

## Comparative Vitellogenic Responses in Three Teleost Species: Extrapolation to *In situ* Field Studies

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### ABSTRACT

*Induction of vitellogenin (VTG) was compared among three teleostean species to determine their relative sensitivity of exposure to 17 $\beta$ -estradiol (E2). Japanese medaka (*Oryzias latipes*), hybrid striped bass (*Morone saxatilis* x *Morone chrysops*) and channel catfish (*Ictalurus punctatus*) were exposed to aqueous concentrations of E2 ranging from 10 to 100,000 ng/L for 21 days. Respective EC<sub>50</sub> values for plasma VTG detected by western blot in medaka, catfish and bass were 200, 170 and 1560 ng E2/L. These data demonstrate sensitivity differences in VTG induction suggesting that this indicator of estrogen exposure cannot be directly compared across species. Catfish and bass VTG responses obtained in laboratory exposures were compared to VTG responses previously observed with 21 day wastewater treatment plant effluent exposures. Plasma VTG induction in effluent exposed fish ranged from 14 to 82 percent above reference values depending on species. Extrapolation of field responses with laboratory exposed fish indicate catfish and bass were exposed to the equivalent of 27 to 240 ng E2/L in sewage effluent.*

Researchers in the U.K. and the U.S. have reported observations in fish implicating reproductive and developmental toxicity as a result of exposure to

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endocrine active substances (Folmar et al. 1996; Harries et al. 1997). Intersex was recently observed in feral fish populations of U.K. river systems that receive estrogenically active effluent from wastewater treatment plants (WWTPs) (Jobling et al. 1998). Concentrations of various estrogens, including 17 $\beta$ -estradiol (E2), its metabolite estrone and the synthetic estrogen ethinylestradiol have been measured in WWTP effluents in concentrations ranging from 2.7 to 48, 1.4 to 76 and 0.2 to 7.0 ng/L, respectively (Desbrow et al. 1998). Induction of vitellogenin (VTG), a critical phospholipoprotein in female egg production, was measured in caged male trout in U.K. river systems and has been established in many fish species as a sensitive biomarker of exposure to environmental estrogens (Harries et al. 1997; Panter et al. 1998; Nimrod et al. 1995).

There is some question, however, regarding the comparative nature of VTG responses among various teleost species given that life histories and reproductive strategies vary greatly. The present investigation compares plasma VTG induction among three species commonly used in endocrine disruption research. Further comparison of VTG responses obtained from field exposed fish with those observed in laboratory E2 exposed fish provide a means to interpret field responses in terms of relative E2 equivalents. This investigation was conducted as part of a larger study to compare biochemical responses among fish species resulting from exposure to estrogenic chemicals.

Adult male Japanese medaka (4 to 6 mos.), adult male channel catfish (12 to 18 mos.) and juvenile hybrid striped bass (6 mos.) were exposed to aqueous concentrations of E2 (n=6) ranging logarithmically from 10 to 100,000 ng/L for 21 days. Vehicle control fish were exposed to ethanol. Fish were held in silanized aquaria at a loading capacity of 6 to 9 g/L on a 16:8 hr light/dark cycle. Optimal water quality was maintained for each species with daily 80% static-renewal.

Exposure water was sampled two times per week to determine actual E2 concentrations. Samples were concentrated utilizing Waters Sep-Pak C-18 columns and E2 concentrations were determined by a competitive EIA with an E2 specific MAb (Munro et al. 1988). Actual E2 concentrations were determined to be 85 to 115% of nominal concentrations 20 minutes post water change.

Blood was obtained from the gill arch of medaka and the caudal vein of catfish and bass, centrifuged at 6,000xg (4°C) for 10 minutes and plasma stored at -80°C until further analysis. For medaka and striped bass, plasma VTG was determined by western blot analysis using a universal MAb (HL1364-IC8) provided by Dr. Nancy Denslow (University of Florida). Catfish VTG was detected by similar methods with a catfish MAb provided by Dr. Charlie Rice (Clemson University). VTG was quantified as percent control by densitometry with Scion Image (1998 Scion Corporation). Statistical differences were determined by one-way ANOVA ( $p < 0.05$ ). Further analysis was performed using Tukey's test and Dunn's Method for multiple comparisons.

Exposure to E2 for 21 days resulted in a concentration dependent induction of plasma VTG for all species (Fig. 1). Juvenile striped bass are the least sensitive species to E2 exposure based on threshold and EC<sub>50</sub> values for VTG expression. Bass demonstrated a significant response above control values at 10,000ng E2/L, while medaka and catfish had lower threshold values of 1,000ng E2/L ( $p < 0.001$ ). EC<sub>50</sub> values for bass, medaka and catfish were 1560, 200 and 170 ng E2/L, respectively, indicating that similar responses among these species occur at significantly higher levels of exposure in bass ( $p < 0.001$ ). In contrast, bass had the most efficacious response to E2 exposure exhibiting greater maximal VTG expression of 670 percent above control values compared to only 230% for medaka and catfish ( $p < 0.001$ ). These data demonstrate that this commonly used indicator of estrogen exposure in laboratory and field studies

cannot be directly compared across species due to differences in sensitivity. This supports previous evidence that factors such as species, age and sensitivity need to be critically evaluated prior to selection of an animal model for studies with estrogenic compounds (Routledge et al. 1998).

Comparing VTG expression in field exposed fish with laboratory derived E2-VTG dose response curves provides a means to describe field exposures in terms of relative E2 equivalents. Channel catfish previously exposed *in situ* to WWTP effluent for 21 days expressed plasma VTG 14 to 38 percent over control fish. Using the laboratory-based E2-VTG dose response data for catfish as a standard curve (Fig. 1), the VTG responses of field-exposed catfish were found to correspond to E2 equivalent concentrations between 27 and 115 ng E2/L (Table 1). Striped bass previously exposed to 75 percent WWTP effluent for 21 days exhibited induction of plasma VTG 82 percent above reference values, which correlates to 240 ng/L E2 equivalents by the same method. E2 concentrations previously reported in sewage effluent range from 2.7 to 48 ng E2/L (Desbrow et al. 1998) indicating that VTG responses in field fish may result from exposure to a combination of estrogenic compounds in these WWTP effluents. It is likely that other natural (e.g. E2, estrone), synthetic (e.g. ethynylestradiol) and xenoestrogens (e.g. *p*-nonylphenol) increase the E2 equivalent value calculated for sewage effluent. Studies are currently underway to determine the chemical source(s) of the observed estrogenic activity.

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