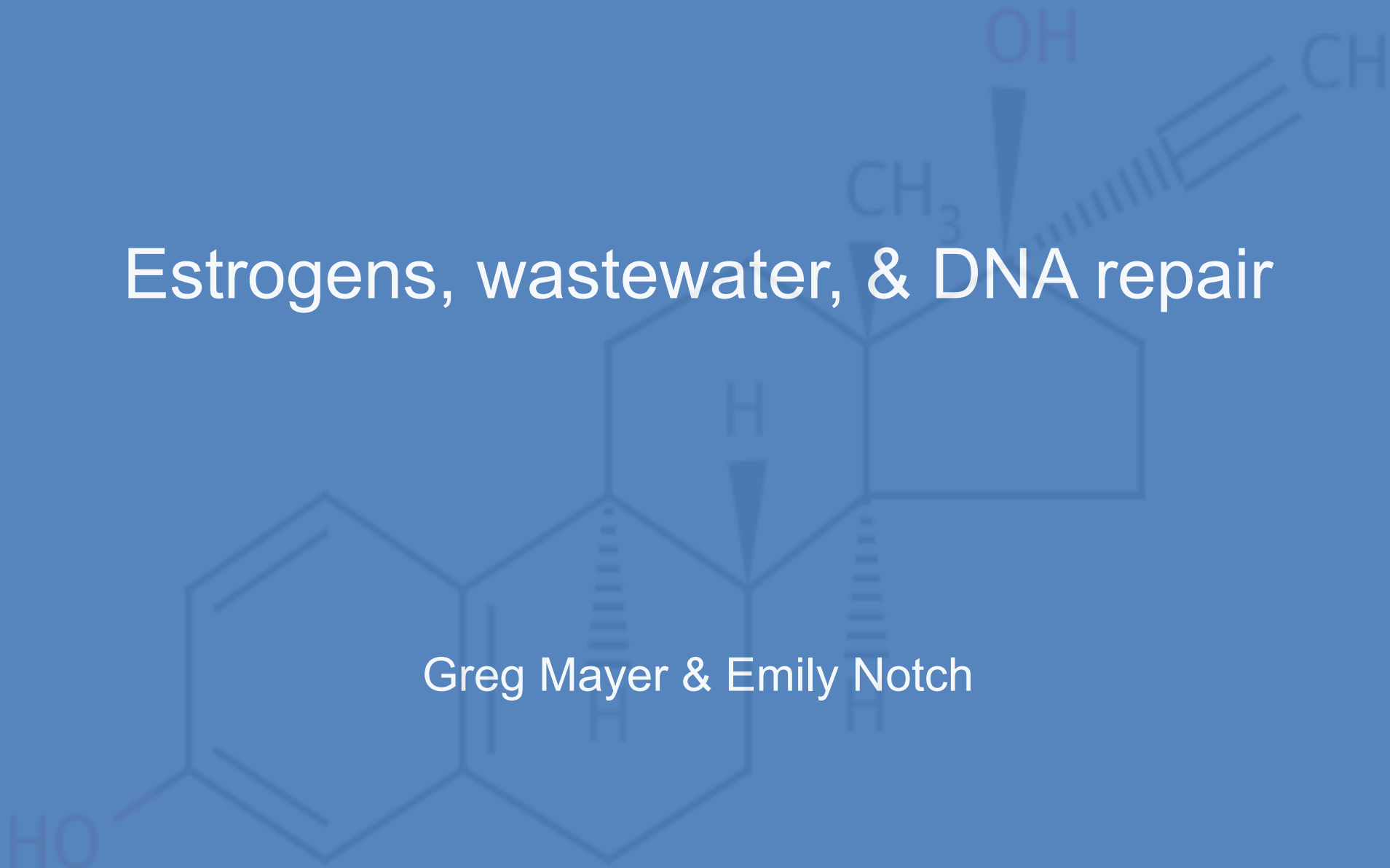


Estrogens, wastewater, & DNA repair

Greg Mayer & Emily Notch

HO



Why study hepatic DNA repair in zebrafish?

- Model of hepatocellular carcinoma
- Transcriptome of zfish very similar to human
- Elevated hepatic estrogen levels in cirrhosis
- Bulky adducts repaired by NER
- Concern of estrogenic chemicals in the environment

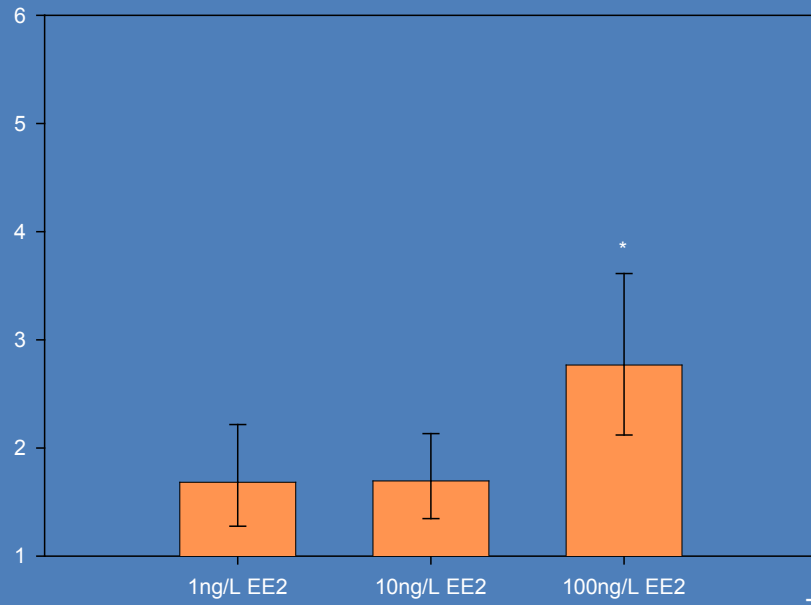
Zebrafish exposures

- 7 day
- Static water renewal daily
- Male and females separately
- RNA isolation – livers

