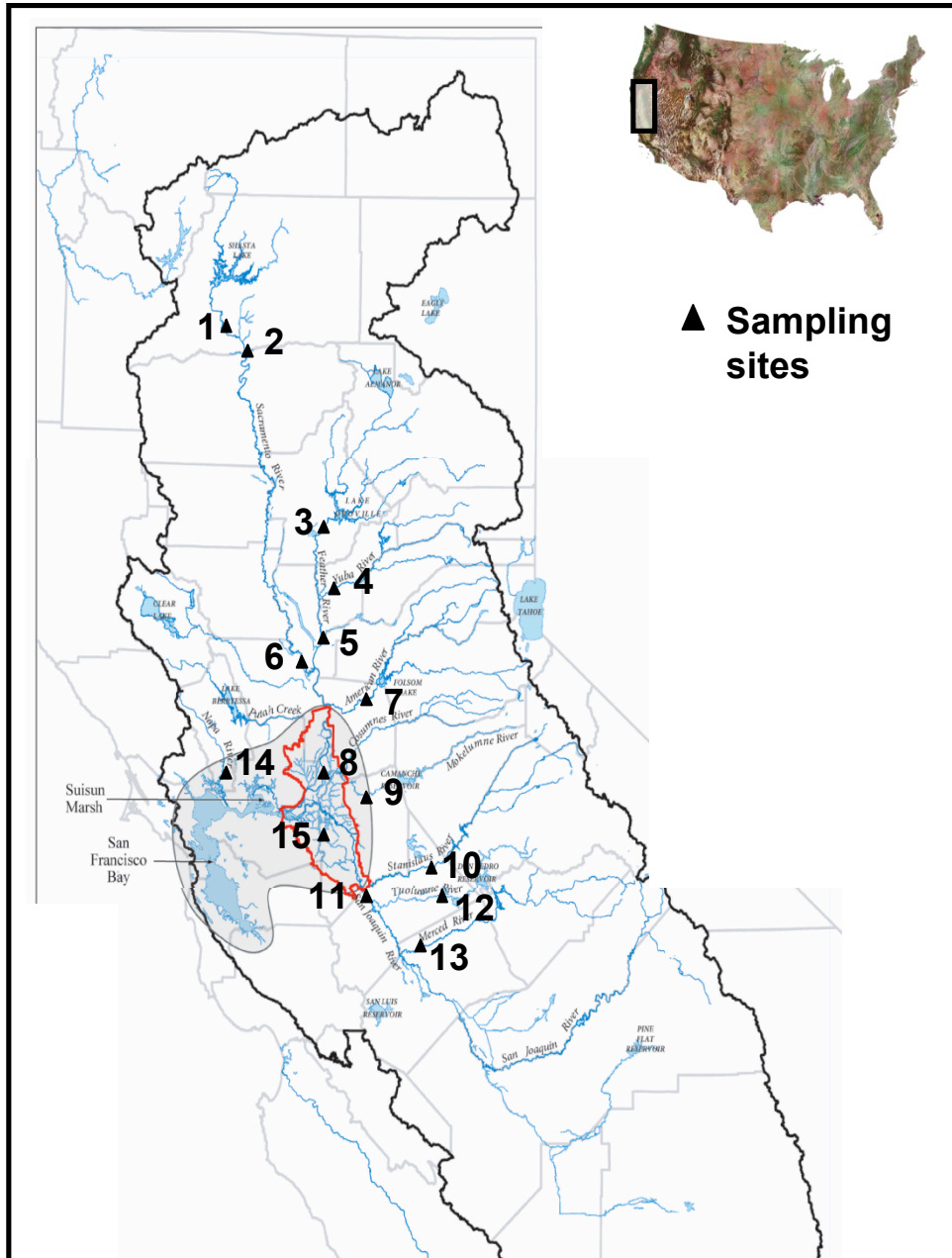


**RMP PROPOSAL**

**Evaluation of Reproductive  
Endocrine Disruption in San  
Francisco Bay Fish**

Daniel Schlenk  
UC Riverside

# Material & Methods: Sampling sites



Site	Site name
1	Upper Sacramento River
2	Battle Creek
3	Upper Feather River
4	Yuba River
5	Lower Feather River
6	Lower Sacramento River
7	Lower American River
8	Sacramento River in Delta
9	Mokelumne River
10	Stanislaus River
11	San Joaquin River
12	Tuolumne River
13	Merced River
14	Napa River
15	Clifton Court Forebay



