Environmental Monitoring and Assessment of Environmental Estrogens in Marine Sediments of California

D.Schlenk
Environmental Toxicology Program
University of California, Riverside

Overview

Phase I Monitoring and identification of Environmental Estrogens in Marine Sediments of California

The specific aims of this study were to

1. Evaluate the Southern California Bight for estrogenic activities in feral fish populations
2. Evaluate sediments of the Southern California Bight for estrogenic activities
3. Determine the source(s) of estrogenic activity within sediments
4. Determine the relationships between exposure to estrogenic chemicals and reproductive, developmental, and population changes in feral flatfish in Southern California.

Sediments were collected from wastewater outfalls of Orange County, Los Angeles County and San Diego Metropolitan Water Districts during the summer and fall of 2002. Sediments were sampled using Van Veen grabs taking the top 2 cm and placed in storage bags at 4 deg C until used for either exposures or extractions.

Initial screening of animals from the Orange County outfall indicated exposure to environmental estrogens during the summer of 2000 (Roy et al., 2003) and 2001. Sediment-only exposures were carried out with OCSD outfall and reference sediments as well as sand, using sexually mature California Halibut cultured in the Hubbs Seaworld facility in Carlsbad and Mission Bay. Halibut were transported to the SEA Laboratory in Redondo Beach, Ca and held in free-flowing seawater tanks of 5 meter diameter and 2 meter depth. Animals were fed daily at 5% of body mass.

For exposures, animals were held for 7 days in 40 L aquaria containing 3 L of sediments and flow-through seawater at ambient conditions. Animals were fed throughout the exposure. Although a statistically significant increase in Vitellogenin was observed (Table 1) in animals exposed to Orange County Outfall sediments, the magnitude of response was not adequate to carry out fractionation studies. Consequently, sediments from Los Angeles and San Diego were evaluated.

Since there were mass limitations of sediments, fish were exposed to Methanol:acetone extracts of sediments from San Diego, Los Angeles and Orange County. Extracts were injected into fish and 7 days later the fish were bled with subsequent evaluation of

vitellogenin. Extracts were also evaluated using the Yeast Estrogen Screen assay for the presence of estrogen receptor ligands. Table 1 demonstrates the relative comparisons of estrogenic activities among each of the sediments.

Table 1. In vitro (YES) and In vivo (California Halibut) evaluations of sediments from the outfalls of Los Angeles County, Orange County and the City of San Diego. The column on the far right represents "sediment only exposures".

	YES EEQ (ng/g)	CH Vtg EEQ (ng/g)	CH Vtg (OD/g)
LAC	1.1	90.4 ± 48.3	NM
SD	0.1	13.7 ± 2.9	NM
OC	0.1	1.3 ± 0.12	0.483 ± 0.04*
Reference	< 0.1	< 1.0	0.443 ± 0.005
Sand	< 0.1	< 1.0	< 1.0

Table 2 shows the chemical evaluation of these sediments for "known" estrogenic compounds including 17 b-estradiol, estrone and a host of nonylphenol and octylphenol compounds. Analyses conducted by Dr. Bruce Brownawell at Stony Brook University indicated that sediments from OCSD had greater concentrations of known estrogens relative to LACSD and the City of San Diego.

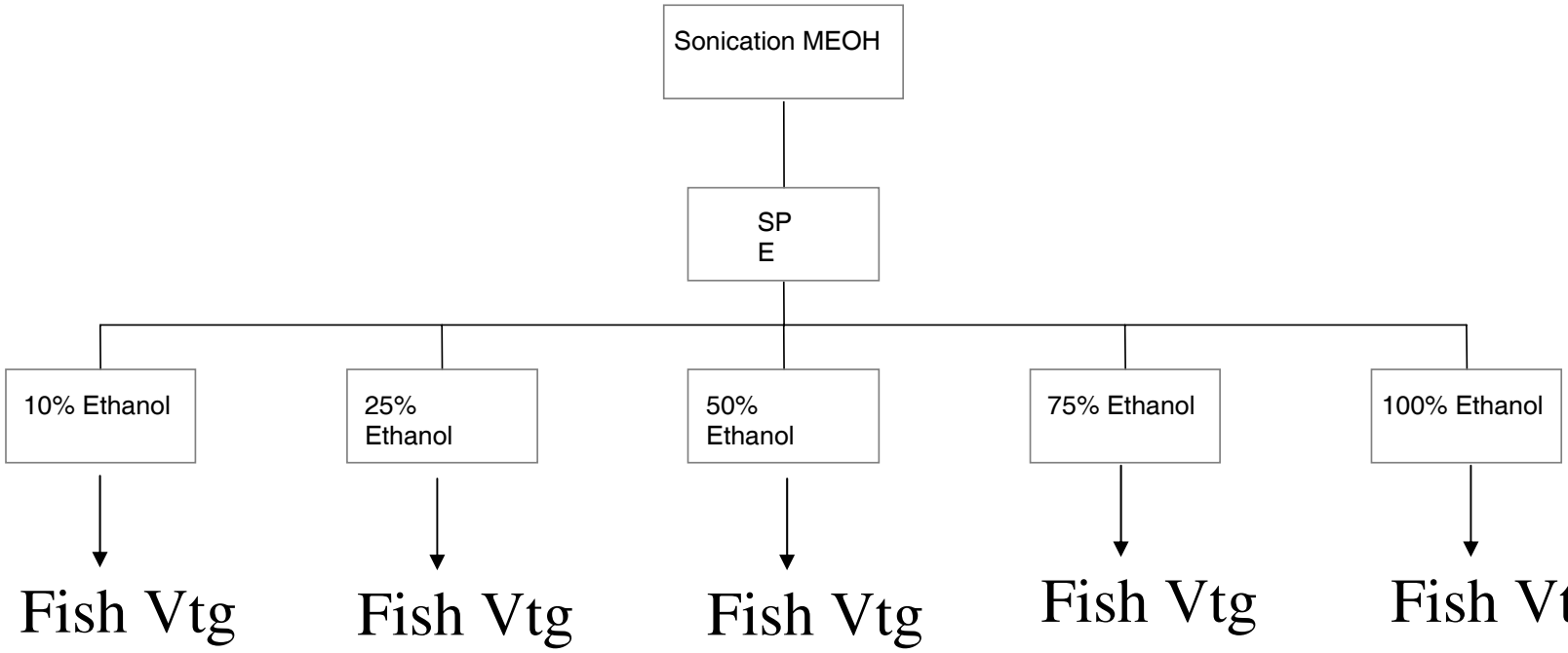
Table 2. E2, E1, AP and APE concentrations (ng/g) of sediments collected from the SCB in 2002.

