Evaluation of Endocrine Disruption in Benthic Flatfish of Southern California Collected Near A Publicly Owned Treatment Works Outfall

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  - City of LA
  - City of San Diego
  - LACSD
  - OCSD
  - CSULB
  - UCSD
- Orange County Sanitation District
- CEQI-UC-Marine Council

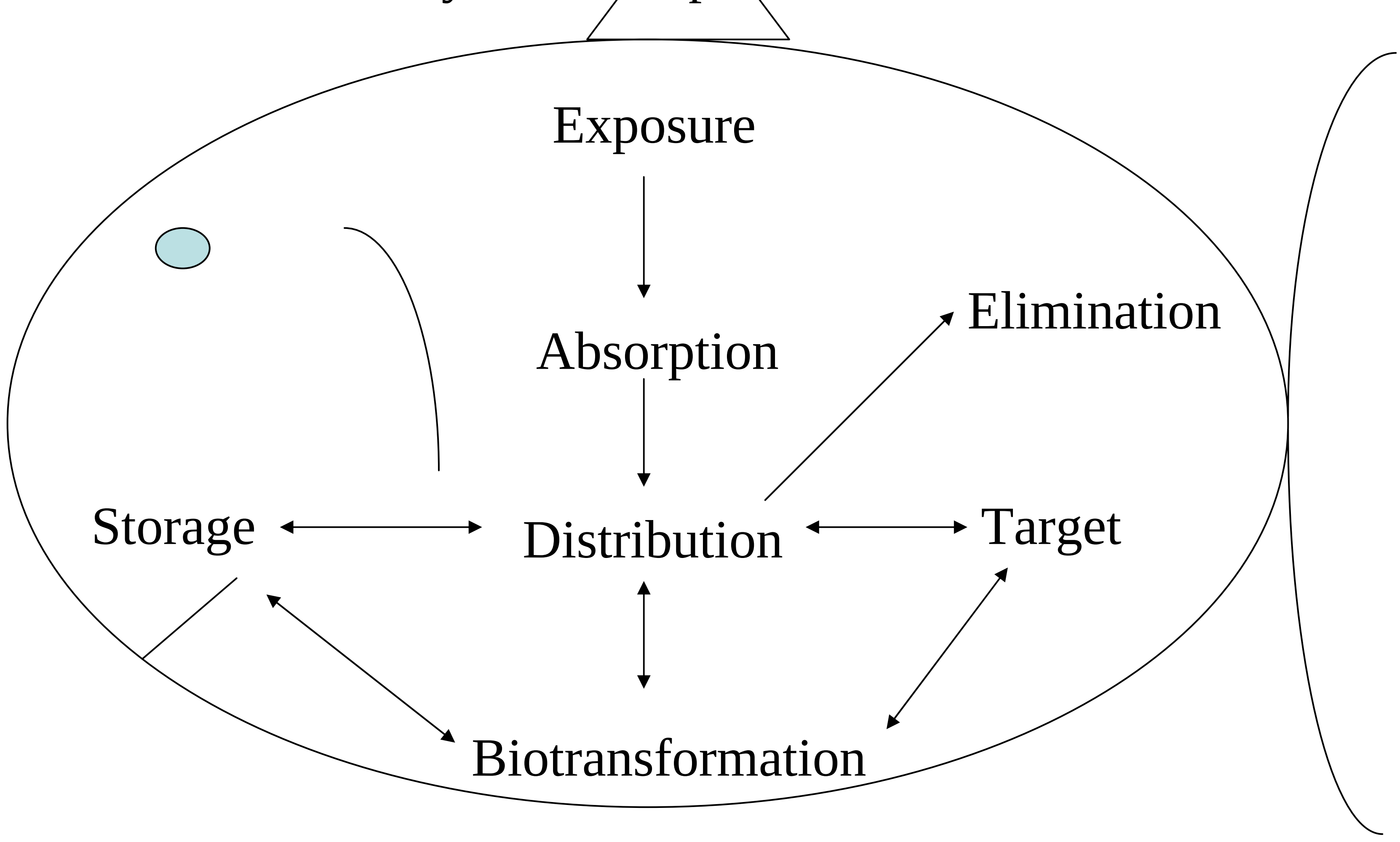
Environmental Estrogens

Natural Products	POPs	Pharmaceuticals	Industrial Compounds
Genistein	DDT	Ethinyl estradiol	Bisphenol A
Naringenin	Kepone	DES	Phthalate esters
Coumestrol	PAHs		Alkylphenols
Zearalenone	PCBs/OH-PCBs		Endosulphan
B-sitosterol	Dioxins		Triazines

Mechanisms of Feminization

- Direct Acting
  - Receptor Based (ER)
- Indirect Acting
  - ↑ Estradiol synthesis
    - Central-HPG axis (gonadotropin, aromatase)
      - Anti-androgens
  - ↓ Estradiol elimination
    - CYP or Phase II inhibition

Pharmacodynamic Dispositional Processes



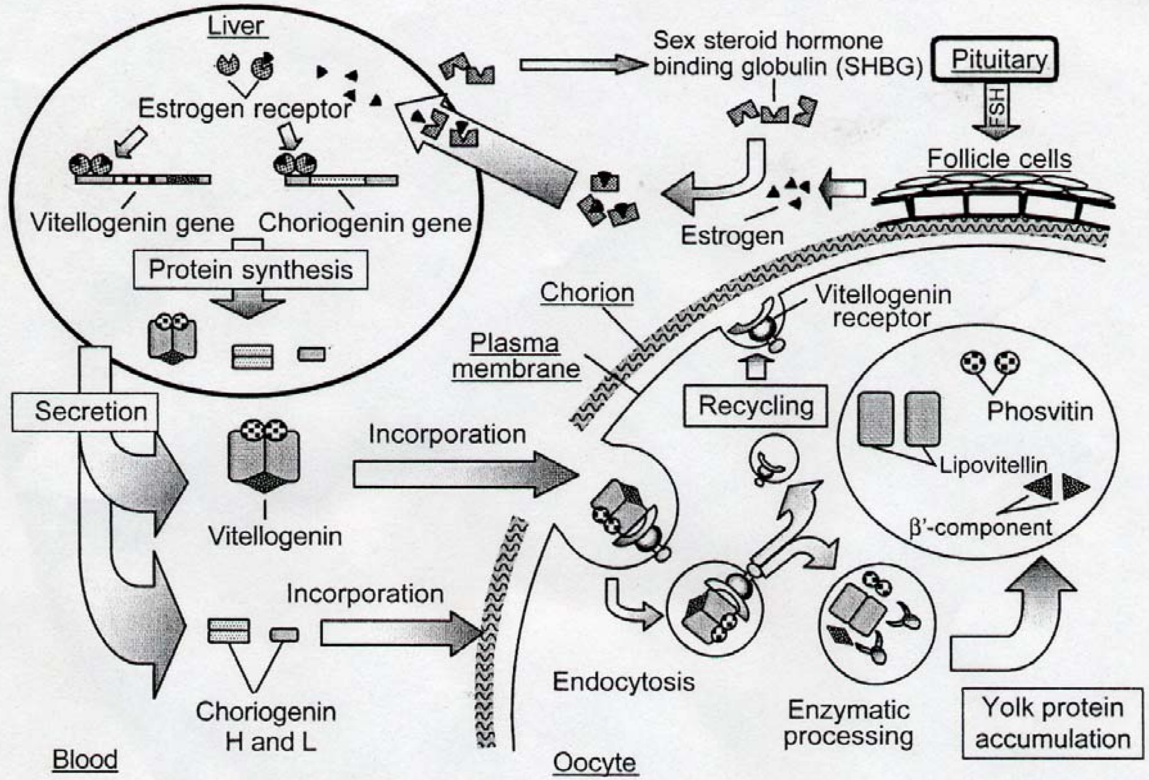


Fig. 1. Outline of the processes of vitellogenesis and choriogenesis in teleost fish. For discussion of vitellogenesis, choriogenesis, and yolk protein formation, see section II. Although only a single form of Vg is illustrated in this figure, multiple forms of vitellogenin (Vg) and choriogenin (Chg) have been discovered in several teleost species (see Fig. 2). Choriogenins produced by the liver include high molecular weight choriogenin (choriogenin H) and low molecular weight choriogenin (choriogenin L). In some teleosts, Chgs of ovarian origin (homologues of mammalian zona pellucida proteins) also are involved in the formation of chorion.

