecundity was similar among all the study sites in 2009
- In contrast to 2009, there were no significant differences in the reproductive condition of female bluegill sunfish upstream or downstream of the spill site at the beginning of the 2010 breeding season (*recently-acquired data not shown*)

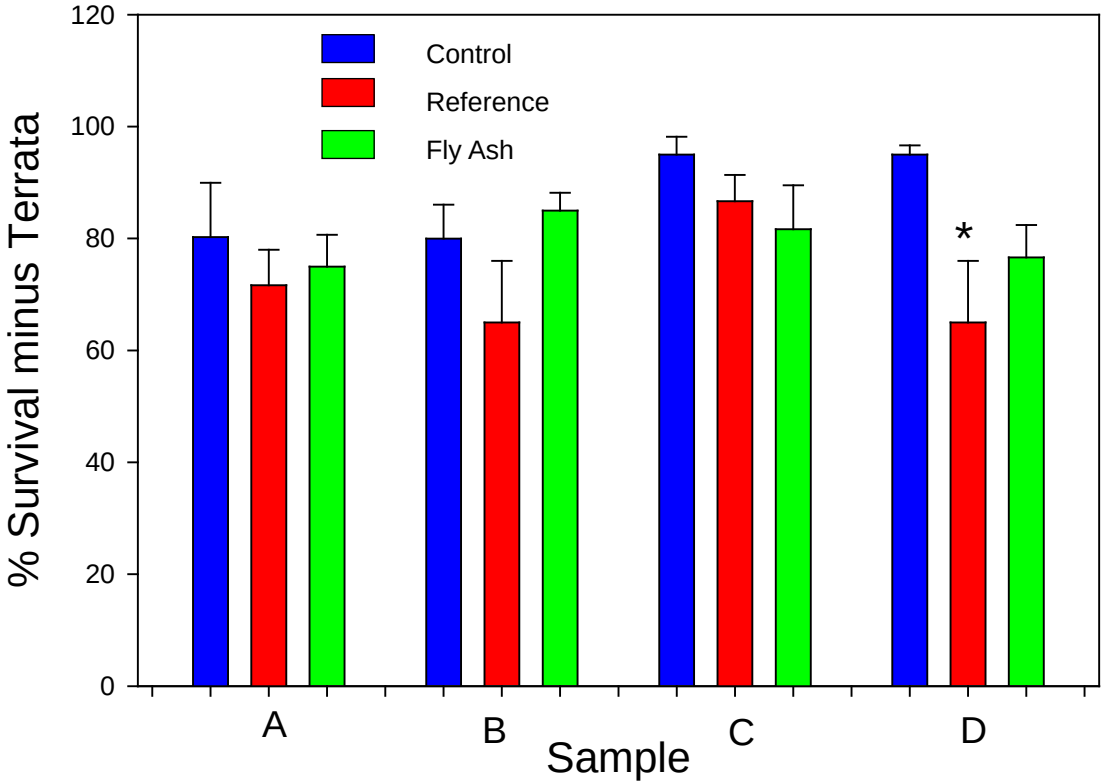
Short-term Laboratory Tests of the Effects of Fly Ash on Fish Early Development

- Tested effects of direct contact exposure to fly ash on fish early development in experimental exposures
- Tests were modified fathead minnow 7-day embryo-larval toxicity tests
- Samples included 4 sediment samples dredged from the Emory River consisting of various percentages of pure ash
- Control treatment consisted of filtered Emory River water collected from ERM 8.0



