Linking the Benchtop to the Bay: *Menidia* Species as Indicators of Endocrine Disruption

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University of California, Davis
University of North Carolina, Wilmington

funding

CALIFORNIA DEPARTMENT OF FISH & WILDLIFE

ECOSYSTEM RESTORATION PROGRAM

UNCW University of North Carolina Wilmington

Sea Grant
North Carolina
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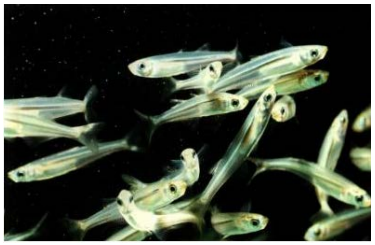
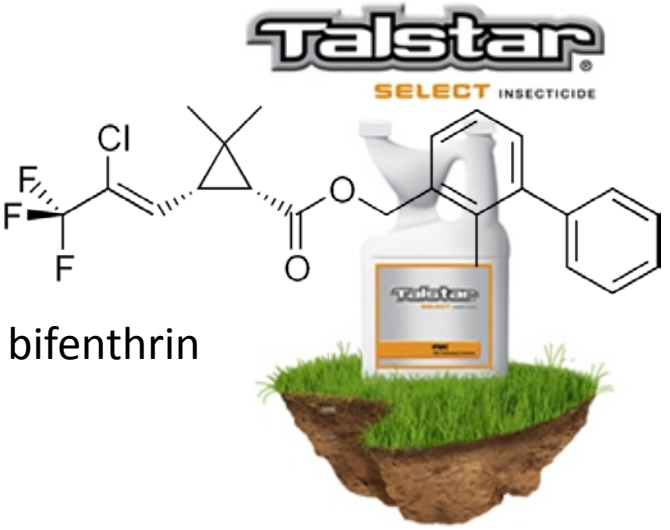
eTEAL

endocrine disruption



endocrine disrupting compounds (EDCs)

- agonize, antagonize, or synergize endogenous hormones
- alter hormonal mechanisms (i.e. steroid metabolism)



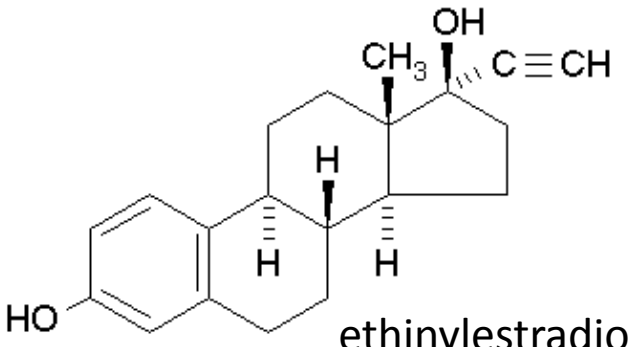
bifenthrin

aquatic organism susceptibility

- exposed at early life stages
- continuous / repeated exposure

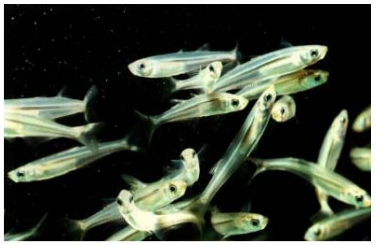
sources

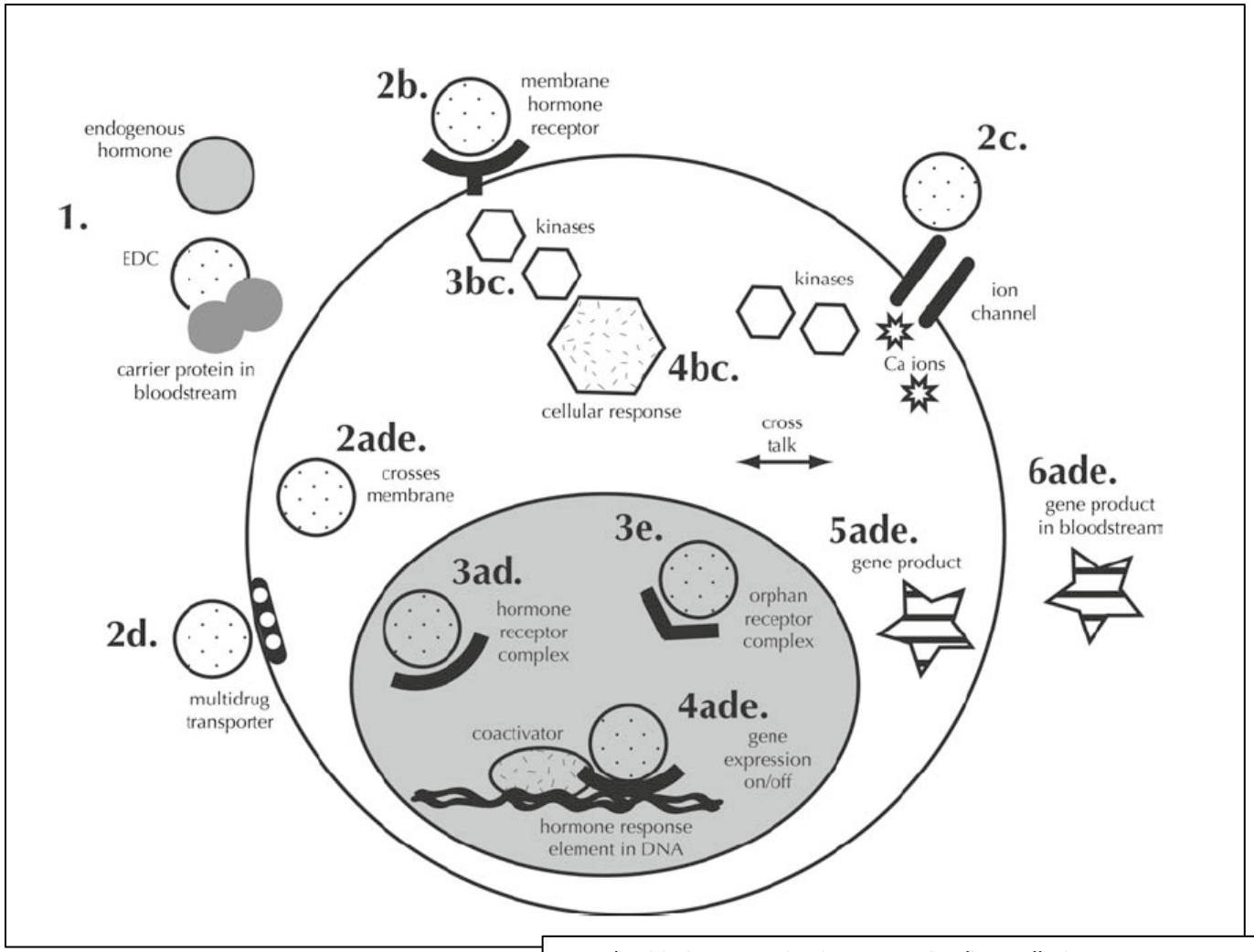
- treated wastewater effluent
- urban run-off
- agricultural or ranch run-off



ethinylestradiol

mechanisms



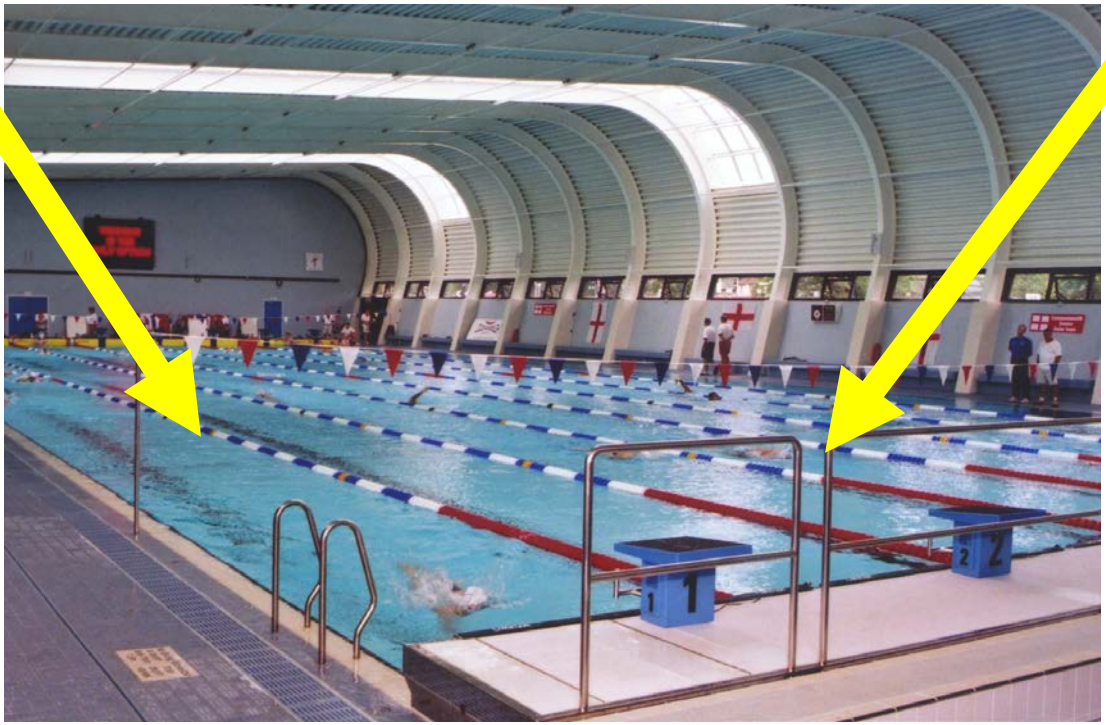


Brander 2013. In: *Monitoring Water Quality: Pollution Assessment, Analysis, and Remediation*

environmental relevance

ibuprofen

parts per billion



bifenthrin

parts per trillion

slide courtesy of N. McGinn

approach

Why *Menidia*?

- Life history / biology well-known

- Ubiquitous in N. American estuaries

- Commercially available (EPA WET)

- Small home range, high site fidelity

- Sensitive to EDCs

  -(Brander et al. 2012a *Ecotoxicology*, 2012b *Env. Tox & Chemistry*, 2013 *PLoS ONE,* Duffy et al. 2009)

- Temperature sensitive sex determination (Duffy et al. 2009)

Menidia audens

Menidia menidia

Menidia beryllina

outline

1. Responses of an estuarine fish to endocrine disrupting compounds across biological scales.
   (Brander et al. 2013, *PLOS One*)

2. Current projects:

   M. Beryllina reproductive assay development
   (S.M. Brander, UNCW)

   Menidia species as bioindicators in the field
   North Carolina (S.M. Brander, UNCW), SF Bay – Delta (B.J. Cole, UCD)

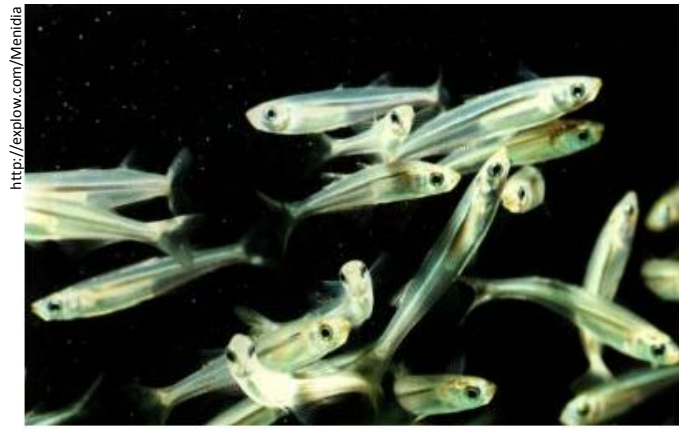
   M. beryllina, M. audens & *M. menidia* transcriptomics
   (R.E. Connon, K. Jeffries, UCD School of Vet Med; S.M. Brander, UNCW)

   Endocrine disrupting effects of pyrethroids in *M. beryllina*
   (S.M. Brander, UNCW)

   Population modeling using *Menidia* species
   (J.W. White, S.M. Brander UNCW)

outline

1. Responses of an estuarine fish to endocrine disrupting compounds across biological scales. (Brander et al. 2013, *PLOS One*)



Evaluated responses to both estrogenic and androgenic EDCs at the site, molecular, organism, and population levels in wild *Menidia (audens/beryllina*) at sites receiving different EDC inputs.