Hiramatsu et al. 2005

Potential Spatial and Temporal Impacts of Environmental Stressors

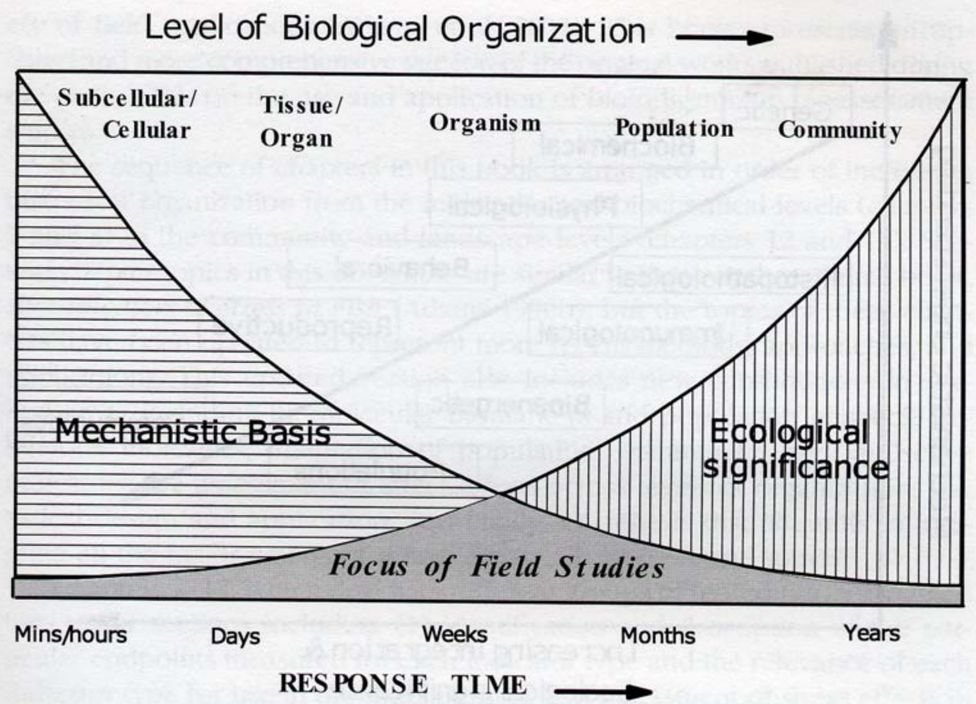
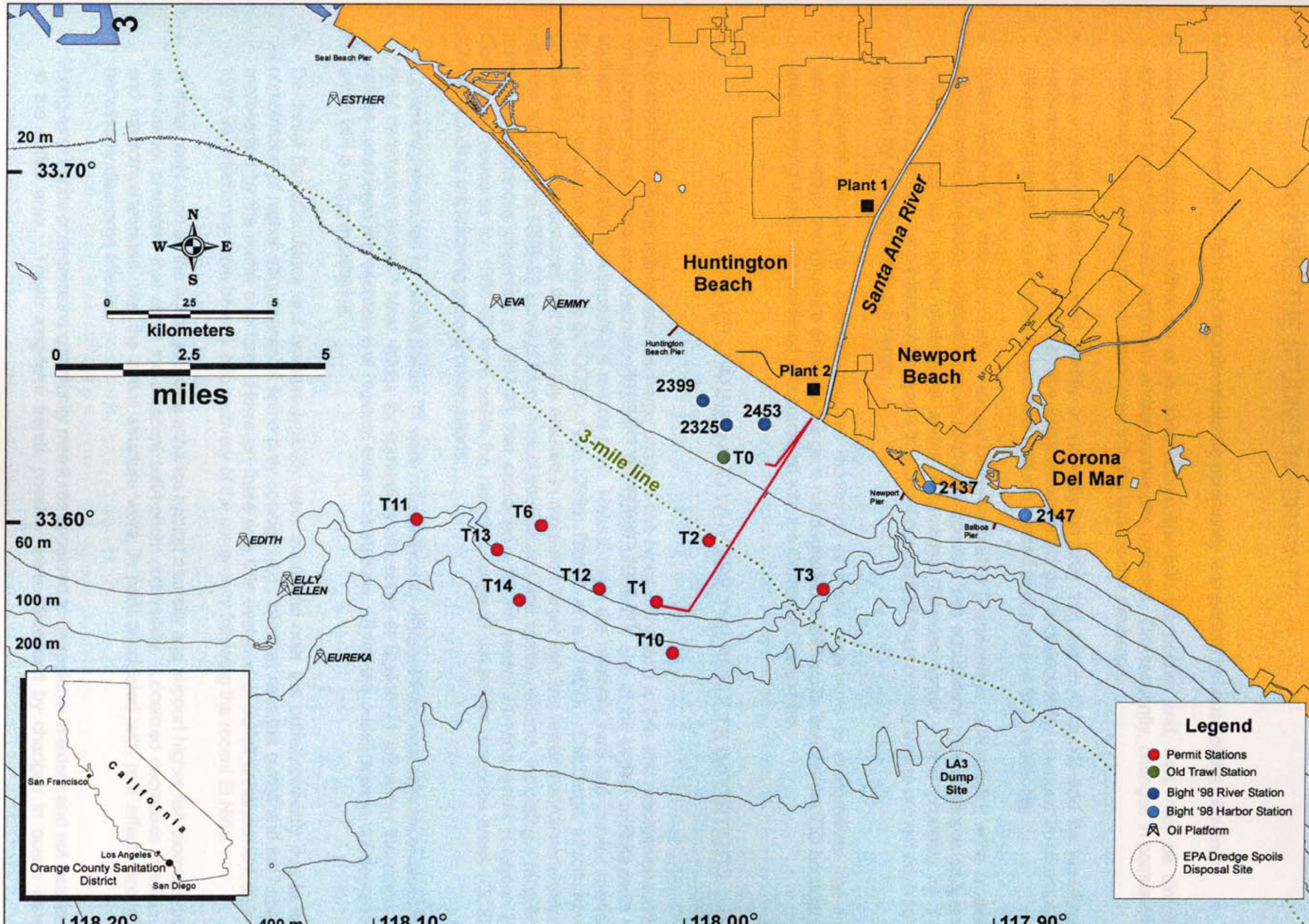


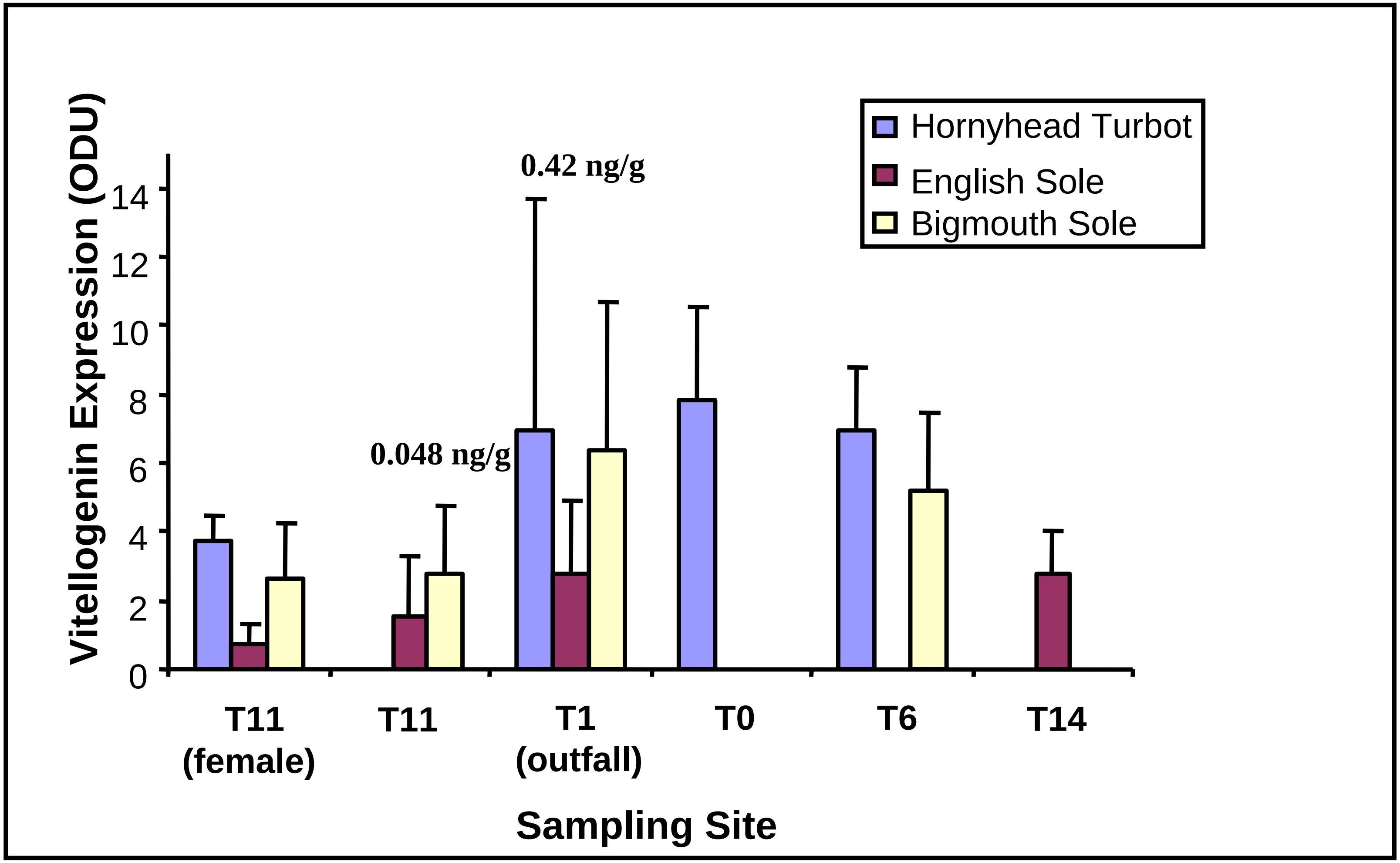
Figure 1. Field bioassessment studies should include a combination of both rapidly responding sensitive biomarkers and the more ecologically relevant bioindicators with a focus at the individual organism level. Organism-level responses provide a pivotal point through which mechanistic understanding and the ecological consequences of stressors can be linked.