Results of 7-d Fathead Minnow Embryo-Larval Tests





No differences were observed between controls and fly ash-exposed embryos

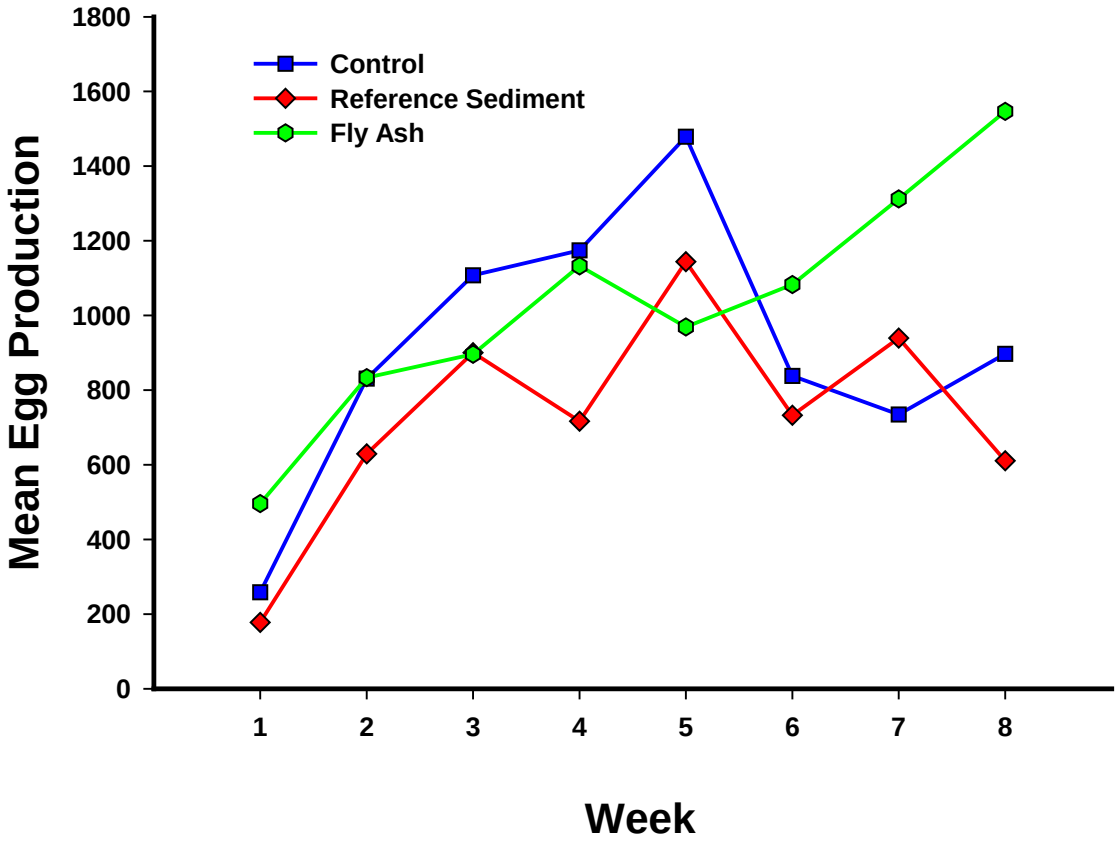
Note: Test based on EPA method 1001.0, modified for fly ash testing;
terrata = embryos/larvae with obvious developmental abnormalities;
in test, developmental abnormalities are assumed to eventually lead to death.

Long-term Experimental Exposures of Fish to Fly Ash in the Laboratory

- Evaluate the feasibility of conducting long-term laboratory exposures of fish to fly ash with reproduction as a target end-point (can fish survive and reproduce on fly ash?)
- Evaluate the chronic toxicity of direct exposures of adult fish and their young to ash collected from the original impoundment
- Bluegill sunfish and fathead minnows were species under consideration for these direct exposure studies and potential follow-up food-chain studies
- Direct exposures of fathead minnows to fly ash are currently underway (5 tanks each of controls, reference sediment and fly ash)
- Preliminary results of this ongoing experiment include measures of egg production and egg viability