approach

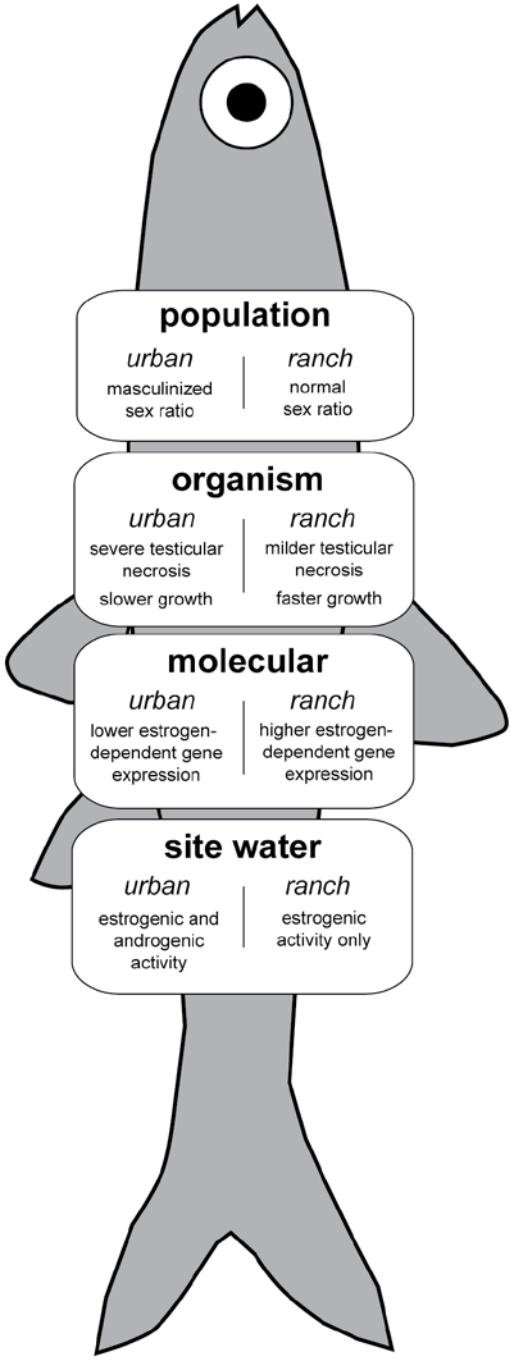
Links between gene & protein expression and gonad histology to population decline in response to one EDC (Kidd et al., 2007, Ankley et al., 2008)

Effect of complex mixtures on gene expression conducted in laboratory (Filby et al., 2007, Lukosky et al., 2008)

however ...

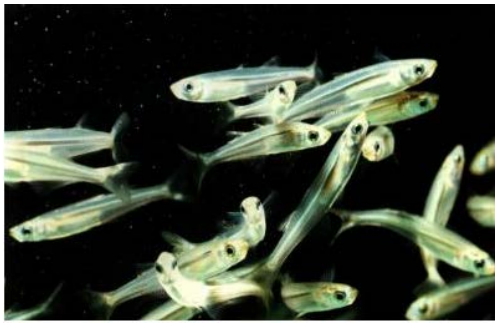
Lack of studies evaluating impacts of environmental mixtures on wild fish populations at multiple biological scales (Vines et al. in prep, Sumpter 2005).

Lack of studies evaluating growth, which is known to be influenced by steroid hormones and to differ between males and females (Reinecke 2010, Pottinger et al., 2010).



Brander et al. 2013, *PLoS ONE*

conclusions



May see impacts at the molecular level, but
doesn't necessarily mean they scale up
to organism / population (ranch site)

Necessary to evaluate multiple biological
scales to gauge impact

Although fish feminization downstream of outfalls remains a concern,
masculinization also an issue (ER antagonism, GR, MR and PR agonists, hypoxia?)
- possible that larger males seen at urban site are masc. females?
    - would have needed to genotype these fish to confirm

Silversides appear to be highly sensitive to EDCs and could be used as
bioindicators / surrogates in estuaries nationwide

Brander et al. 2013, *PLoS ONE*

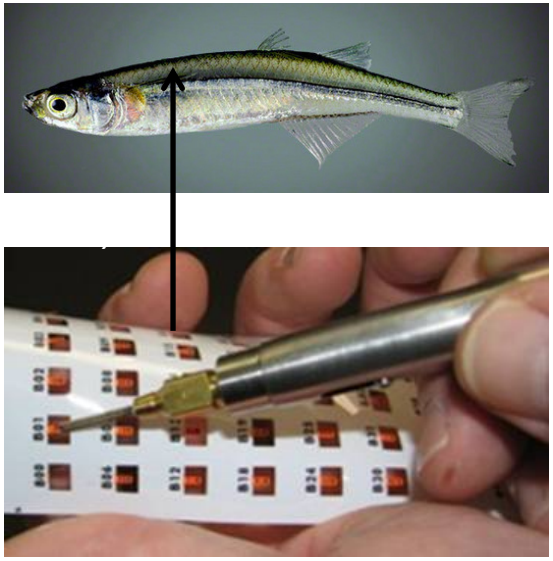
current projects: reproduction

Do observed skewed sex ratios impact reproduction?

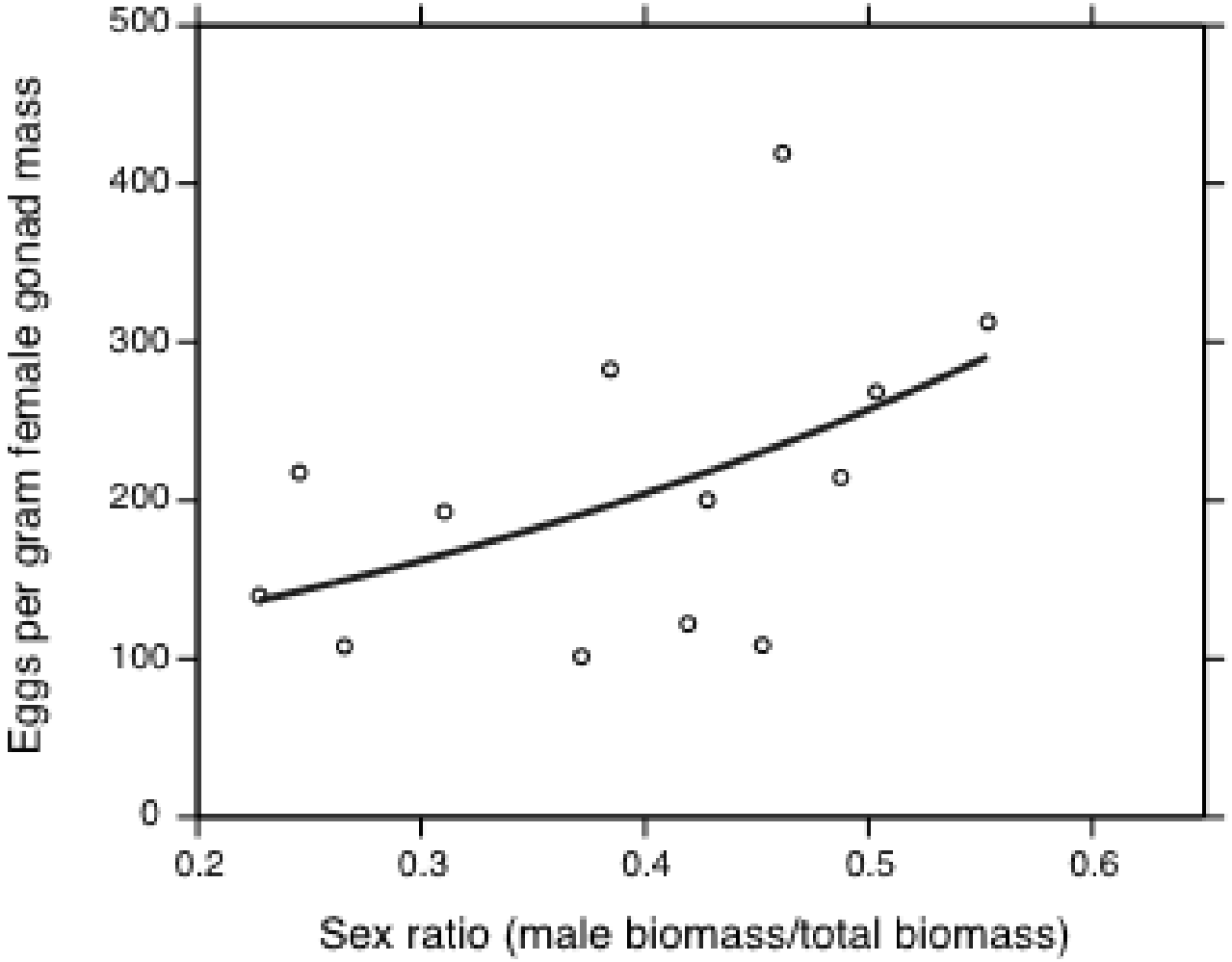
Randomized groups of tagged fish spawn for 48 hours

- sex ratios & reproductive output, fertilization rate quantified at end of trial series

Currently conducting exposures followed by spawning trials



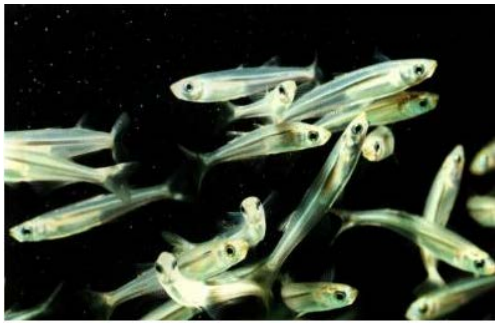
reproduction



Tagged fish (age 6-8 months at start of study) randomly selected to spawn for 48 hours in a group of 25, every 2 weeks.

Nonlinear regression
$p < 0.05$

current projects



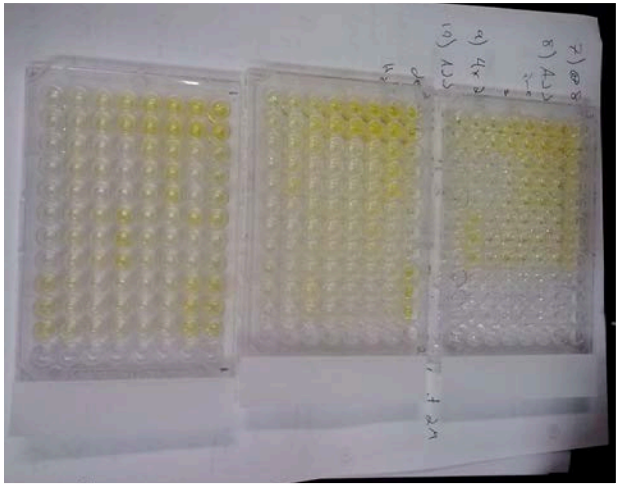
How do environmental EDC mixtures and specific compounds affect molecular markers in *Menidia* species?