	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

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

Since sediments from the LACSD outfall had the highest YES and fish vitellogenin responses, sediments were collected in 2004 and fractionated using fish vitellogenin in an attempt to identify the causative agent(s) responsible for the estrogenic activity. The following scheme was utilized (Figure 1).

Figure 1. Fractionation scheme of sediments for identification of causative agents.



To determine where steroid estrogens might elute using this fractionation method, samples were evaluated by the laboratory of Dr. David Sedlak. Table 3 shows that the majority of steroids eluted in the 50% ethanol wash of the solid phase extraction procedure.

Table 3. Elution profile of steroids following fractionation strategy of Figure 1.

Sample	17α E2 [ng]	17β E2 [ng]	E1 [ng]	E3 [ng]	T [ng]	AD [ng]	P [ng]	MP [ng]
LA Sediment	ND	ND	ND	ND	ND	ND	ND	ND
10% EtOH	ND	ND	ND	ND	ND	ND	ND	ND
25% EtOH	ND	ND	ND	ND	ND	ND	ND	ND
50% EtOH	ND	14.4	ND	ND	0.5	ND	8.8	ND
75% EtOH	ND	2.0	ND	ND	ND	ND	ND	ND
75% MeOH (cleaned)	ND	2.4	1.2	ND	0.4	BQ	6.5	ND
100% EtOH	ND	ND	ND	ND	ND	ND	ND	ND

Legend: ND = No detect; BQ = below limit of quantification (low S/N ratio for detected peak); E2 = estradiol; E1 = estrone; E3 = estriol; T = testosterone; AD = androstenedione; P = progesterone; MP = medroxyprogesterone.

As Figure 2 indicates, most of the estrogenic activity eluted in the 75% ethanol fraction which did not contain steroids. Consequently, the 75% fraction underwent additional fractionation (Figure 3) which subsequent fractions evaluated for estrogenic activity.

Polar fractions (F2 and F3) were sent to Dr. Shane Snyder at the Southern Nevada Water Authority for unknown analysis. The fractions were screened against 62 know estrogens and 3500 pharmaceutical and personal care product chemicals.

Figure 2. Vitellogenin expression following treatment of fish with sediment fractions.