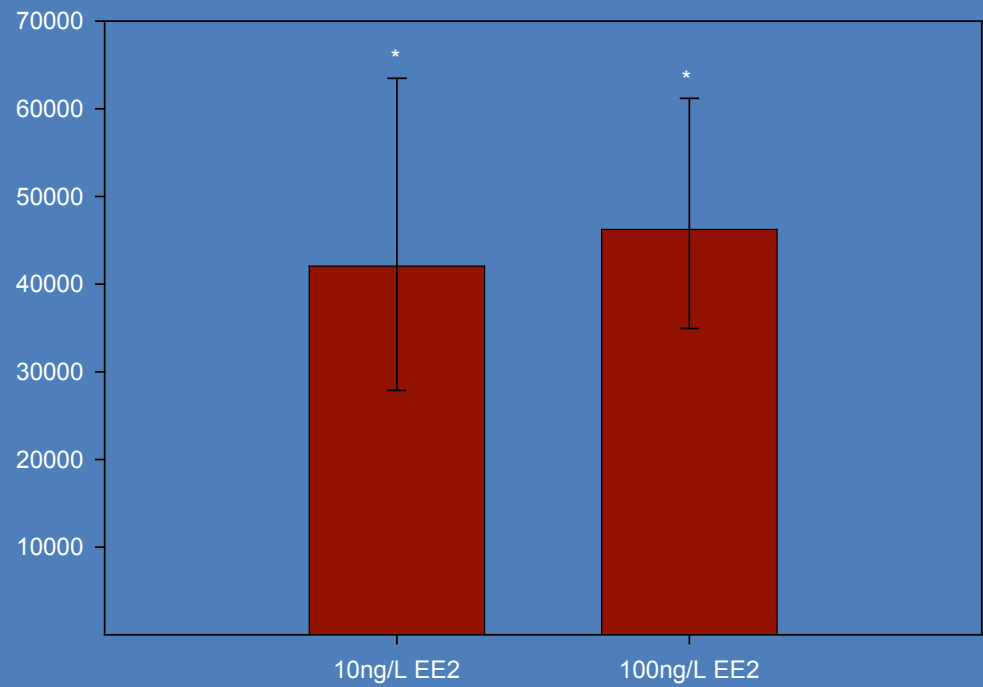


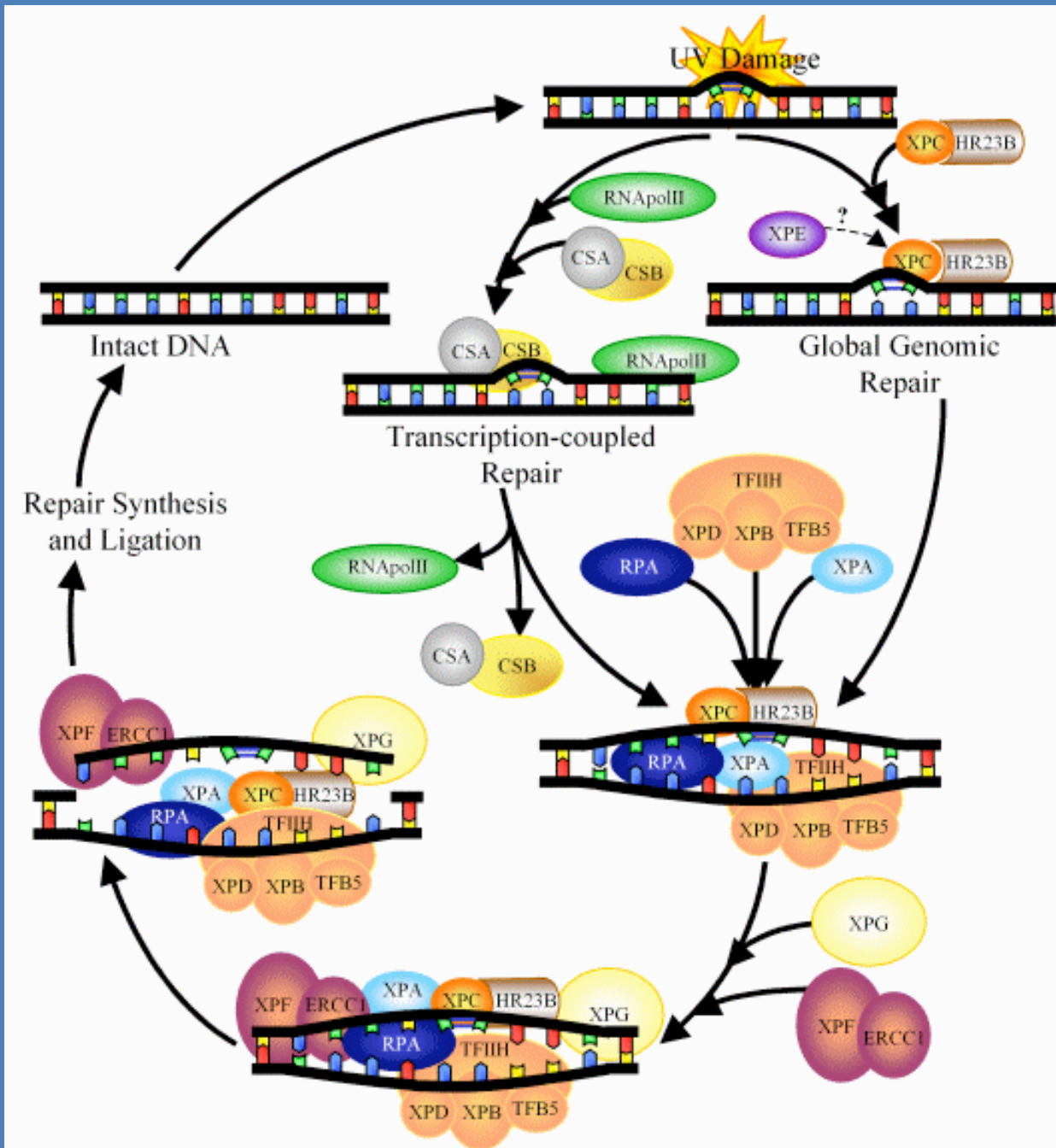
vtg

Adult females



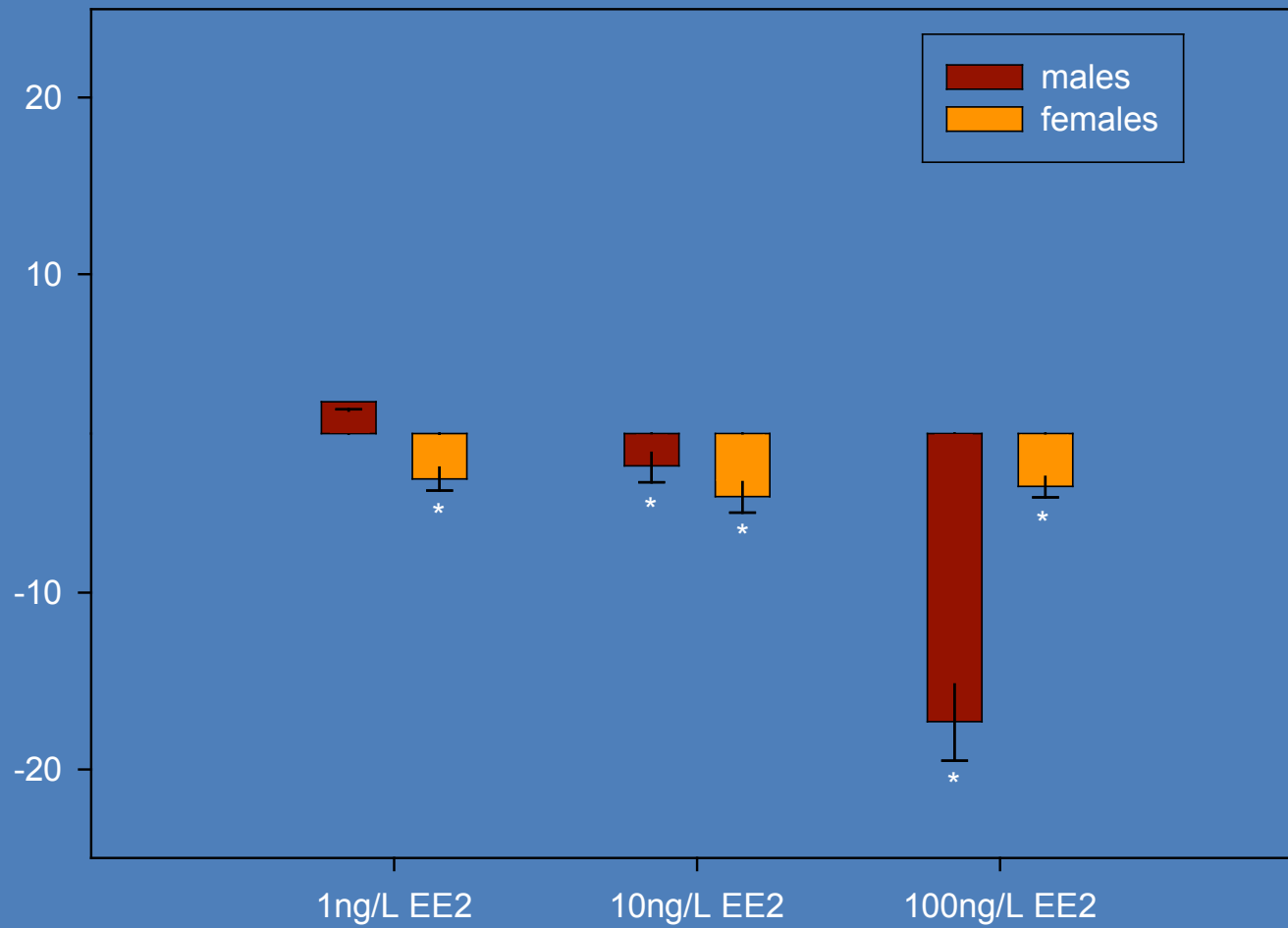
Adult males



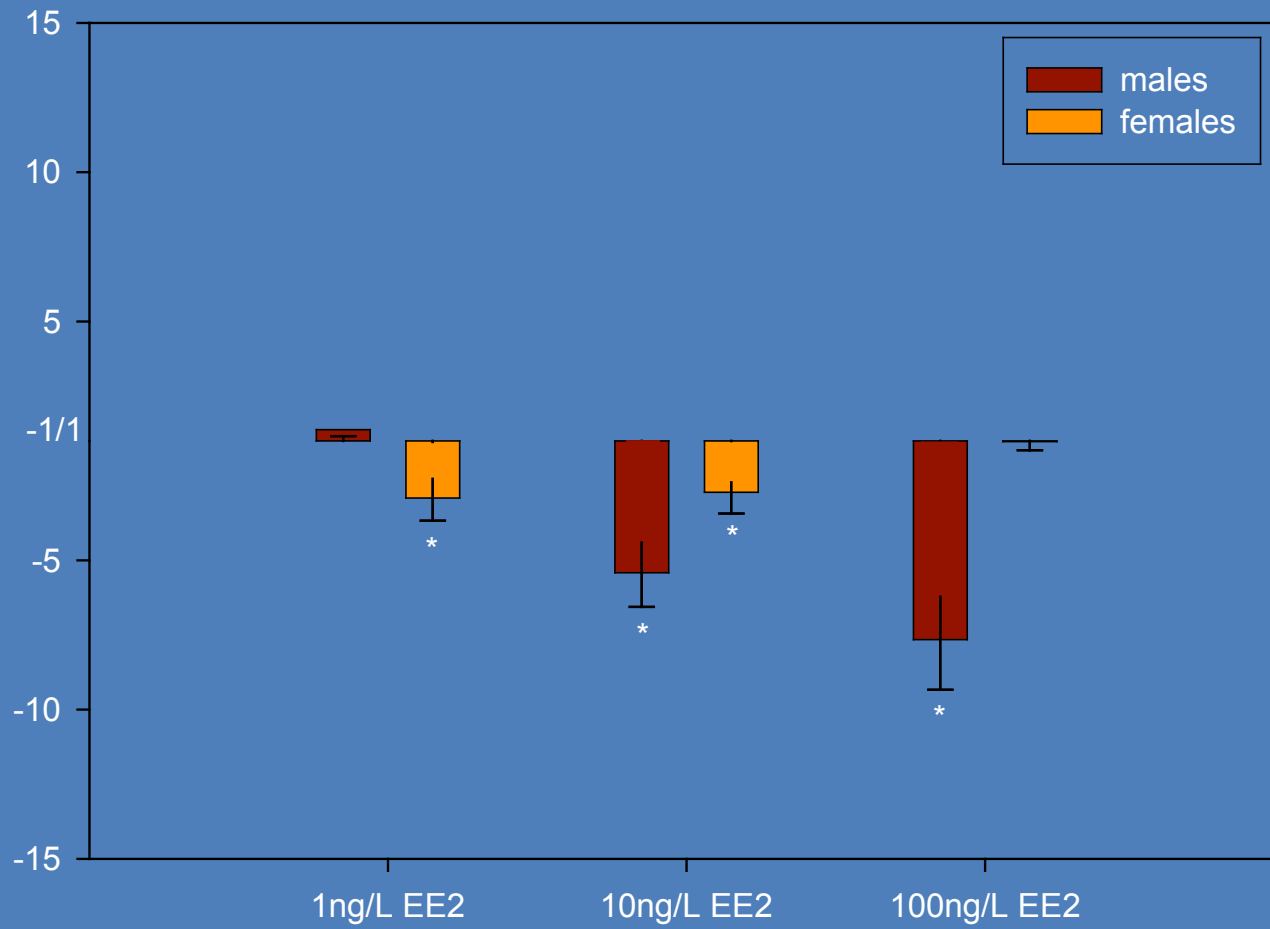