Effects of selected EDCs on gene expression and estrogen-dependent protein expression in *Menidia*

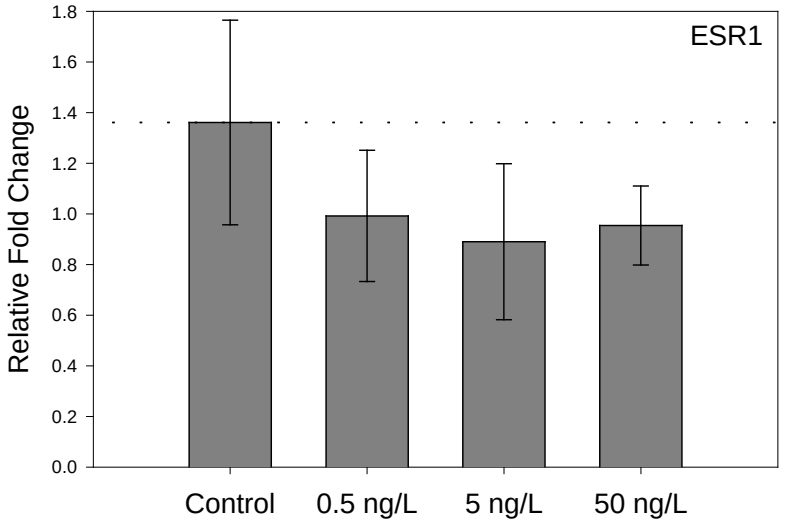
- *Menidia* qPCR (K. Jeffiries, S.M. Brander, R.E. Connon)
- choriogenin ELISA (S.M. Brander)

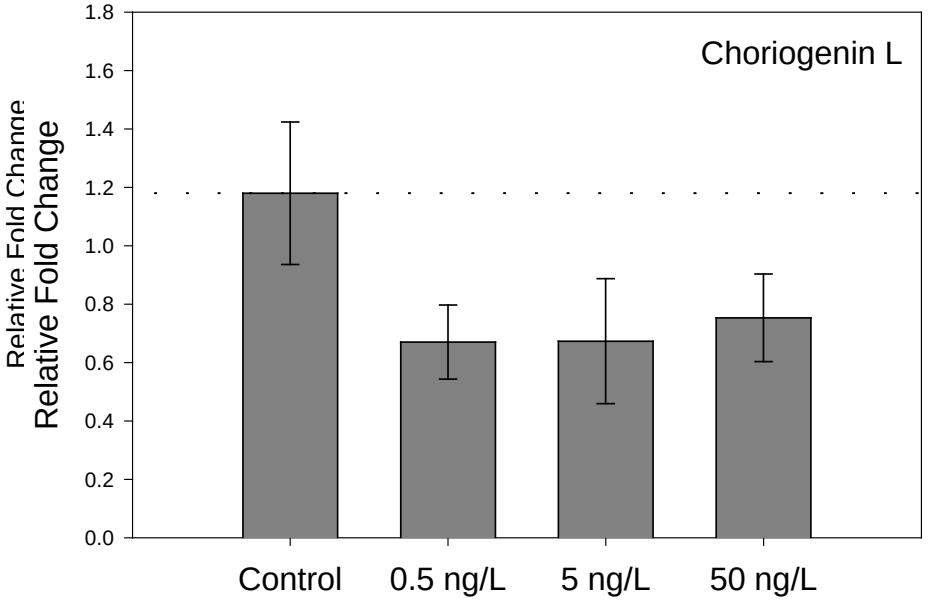
Environmental mixtures

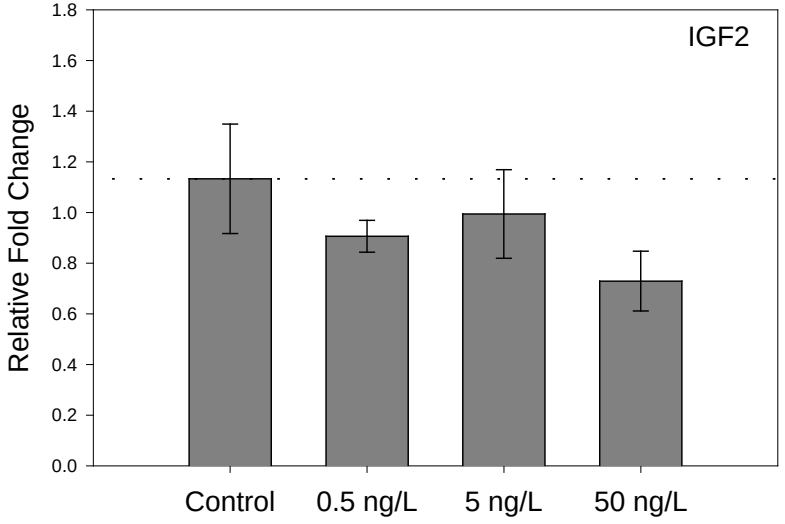
- SF Bay Delta, gene and protein expression (B.J. Cole, UCD)