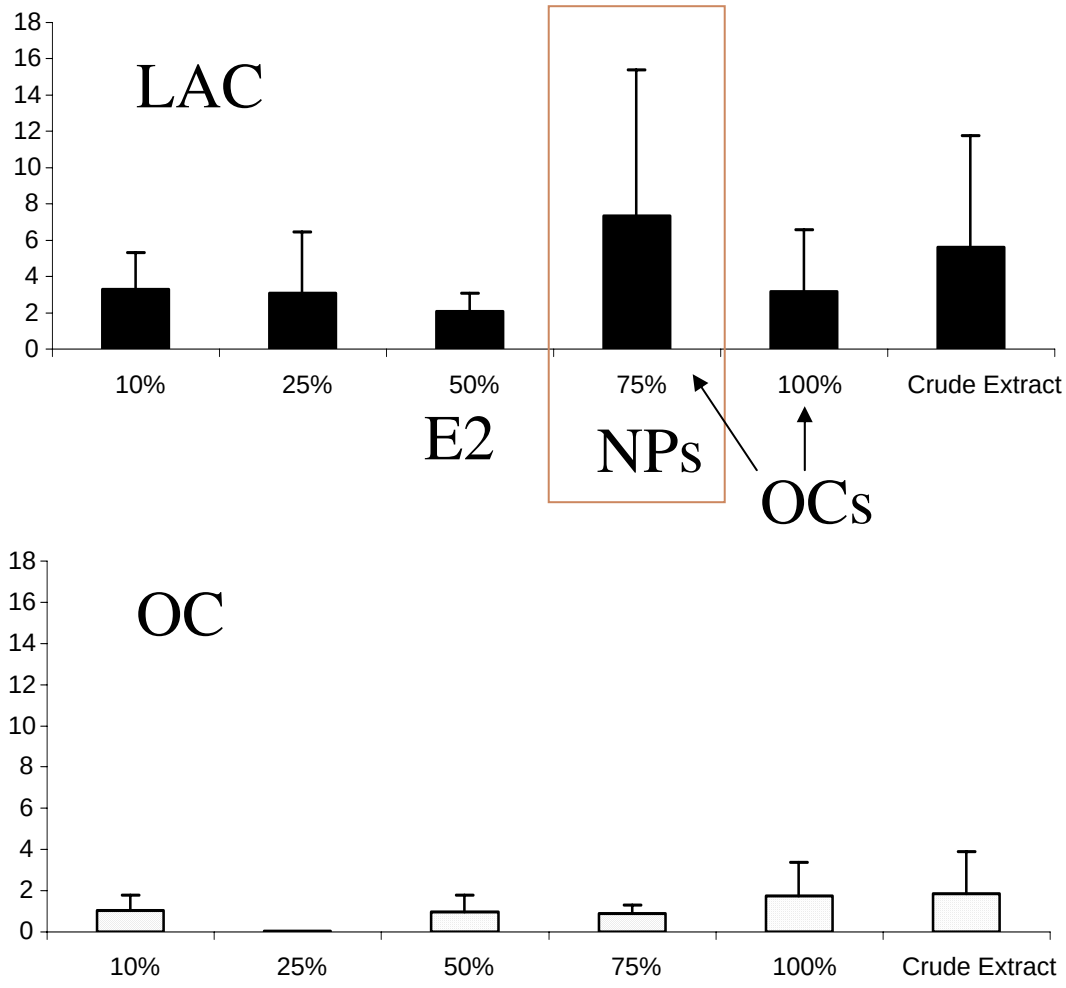
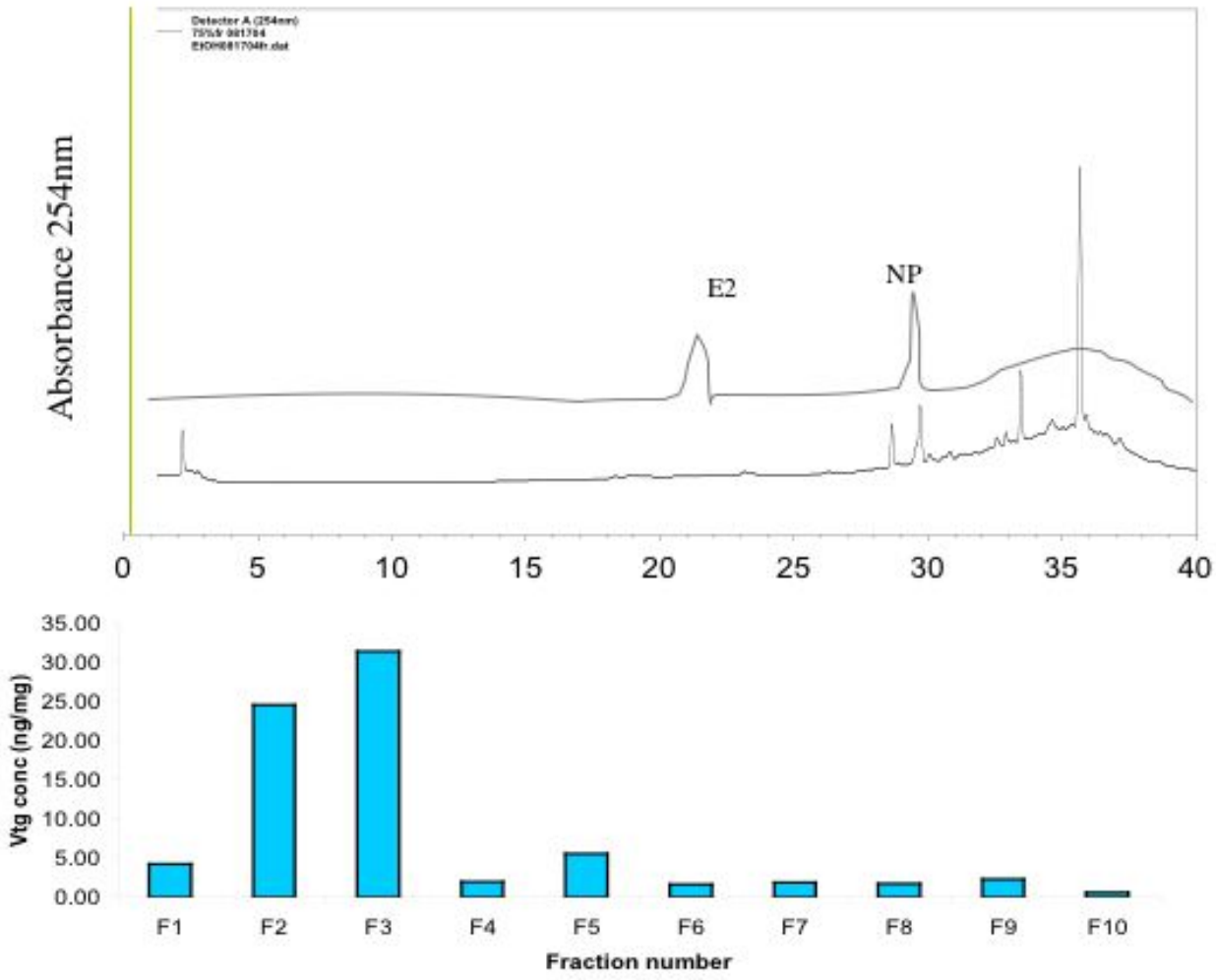


Figure 3. HPLC fractionation of the 75% ethanol fraction of LACSD sediment extracts.



The only compound identified in the estrogenic fractions was the sunscreen agent oxybenzone (8 ng/g). Other compounds were present, but could not be unequivocally identified.

Summary

- Estrogenic Activity was observed in all sediment extracts with sediments from the LA County wastewater outfall having the highest activity. Fish exposed to sediments (not just extracts) from OCSD also caused estrogenic activity.
- Chemical analysis of sediment extracts indicated a higher concentration of estrogenic chemicals in the OCSD sediments which did not correlate with biological activity (i.e. LACSD > OCSD).
- Bioassay guided Fractionation of LACSD sediment extracts failed to identify steroids or nonylphenols as causative agents
- Oxybenzone (8 ng/g) was the only compound identified after screening biologically active extract fraction for 62 pharmaceutical and estrogenic compounds.

Phase II. Environmental Impact of Estrogenic Activity within Feral Flatfish

The specific aims of this study were:

1. Evaluate 2 demersal flatfish species for the occurrence of estrogenic activity off the coast of California
2. Evaluate the relationships between estrogenic activity, development, reproductive status, gender and abundance in each species

The OCSD outfall is located 7 kilometers offshore on the San Pedro Shelf within the Southern California Bight at a nominal depth of 60 m (Lat 33 34.641'; Long 118 00.567'). The reference sampling location (Lat 33 36.055'; Long 118 05.199') was 7.7 km north of the outfall at a nominal depth of 56 m. The reference location was based on 25 years of monitoring data and confirmed by USEPA Region IX. The prevailing surface current is upcoast (north). Trawling paths were determined using differential Global Positioning System (dGPS) navigation to accurately locate the area sampled and to control the speed of the trawl (2–2.5 knots).