<http://ccr.coriell.org/nigms/pathways/ner.html>

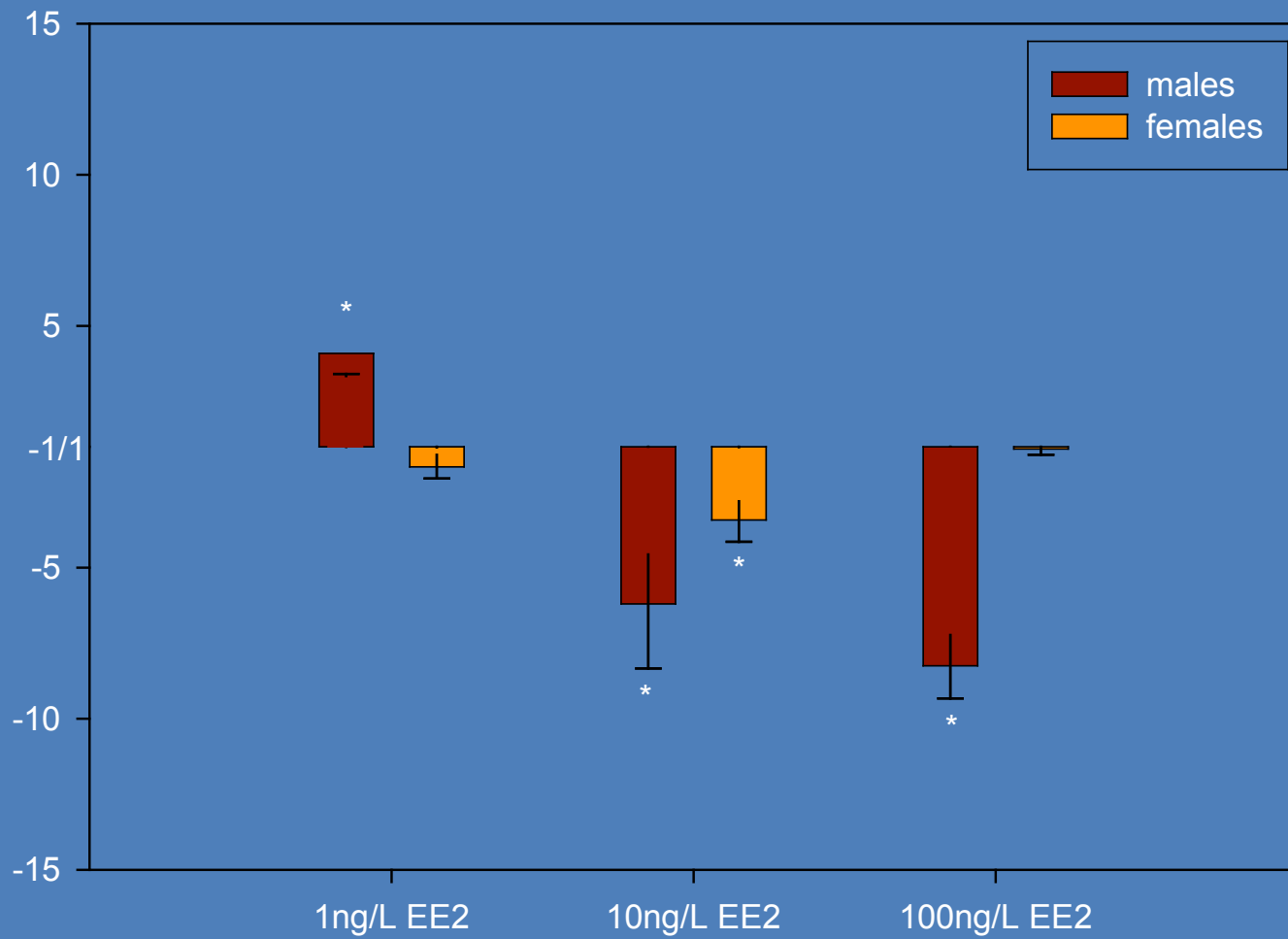
Zebrafish XPC



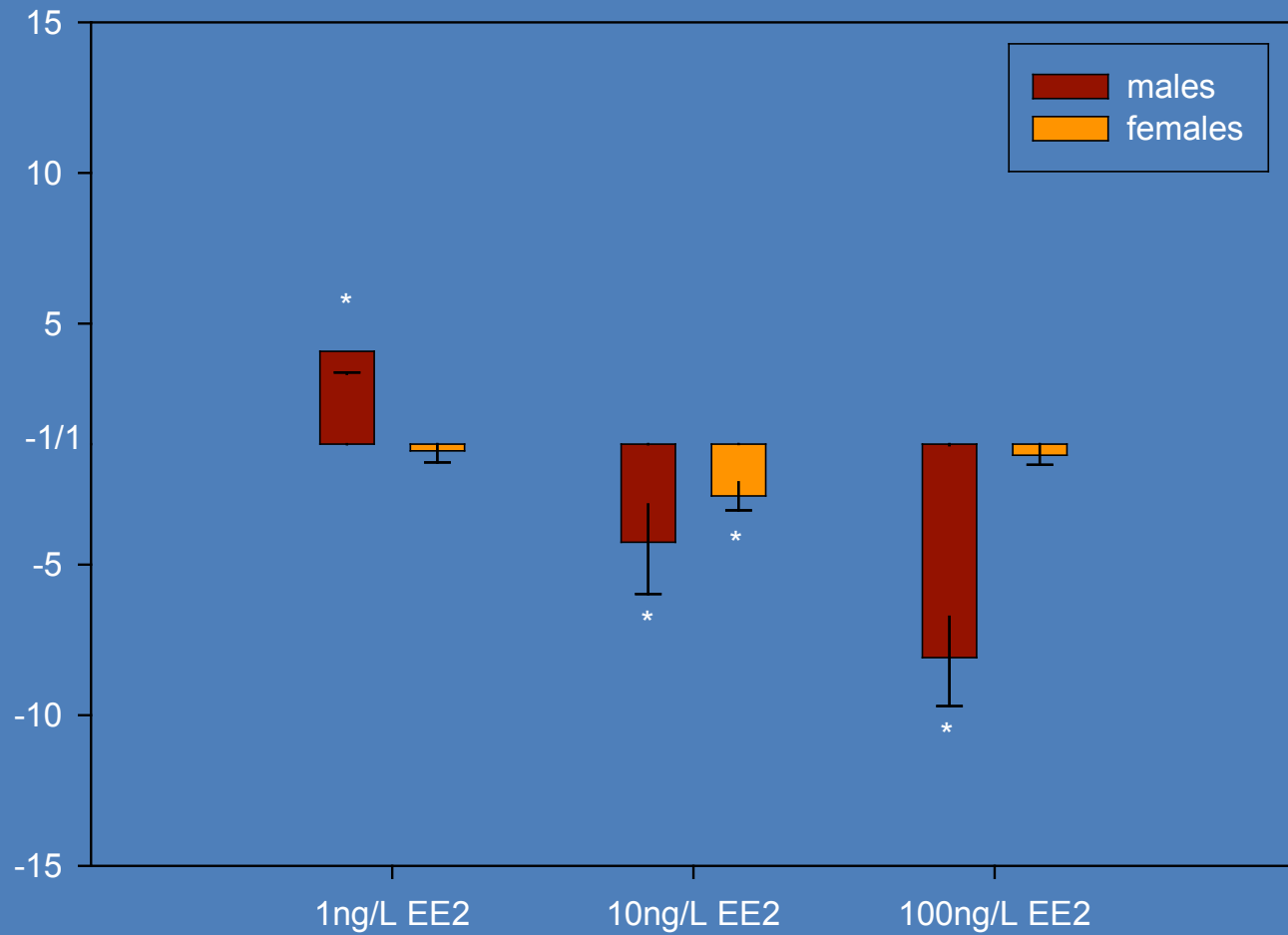
Zebrafish XPA

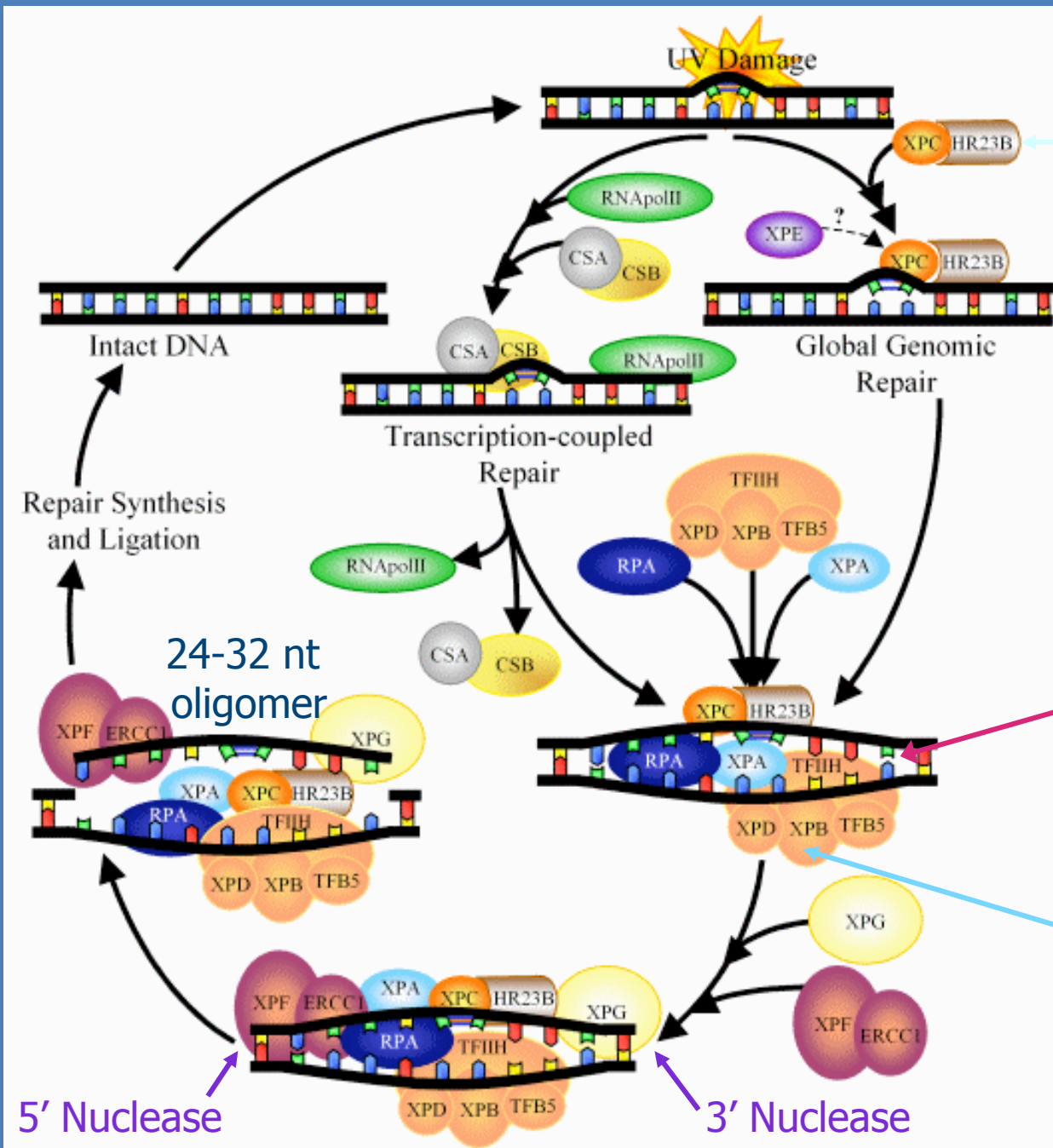