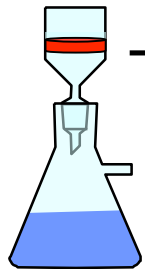
# Material & Methods: Extracts & Exposures

## Water extracts



Water (1 L)

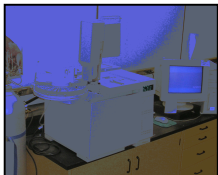
Solid-phase  
Extraction



C18 Disks

Elution  
10 mL MetOH

Hormone  
determination  
(GC-MS)



Exposures *in vitro/in vivo*



## Extract exposures

*In vitro* hepatocytes  
exposure



Primary Hepatocytes  
isolation and culture

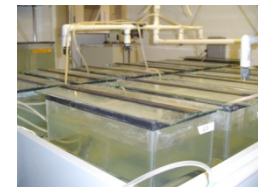
Extract added in the  
media (0.6% v/v)

Incubation  
24 h

Total mRNA extract

VTG mRNA  
determination

*In vivo* whole fish  
exposure

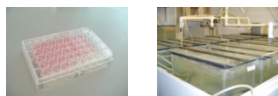


Intraperitoneal  
extract injection (x2)

Incubation  
7 days

Plasma collection

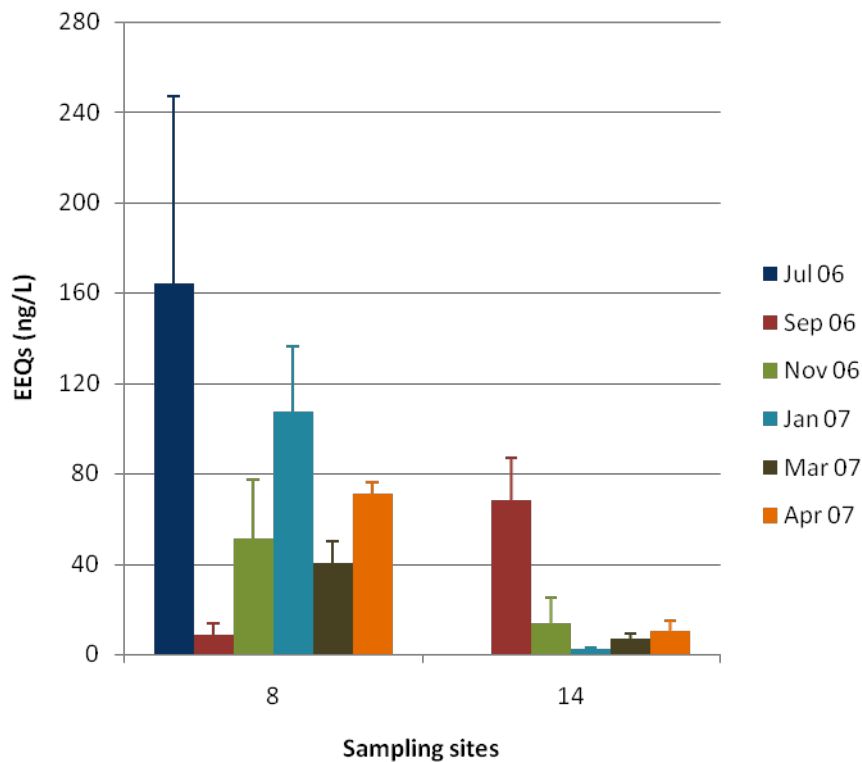
VTG protein levels  
determination



## Results: Water extracts

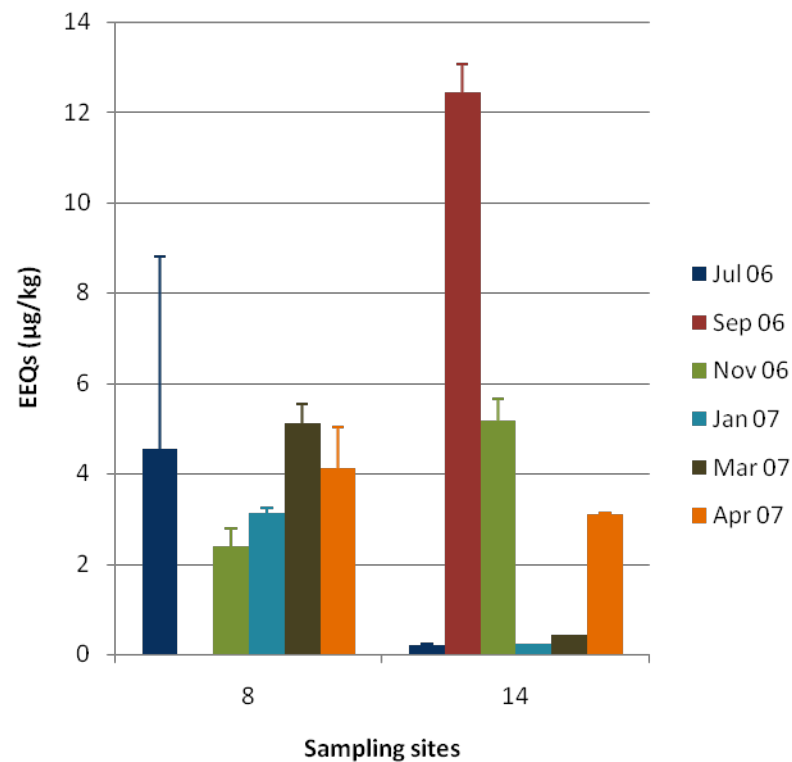
### High Estrogenicity Areas (both methods)