Fish Sampling

English Sole (*Pleuronectes vetulus)*, and Hornyhead Turbot (*Pleuronichthys verticalis)* were collected in January 2003, July 2003, January 2004, and August 2004 utilizing a 7.6 meter wide semiballon otter trawl. Three replicate samples for population analysis were collected. All specimens were taxonomically identified, counted, and individually weighed and measured. Gender ratio data were collected by OCSD biologists from 1988 through 2004 for Hornyhead Turbot, and 2001 through 2004 for English Sole. Sex was determined by gross morphology of the gonads. Blood (<1 ml) was collected via heparinized 22g

syringe from the dorsal aorta and immediately spun with a portable centrifuge unit for two mins at 5000 rpm. The plasma was removed and stored on dry ice until transport to a –80 °C freezer where it was stored until analysis. Only one-half of the gonad was removed for GSI determination, as the other half was collected for use in other studies. Therefore all GSI data presented are reported as ½ GSI. Fish were size-classed and aged using age/length regression equations reported by the National Marine Fisheries Service. The regressions were developed for each target species by geographic area. Regression equations from the geographic area either encompassing or nearest to the OCSD study site were used to determine the age of the study fish. The equation used for English Sole was $y = 3.126 - 0.021x + (8.9136E\text{-}5)x^2$, where y = age and x = length in mm, developed for the Monterey-Moss Landing geographic area. The equation used for Hornyhead Turbot was $y = 1.526 – 0.015x + (2.0965E\text{-}4)x^2$, developed for the California geographic area.

Vitellogenin measurement

In order to carry out temporal and spatial comparisons of estrogenic activity, it was necessary to develop a quantitative method for vitellogenin measurement. In collaboration with Dr. Tjeerdema, who provided purified halibut vitellogenin, Dr. Schlenk's lab developed an ELISA for the measurement of vitellogenin in California halibut, English sole, and Hornyhead turbot (see Rempel et al. 2006 for details).

Other Endpoints

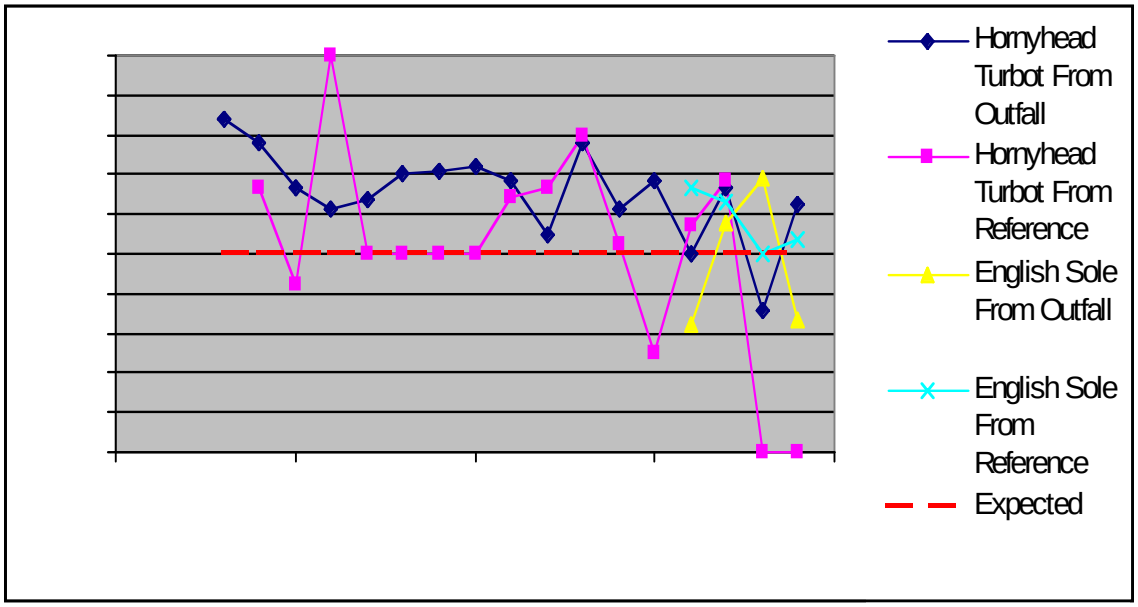
In addition to vitellogenin, plasma estradiol and sperm DNA damage was measured in each animal and correlations were evaluated between each endpoint and vitellogenin expression.

Results

Gender ratios for Hornyhead Turbot since 1988 have been significantly male oriented at the outfall (Figure 4 $p \leq 0.005$). In 11 of 16 years where animals from both sites have been collected, the male sex has significantly ($p<0.05$) predominated at the outfall relative to the reference site (Figure 4). Gender ratios for English Sole from 2001 through 2004, however were not male oriented, nor was there a significant trend towards masculinization at the outfall.

Fish collected in July 2003, January 2004 and August of 2004 were analyzed for sexual maturity. Hornyhead Turbot samples were collected from the outfall during all sampling periods, however only females were collected from the reference location during the January 2004 sampling event. English sole were collected from both locations during all three events. Hornyhead Turbot are estimated to be sexually mature at 150 mm, or 4.0 years of age. English Sole, on the other hand, are sexually mature at 210 mm (2.6 years) for males, and 260 mm (3.7 years) for females. Age estimates ranged from 2 to 10 years for Hornyhead Turbot. The majority (67 to 100%) were sexually mature, regardless of sex or where they were sampled (Table 4). In contrast the majority of

Figure 4. Gender ratios of Hornyhead Turbot and English Sole from the OCSD outfall and Reference station from 1987 to 2004.



English Sole (0 to 24% mature) were immature, with the exception of the males collected at the outfall during July of 2003 (54% mature). Age estimates for English Sole ranged from 1 to 4 years of age. Despite the observed variation in maturity in both species, there were no clear trends between sampling location over the entire study period.