Zebrafish XPD



Zebrafish XPF





Initial
Damage
Recognition

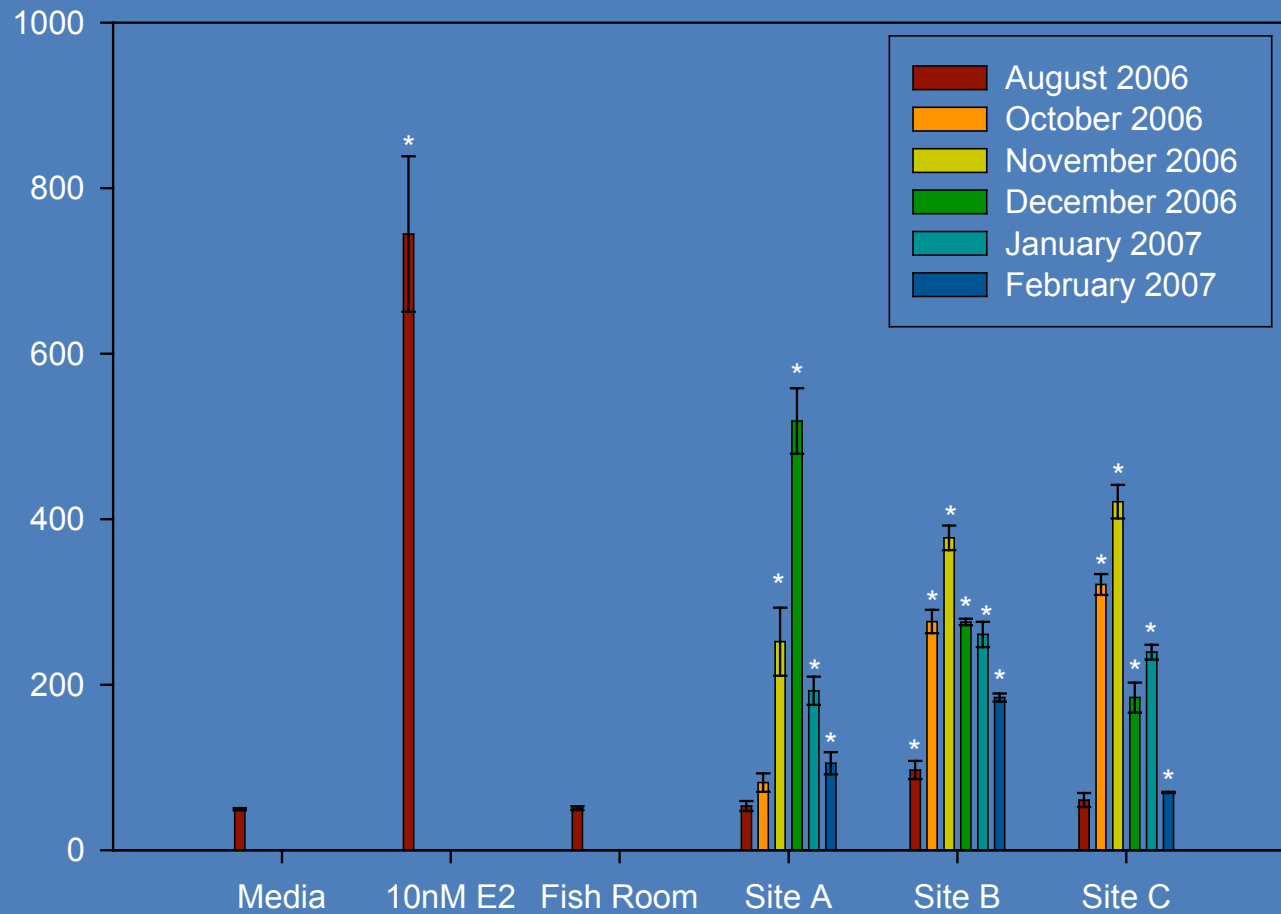
Damage
Verification

Helicases

5' Nuclease

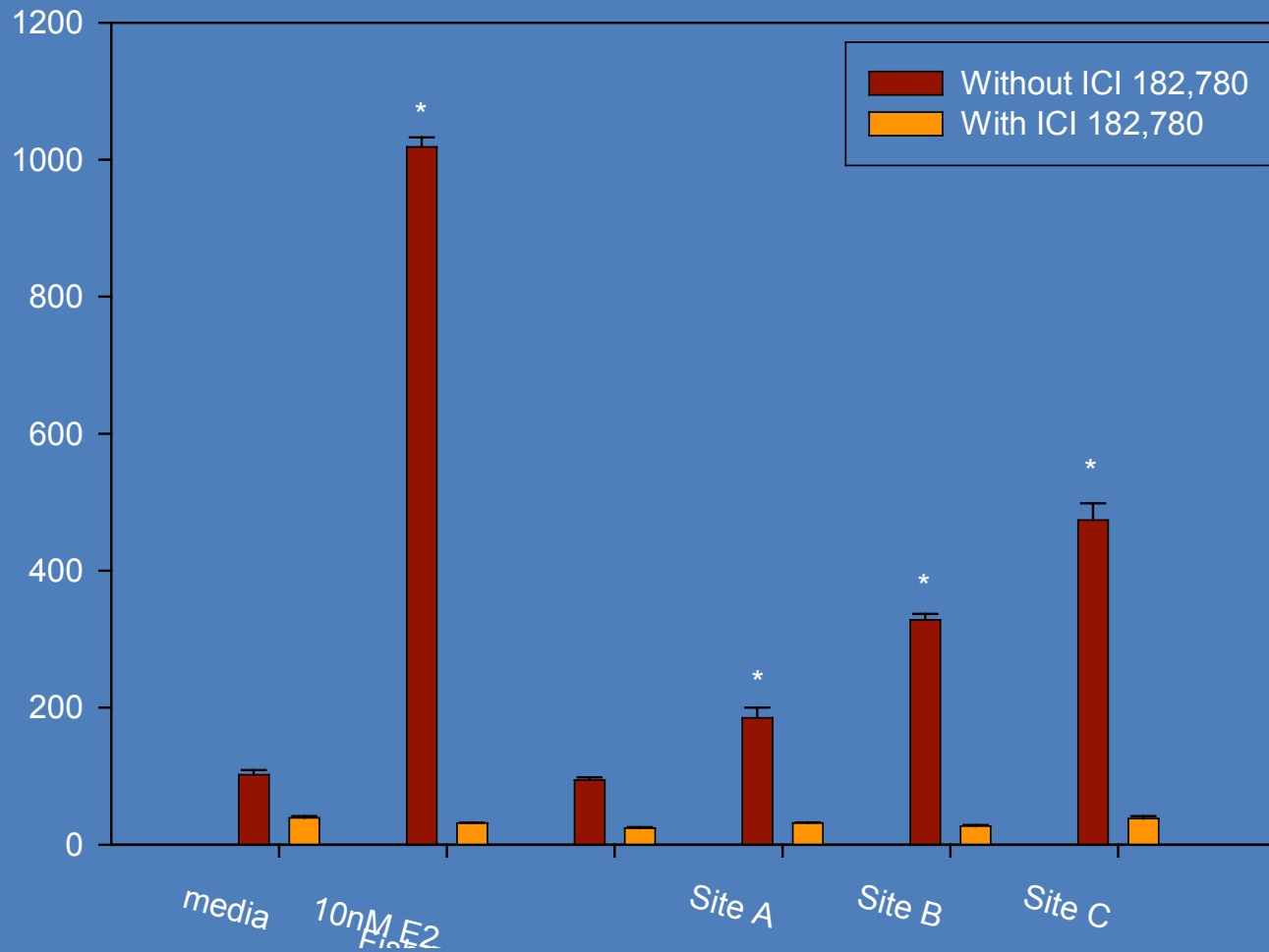
