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**Uptake of estradiol from sediment by hornyhead turbot
(*Pleuronichthys verticalis*) and effects on oxidative DNA
damage in male gonads**

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Abstract

Male and female hornyhead turbot (*Pleuronichthys verticalis*) were exposed to four concentrations (0, 0.75, 14.7 and 46.5 ng/g dry weight) of E2-amended sediment for 7 days. Sediment-derived E2 was bioavailable to the flatfish, though the route of uptake was unclear. A concentration of 46.5 ng/g E2 in sediment led to a significant increase in vitellogenin concentrations in the plasma in both sexes after seven days of exposure. Though plasma E2 concentrations increased significantly in males at sediment E2 concentrations of 0.75 ng/g dry weight and above, a dose-dependent increase was not observed. There was also no correlation between sediment E2 concentrations, plasma E2 concentrations, and oxidative DNA damage in male gonads. The results suggest that the DNA damage previously seen in the gonads of feral hornyhead turbot at a sewage outfall is likely not caused by acute exposure to exogenous E2 from sediments.

Keywords: Estradiol; DNA damage; Oxidative Stress; 8-oxoDG; Vitellogenin

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A correlation between plasma 17 β estradiol (E2) and DNA damage using the comet assay in germ cells of male hornyhead turbot (*Pleuronichthys verticalis*) was observed at an oceanic wastewater outfall in southern California (Rempel et al., 2006). E2 concentrations in sediments around the outfall were roughly 3-fold higher than those at a reference location (0.45 ng/g vs. 0.16 ng/g dry weight), where no correlation between plasma E2 levels and DNA damage was demonstrated (Schlenk et al., 2005). Mammalian cytochrome P450 1B1 catalyzes production of 4-hydroxy estradiol, which is further metabolized to semiquinones and quinones causing oxidative DNA damage due to redox cycling (Han and Liehr, 1994). The purpose of this study was to determine whether acute exposures to E2 within sediment could cause oxidative DNA damage in turbot testes, as measured by 8-oxodeoxyguanosine (8-oxodG) levels.

Hornyhead turbot were collected by otter trawl from a reference location identified by the Orange County Sanitation District (OCSD) for their ocean monitoring program (latitude 33°36.055', longitude 118°05.199'). Fish were collected in February 2005; none of the females were gravid, but sperm was observed in males. The fish were held in artificial seawater (34 ppt) at 14 \pm 1°C for 4 days and fed once daily with earthworms.

Sediments from the USEPA-designated LA-3 reference location off southern California were treated with E2 in acetone and mixed overnight in a tumbler at room temperature. The amended sediment was then allowed to equilibrate for 8 days in the dark at 4°C based on earlier studies (Lai et al., 2000). Blood and gonads were collected from animals and frozen at -80 °C. Water and sediments were collected for each treatment at the beginning and at the completion of the exposure, with water samples

filtered with 1.0 and 0.45 μm filters in tandem. Analysis of E2 by GC/MS followed the method outlined Ternes et al. (2002). Recovery for sediment and particulates was $88\% \pm 22\%$, and for water $114\% \pm 20\%$. The detection limit was 0.6 ng/g for sediment and particulates, and 0.15 ng/L for water. Initial sediment concentrations were 0.75, 14.7 and 46.5 ng/g. Vitellogenin and E2 as were measured as described previously (Rempel et al., 2006). Analysis of 8-oxodG by LC/MS-MS followed those outlined in Hong et al. (2006) using UV treatment in MCF-7 cells as a positive control. Data was analyzed for normality of distribution by Shapiro-Wilk's test, for equality of variance by Bartlett's test and for significance by Dunnett's and Bonferroni tests ($p < 0.05$).

Evaluation of the exposure system revealed that the majority of the E2 remained associated with sediments and particulates with some movement into the water column. Water values ranged from 55.7 ng/L in the highest exposure concentration at $t = 0$ to 3.32 ng/L on day 7. Particulate fractions ($>0.45 \mu\text{m}$) had final concentrations ranging from 2-15 ng/g and final sediment concentrations ranging from below detection to 17 ng/g. With a log K_{ow} of 3.9, it was expected that the affinity of E2 for particulates would be relatively high. Data from this study supported this hypothesis with the majority of the E2 remaining associated with both sediments and suspended particulates.