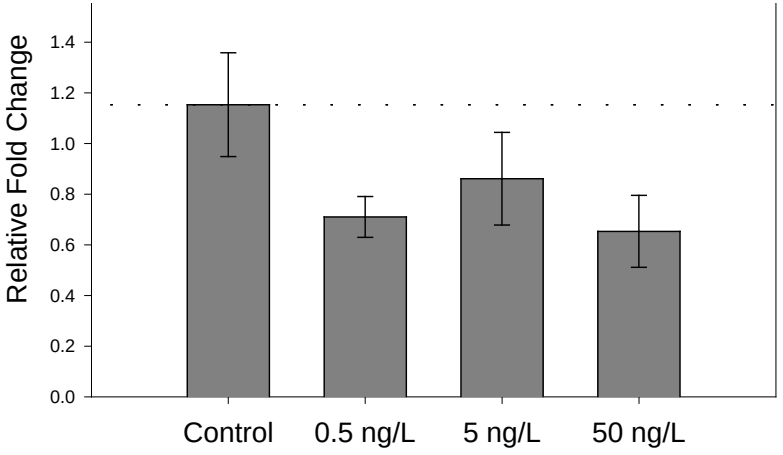


qPCR: bifenthrin



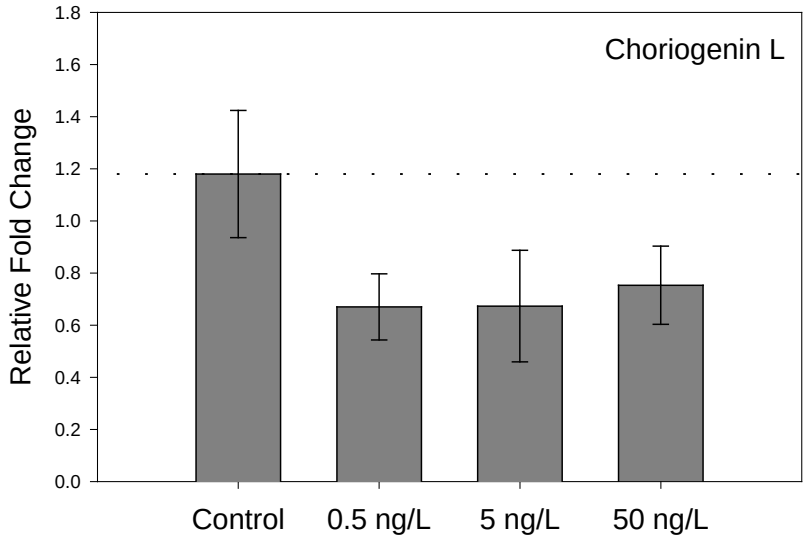


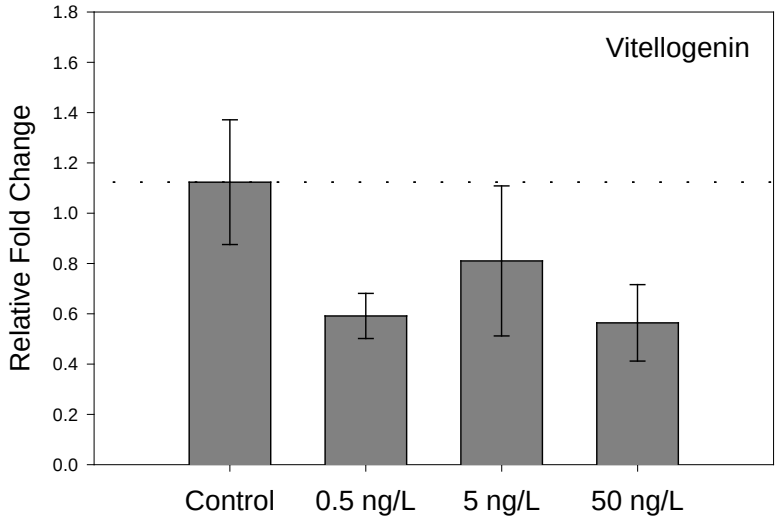




Brander et al. *(in prep)*

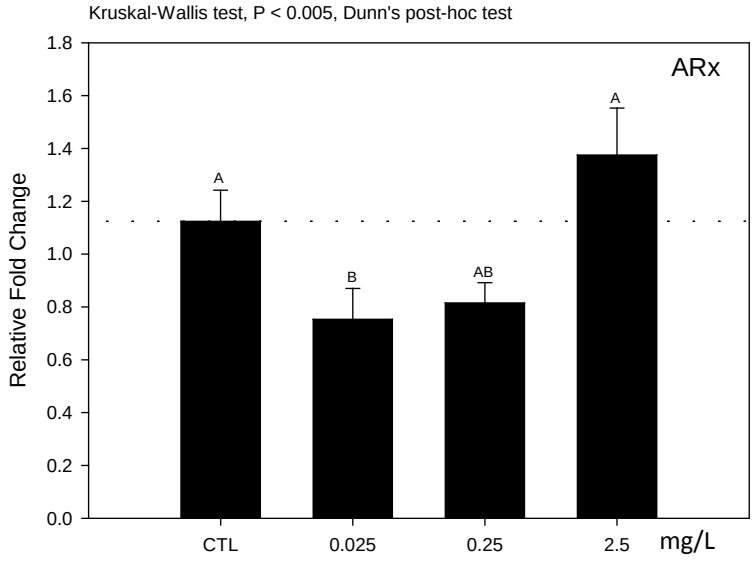
qPCR: bifenthrin

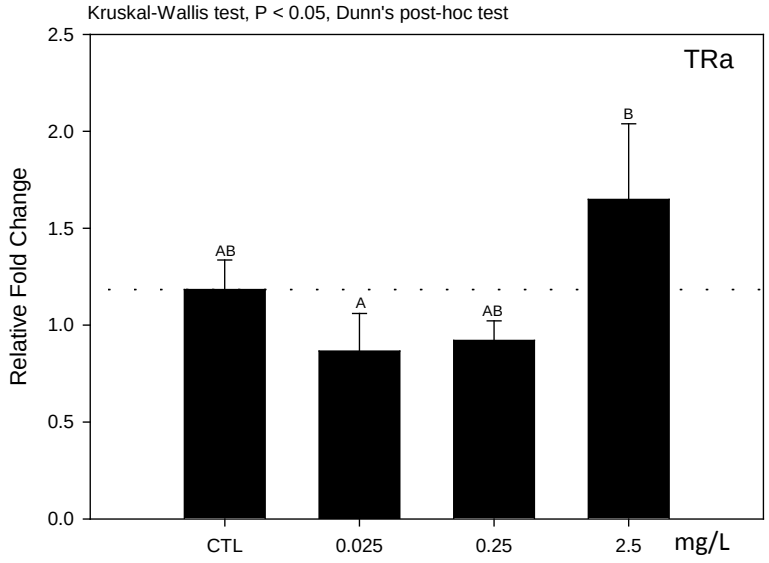


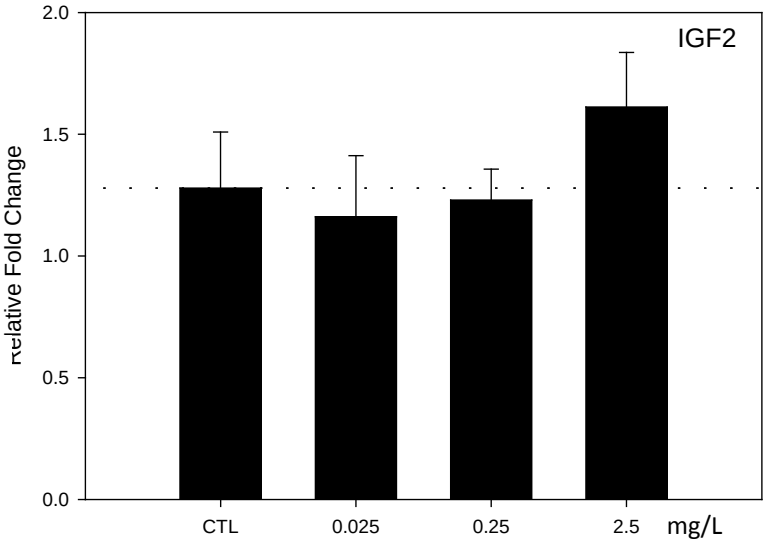


Trend towards down-regulation of estrogen-responsive genes

qPCR: ibuprofen

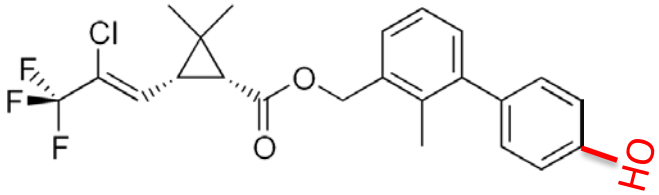






Jeffries et al. *(in prep)*

protein: bifenthrin



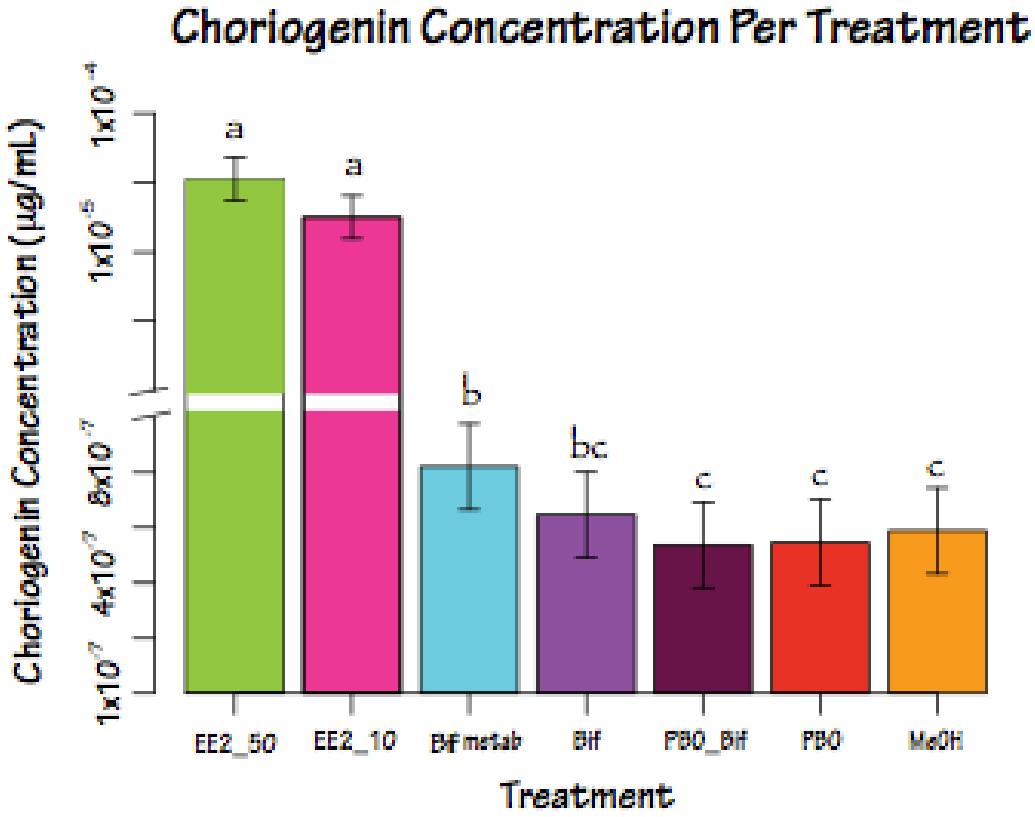


Figure 6: Effect of 10 ng/L EE2, 50 ng/L EE2, 10 ng/L 4-hydroxy bifenthrin (bifenthrin metabolite), 10 ng/L bifenthrin, and 10 ng/L bifenthrin+ 25 µg/L PBO on choriogenin expression in juvenile Menidia beryllina. Negative controls included 10 ng/L PBO and 10 ng/L MeOH. Fish were exposed to indicated concentrations in the water with daily 80% water renewals. Significant differences between treatments were determined by a Tukey test. Treatments that were not significantly different from each other are denoted by the same letter.

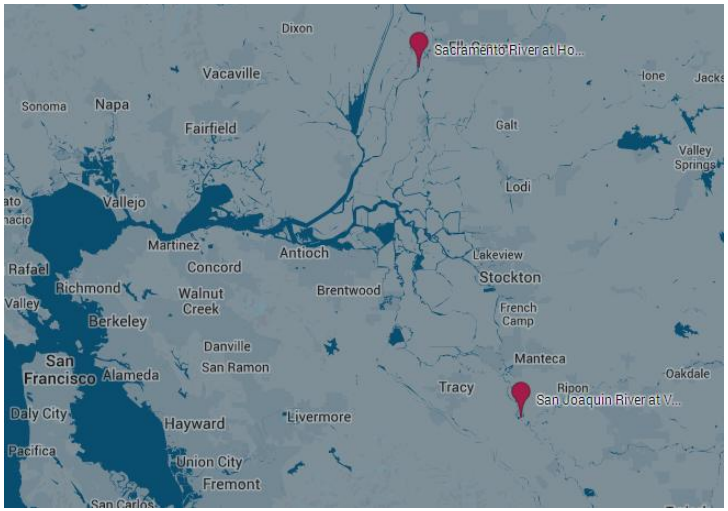
Previous work found that pesticides bifenthrin and permethrin are estrogenic *in vivo*, but anti-estrogenic *in vitro*

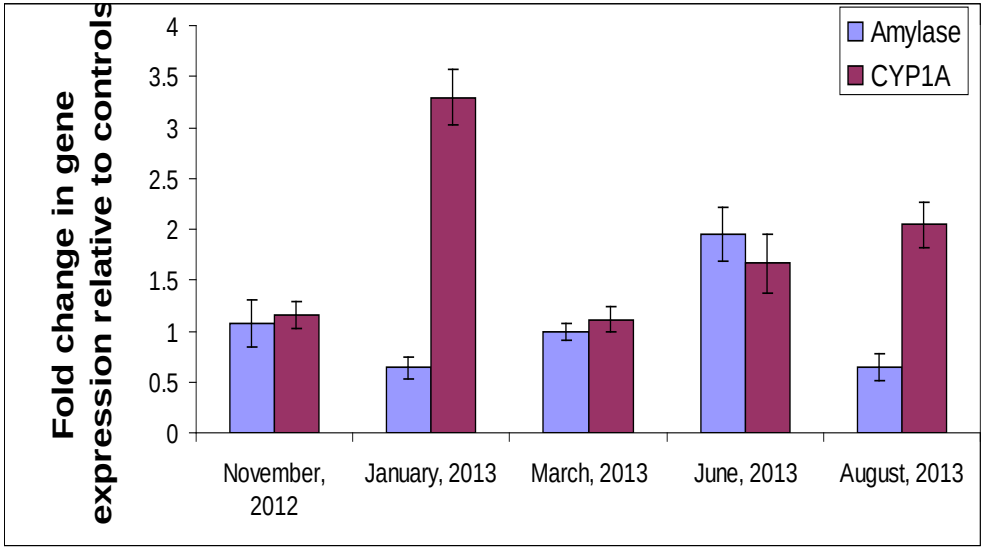
Results suggest that P450 metabolism may play a role in increasing bifenthrin's estrogenicity.

Brander et al. 2012, *ET&C*
DeGroot and Brander *(in prep)*

field studies

- 90 day old lab-reared Menidia
- Outplanted in flow-through aquaria at Hood and Vernallis stations, and in matched temp. control aquaria
- Held at outplant sites for 7 days, bimonthly
- Whole-body homogenate of outplanted fish is collected

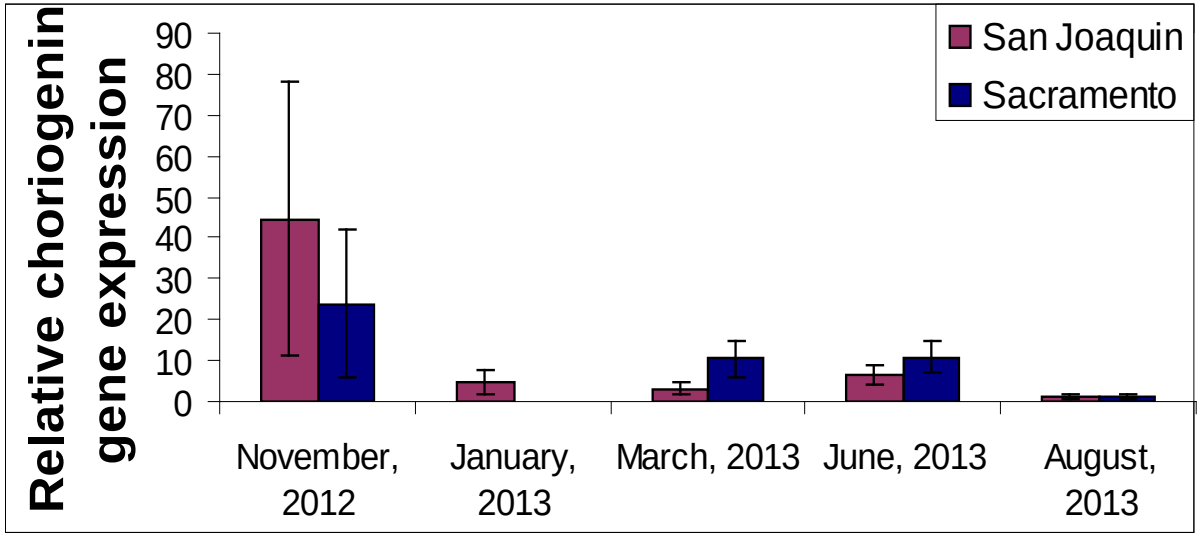


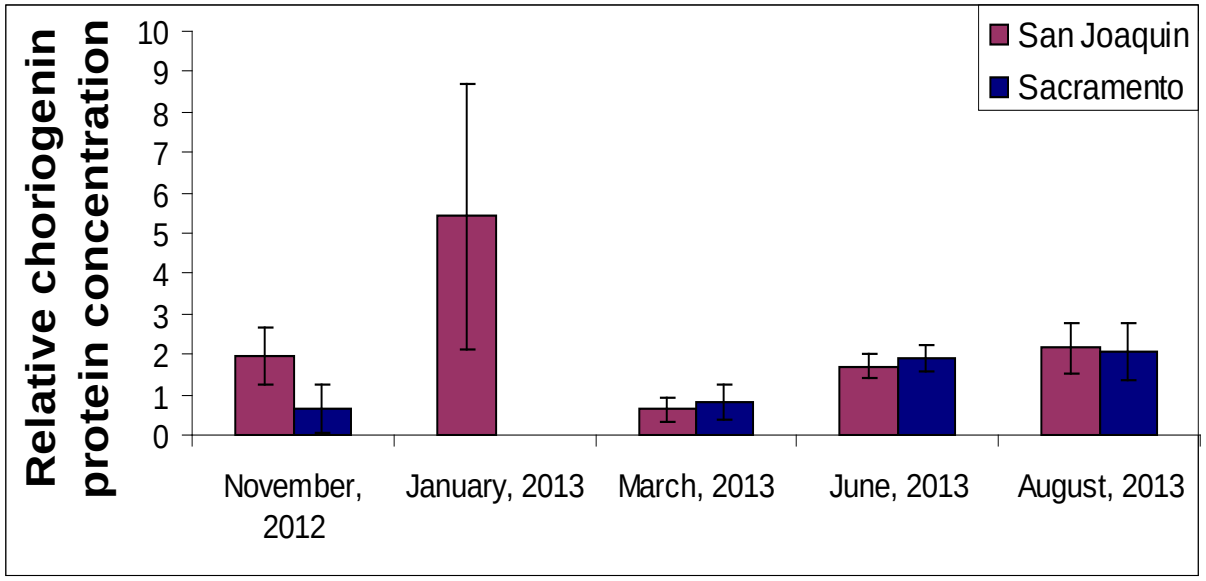


San Joaquin River at Vernallis

Cole et al. (in prep)