3' Nuclease

Estrogenic effects of POTW effluent

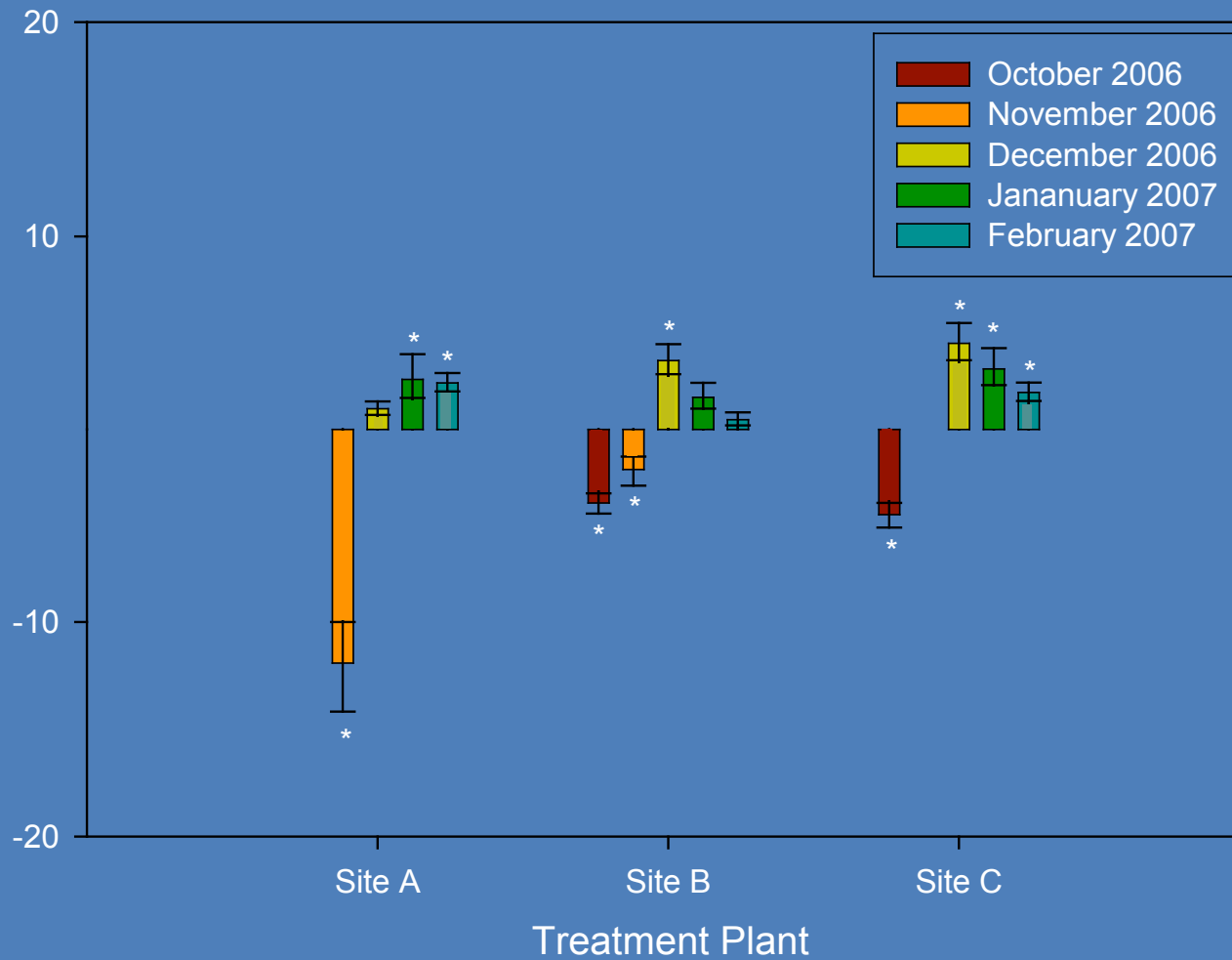


ER mediated

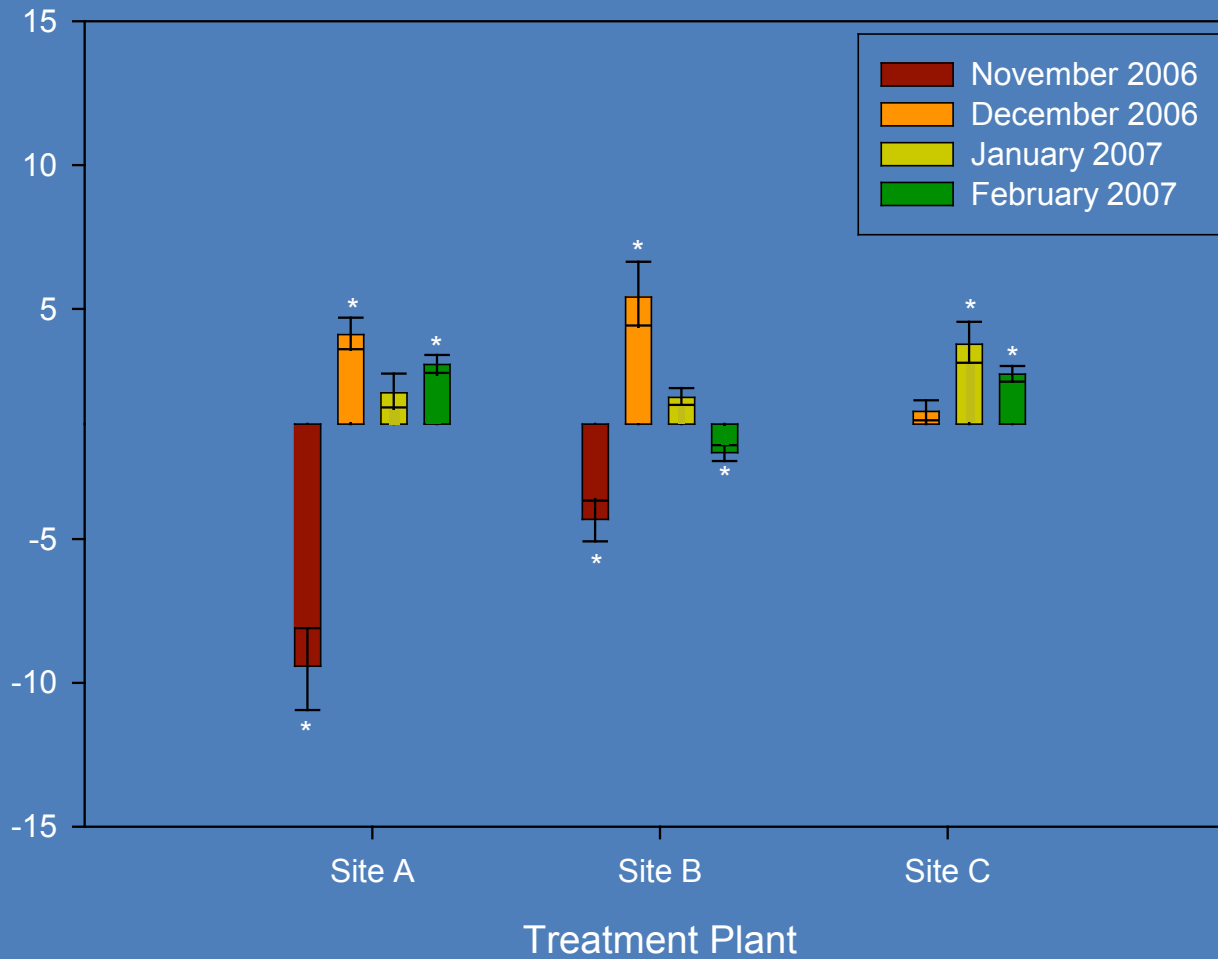
November 8, 2006 Grab Sample Effluent



WWTP Effluent – XPC