Adams et al. 2002



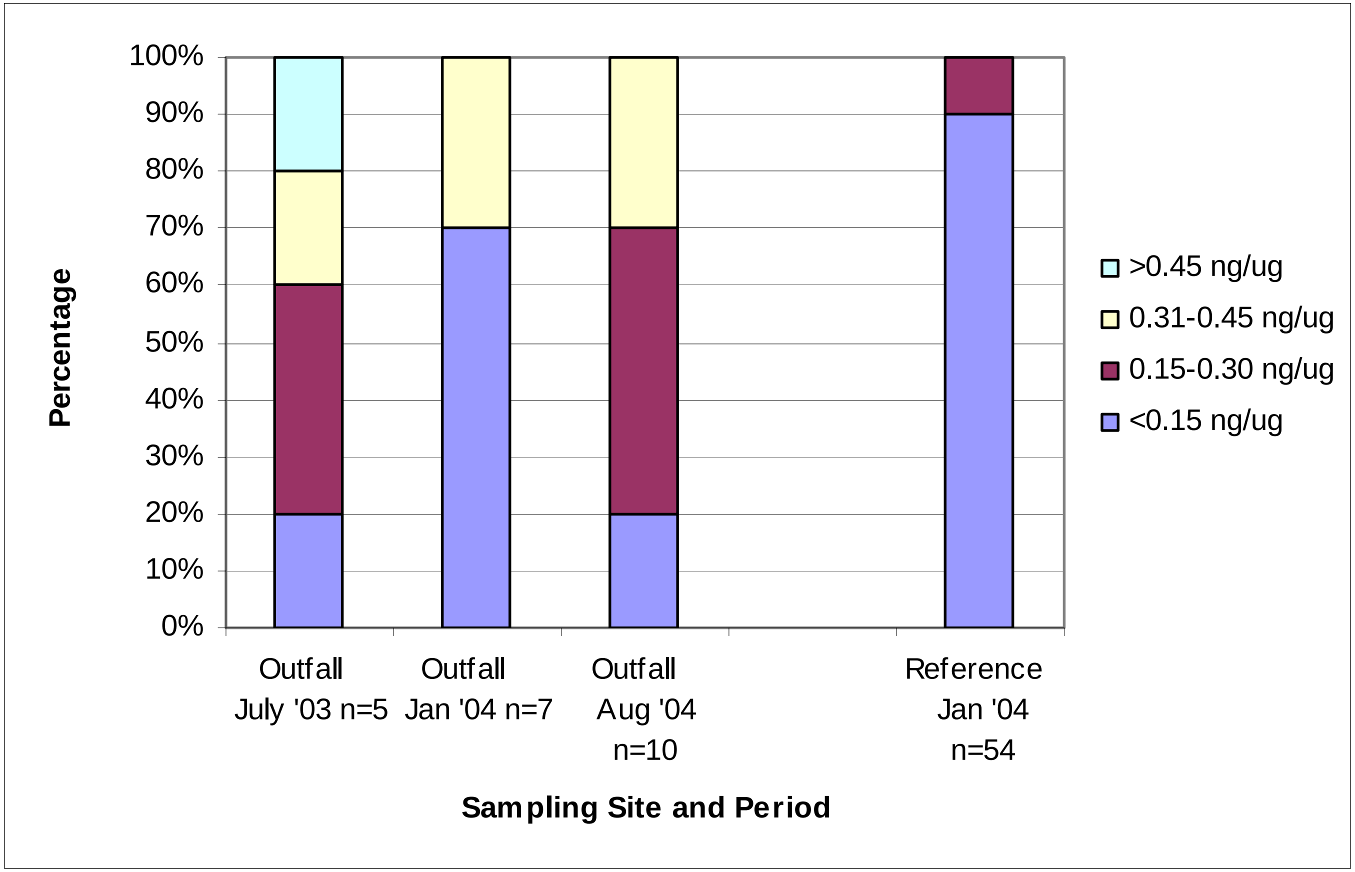
Seal Beach Pier
ESTHER
20 m
33.70°
N
W
E
S
0
2.5
5
kilometers
0
2.5
5
miles
EVA
EMMY
Huntington Beach Pier
Huntington Beach
Plant 1
Santa Ana River
Plant 2
Newport Beach
Corona Del Mar
2399
2453
2325
T0
3-mile line
2137
2147
Newport Pier
Balboa Pier
T11
T6
T13
T2
T3
33.60°
60 m
EDITH
ELLY
ELLEN
T12
T1
T14
100 m
200 m
T10
EUREKA
LA3 Dump Site
Legend
Permit Stations
Old Trawl Station
Bight '98 River Station
Bight '98 Harbor Station
Oil Platform
EPA Dredge Spoils Disposal Site
California
San Francisco
Los Angeles
Orange County Sanitation District
San Diego
118.20°
400 m
118.10°
118.00°
117.90°

Vitellogenin Expression in Demersal Flatfish of So. Cal Bight



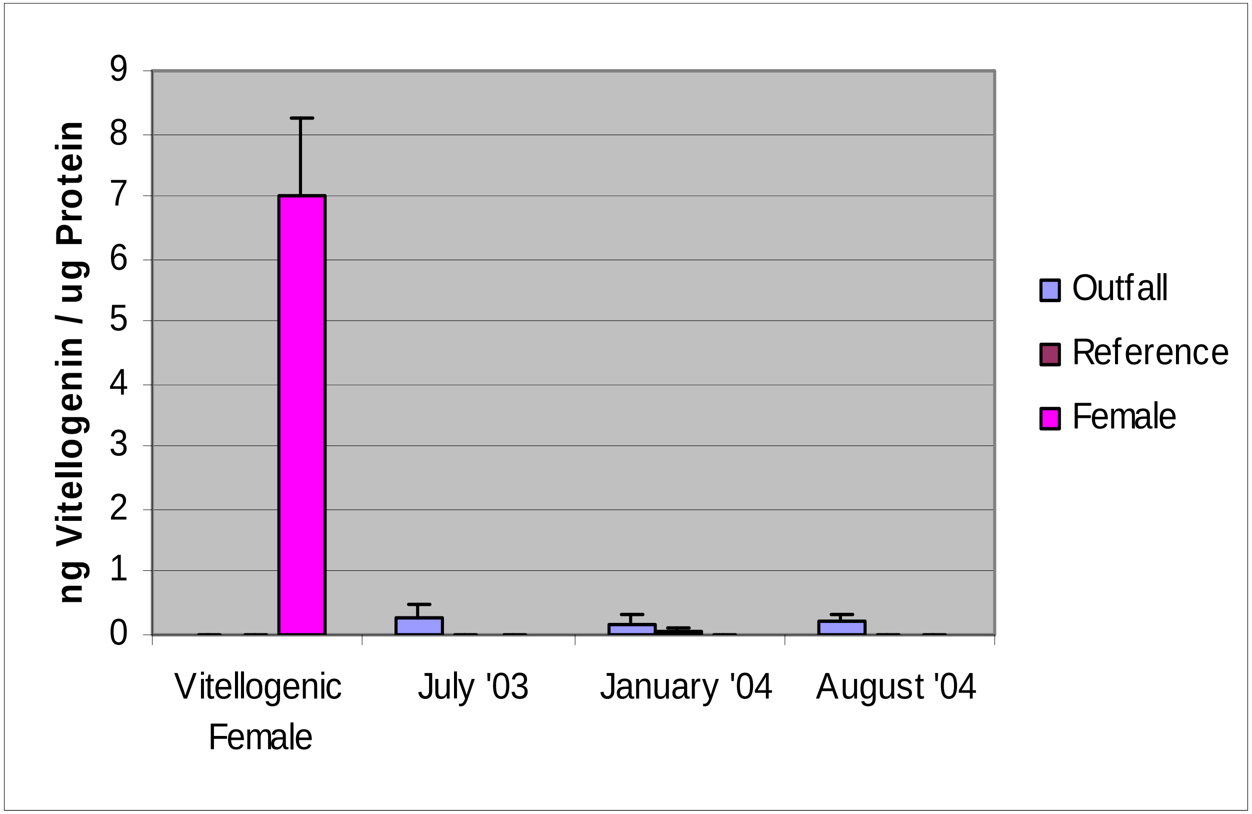
Roy et al. 2003

Proportion of plasma vitellogenin levels at given concentrations in hornyhead turbot



Rempel et al. 2006

Comparison of male hornyhead turbot plasma vitellogenin levels to average vitellogenic female levels