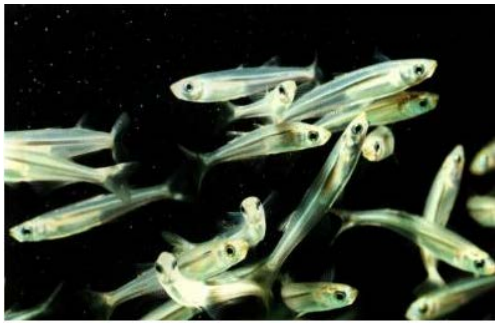
field studies





Cole et al. (in prep)

current projects: modeling



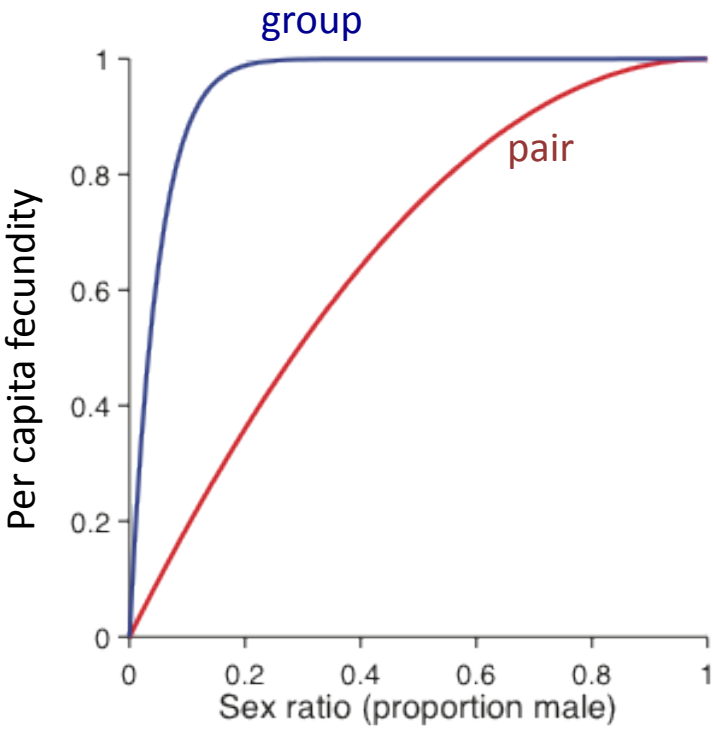
What effect will EDCs have on population size and persistence?
(J.W. White, S.M. Brander, UNCW)

Age-structured population model with carrying capacity

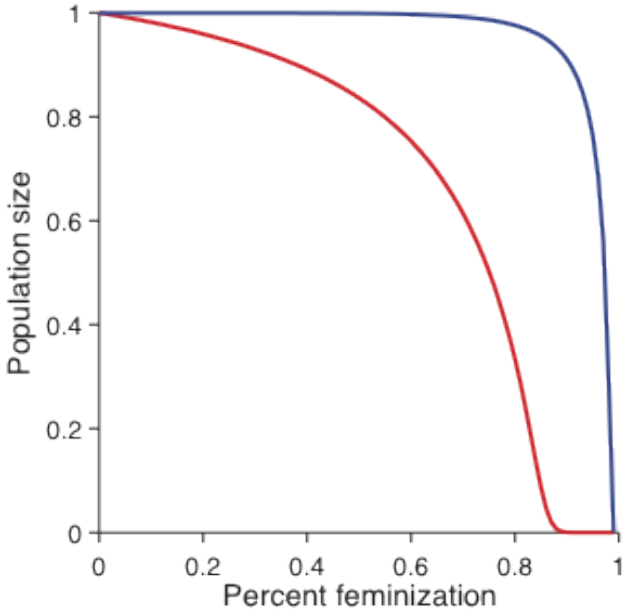
Tracks male and female genotypes independently
- genetic females ➔ phenotypic males
- genetic males ➔ phenotypic females

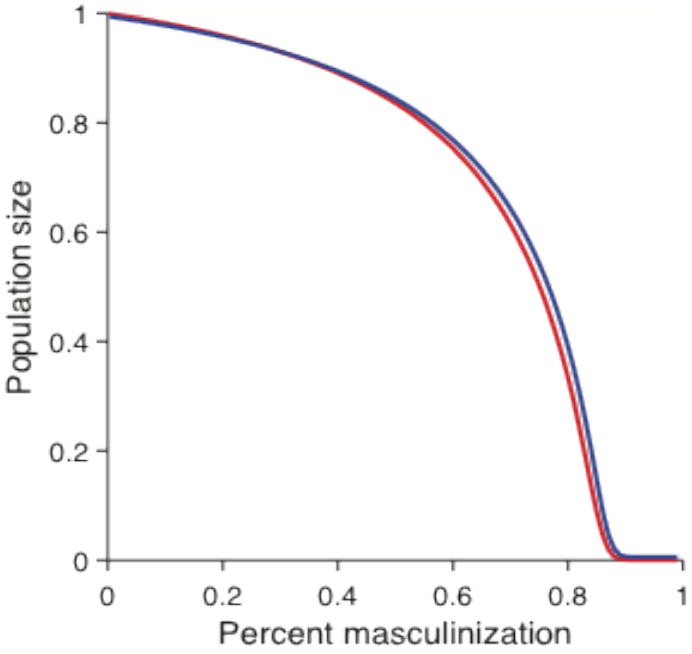
EDC effects in the model:
- Reduced fecundity of sex-changed fish
- Altered sex ratio reduces fertilization success
- EDCs alter growth (and thus may change individual fecundity)

modeling



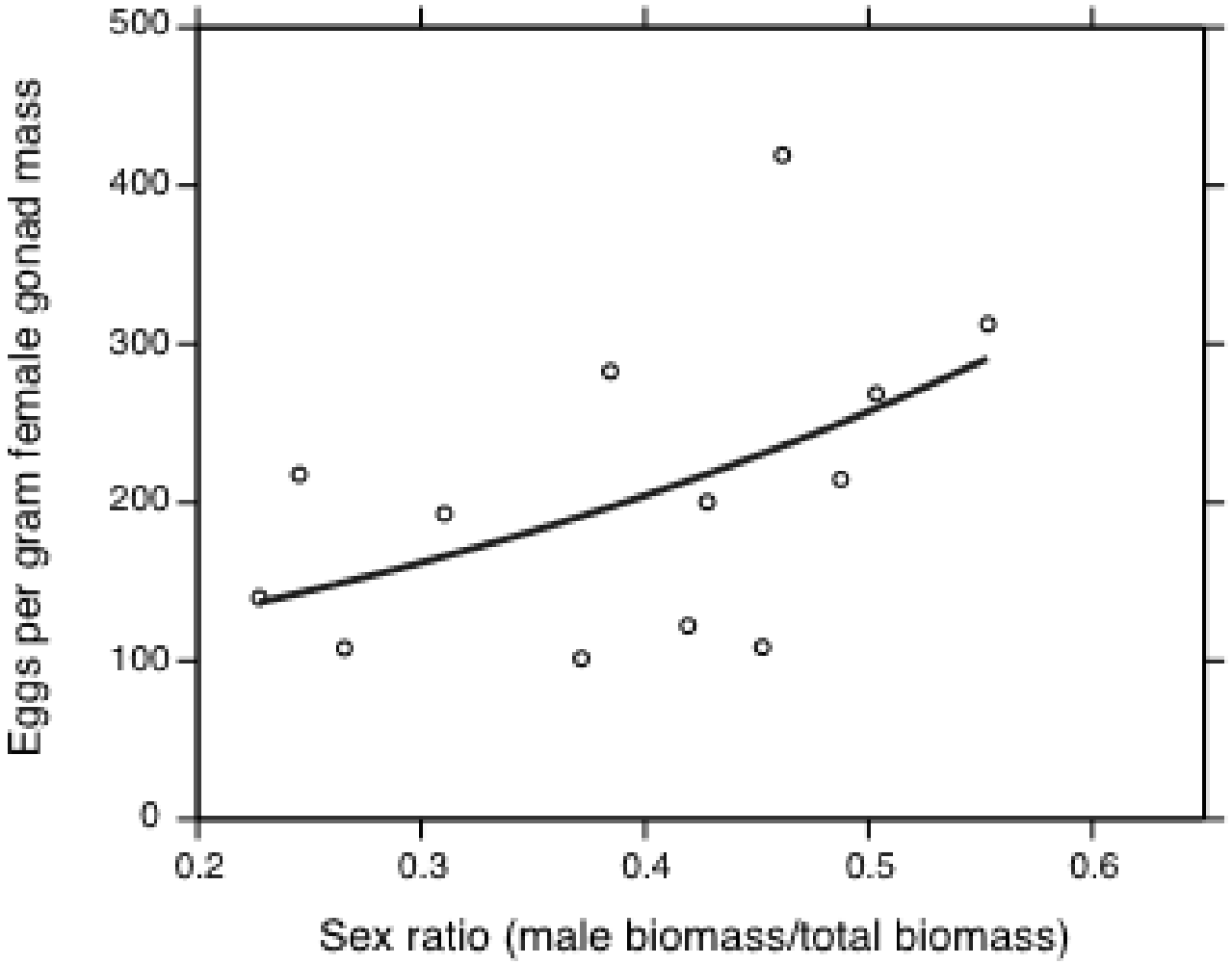
parameters model is based on





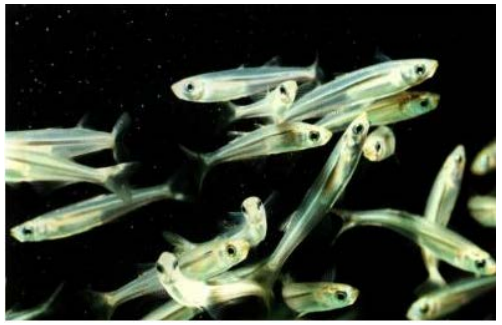
Brander, White, Cole and Connon (in prep)

modeling

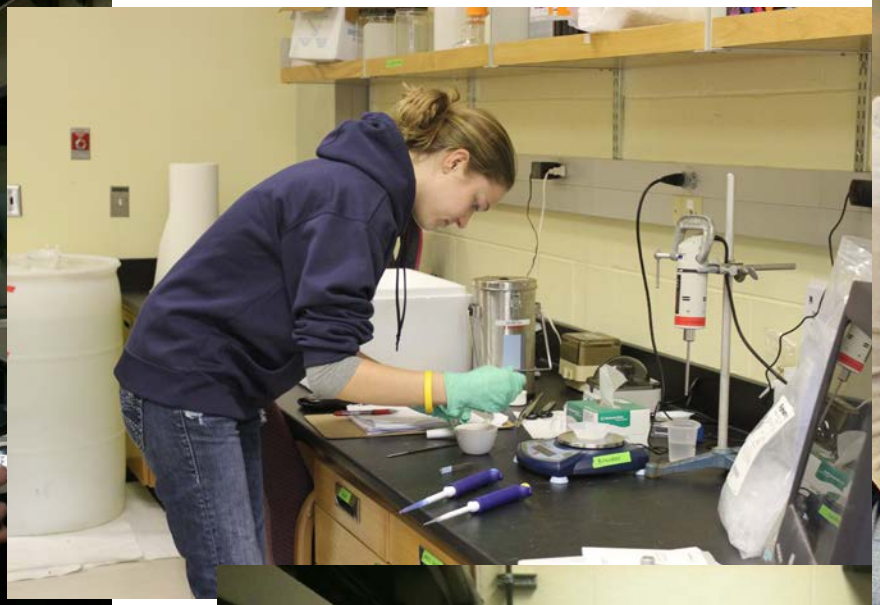


Sigmoidal-shaped spawning functional response

summary



- *Menidia* species are highly informative and sensitive EDC bioindicators
- Endpoints at multiple biological scales
- Can be sampled nationwide
- Next steps ...
  - Microarray with selected samples, determine AOPs, qPCR
  - Testing effects of early life exposure on sex ratio and reproductive output later in life
  - Effects of multiple stressors (salinity, temperature, EDCs)
  - Effects of environmental mixtures in CA and NC
  - Epigenetics, i.e. role of aromatase in sex determination



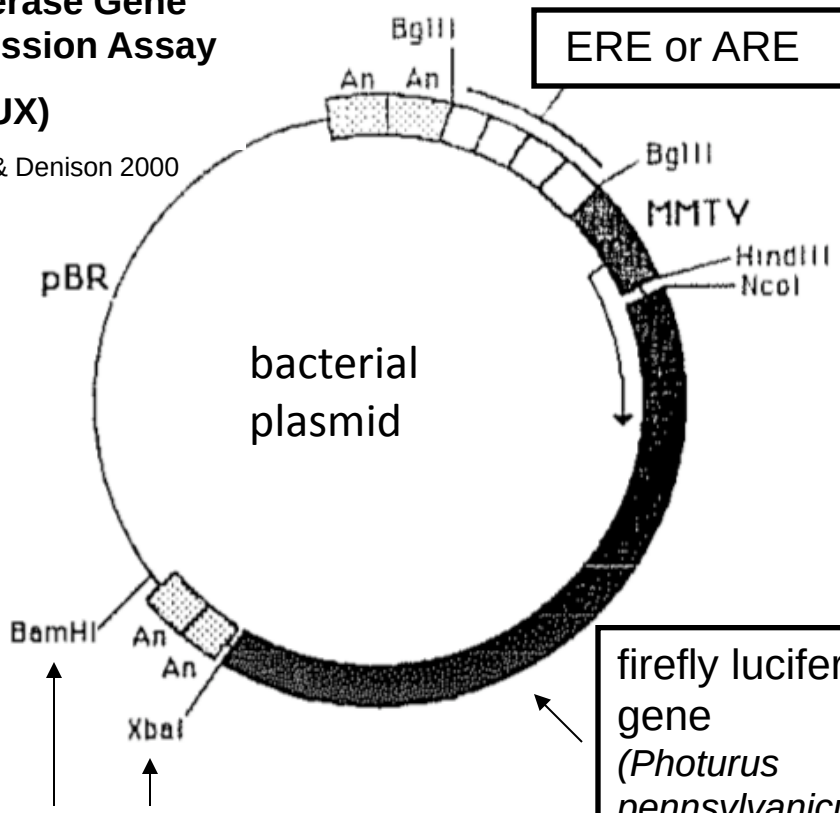
questions?