WWTP Effluent - XPA



What does this mean?

In vitro - ZFL cells

24 hour pre-treatment

EE₂ exposure

Control exposure

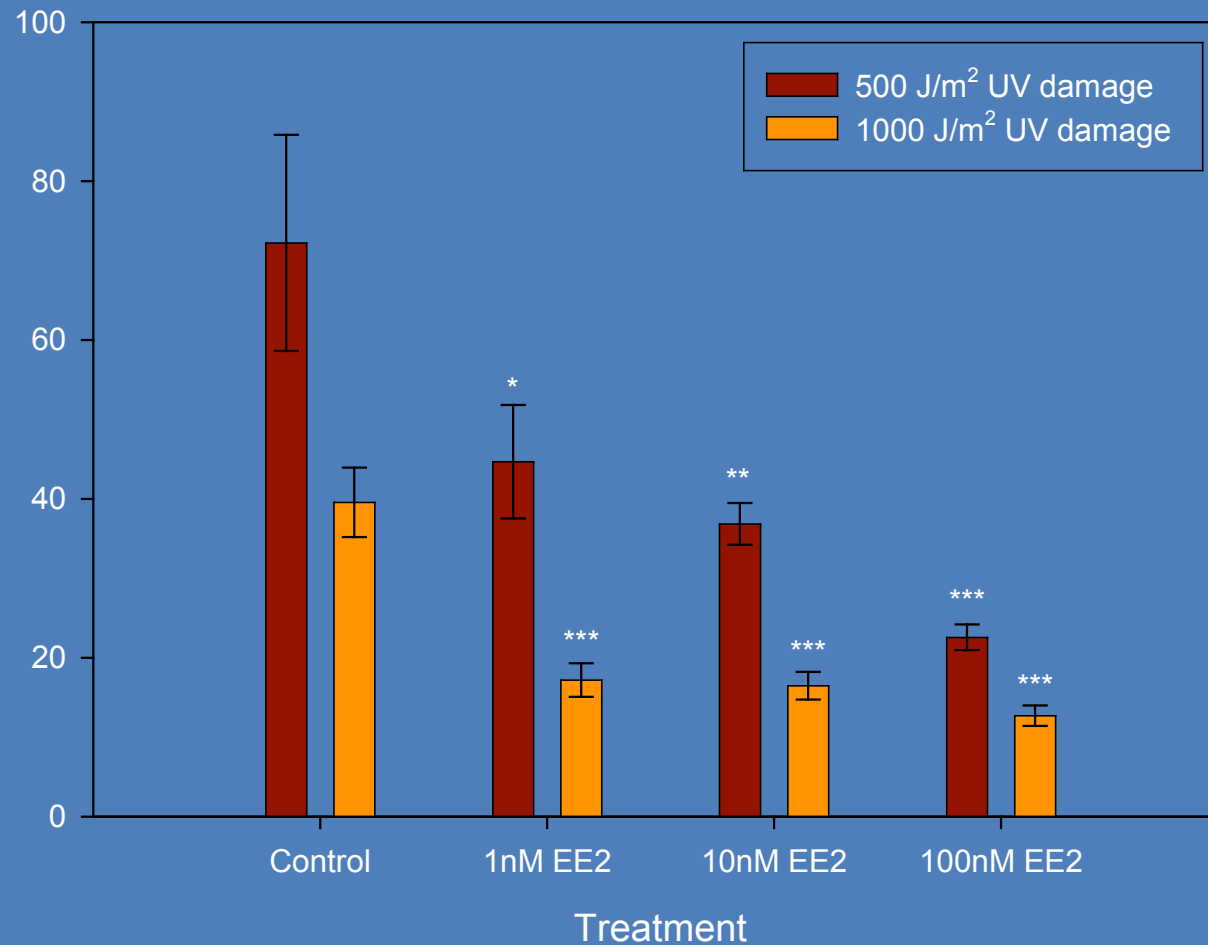
Undamaged plasmid (control)