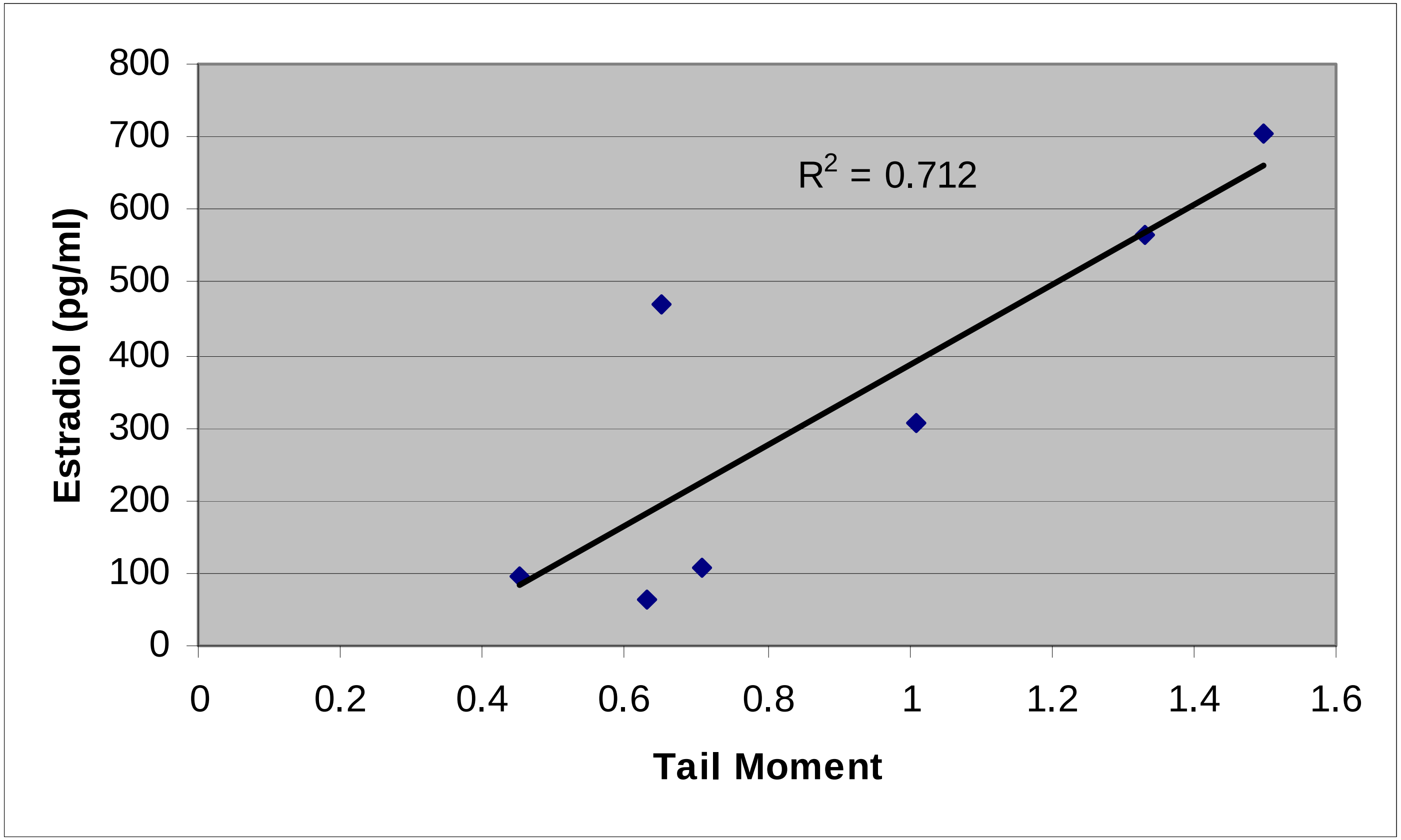
Rempel et al. 2006

Plasma estradiol concentrations (pg/ml) of hornyhead turbot and English sole collected from the outfall and reference sites

Species	Sampling Period	Outfall		Reference	
		Male	Female	Male	Female
Hornyhead Turbot	January 2003	201.5±3.7 (n=5)	206.6±6.4 (n=4)	208.0±8.2 (n=5)	212.9 (n=1)
	January 2004	329.9±255.6 (n=7)	302.6±165.2 (n=5)	388.6±215.0 (n=17)	245.0±143.3 (n=2)
English Sole	January 2003	191.4±2.8 (n=2)	184.0±1.1 (n=3)	205.2±10.3 (n=4)	200.5 (n=1)
	January 2004	142.1±206.3 (n=8)	209.4±282.3 (n=11)	169.4±92.0 (n=12)	108.9±94.5 (n=6)

Rempel et al. 2006

Regression analysis of sperm DNA damage (expressed as tail moment) versus estradiol plasma levels in male hornyhead turbot from the outfall



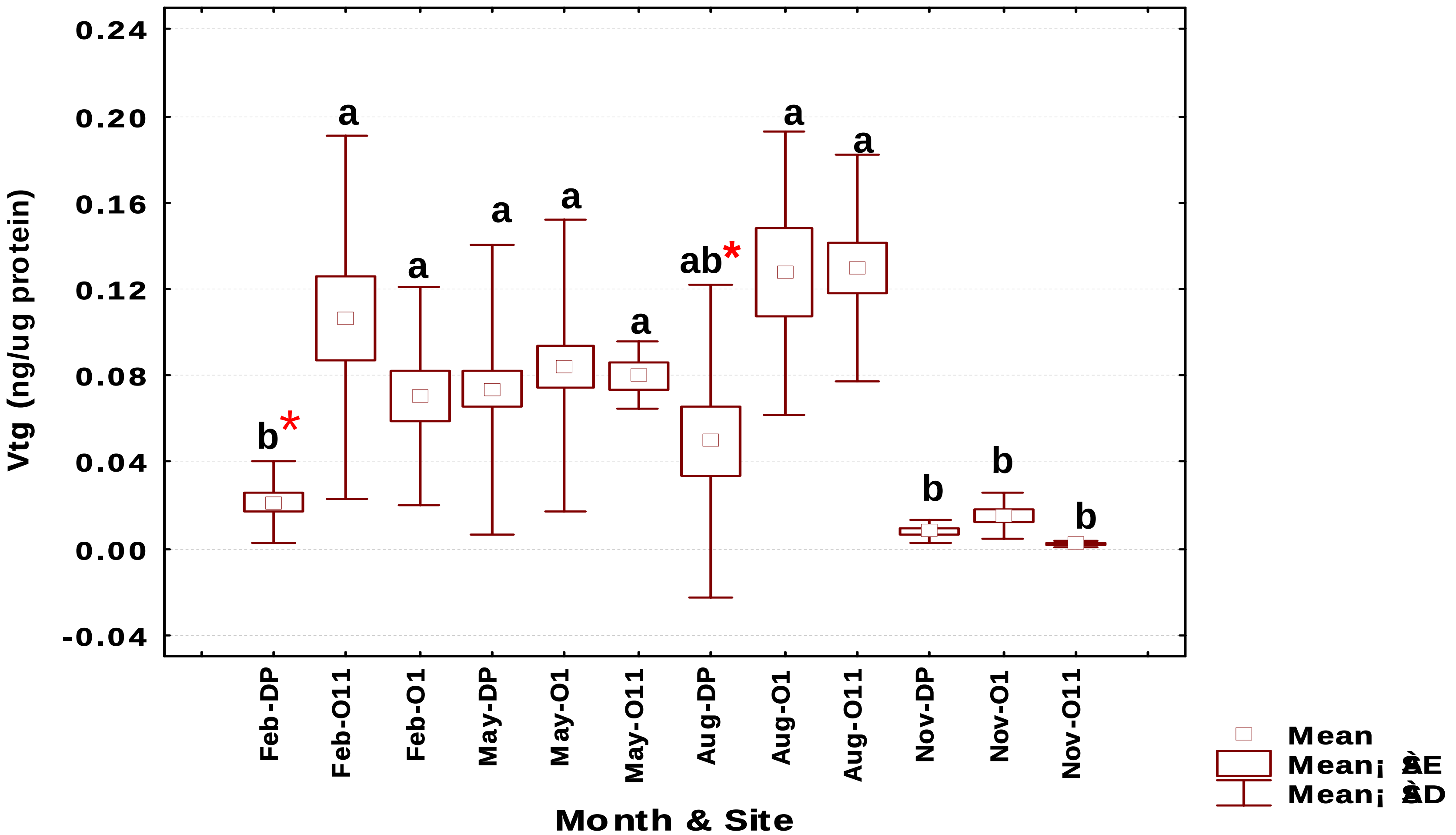
Rempel et al. 2006

$p < 0.05$

Males with detected Vtg levels

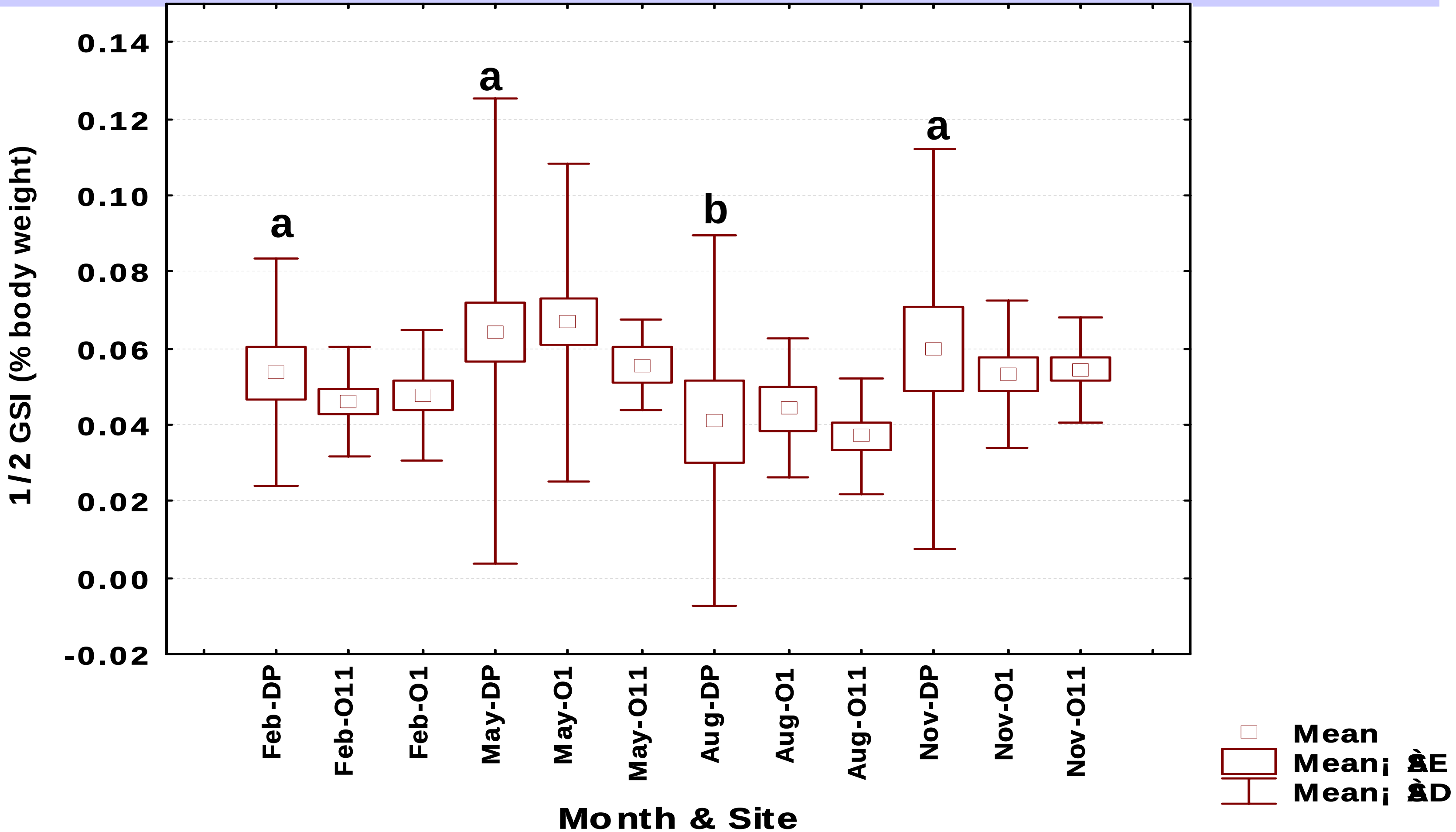
Month	Site	Number of Males	Number of Males with Vtg induction	%
Jan	DP O1 O11	19 18 20	0 3 6	0 15 33
May	DP O1 O11	31 24 5	5 6 0	16 25 0
August	DP O1 O11	20 10 20	2 9 14	10 90 70
November	DP O1 O11	23 18 23	0 0 0	0 0 0
Total	DP O1 O11	74 52 48	7 15 14	9.5 29 29