#### Cells Exposure



Data presented as Mean  $\pm$  SEM

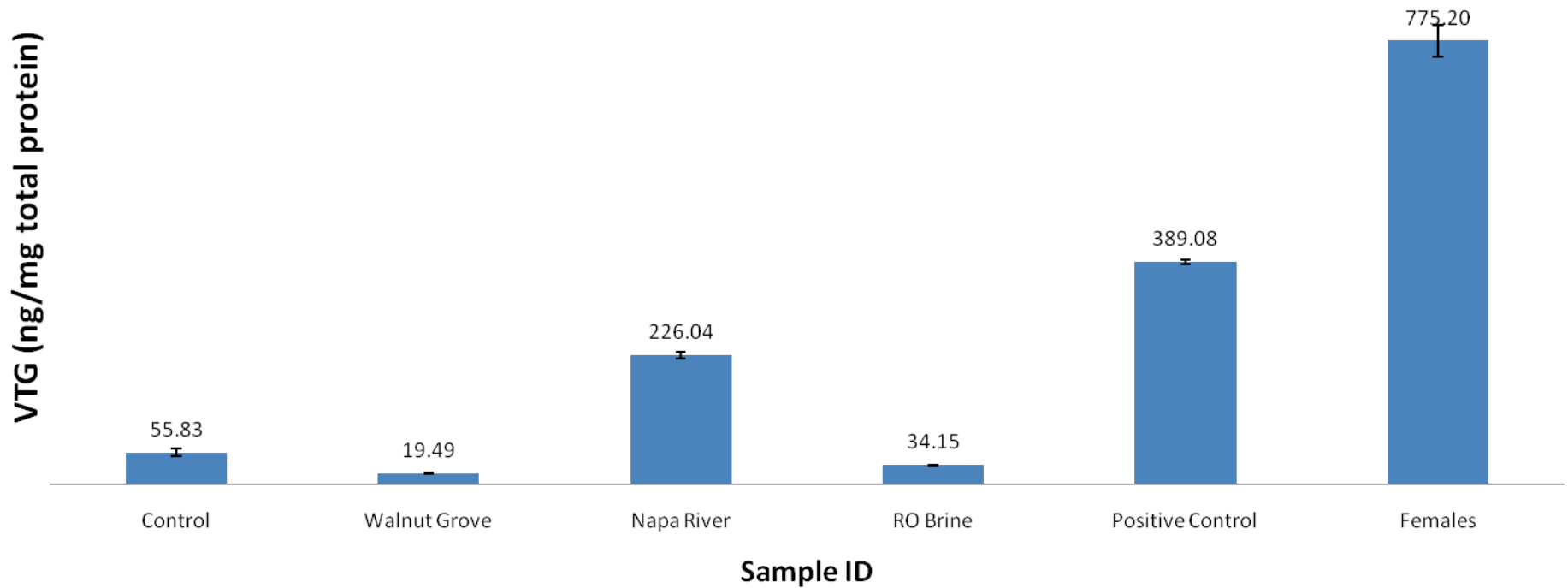
#### Whole Animal Exposure



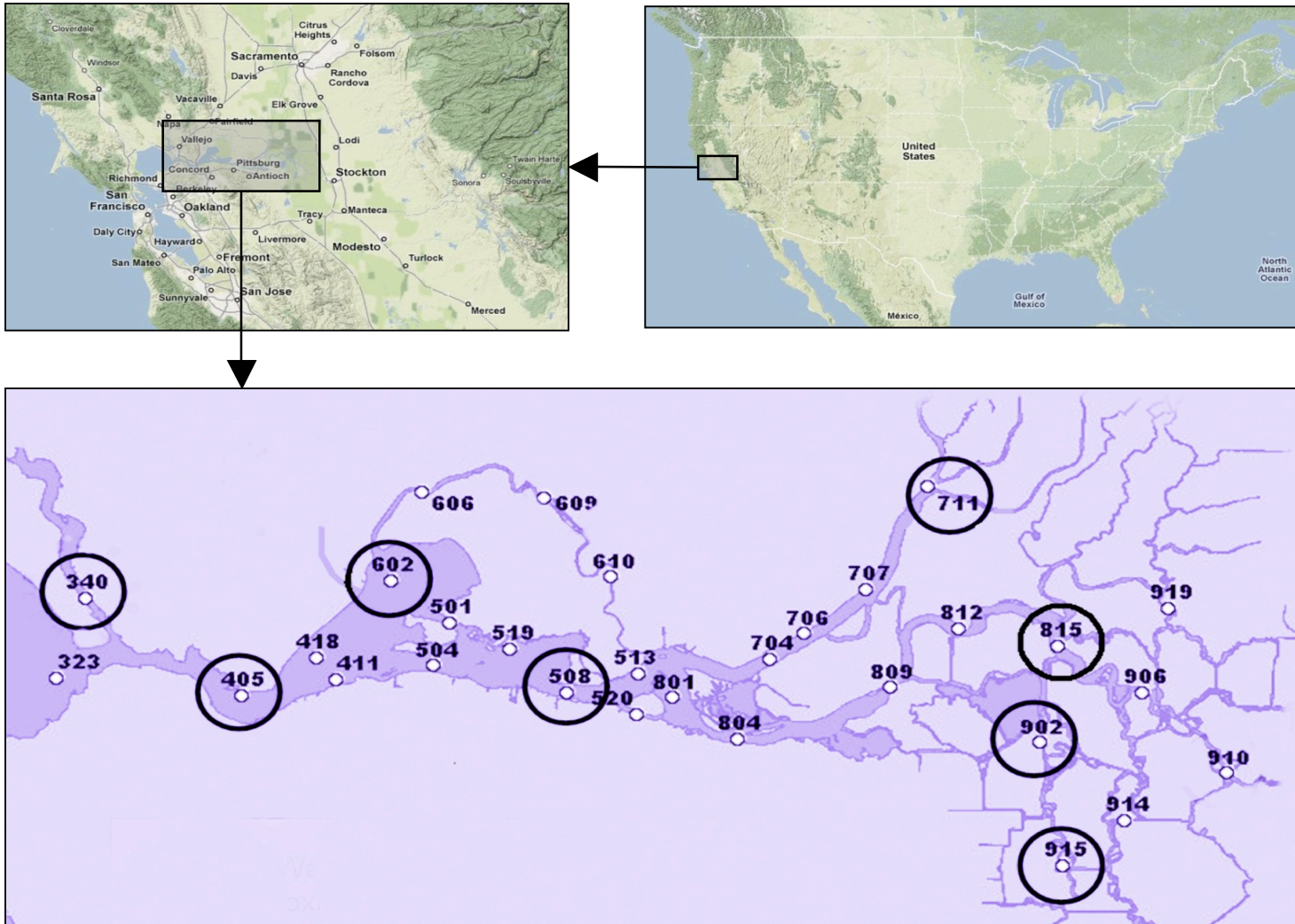