UV damaged plasmid

Measure luminescence at 12, 24 and 48hrs

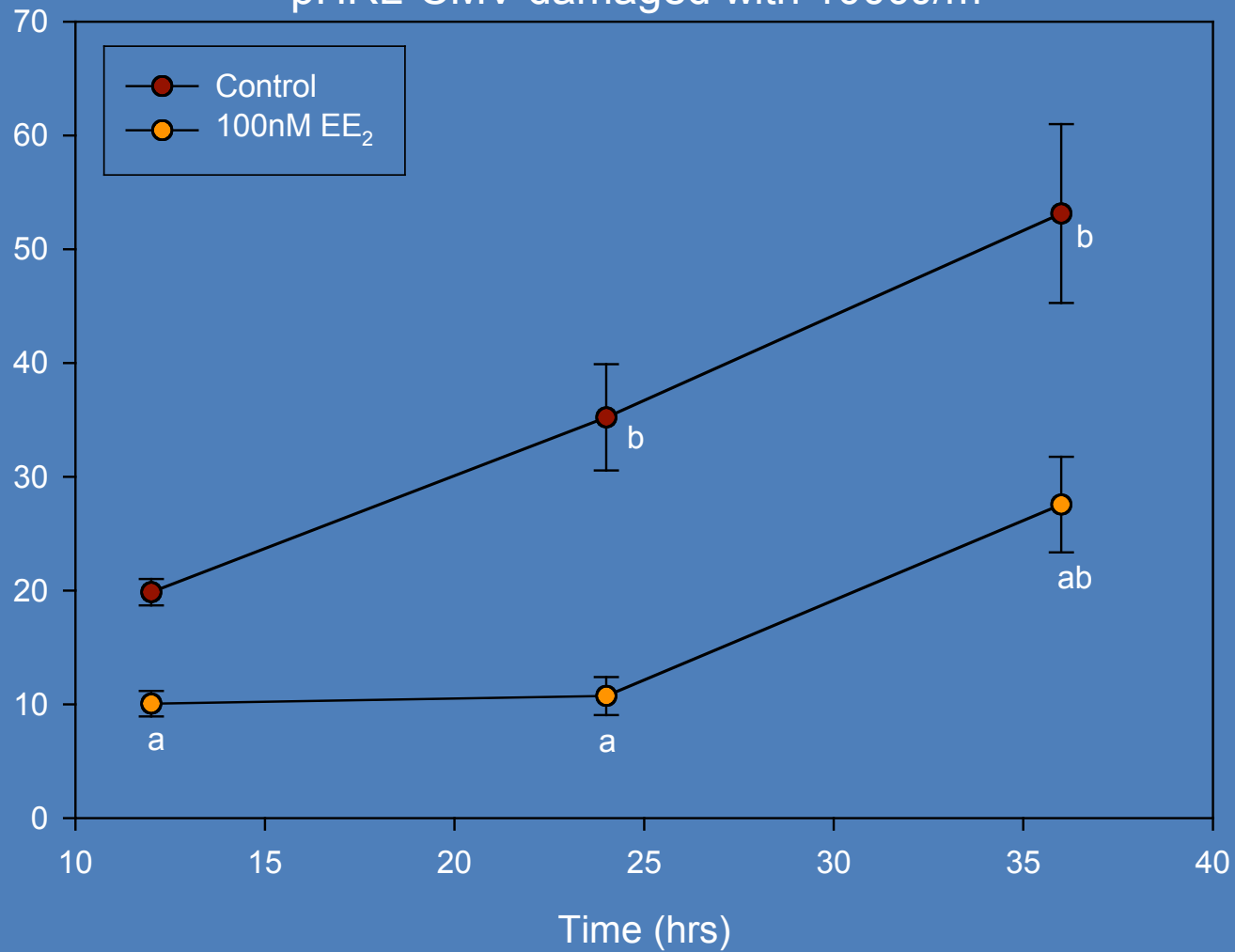
Host Cell Reactivation

Host Cell Reactivation - ZFL cells
24hr timepoint



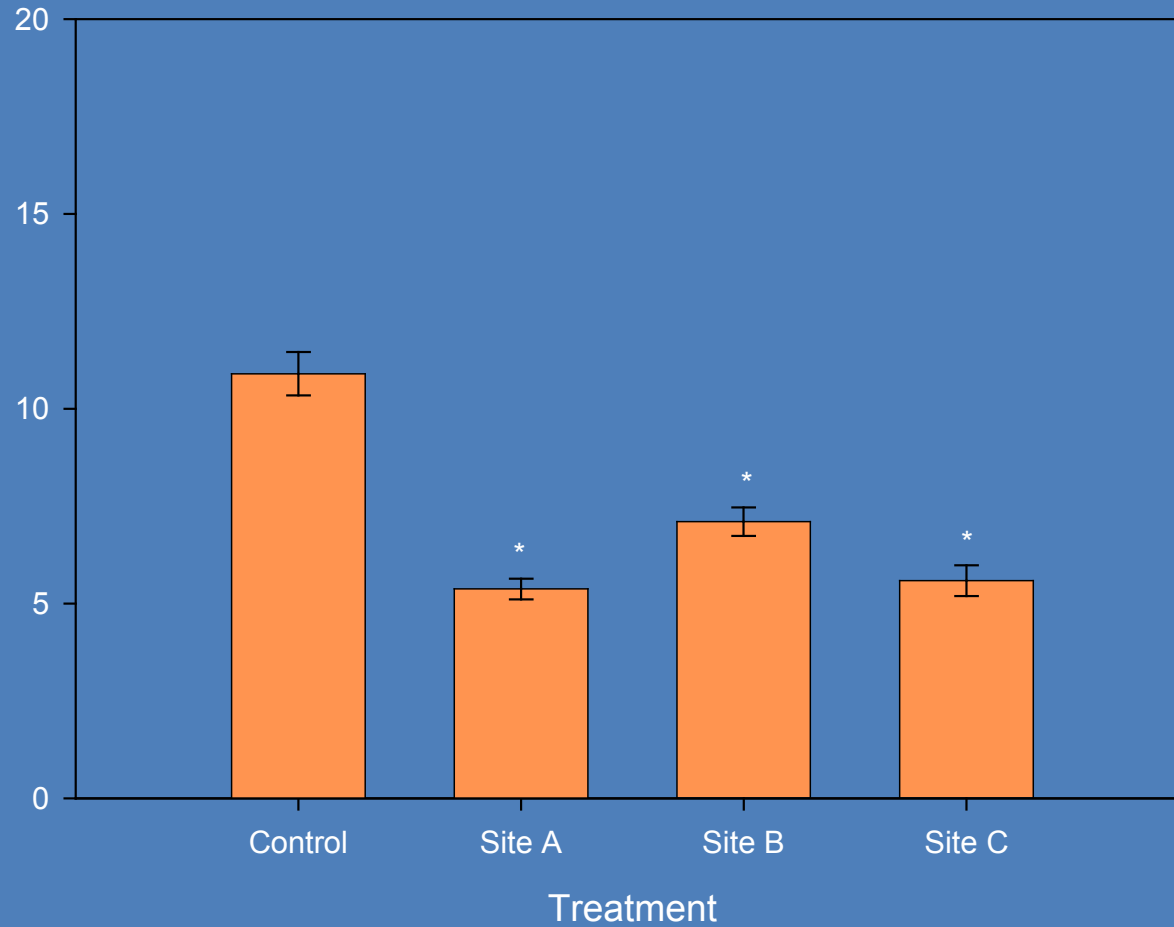
Host Cell Reactivation

ZFL Host Cell Reactivation
pHRL-CMV damaged with 1000J/m²



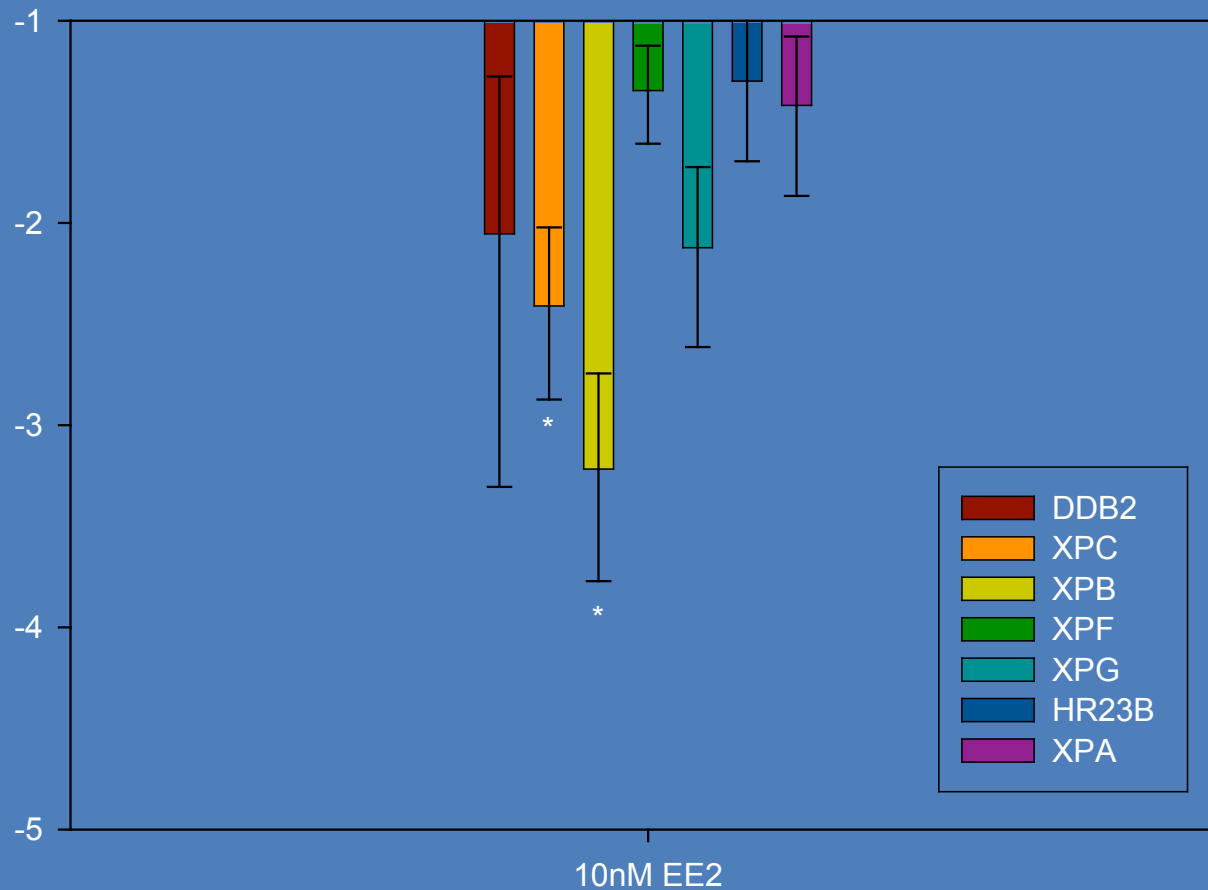
Host Cell Reactivation