Irrespective of their differences in reproductive status (mostly mature Hornyhead Turbot, mostly immature English Sole), measured values of ½ GSI in males of both species were consistently higher at the outfall as compared to the reference location during all sampling periods in which males were collected from both locations (Table 5). The increase was significant in Hornyhead Turbot males in January 2004 and in English Sole males in July 2003 and August 2004. In contrast, Hornyhead Turbot females tended to have reduced ½ GSI at the outfall location, but the differences were not significant, while English Sole females showed no obvious differences between the two locations (Table 5).

Table 4. Proportion of sexually mature fish at the OCSD outfall versus reference location.

Species	Sampling Period	Outfall	Outfall	Reference	Reference
		Male	Female	Male	Female
Hornyhead Turbot	July 2003	1.00 (n=5)	0.89 (n=9)	ND	1.00 (n=2)
Hornyhead Turbot	January 2004	1.00 (n=7)	1.00 (n=5)	0.98 (n=56)	1.00 (n=4)
Hornyhead Turbot	August 2004	0.80 (n=10)	0.67 (n=6)	ND	1.00 (n=2)
English Sole	July 2003	0.54 (n=26)	0.07 (n=14)	0.07 (n=30)	0.03 (n=30)
English Sole	January 2004	0.13 (n=8)	0.00 (n=12)	0.08 (n=13)	0.00 (n=9)
English Sole	August 2004	0.13 (n=16)	0.00 (n=34)	0.24 (n=25)	0.05 (n=20)

Concentrations of E2 were measured in plasma samples of Hornyhead Turbot and English Sole available from the January 2003 and January 2004 trawls. In both years, males of either species exhibited no significant differences in plasma E2 concentration between the outfall and reference locations (Table 6), although there did appear to be a slight trend toward reduced plasma E2 at the outfall site. Similarly, in females of both species there were no significant differences in plasma E2 concentration in 2004; unfortunately, only one female sample of each species was available from the reference location in 2003, making site comparisons impossible. The lack of sex differences in E2 levels observed in these data reflect the reproductive status of the two species under study as indicated in Table 4: the majority of the English Sole were immature, whereas the Hornyhead Turbot were out of breeding season in January.

Table 5. 0.5 GSI (g) of Hornyhead Turbot and English Sole collected from the OCSD outfall and reference sites.

Species	Sampling Period	Outfall	Outfall	Reference	Reference
Species	Sampling Period	Male	Female	Male	Female
Hornyhead Turbot	July 2003	0.051±0.020 (n=5)	1.856±1.297 (n=8)	ND	1.106±0.577 (n=2)
Hornyhead Turbot	January 2004	0.076±0.023 (n=7)	0.794±0.480 (n=4)	0.060±0.038 (n=55)	1.378±0.890 (n=4)
Hornyhead Turbot	August 2004	0.057±0.021 (n=10)	1.346±0.992 (n=6)	ND	1.659±0.200 (n=2)
English Sole	July 2003	0.067±0.034 (n=26)	0.513±0.220 (n=13)	0.048±0.021 (n=30)	0.461±0.176 (n=30)
English Sole	January 2004	0.684±0.338 (n=8)	1.428±1.452 (n=12)	0.597±0.319 (n=13)	1.238±1.775 (n=9)
English Sole	August 2004	0.041±0.017 (n=16)	0.374±0.149 (n=32)	0.024±0.015 (n=23)	0.329±0.182 (n=20)

The sampling pattern for sperm vtg was the same as described above for sexual maturity. Vtg concentrations were detected in 73% of the male Hornyhead Turbot samples from the outfall and 83% of the male Hornyhead Turbot samples from the reference station. The majority of the samples in which vtg was detected were near detection limits. There was, however an overall trend toward more individuals expressing higher concentrations of vtg at the OCSD outfall (Figure 5). Vtg concentrations reached a maximum of 0.27 ng vtg /µg plasma protein in the reference site fish and 0.55 ng/µg in the outfall site fish (July 2003 sampling period).

Sixty percent of the male English Sole collected at the reference station, and 62% of the male fish collected at the outfall expressed vtg above detection. Again there was an overall trend toward higher levels in animals collected at the OCSD outfall (Figure 6). Vtg concentrations reached a maximum of 5.34 ng/µg in the reference site fish (August

2004 sampling period), and 15.62 ng/µg in the outfall site fish (January 2004 sampling period). Despite this trend toward higher vtg levels in fish from the outfall site, no significant differences between sites could be established for plasma vitellogenin expression in either species. Vtg expressions in males of both species between sites were also much lower than found in vitellogenic females (Figures 7 and 8).

The sampling pattern for sperm DNA damage was the same as described above for sexual maturity. With the exception of the August 2004 English Sole, a trend toward higher DNA damage at the outfall was apparent (Table 7). The damage was significantly elevated in Hornyhead Turbot collected at the outfall during the January 2004 sampling ($p<0.01$). This trend has been observed consistently in both flatfish species since DNA damage monitoring was initiated in 2000.

Table 6. Plasma Estradiol concentrations (pg/ml) of Hornyhead Turbot and English Sole collected from the OCSD outfall and Reference locations.

Species	Sampling Period	Outfall	Outfall	Reference	Reference
Species	Sampling Period	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)
Hornyhead Turbot	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)
English Sole	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)

Regression analyses of all metrics within individual fish (i.e. E2 values vs. vtg, DNA sperm damage in males, etc.) were performed on both overall data and individual location data. The regression analysis of E2 values vs. other metrices could only be performed for the January 2004 sampling period as this was the only period during which corresponding data from individual fish were collected. There was a positive correlation between E2 values (pg/ml) and vtg levels (ng/ml) in English Sole females ($R^2 = 0.51$, $p = 0.01$, d.f. = 12). and Hornyhead Turbot females ($R^2 = 0.64$,$p = 0.1$, d.f. = 4). A relationship between E2 and vtg levels was not demonstrated in English Sole males or Hornyhead Turbot males. There was a positive correlation in Hornyhead Turbot males between sperm DNA damage and E2 ($R^2 = 0.71$, $p < 0.05$, d.f. = 6) (Figure 9). This correlation was only seen in fish from the outfall, not in fish from the reference location.