Data presented as Mean  $\pm$  SEM

# In Vivo Estrogenicity

In vivo vitellogenin induction in Japanese medaka exposed for 7 days,  
measured by ELISA



# Delta Sampling in 2008 (POD samples)



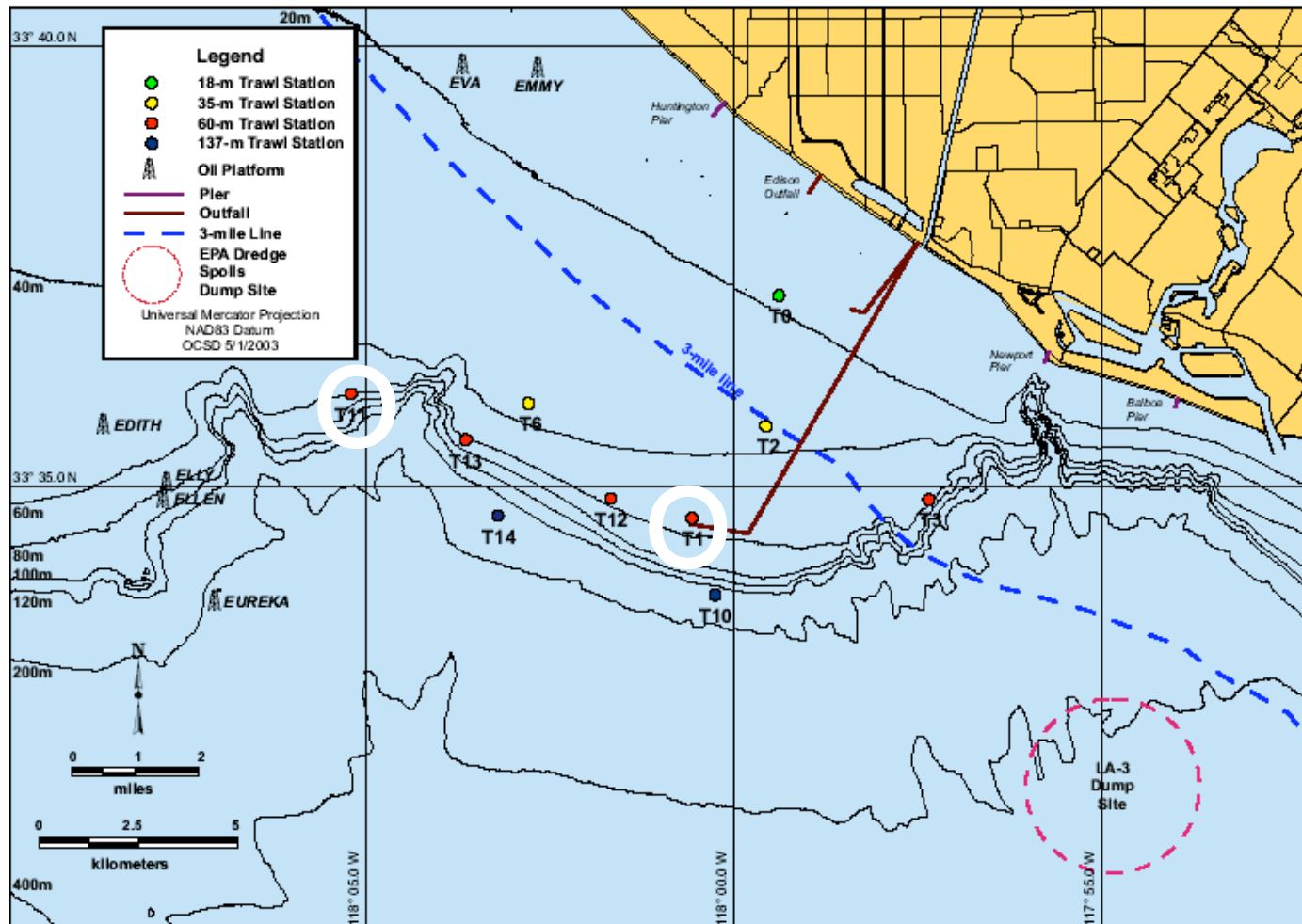
# In vivo Estrogenic Activities of 2008 POD samples