The E2 in the sediment was bioavailable, as evidenced by increased vitellogenesis (Figure 1A). Though the level of vitellogenin found in the plasma of males exposed to 14.7 ng/g E2 was nearly statistically different from the control ($p = 0.052$) significant increases in vitellogenin were observed in the 46.5 ng/g concentration for both sexes. Correlations between vitellogenin and E2 concentrations in sediment, particulate, and water were observed, with the strongest correlations associated with sediment

concentrations ($R^2 = 0.8011$, $p = 1.1 \times 10^{-11}$) and final water concentrations ($R^2 = 0.7506$, $p = 3.0 \times 10^{-10}$).

Plasma E2 levels were significantly increased in males for all treatments, but not in females (Figure 1B). E2 levels in general were higher in males than in females, as previously shown in feral turbot caught during winter months (Rempel et al., 2006). Though there was a dose-dependent increase in plasma vitellogenin, the plasma E2 concentrations did not show similar patterns. The combined effect of natural fluctuations in E2, and efficient metabolic clearance of E2 may have masked the effect of E2 exposure, especially as E2 was measured only transiently.

Despite increase in plasma E2 in the males, there was no concomitant increase in oxidative DNA damage or significant correlation between plasma E2 levels and 8-oxodG in their testes after 7 days. Aqueous exposure to 4 µg/L E2 caused a transient increase of genotoxicity (as measured by ethythrocytic nuclear anomalies) in gilthead seabream (*Sparus auratus*) after 8 hours of exposure and correlated with higher plasma E2 concentrations (Teles et al., 2005), whereas after 12 hours no genotoxicity was observed. It is possible that any oxidative damage that may have occurred in turbot testes was transient, and therefore could not be discerned after 7 days.

In conclusion, it is unlikely that DNA damage previously observed in wild flatfish was due to acute exposure to sediment-derived E2. It is unclear whether chronic exposure to E2 or other compounds that have been identified in sediments may be responsible for the impacts at the OCSD outfall.

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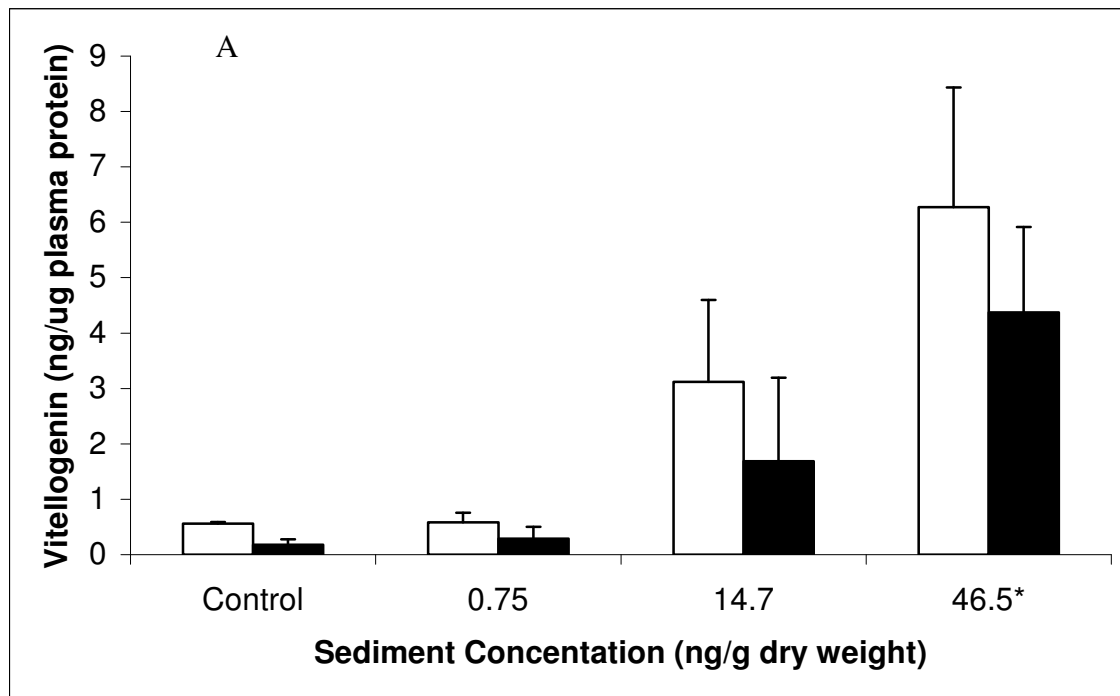
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Figure captions:

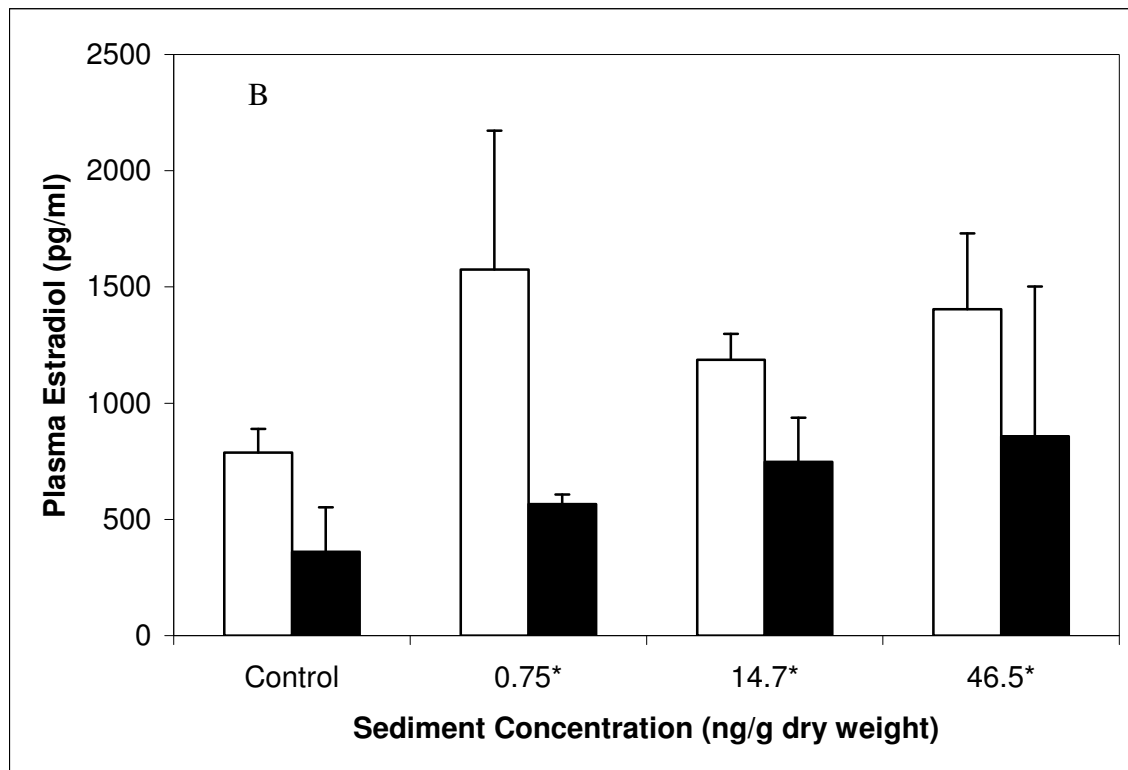
Fig. 1. (A) Vitellogenin levels in Hornyhead Turbot exposed to 17β -estradiol for seven days. White bars = males, black bars = females. Treatments are initial sediment concentrations. * = significant at $p < 0.05$. $n=3$ for each treatment for males. $n=3$ for control and 14.7 ng/g treatments, $n=2$ for 0.75 and 47 ng/g treatments for females. (B) Plasma estradiol levels in Hornyhead Turbot exposed to 17β -estradiol for seven days. White bars = males, black bars = females. Treatments are initial sediment concentrations. * = significant at $p < 0.05$ for males only. No significant difference for the females. $n=3$ for each treatment for males. $n=3$ for control and 14.7 ng/g treatments, $n=2$ for 0.75 and 47 ng/g treatments for females.

Figure 1A



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Figure 1B



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