Table 7. DNA damage expressed as tail moment in sperm of fish collected from the OCSD outfall and reference station

Species	Sampling Period	Outfall	Reference
Hornyhead Turbot	July 2003	0.75±0.78 (n=5)	ND
Hornyhead Turbot	January 2004	0.81±0.45 (n=8)	0.37±0.31 (n=20)
Hornyhead Turbot	August 2004	0.38±0.51 (n=5)	ND
English Sole	July 2003	2.33±3.06 (n=16)	1.12±1.24 (n=17)
English Sole	January 2004	0.50±0.74 (n=7)	0.46±0.82 (n=12)
English Sole	August 2004	0.07±0.13 (n=4)	0.18±0.34 (n=11)

Figure 5. Plasma Vitellogenin concentrations in male Hornyhead Turbot collected from the OCSD outfall and reference site.

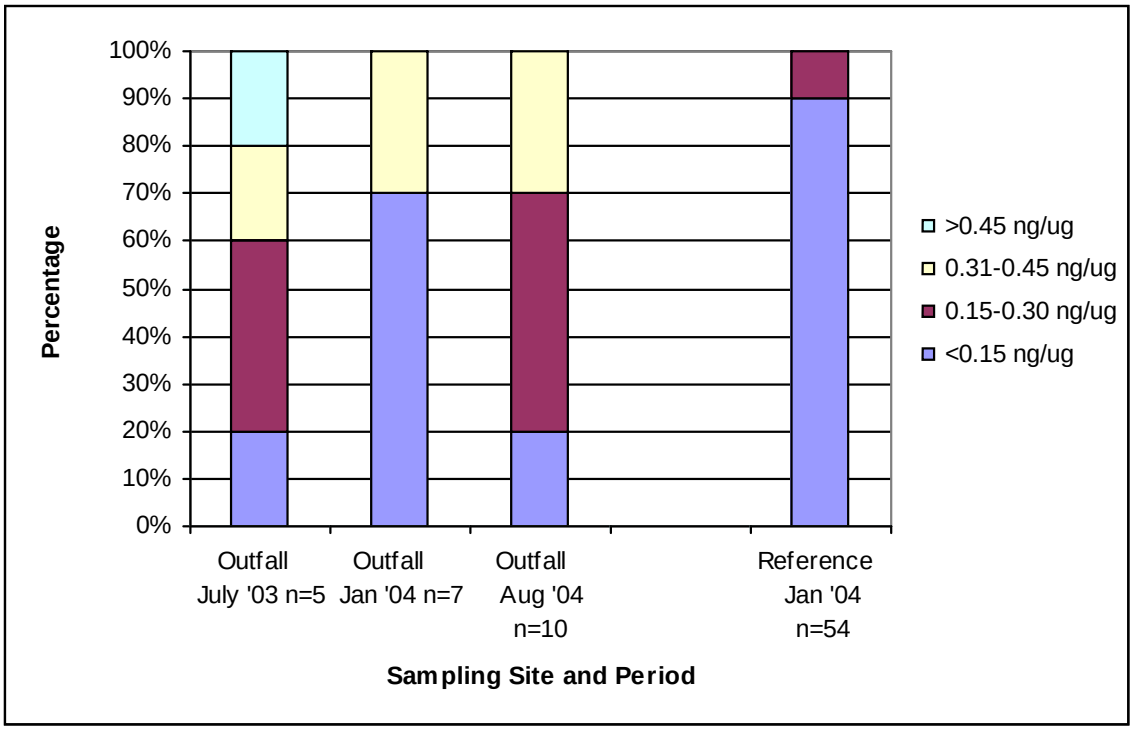


Figure 6. Plasma Vitellogenin concentrations in male English Sole collected from the OCSD outfall and reference site

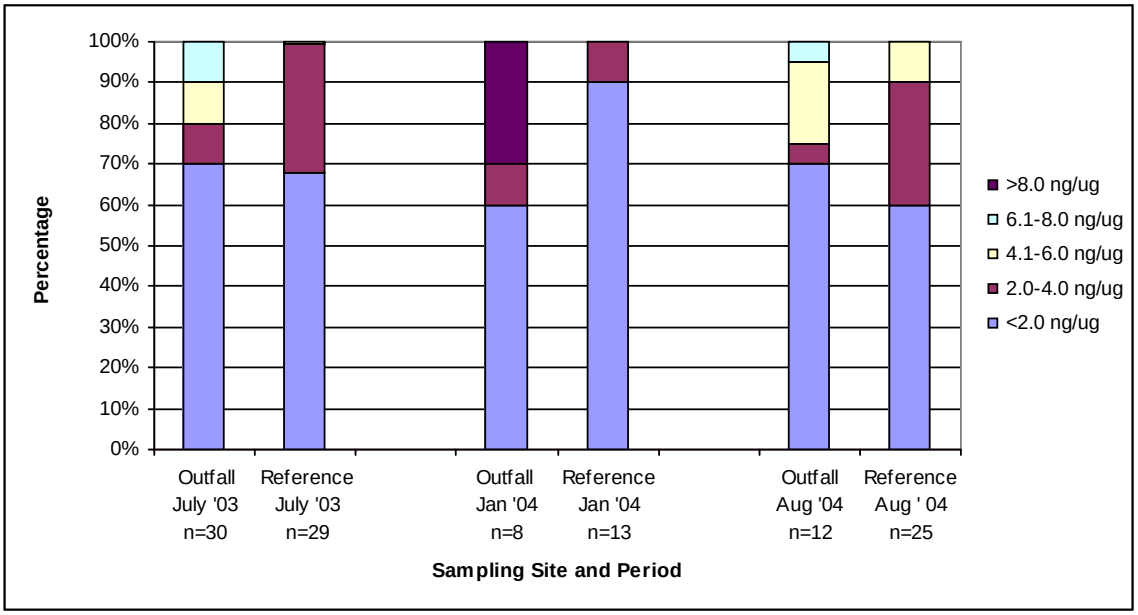


Figure 7. Comparison of male Hornyhead turbot plasma vitellogenin levels to average vitellogenic female levels