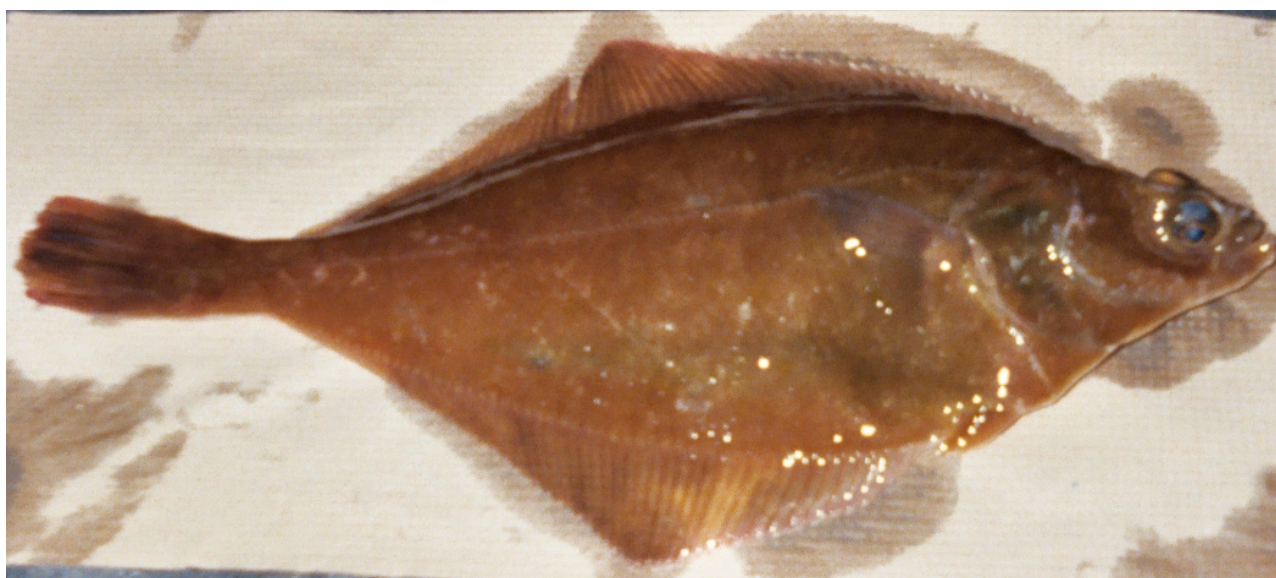
Sample	<i>In vivo</i> EEQs (ng/L)
340	0.90 ± 0.03
<b>405</b>	<b>25.65 ± 4.01</b>
508	<i>bdl</i>
602	1.05 ± 0.10
<b>711</b>	<b>12.79 ± 1.65</b>
815	0.80 ± 0.12
902	<i>bdl</i>
915	2.02 ± 0.32

Are feral animals affected?