Vitellogenin levels--Males



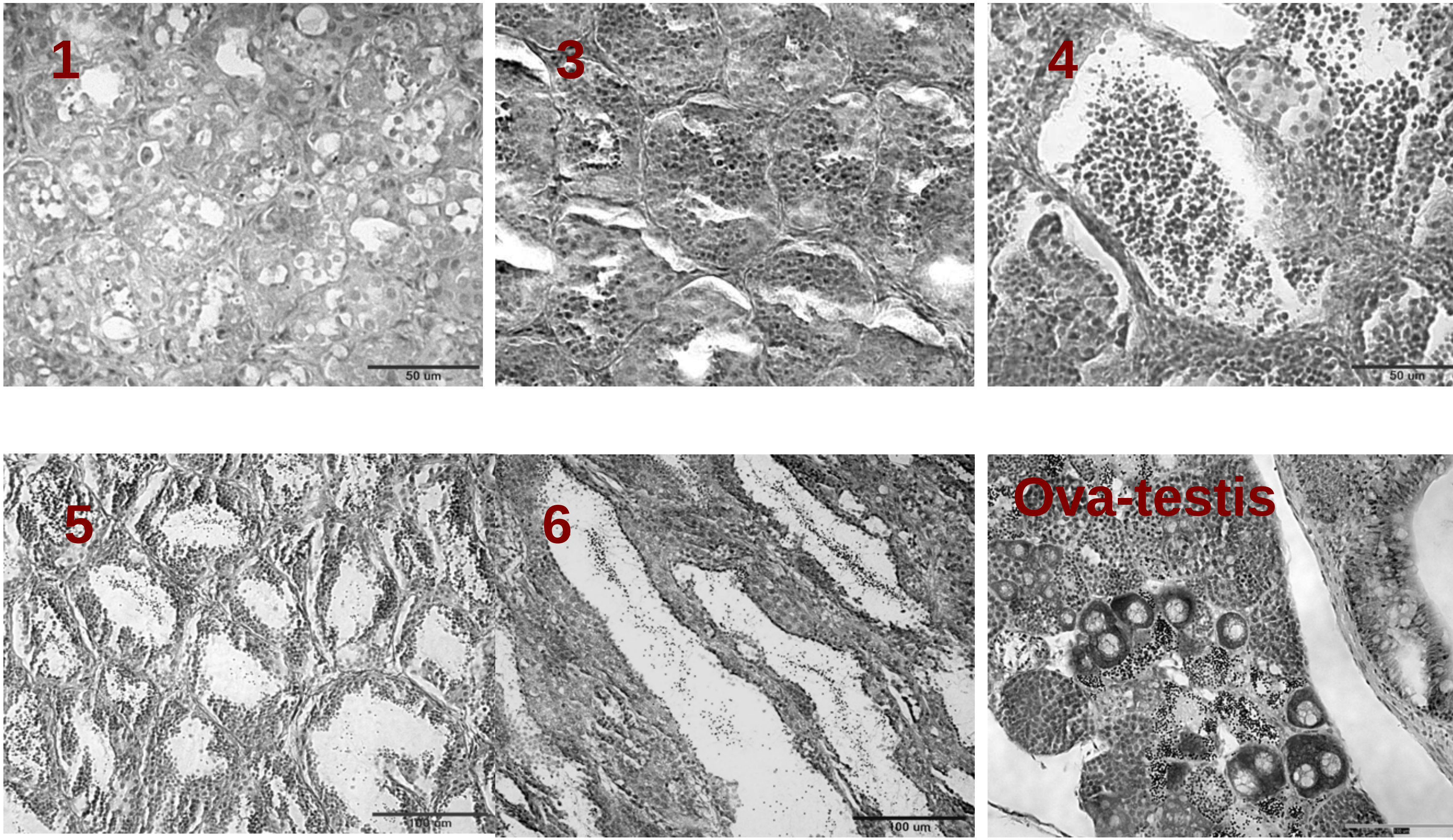
Wilcoxon / Kruskal-Wallis Tests P < 0.0001, different letters denote significantly seasonal difference. * indicates significant site difference of the same season.

Gonadosomatic Index--Male

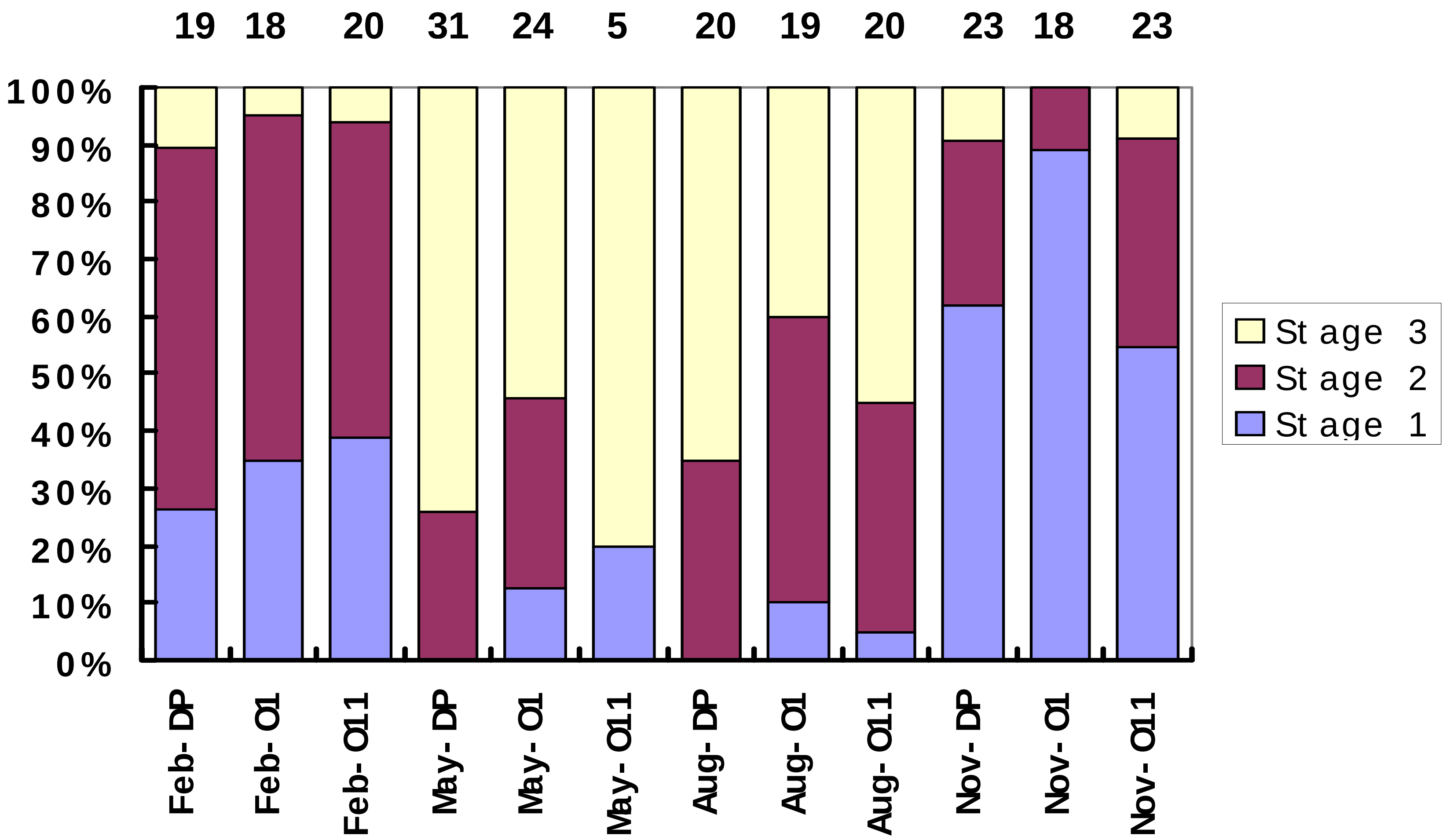


ANOVA, P< 0.0001, different letters denote significantly seasonal difference.