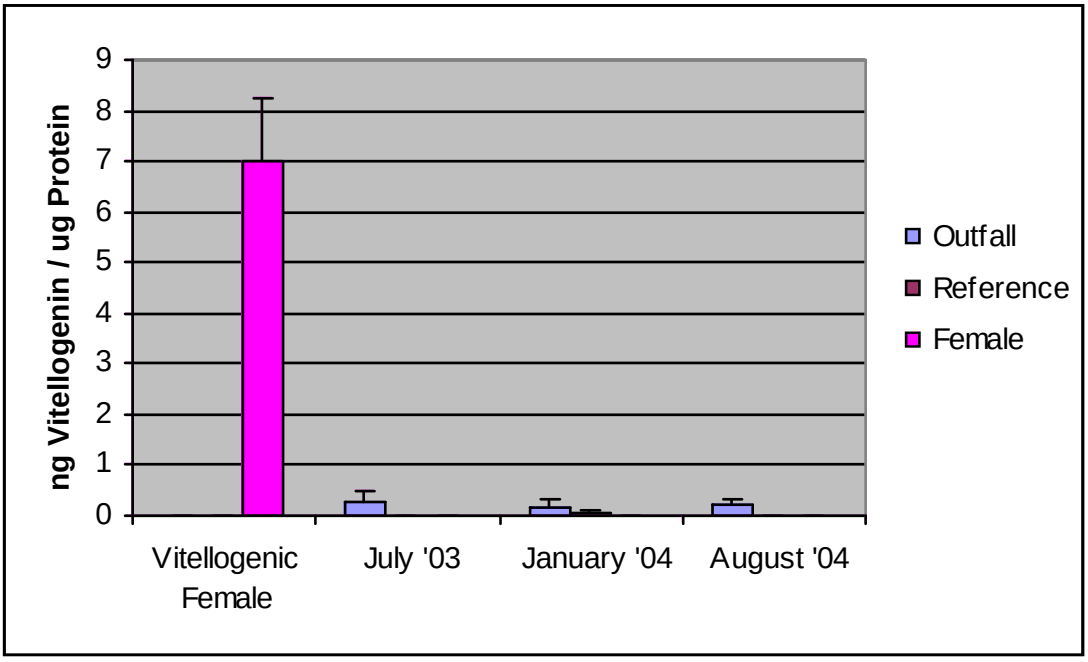


Figure 8. Comparison of male English sole plasma vitellogenin levels to average vitellogenic female levels

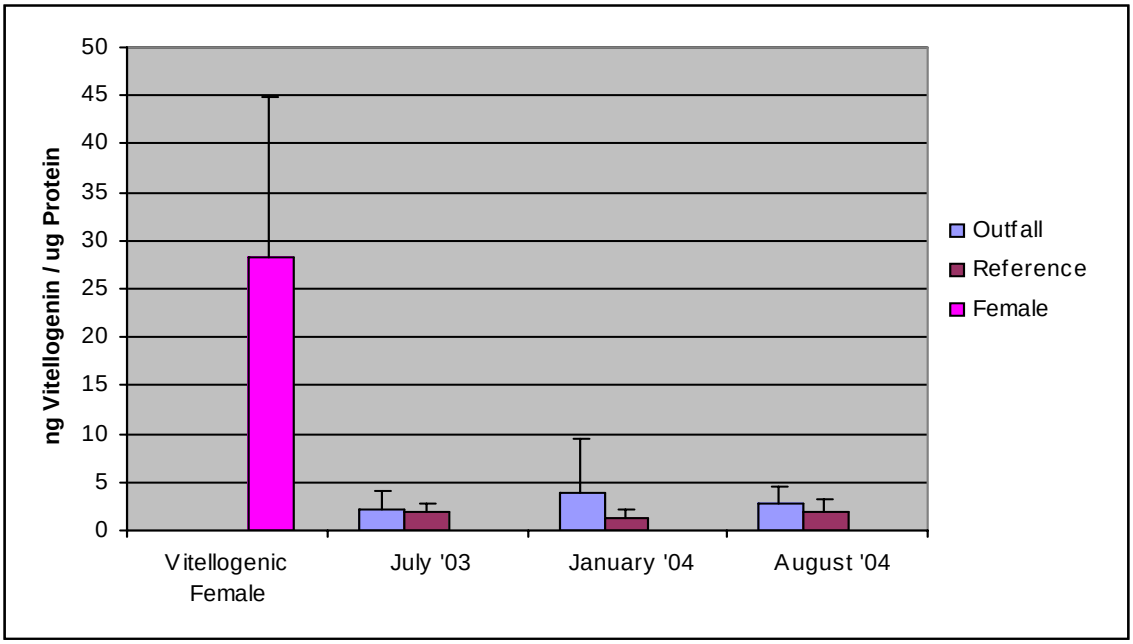
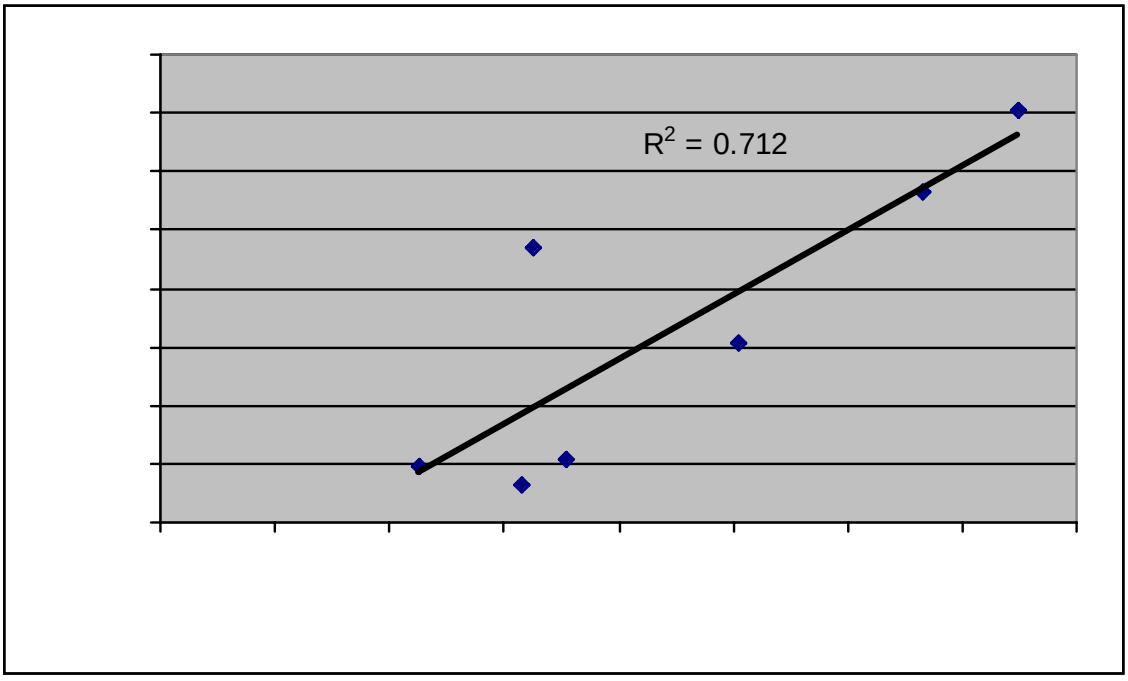