# Materials and Methods





# Sampling

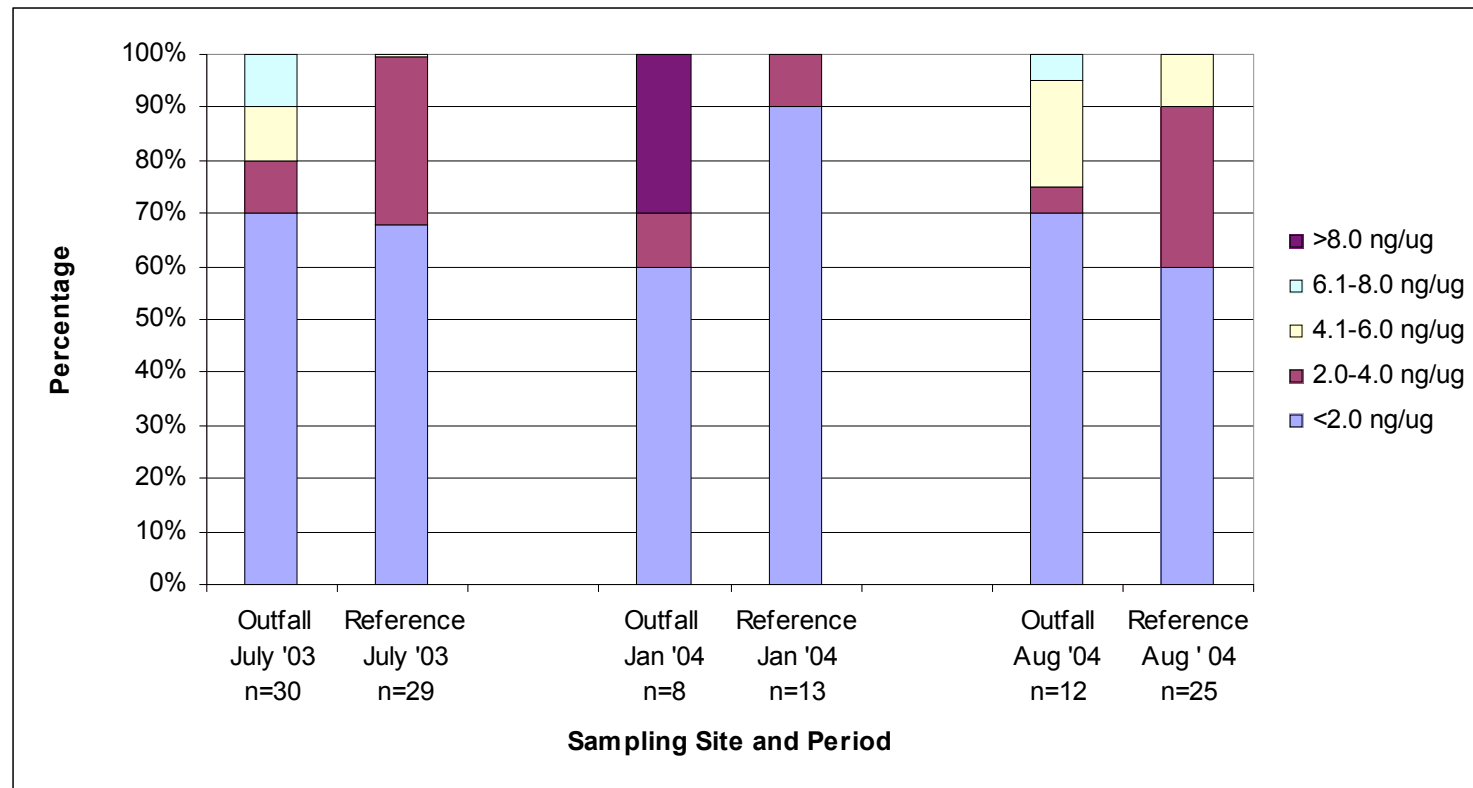




# Sampling



# Proportion of plasma vitellogenin levels at given concentrations in English sole





*Aquatic Toxicology* 77 (2006) 241–249

**AQUATIC  
TOXICOLOGY**

[www.elsevier.com/locate/aquatox](http://www.elsevier.com/locate/aquatox)