cell line – site water

Chemical-Activated Luciferase Gene Expression Assay

(CALUX)

Rogers & Denison 2000



how it works …

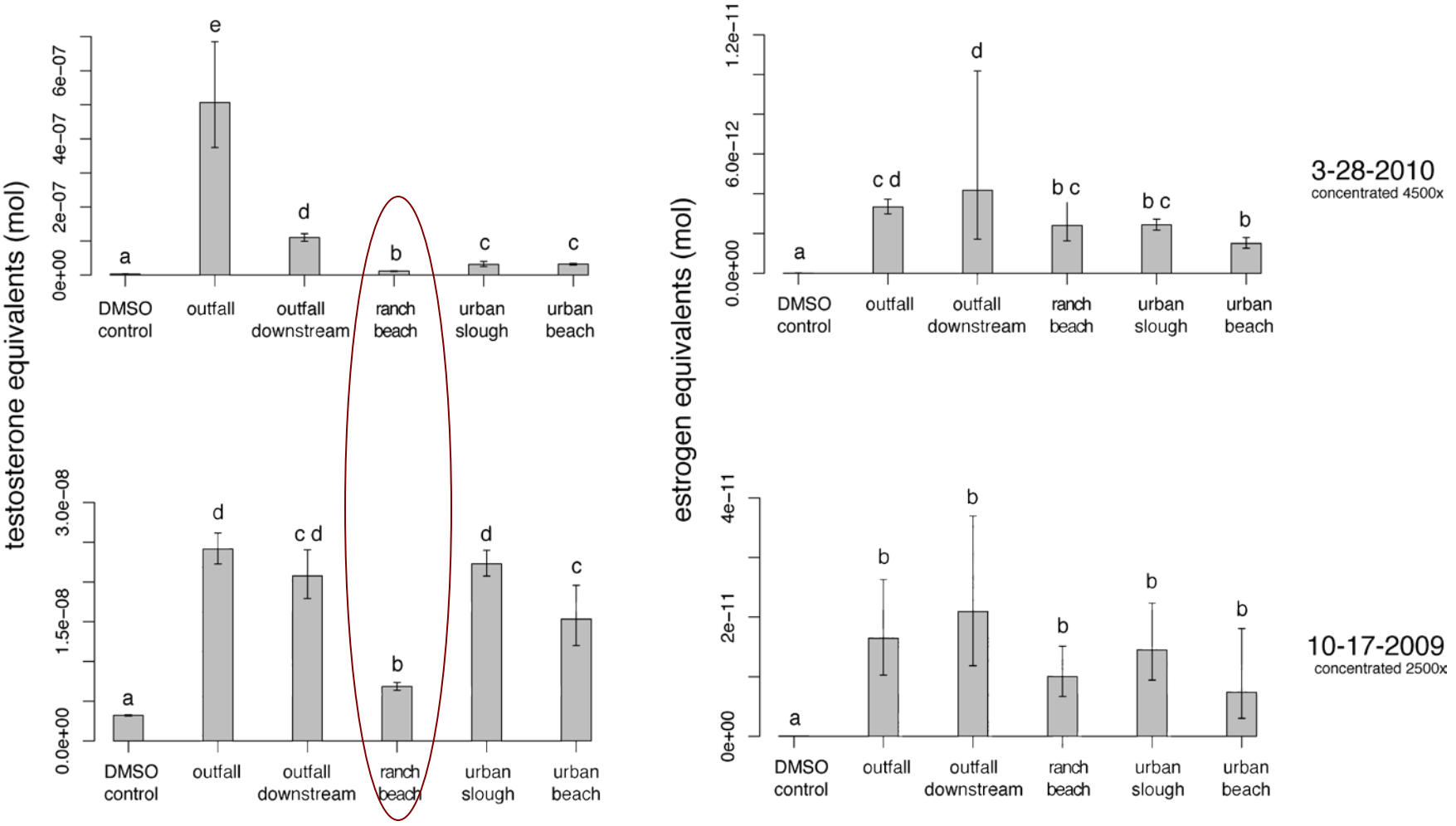
Transfected plasmid contains:
- ERE, ARE
- firefly luciferase gene.

plasmid transfected into cell line that has ER or AR

cells are incubated with extracted water samples for 24 hours

luciferase induction is proportional to activation of ERE or ARE

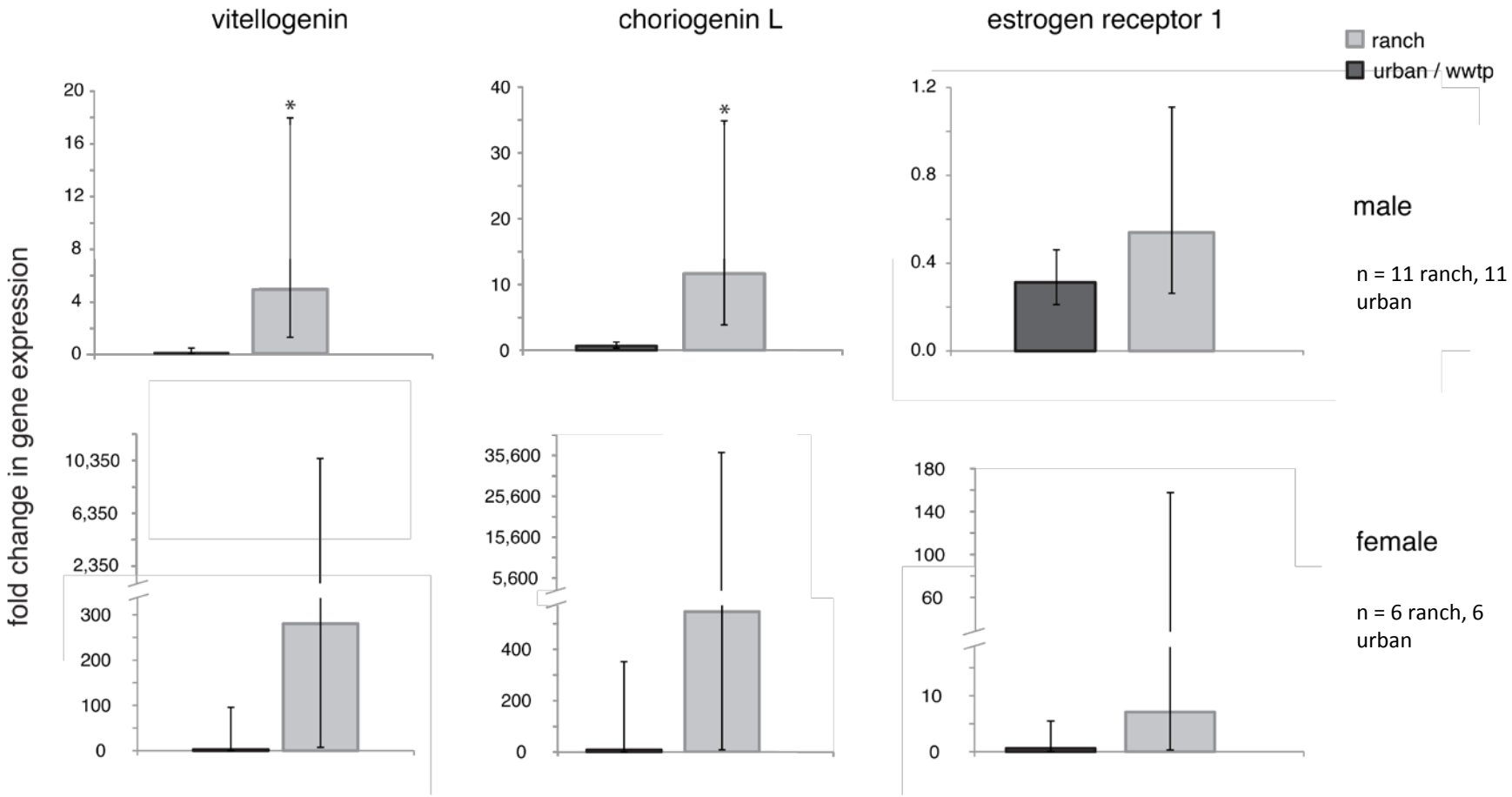
cell line – site water



Brander, Connon, He, Hobbs, Smalling, Teh, White, Denison, Werner, Cherr. From omics to otoliths: responses of an estuarine fish to endocrine disrupting compounds across biological scales (in prep).

Tukey test, $p < 0.05$

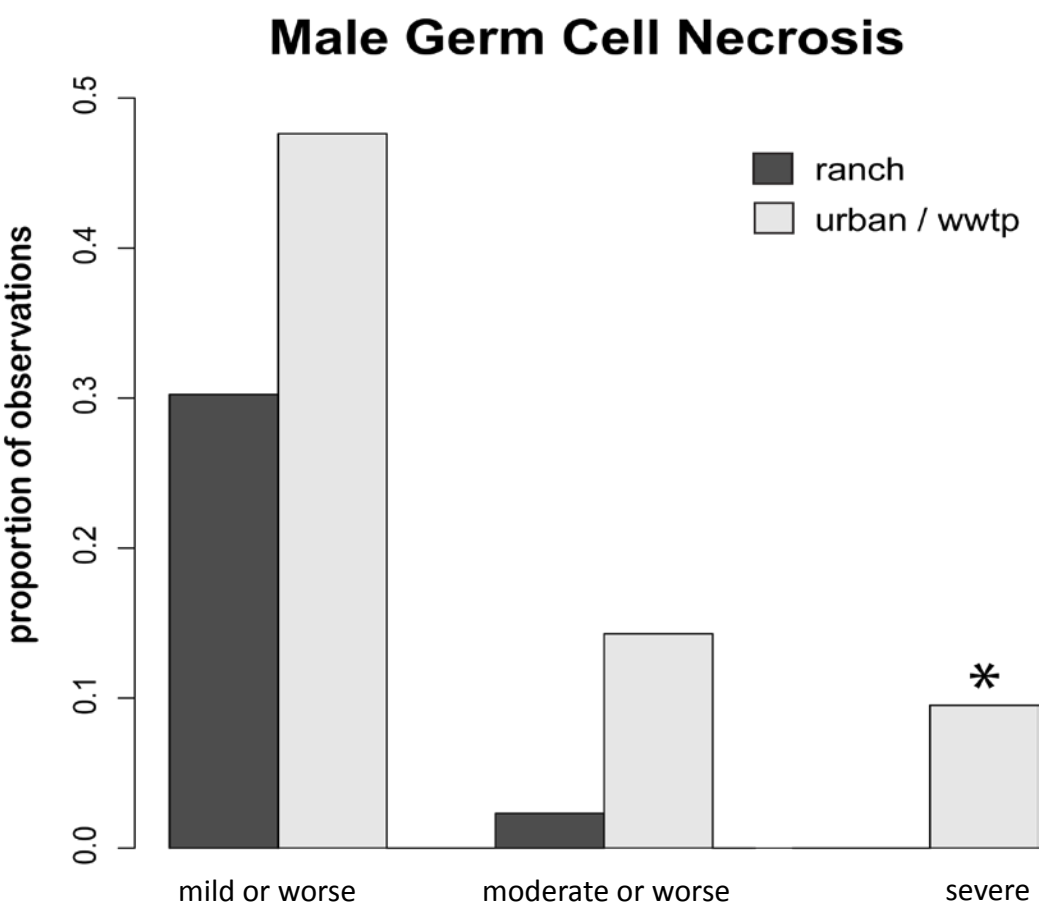
molecular level

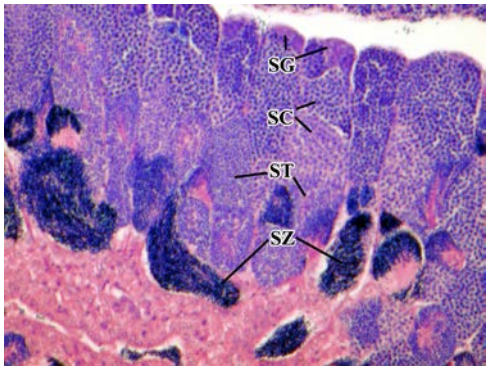


**t-test, $p < 0.05$*

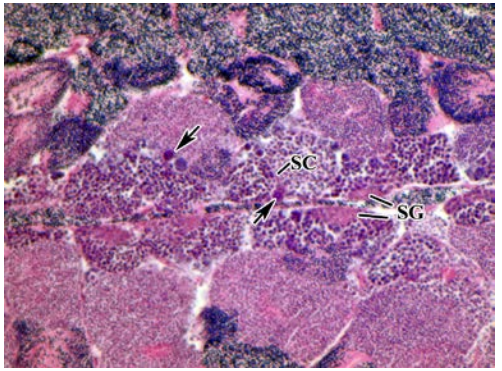
Brander et al. 2013, *PLoS ONE*

organism level: gonad histology





healthy S.J. Teh



necrotic S.J. Teh

Male GSI significantly lower at urban beach.