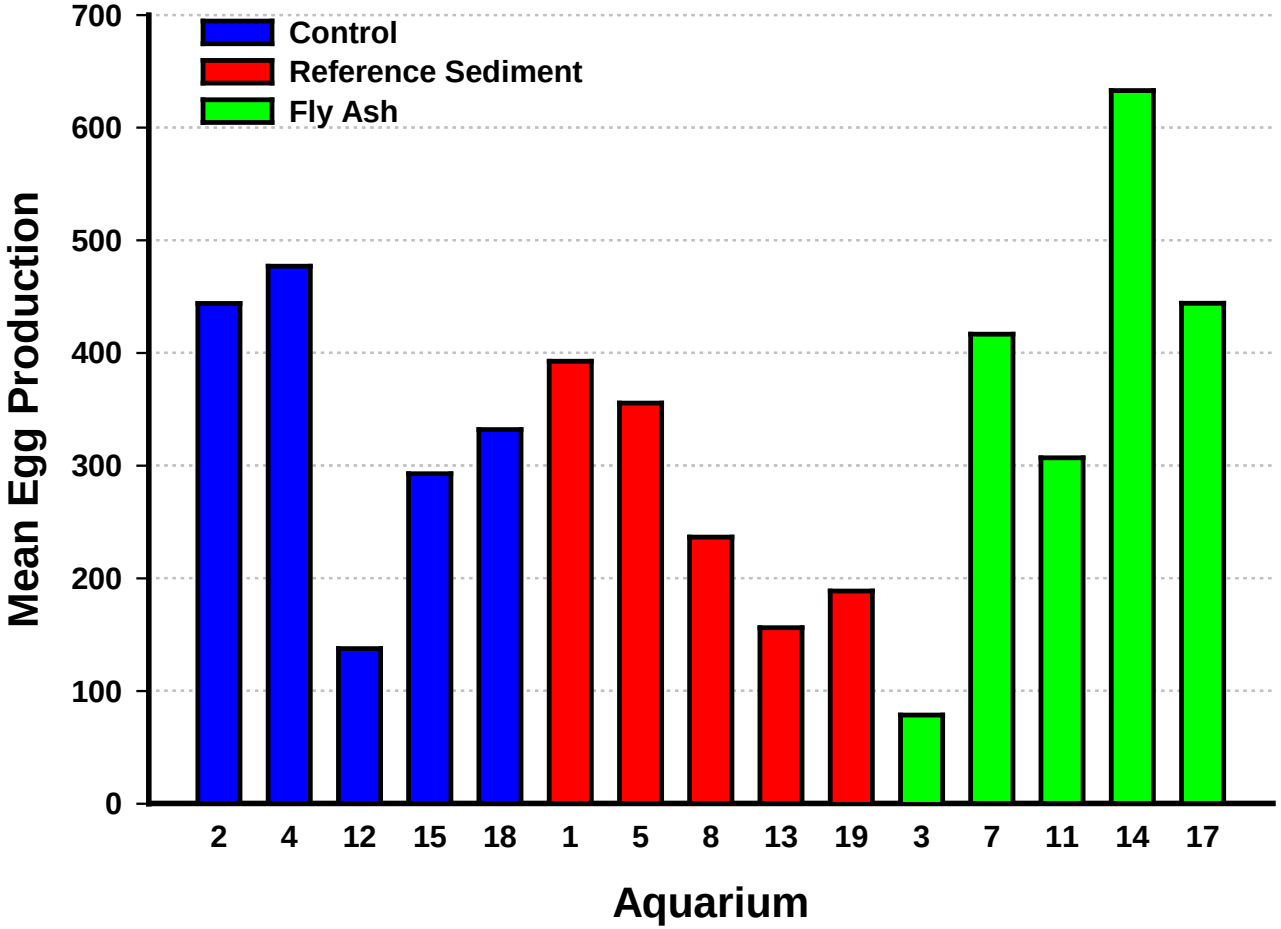
Weekly Egg Production per Treatment in Long-Term Fathead Minnow Exposures

(Preliminary Results of Ongoing Study)