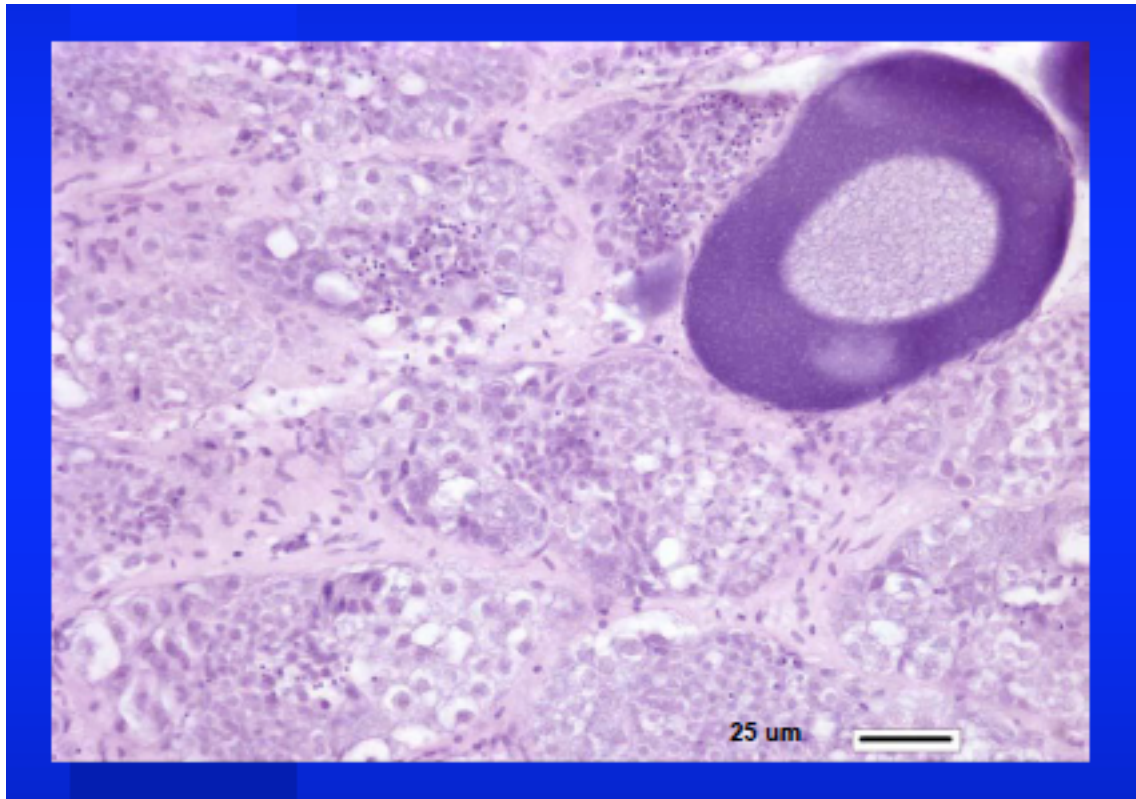
## Evaluation of relationships between reproductive metrics, gender and vitellogenin expression in demersal flatfish collected near the municipal wastewater outfall of Orange County, California, USA

Mary Ann Rempel<sup>a</sup>, Jesus Reyes<sup>b</sup>, Scott Steinert<sup>c</sup>, Wendy Hwang<sup>a</sup>, Jeff Armstrong<sup>d</sup>,  
Ken Sakamoto<sup>d</sup>, Kevin Kelley<sup>b</sup>, Daniel Schlenk<sup>a,\*</sup>

# Tasks

- Evaluate blood of English Sole for vitellogenin in 3 locations
  - (2 reference; EBMUD)
- Evaluate gonads of males and females
  - GSI
  - Ova-testes/atresia

# Ova-testes





# Methods

- Collections by outer trawl (Late Aug/Sept)
  - Rusty Fairey (CFG/ Moss Landing)
- Minimum 10 males and females from each location
- Bleed on site; freeze plasma/serum
- Remove gonads for histopath/GSI

# Budget Justification

- Collection (subsidized by collaboration)
- Travel for collection
- Supplies/consumables
- Student stipend/fees