Developmental Stages of Males



Developmental Stages-Male(1)

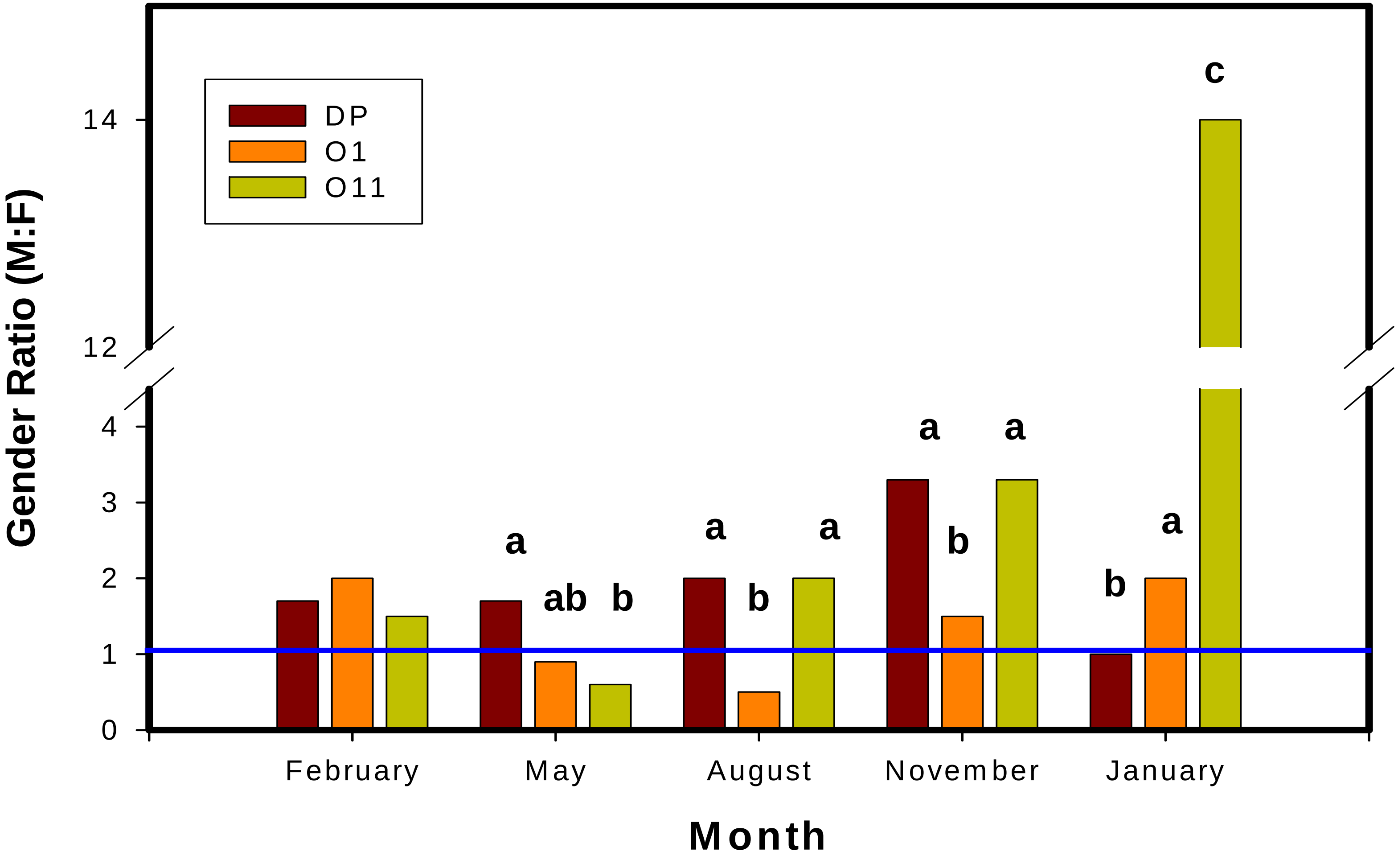


Chi-square test, P<0.0001

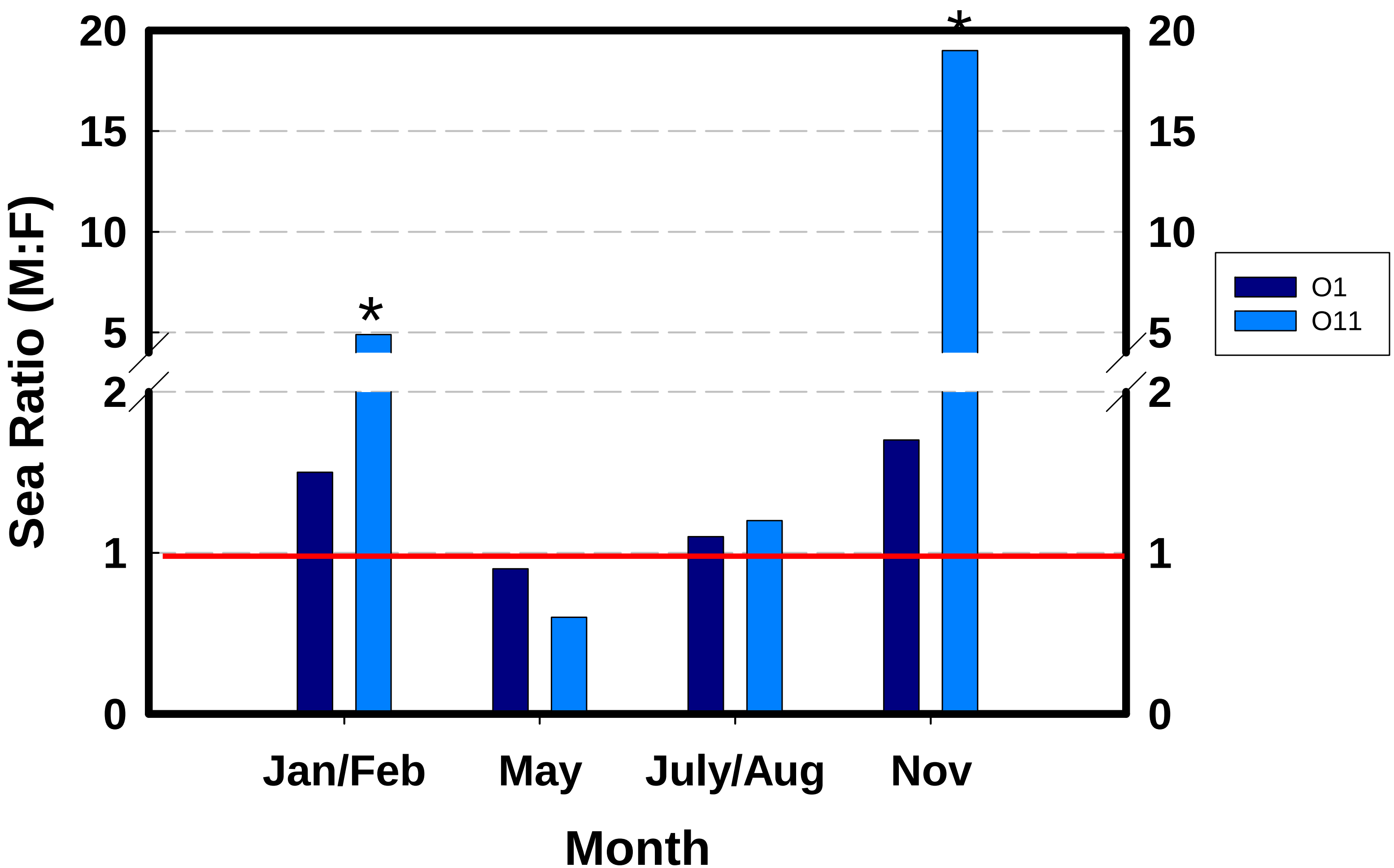
Ova-testis

Site	Feb	May	Aug	Nov
DP	0	0	0	0
O 1	0	0	0	1
O 11	0	0	1	2

Sex Ratio (M:F) in 2006-07

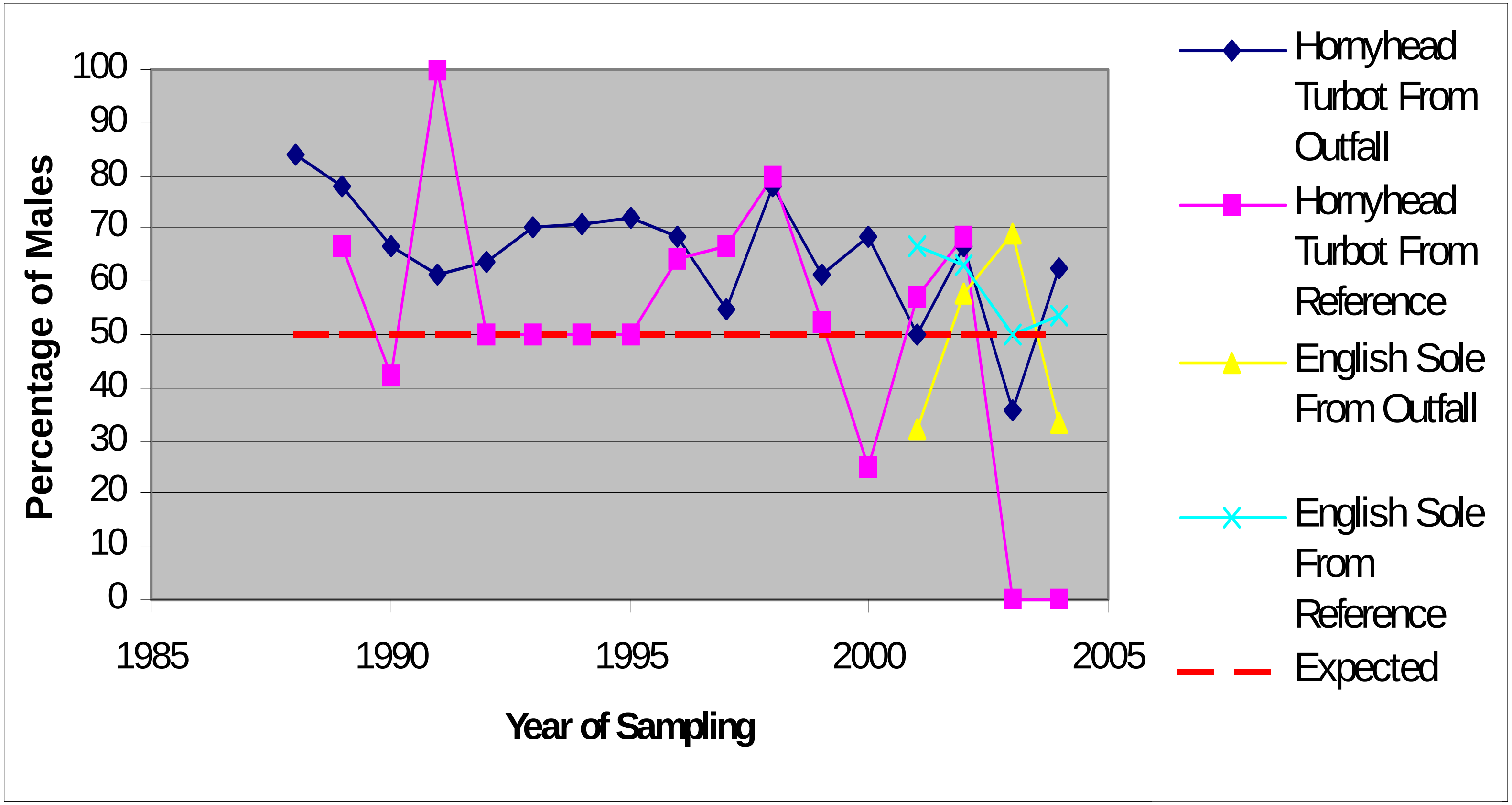


Sex Ratio (M:F) at O1 and O11(2003-2006)

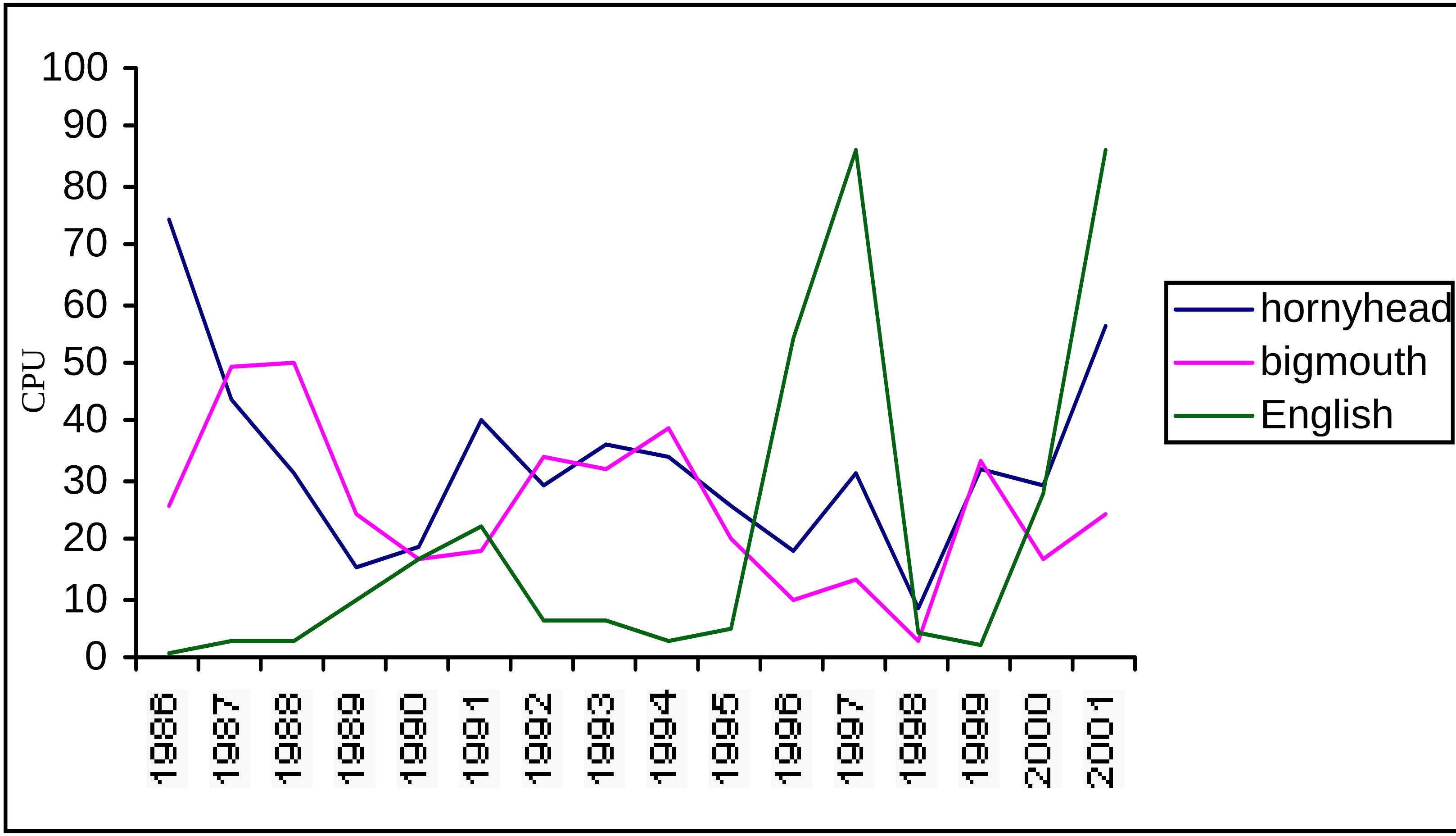


Proportion of hornyhead turbot and English sole males collected at the outfall and reference site

1988 to 2004



Abundance of 3 Flatfish Species Collected Near the outfall of OCSD (T-1) 1986-2001