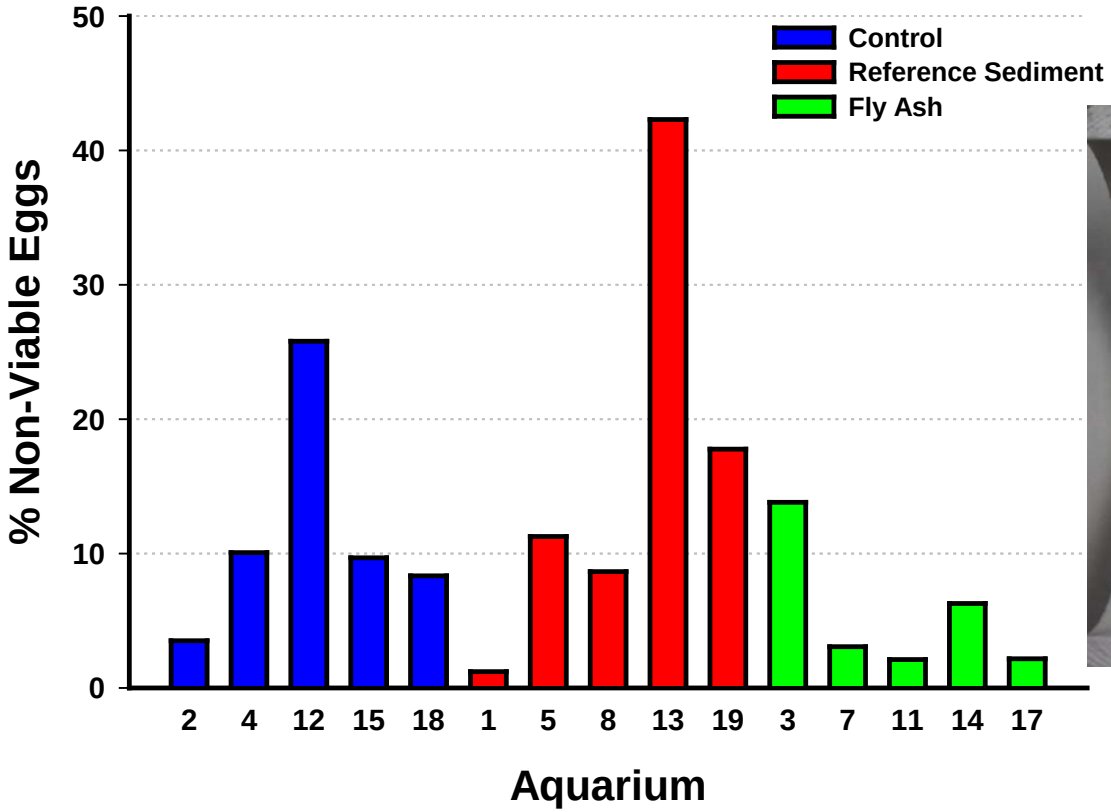
Egg Production per Aquaria per 2 to 3-Day Collection Period

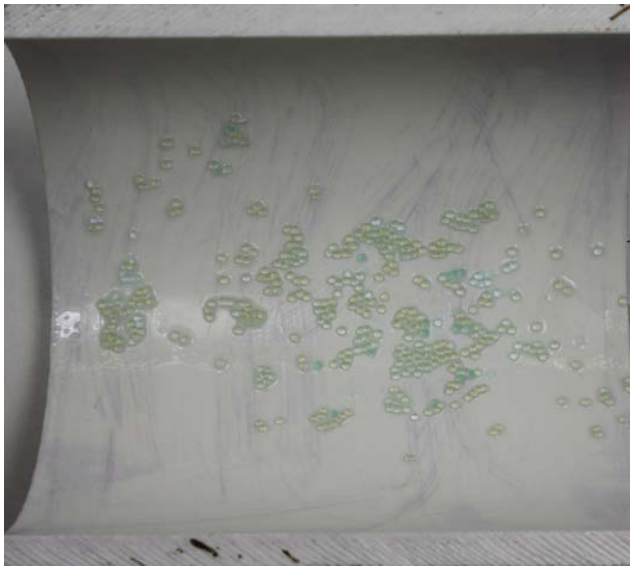
(Preliminary Results of Ongoing Study)



