9-25-2013

Effects of 17β-Estradiol Exposure on Gamete Development and Viability in Freshwater Unionids

David Sovic
The Ohio State University

Kody F. Kuehnl
Franklin University, kody.kuehnl@franklin.edu

Roman Lanno
The Ohio State University

G. Thomas Watters

Effects of 17β-Estradiol Exposure on Gamete Development and Viability in Freshwater Unionids

David Sovic[1], Kody F. Kuehnl[2, 3], Roman Lanno[1,3]
1. Environmental Science Graduate Program, The Ohio State University, Columbus, OH
2. Department of Social and Natural Sciences, Franklin University, Columbus, OH
3. Dept. of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, OH

STUDY OBJECTIVES

1)To investigate the effects of 17β-estradiol (E2) exposure on gamete development and viability in Unionid mussels.

2)To determine the value of a nonlethal gonad biopsy in assessing reproductive development in Unionids.

3)To apply nonlethal methods of reproductive assessment to natural populations of Unionids (*Elliptio complanata, Pleurobema clava)*

4)To determine whether any correlations exist between the estrogenic potency of surface waters near mussel populations and atypical reproductive development in these populations.

5)To investigate the potential of Unionid hemolymph estrogenicity as a biomarker of estrogenic compound exposure.

EXPOSURE STUDY DESIGNS

Three groups of adult male and female *Lampsilis cardium* (n = 4/group/sex) were injected at two week intervals from October 2011 to April 2012 with 10 µL E2 at one of three exposure levels (540 ng/µL, 0.54 ng/µL, and 0.00054 ng/µL). Exposure was via direct injection of E2 solution into the foot. All controls were similarly injected with the pure solvent (200 proof Ethanol). Organisms were held in artificial raceways filled with clean gravel and fed by a continuous flow of water pumped from the Scioto River at the Columbus Zoo and Aquarium Freshwater Mussel Conservation and Research Center (Image 1). Every two weeks, organisms were pulled from the substrate, and valves were gently pried apart to expose the foot/visceral mass. Organisms were immediately returned to the substrate following each injection.

Similarly, three groups of adult male and female *Elliptio insulsa* (n = 5/group/sex) were injected twice (time = 0 and 5 days) with 10 µL E2 at one of three exposure levels (190 ng/µL, 0.19 ng/µL, and 0.00019 ng/µL) in October 2012. Controls were similarly injected with 10 µL solvent (27% Ethanol). Exposure setup and protocols were otherwise identical to those described above for *L. cardium*. At time = 10 days, organisms were pulled from the substrate and sampled for hemolymph and a biopsy of developing reproductive tissue.

FIELD STUDIES

E. complanata: The Cacapon River flows northeast through the Eastern Panhandle of West Virginia (WV). Agricultural activity is more concentrated along the upper reaches of the river (Site 1), while downstream reaches (Site 2) are primarily forested.

Site 1 (left)

Site 2 (right)

P. clava: Recent surveys of the East Fork West Branch St. Joseph River (SE Michigan) Indicated a complete lack of recruitment in this *P. clava* population. A "reference" population exhibiting successful reproduction is located in the Allegheny River near Tionesta, PA.

Site 3 (left)

Site 4 (right)

Image 1: Exposures are conducted at the Columbus Zoo and Aquarium Freshwater Mussel Conservation and Research Center

Image 2: Hemolymph is collected from the posterior adductor of *Elliptio insulsa*.

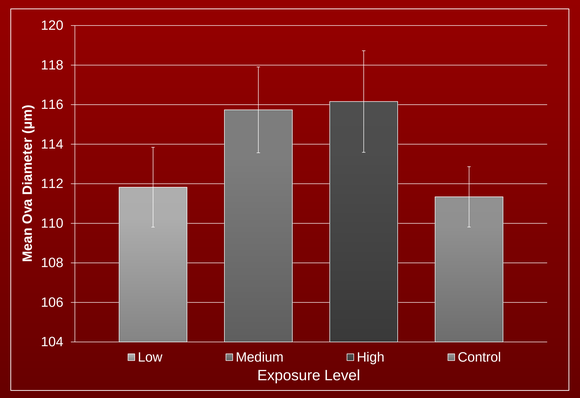

Figure 1. Mean ova diameters from *Elliptio insulsa* biopsies collected following a 10-day exposure to 17β-estradiol. Error bars represent standard error.

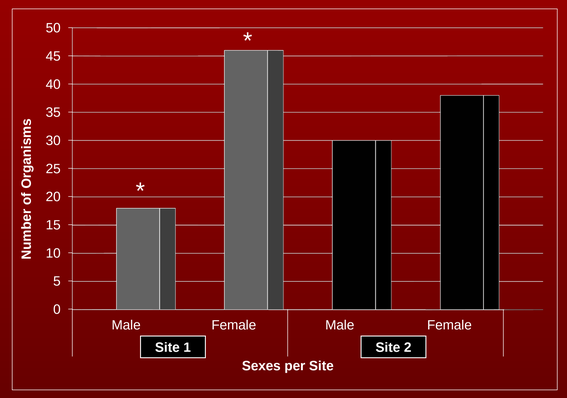

Figure 2. Gender differences observed within the two separate *E. complanata* populations (* indicates significant departure from 1:1).