Summary and Conclusions

- Estrogenic activity is present in demersal flatfish throughout the Southern California Bight.
- Three histological lesions indicative of feminization in 2006-2007 samples
- No significant effects on developmental stage
- Populations tend to display greater than 50% males (seasonal) (2:1) with no relationship to Vtg, histopath endpoints, or overall abundance

Unknowns??

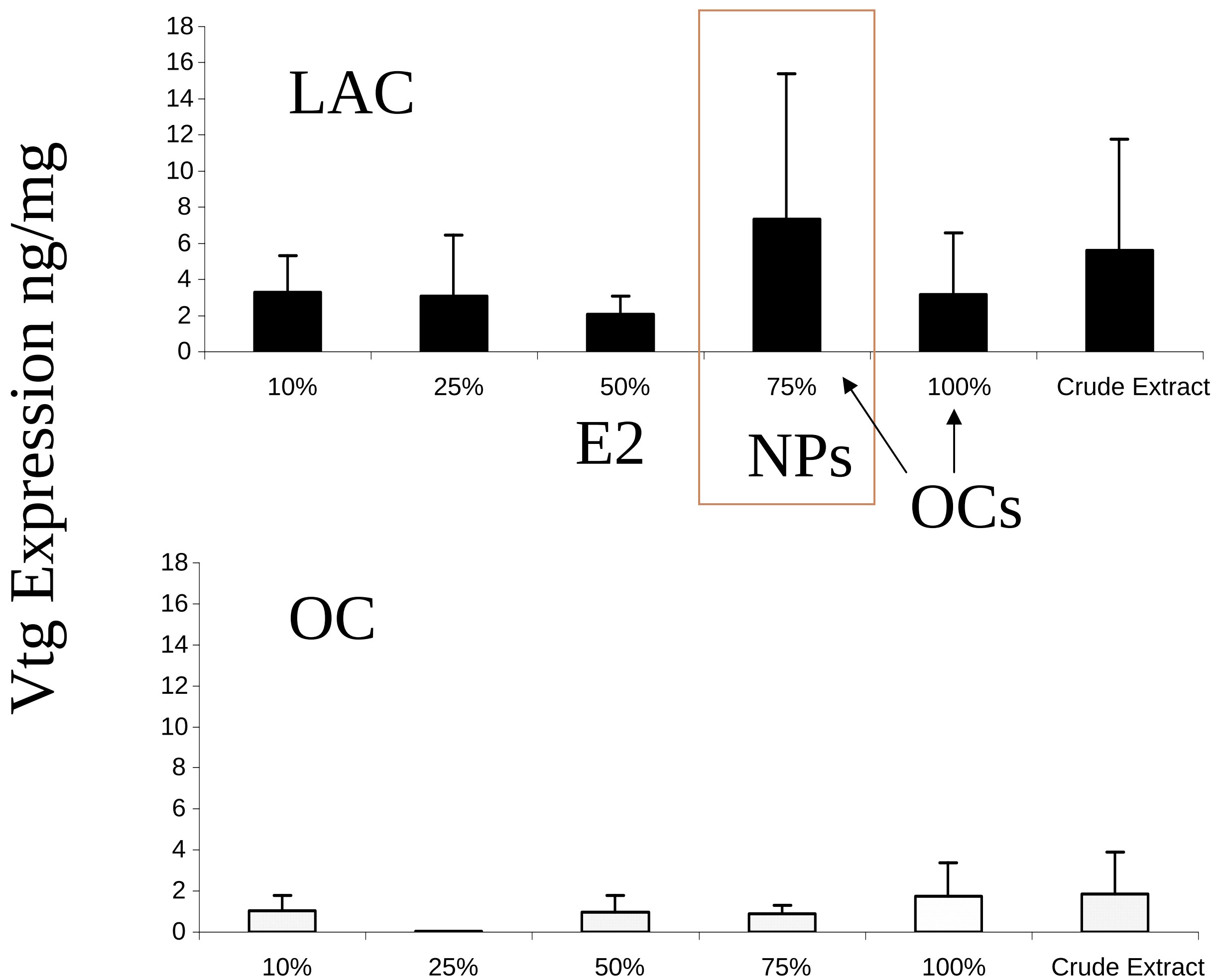
Table 2. E2, E1, AP and APE concentrations (ng/g) of sediments collected from the SCB in 2002.