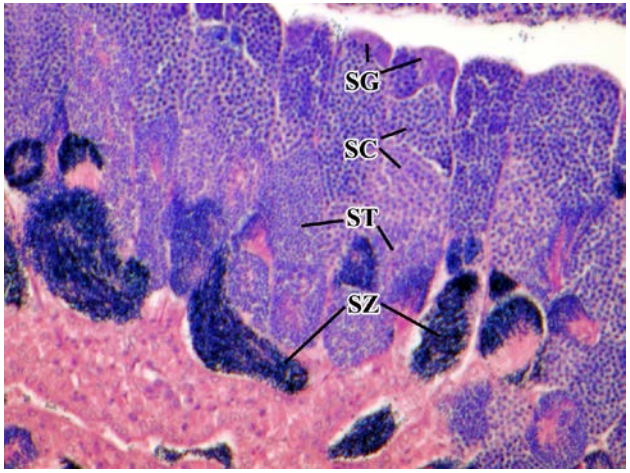
organism level

- Male GSI significantly lower, significantly higher incidence of germ cell necrosis at urban site

- Observed (via otoliths) that growth was significantly slower at urban site ...

- BUT male standard length was significantly greater at urban beach than ranch beach

- At urban beach males were significantly longer than females.



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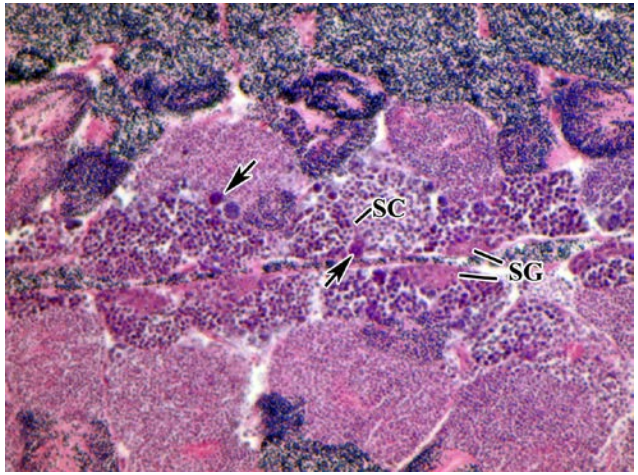
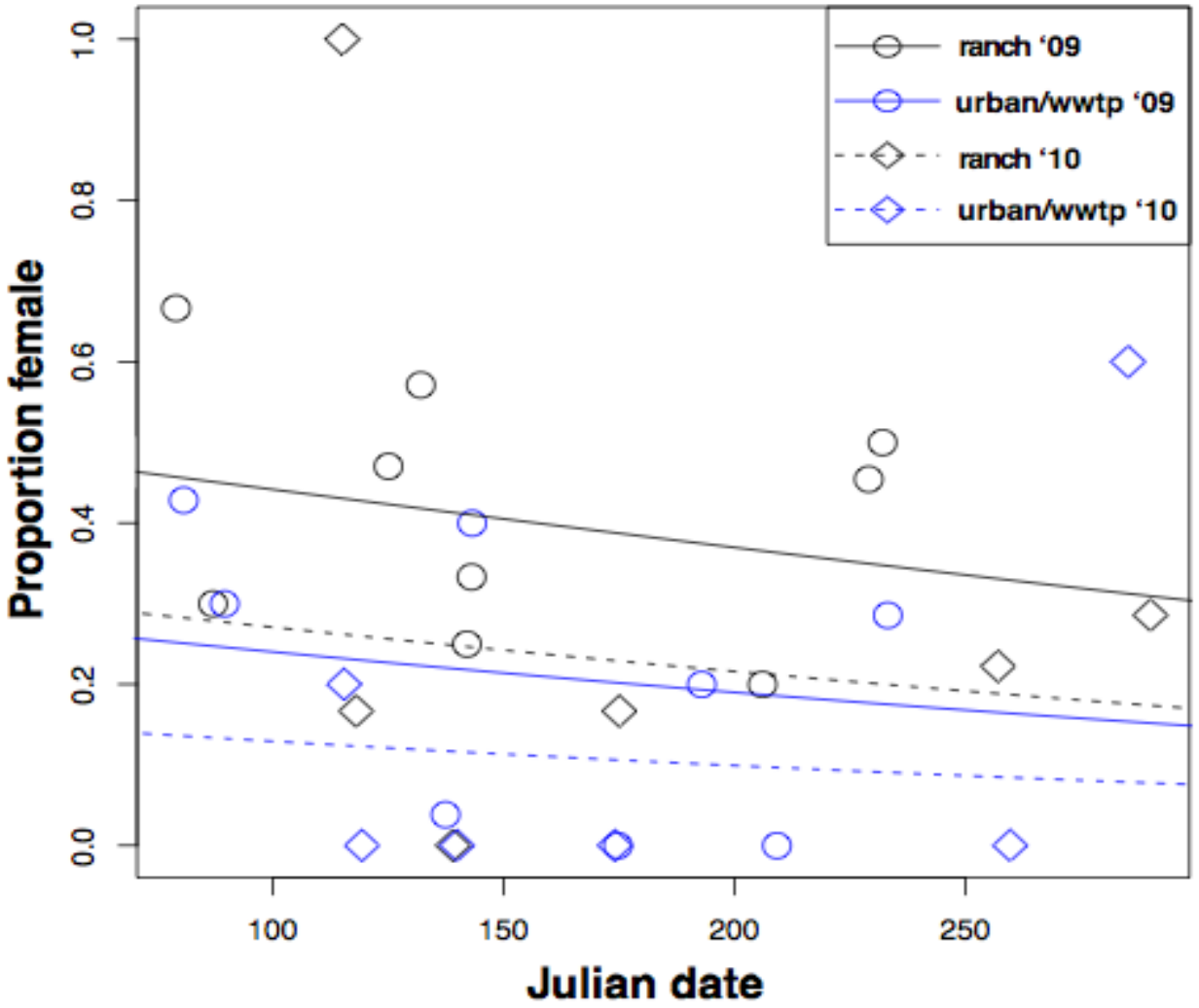


