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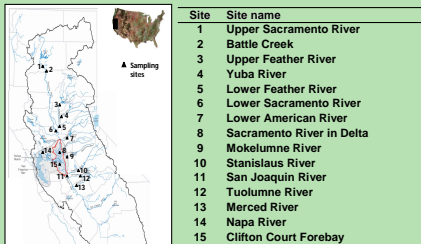
BACKGROUND

Hormonally active steroids with feminizing/estrogenic activity have been measured in surface waters receiving wastewater throughout North America (Snyder *et al.*, 2001). In Central California, recent studies have indicated that steroid hormones are present in municipal wastewater effluent and in agricultural wastes (Huang and Sedlak, 2001) and also, these steroid hormones were detected in water discharged by dairy farms and fish hatcheries along the American and Mokelumne Rivers (Kolodziej *et al.*, 2004). In the last years, several studies showed high feminization frequency of chinook salmon (*Oncorhynchus tshawytscha*) captured in California's Central Valley (Williamson and May, 2002; Chowen and Nagler, 2004).

The objective of this study was to evaluate the estrogenic or feminizing activity of water samples from waterways of the Central Valley of California, by combining chemical analyses with *in vitro* and *in vivo* expression of **vitellogenin** in juvenile rainbow trout (*Oncorhynchus mykiss*).

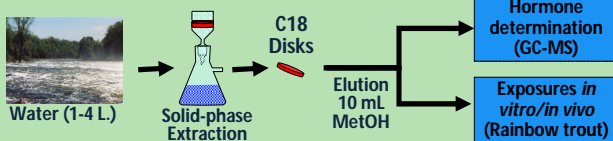
MATERIAL & METHODS

Sampling sites



6 Samplings: Water samples (1 L.) were taken on **July, September, November 2006 and January, March, April 2007**

Experimental procedures



In vitro hepatocytes exposure:

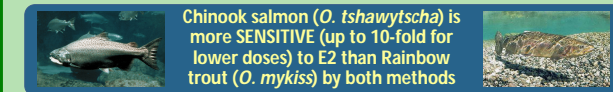
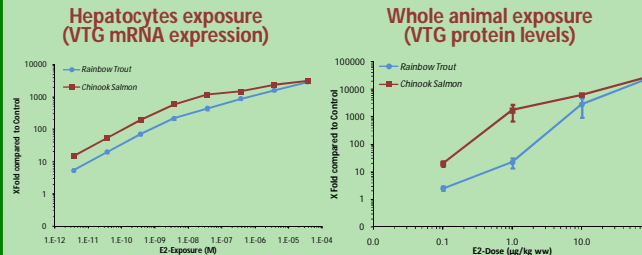
• Primary rainbow trout hepatocytes isolation and culture (1x10⁶ cells/well). Extract added in the media (0.6% v/v). After incubation of 24 h, total mRNA was extracted and transcribed to cDNA. **VTG mRNA** analysed by **PCR amplification**.

In vivo whole animal exposure:

• Juvenile rainbow trout (*O. mykiss*, 16-20 cm length) intra-peritoneally injected with water extracts (0.1% v/v). After 7 days, plasma (1-1.5 mL) was collected and **VTG protein** levels analysed by **Enzyme Linked Immunoassay (ELISA, Biosense™)**.

RESULTS

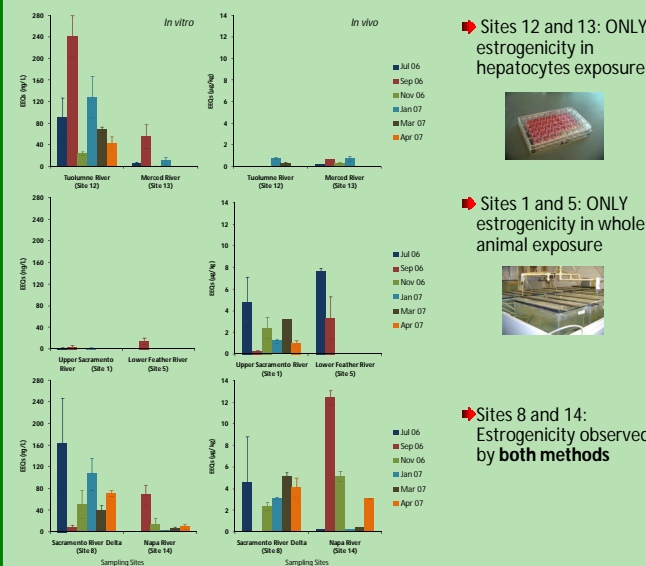
E2 exposure (Model Estrogenic Compound)



Estrogenicity determination in water extracts

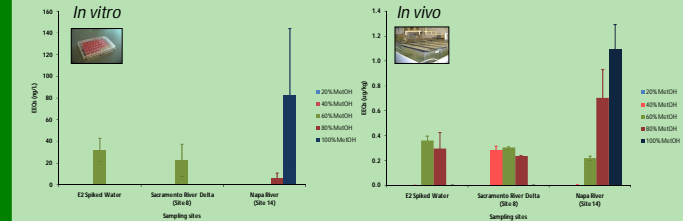
Areas 2, 3, 4, 6, 7, 9, 10, 11 and 15 NOT IMPACTED by estrogenic compounds (Lower EEQs levels or below detection limit levels: <0.12 ng/L for hepatocyte exposure and <0.1 µg/kg for whole animal exposure)

Areas impacted:



RESULTS

SPE Fractionation of areas of interest



- Areas selected: Sacramento River Delta and Napa River (both presented estrogenicity by *in vitro* and *in vivo* methods)
- 17β-Estradiol detected by GC-MS in 60% MetOH fraction in spiked water.
- Estrogenicity observed in different fractions → DIFFERENT COMPOUNDS in Sacramento River Delta (urban area) and Napa River (agricultural area)

Steroid hormone determination

Hormones analysed in water from the 15 sampling sites:

- ▶ 17α-Estradiol
 - ▶ Progesterone
 - ▶ 17β-Estradiol
 - ▶ Medroxyprogesterone
 - ▶ Estrone
 - ▶ Testosterone
 - ▶ Estratriol
 - ▶ Androstenedione
- All hormones analysed were **BELOW DETECTION LIMIT** (<0.25 ng/L) in all areas

CONCLUSIONS

- The induction of VTG (mRNA and protein levels) observed due to exposure to watershed extracts from areas in Central California strongly suggests the **presence of estrogenic compounds**. The negative detection of the main estrogenic natural hormones, further supports the fact that exposure to xenoestrogens or other compounds modulating endogenous steroid levels may be responsible for the **increased synthesis of VTG**. T.I.E. (Toxicity Identification and Evaluation) studies will be conducted to identify them.
- Higher EEQs (>100 ng/L) detected in some areas (Sacramento River Delta & Napa River) could lead to **reproductive problems** in local fauna (Leusch *et al.*, 2006).
- Lack of consistency between *in vitro* and *in vivo* responses in some areas (i.e. *in vitro*-*in vivo* in Tuolumne River; *in vitro*-*in vivo* estrogenicity in Upper Sacramento River) indicate the presence of **potential "indirect" acting compounds** with varied mechanisms of action.

References

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Acknowledgements

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