ZFL Host Cell Reactivation - WWTP Effluent
24hr timepoint



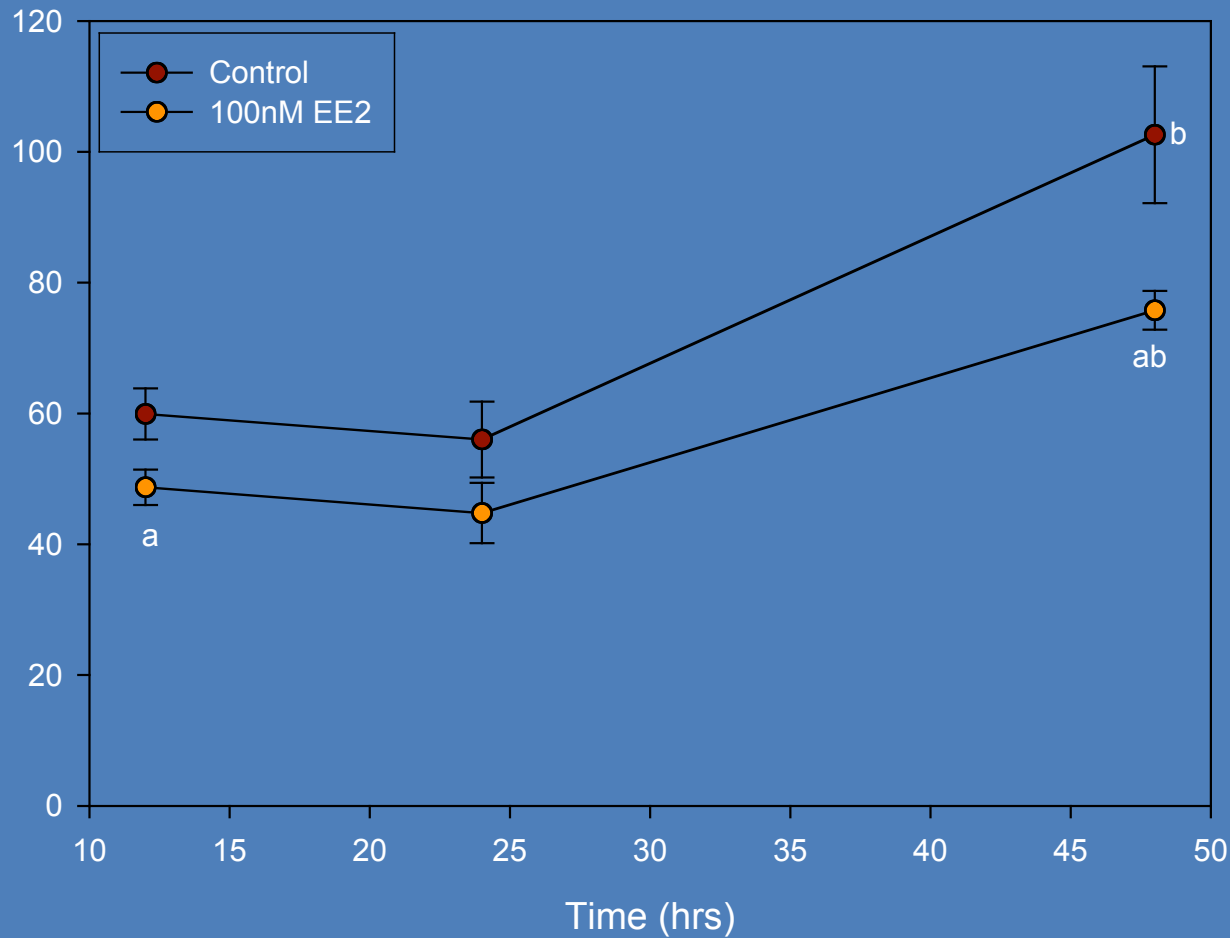
Is this just an issue for
aquatic organisms?

Human Transcript Data



MCF-7 Human Breast Cancer Cells

Host Cell Reactivation



HepG2 cells

Conclusions

- EE₂ exposure decreases NER gene transcription
- EE₂ decreases transcription coupled repair capacity

Acknowledgements

Emily Notch
Danielle Miniutti