Percent Non-Viable Eggs

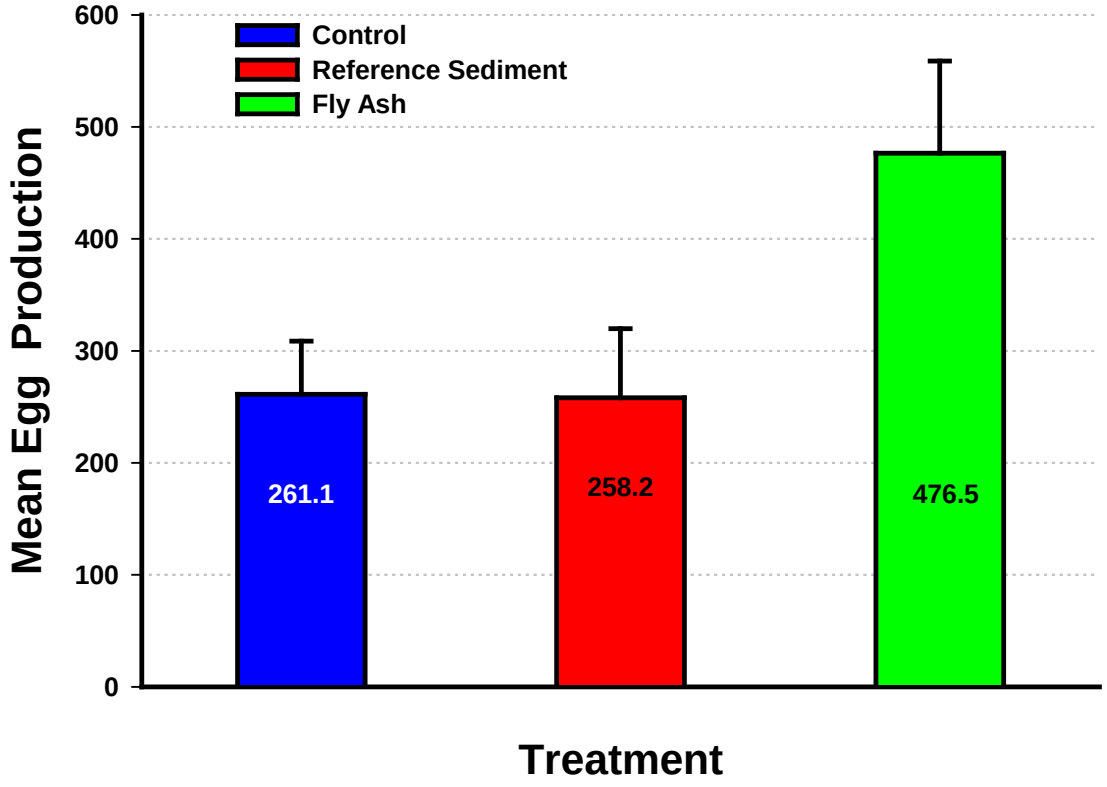
(Preliminary Results of Ongoing Study)





Mean Egg Production per Treatment

(Preliminary results of ongoing study)



% Terrata = 14% 16% 14%

Preliminary Conclusions from Short and Long-Term Fly Ash Exposure Studies

- Direct contact exposures to fly ash to date have had no apparent adverse effects on fathead minnow embryo-larval development
- Also no effects so far on adult fish survival during direct laboratory exposures to fly ash
- Laboratory exposures of adult fathead minnows may actually stimulate egg production
- No adverse effects observed to date on the viability of fertilized eggs
- No observable increases to date in developmental abnormalities
- Preliminary findings of this study suggest an absence of acute or chronic toxicity to fish from contact exposures to fly ash
- Exposures will continue for at least another 4 – 6 weeks following SETAC meeting
- Metals will be measured in adult fish tissues, eggs and exposure water at the conclusion of the study

Field Evaluation of Incidences of Developmental Abnormalities in Larval Fish

- Incidences of developmental abnormalities are also being assessed in larval fish sampled from the Emory and Clinch Rivers
- Adds a field-based food-chain component to current laboratory studies
- Sampling conducted by TVA biologists in spring 2010
- Focus is on the detection of non-lethal developmental abnormalities in fish larvae that are able to survive until sampling
- Target fish species (i.e. sunfish, bass, crappie) are the same as in fish health and reproductive condition studies
- Analyses of larvae samples from spring 2009 collections are ongoing

Potential Future Research Directions

- The primary reproductive risk to fish from the selenium in fly ash lies in maternal transfer through the eggs to the developing young
- Following assessments of the direct toxicity of fly ash to adult fish and embryos, focus is now shifting to potential food-chain effects
- As one approach to evaluate potential maternally-transferred metal toxicity, we will attempt to *in vitro* spawn wild fish (bluegill or redear sunfish) exposed to fly ash in the Emory River during the spring 2011 breeding season (preliminary studies were conducted in spring 2010)
- Additional follow-up laboratory studies involving food chain exposures to ash constituents are also under consideration

Redear sunfish

Acknowledgements

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K. Ward and L. Elmore

J. Tenney

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G. Morris