necrotic S.J. Teh

Brander et al. 2013, *PLoS ONE*

population level



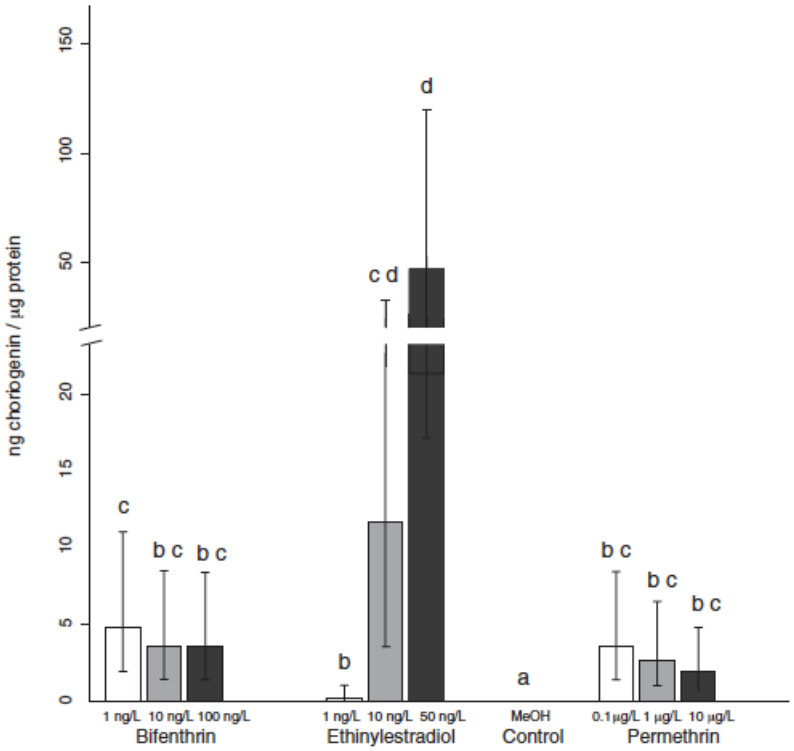
J. Wilson White

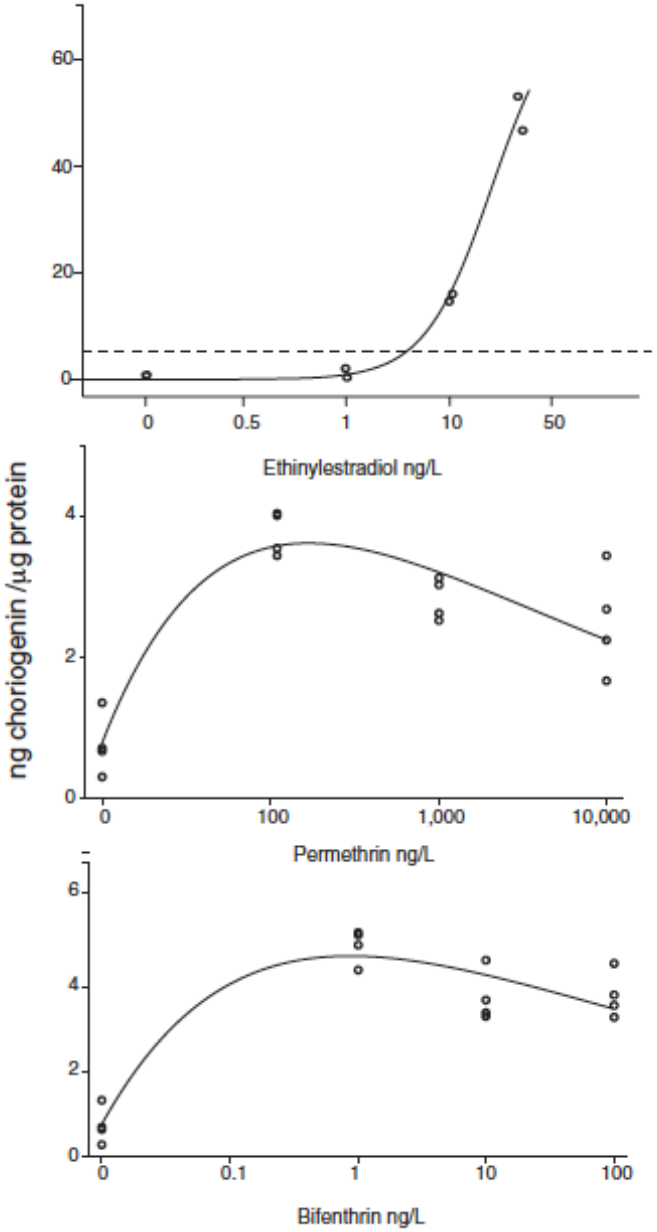
S. Brander

Brander et al. 2013, *PLoS ONE*

current projects

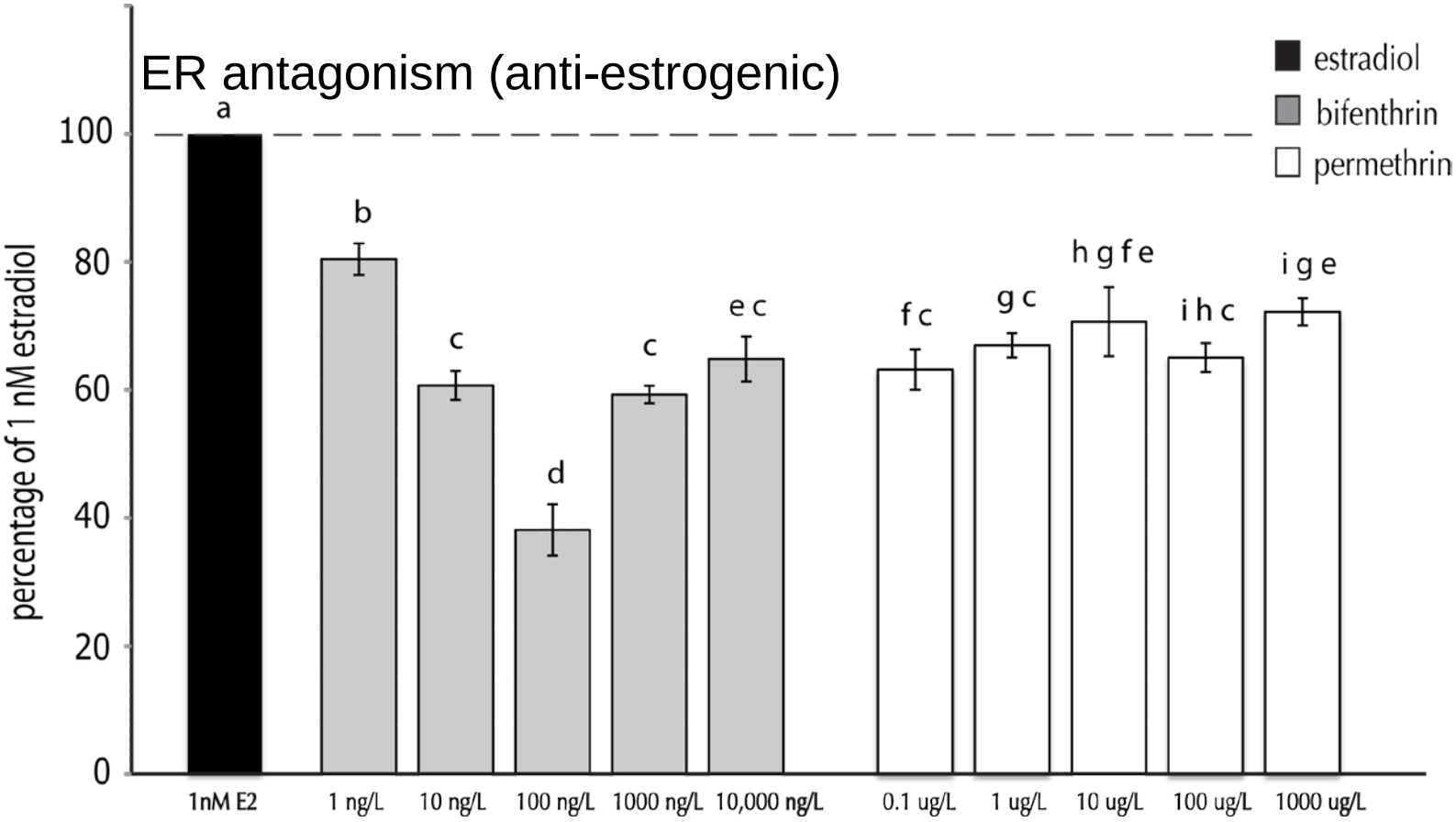
- Previous work found that pesticides bifenthrin and permethrin are estrogenic *in vivo*, but anti-estrogenic *in vitro*





Brander et al. 2012, *ET&C*

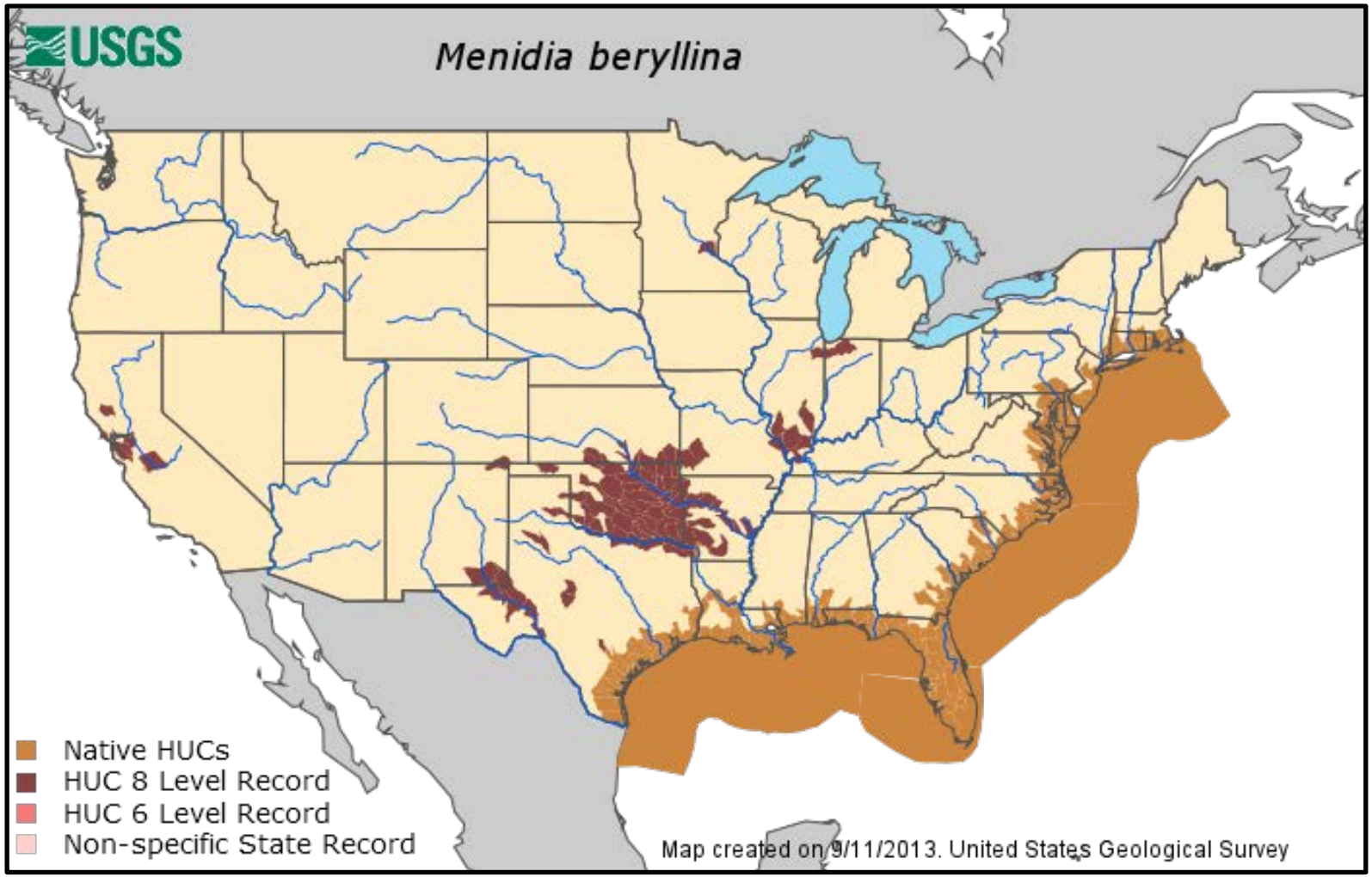
pyrethroid effects

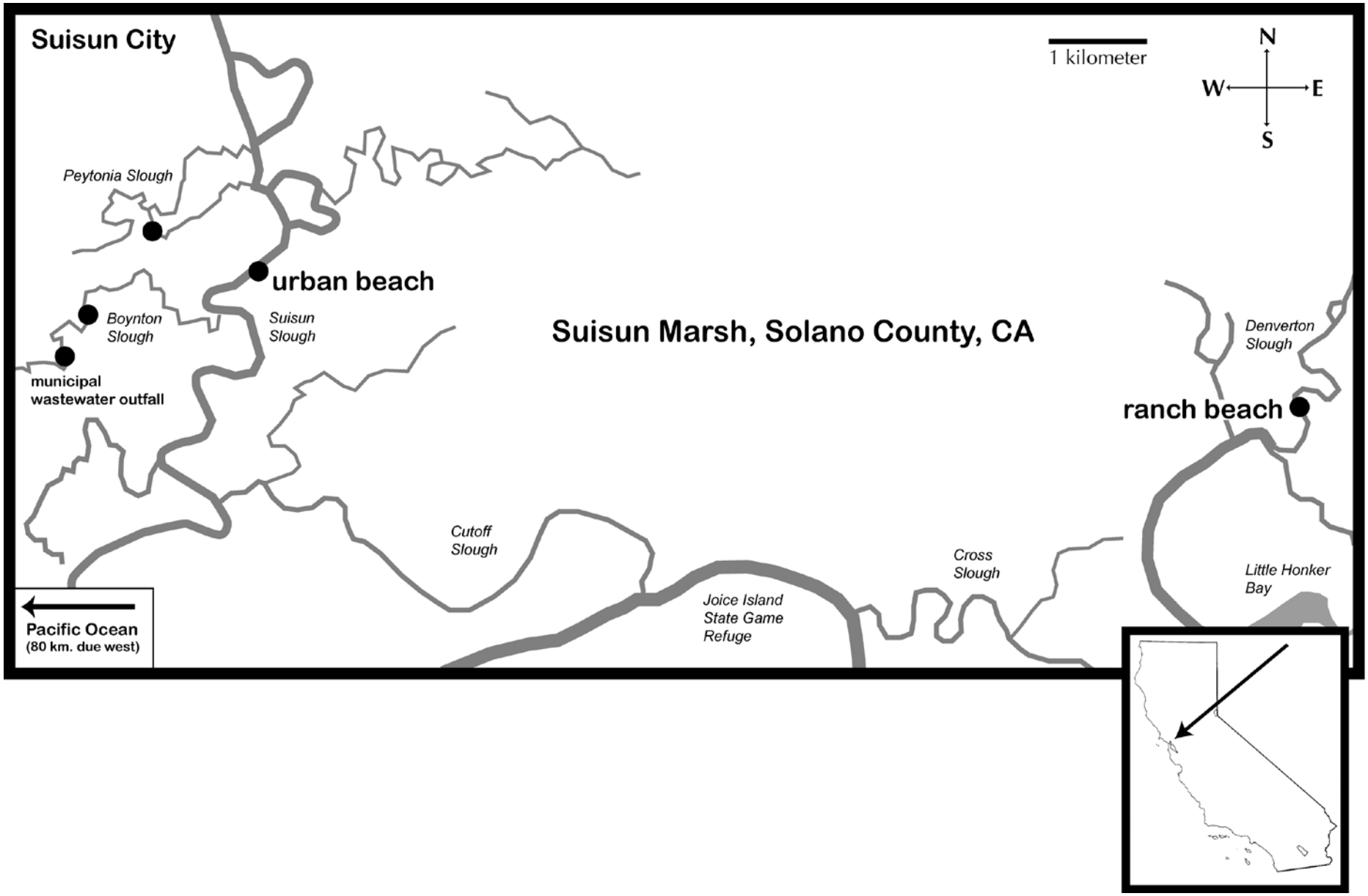


estrogenic, androgenic and anti-androgenic activity not significantly different from control

Brander et al. 2012. *Env. Tox & Chemistry*

approach


USGS
Menidia beryllina
Native HUCs
HUC 8 Level Record
HUC 6 Level Record
Non-specific State Record
Map created on 9/11/2013. United States Geological Survey


Suisun City
1 kilometer
N
W
E
S
Peytonia Slough
urban beach
Boynton Slough
Suisun Slough
municipal wastewater outfall
Suisun Marsh, Solano County, CA
Denverton Slough
ranch beach
Cutoff Slough
Cross Slough
Little Honker Bay
Joice Island State Game Refuge
Pacific Ocean
(80 km. due west)