	LAC	SD	OC	Reference
E1	0.6	< 0.03	< 0.03	< 0.03
E2	0.3	0.3	0.45	0.16
NP	198	122	3200	130
NPCl	<DL	<DL	100	<DL
NPBr	<DL	<DL	<DL	<DL
NP 1EO	36	11.6	330	76
NP 2EO	19.2	3.2	600	92
NP 3EO	15.6	1.9	3900	92
OP	8.2	1.9	<DL	<DL
OP 1EO	<DL	<DL	<cal	21
OP 2EO	<DL	<DL	<cal	8
OP 3EO	<DL	<DL	42	5.8

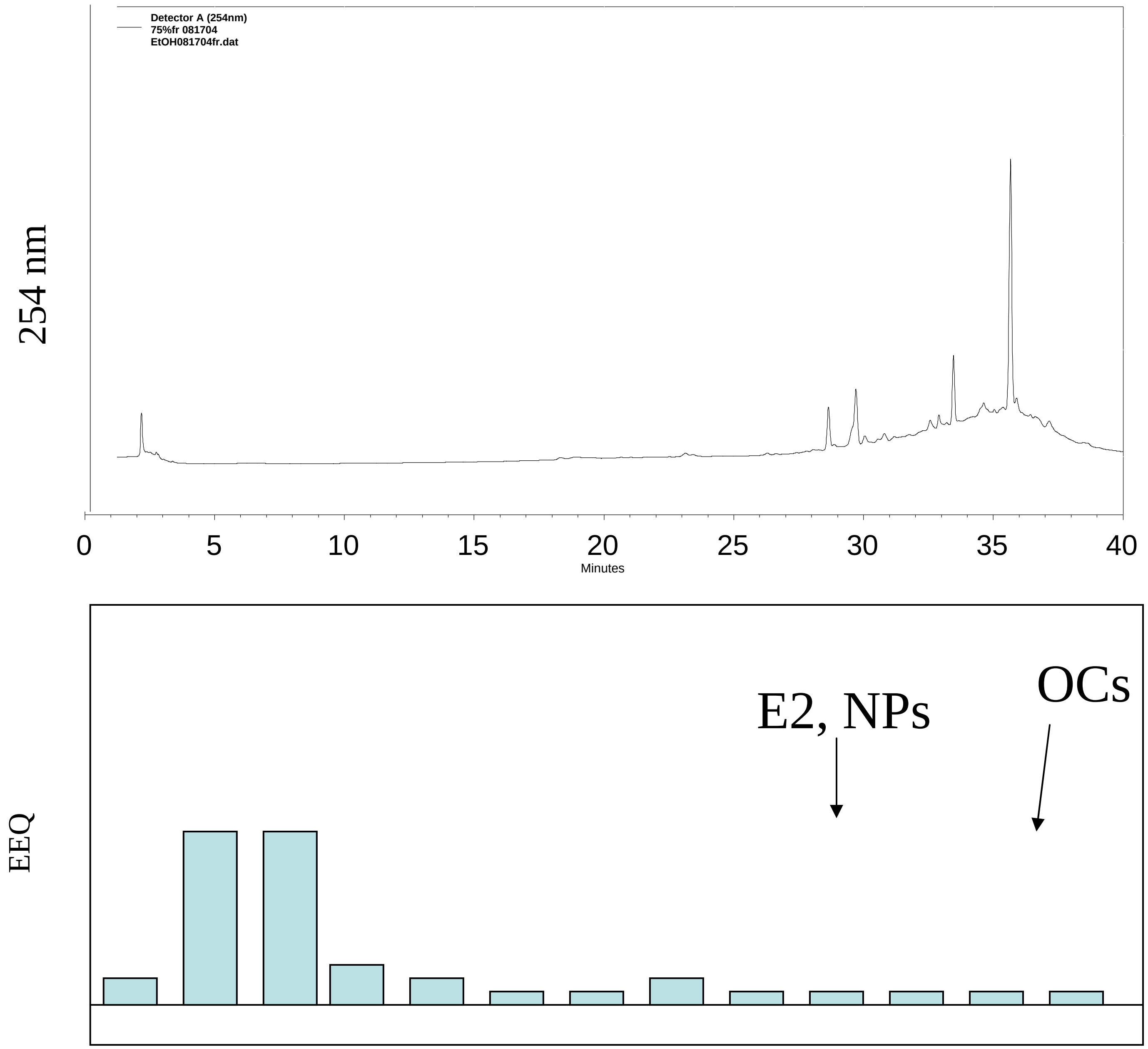
Schlenk et al. 2005

< cal= detected by instrument, but below calibration curve.

Bioassay guided fractionation of sediment extracts from LAC and OC



Schlenk et al. 2005

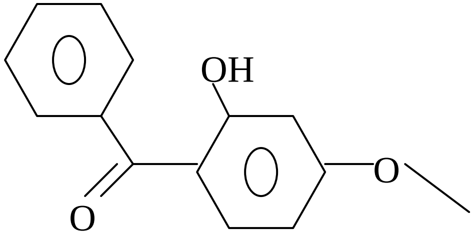


Schlenk et al. 2005

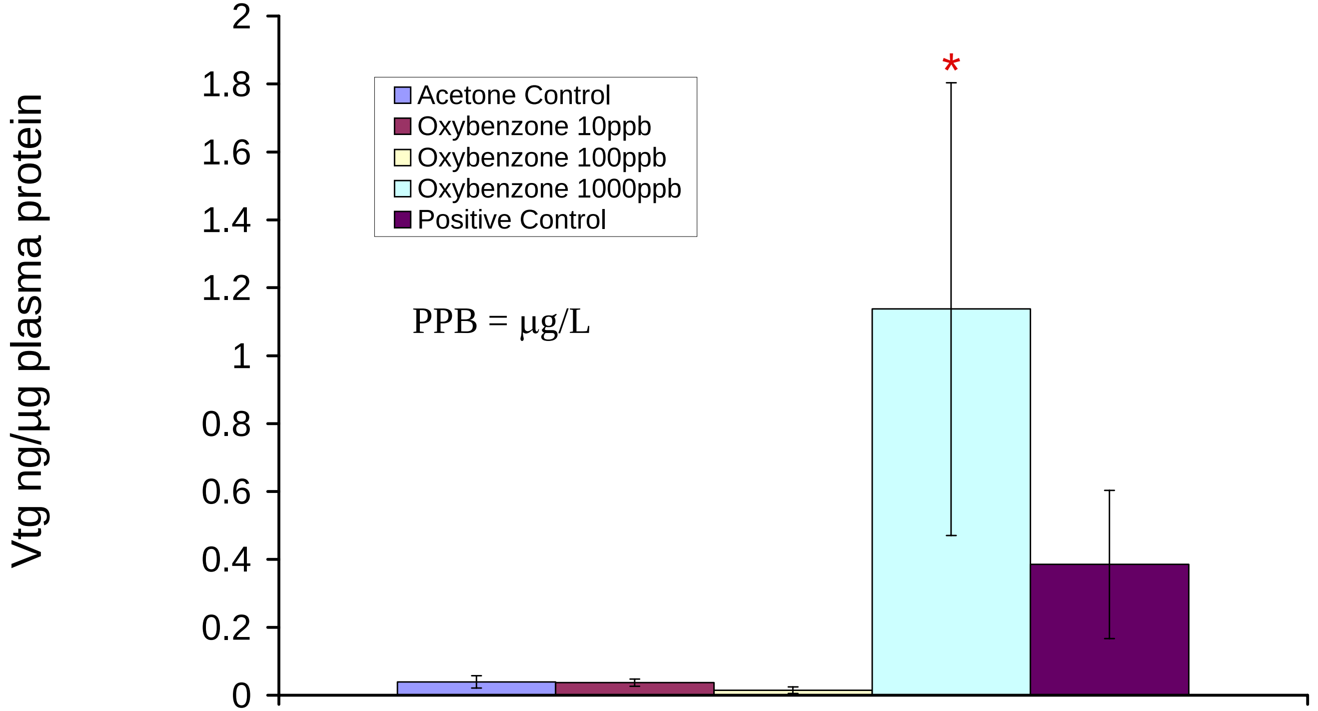
Oxybenzone

- New York City wastewater
  - Bioassay guided fractionation for estrogenic activity
  - 19 ng/L oxybenzone

- LA County Sanitation District (LACSD) Sediment extracts
  - Bioassay guided fractionation for estrogenic activity
  - 8 ng/g oxybenzone



Effect of Oxybenzone on the Expression of Rainbow Trout Vitellogenin after 14 day Aqueous Exposure.



Summary and Conclusion-Cont.

- In vivo bioassays can be used for the determination of matrix estrogenic activity and identification of estrogenic compounds
  - In vitro responses tend to underestimate “total” estrogenic activity (Industrial input)
  - Should be used in concert with cell-line screening and AC if possible
- Estrogenic compounds (known and unknown) are present in sediments near SC municipal facilities at concentrations that elicit feminizing effects in laboratory fish
  - Acute responses do not appear to be due to steroidal exposures but likely due to exposures to multiple compounds at $\leq$ ppb concentrations
  - Chronic responses??

Questions??