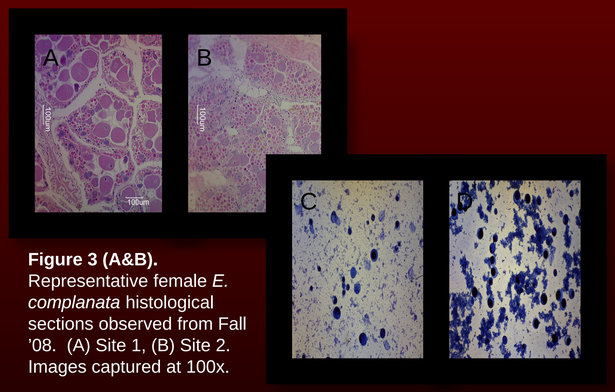

Figure 3 (A&B). Representative female *E. complanata* histological sections observed from Fall '08. (A) Site 1, (B) Site 2. Images captured at 100x.

Figure 3 (C&D). Representative female *E. complanata* tissue biopsy samples observed from Spring '10. (A) Site 1 (B) Site 2. Images captured at 40x.

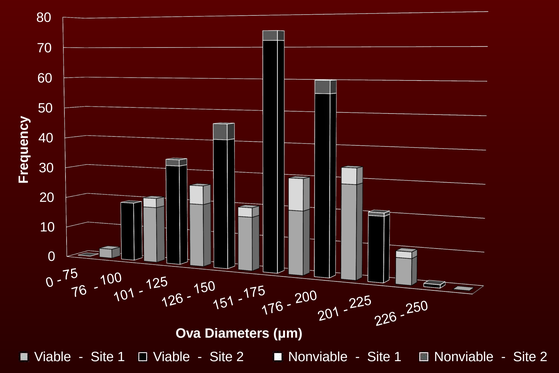

Figure 4. Frequency of viable and nonviable ova and corresponding diameters from gonad biopsies collected in Spring '10 (n = 13 and 11 biopsied females from Sites 1 & 2, respectively).

RESULTS & DISCUSSION

- Overall, high mortality was observed in all *L. cardium* exposure groups (>50%), including controls. It is speculated that this significant die-off may have been due, at least in part, to stress associated with repeated handling of organisms, as well as the use of pure Ethanol as the exposure medium, as this solvent may have been caustic to tissues.
- Mean ova diameters measured from biopsies of female *E. insulsa* appear to exhibit a pattern of increasing ova size with each exposure level (Figure 1), however no significant difference from control could be detected for any group (one-way ANOVA; $P = 0.20$, $\alpha = 0.05$).
- Significance was observed in F:M sex ratios between Site 1 (~ 2.5:1) and Site 2 (~1.3:1). (χ^2, $p = 0.0109$, $\alpha = 0.05$).
- When compared with an expected 1F:1M ratio, significant differences were observed for the Site 1 population (χ^2, $p = 0.0005$, $\alpha = 0.05$), while at Site 2, no difference could be detected . (χ^2, $p = 0.332$, $\alpha = 0.05$) (Figure 1).
- Gametogenic stage assessment for Fall '08 histological samples of *E. complanata* indicate differential reproductive developmental rates between the two populations. Subsequent biopsy analyses of reproductive tissues support this differential development (Figures 3, 4).
- Recent recruitment, as evidenced by observation of numerous small, 3 – 5 year old individuals, was found in the East Fork *P. clava* site. Biopsy of gonad tissue from this population revealed both mature and developing tissue, suggesting that this population, though small, is healthy and reproducing, at least to some extent.
- Extracts of Polar Organic Chemical Integrative Samplers (POCIS) deployed in surface waters near field study sites exhibited low, but variable, estrogenicity, as measured by the Yeast Estrogen Screen (YES). Response ranged from <1 – 7 ng/POCIS. No relationships have been detected between estrogenicity of passive sampler extracts and atypical reproductive development or sex ratios observed in the field (data not shown).
- Preliminary YES analyses of Unionid hemolymph suggest a possible anti-estrogenic activity associated with samples from *E. insulsa*. Further sampling is required to verify this initial observation.

FUTURE

1) Measure of sperm motility from male *E. insulsa* and *L. cardium* tissue biopsies following 10-day E2 exposure.
2) Spring 2013 re-sampling of individuals exposed in Autumn 2012 to evaluate potential effects of exposure on long-term gamete development.
3) Analysis of Unionid hemolymph throughout reproductive cycle, and laboratory investigation of hemolymph estrogenicity as a potential biomarker of EDC exposure.

ACKNOWLEDGEMENTS & CITATIONS

Thanks to The Ohio State Environmental Science Graduate Program, Department of Evolution, Ecology, and Organismal Biology, Department of Entomology, Center for Life Sciences Education, and faculty for the support and resources provided in completion of this work. In addition, thanks to the Robert H. Edgerley Environmental Toxicology Fellowship for the financial assistance and support provided. A portion of this research is also funded by a grant from the Great Lakes Restoration Initiative.