Figure 9. Regression analysis of sperm DNA damage (expressed as tail moment) versus estradiol plasma levels in male hornyhead turbot from the outfall.



Summary

- Variable levels of vitellogenin were continually observed in the plasma samples of fish collected at both locations. Vitellogenin expression and plasma E2 levels were significantly correlated in females but not in males.
- A positive relationship was demonstrated between plasma E2 levels and sperm DNA damage.
- Rather than an expected feminization of populations, a trend toward masculinization was observed particularly at the OCSD outfall, as indicated by gender ratios and significantly higher GSI in males versus females.
- These results were consistent with previous studies showing vitellogenin expression in male flatfish, but no alteration in overall flatfish abundance at the sampled sites.

Partnerships with other Governmental Agencies

Through interactions with each of the San Diego, Los Angeles, and Orange County sanitation districts, fish were sampled throughout the Southern California Bight evaluated for estrogenic activity during the summer of 2003 as part of the Bight-03 project. Three flatfish species were targeted in this study: the hornyhead turbot (*Pleuronichthys verticalis*), the English sole (*Pleuronectes vetulus*) and the California halibut (*Paralichthys californicus*). A total of 202 fish were collected from 33 stations in the Southern California Bight, between Point Conception and the border with Mexico. Only a few California halibut were collected during the survey and the results summarized here only present data on the other two species.

FISH GENDER, MATURITY STAGES AND FISH SIZE

- For hornyhead turbot 42 females and 43 males were collected.
- The majority of male hornyhead turbot (HT) were in immature stages 1 and 2, immature or intermediate stages were fish are not ready for reproduction.
- The majority of female HT were in stages 2 and 3 corresponding to middle and late development respectively. Fish in these maturing stages had eggs developing (level 2) or with some vitellogenin already present (level 3).
- HT male standard length averaged 13.7 cm.
- Average length for female HT was 17.5 cm. Female HT were on average larger than the males.

- For English sole (ES) 42 females and 46 males were collected.
- The majority of male ES fish were in immature stages 1 and 2 corresponding to conditions were the fish are not yet ready for reproduction.
- The majority of female ES fish were in immature stages 0 and 1, which correspond to undeveloped stages.
- ES male standard length averaged 17.34 cm
- ES female standard length averaged 17.49 cm. Female and male English sole standard lengths were similar.

ESTRADIOL

- Male hornyhead turbots had statistically higher estradiol (E2) concentrations than females ($p = <0.05$).
- When related to contaminant concentrations in the sediment, an ANOVA test detected differences in mean E2 concentrations for HT males and females exposed to 4 different DDT concentration-groupings: below detection limits (BDL), 10, 100, and >100 µg/kg.
- A multiple comparison test (Student Newman Keuls test: SNK) showed that males and females HT collected from sediments with DDT values below detection limits had significantly higher concentrations of estradiol ($p = <0.05$).

- Male English sole had on average similar E2 concentrations than females.

- No statistically significant relationships with DDT sediment concentrations and plasma proteins (estradiol, vitellogenin, testosterone, cortisol or IGF-1) were found in English sole (ES) males or females.

VITELLOGENIN

- Levels of vitellogenin (VTG) were statistically different between male and female HT and were significantly different. HT females had on average higher levels of VTG than males ($p = <0.05$).
- The SNK's multiple comparisons showed that fish collected in sediments with DDT concentrations >100 µg/kg had significantly higher concentrations of VTG than fish in the nondetect range of DDTs ($p = <0.05$).

- Levels of vitellogenin between male and female English sole were statistically similar.

TESTOSTERONE

- Levels of testosterone (T) were statistically different between male and female HT ($p = <0.05$). The SNK test showed that males had higher levels of T than females.
- SNK's multiple comparisons showed that fish that were collected from sediments with DDT values below detection limit had significantly higher concentrations of testosterone ($p = <0.05$).

- Levels of T were statistically similar between male and female English sole.

CORTISOL

- Levels of cortisol were statistically similar between male and female HT.

- Levels of cortisol were statistically similar between male and female English sole (ES).

IGF-1

- Levels of IGF-1 were statistically similar between male and female HT.
- No statistically significant differences in IGF concentrations between female and male HT were found.

- Levels of IGF-1 were statistically similar between male and female ES.

<u>PLASMA ANALYSES RELATIONSHIPS</u>

- The Spearman correlation between VTG and E2 was positive and significant in female and male HT.
- The Spearman correlation showed that T and E2 are positively and significantly correlated for female HT.
- The Spearman correlation showed that the relationship between VTG and cortisol is positive and significant for female HT.

- HT females had lower concentrations of E2 at a standard length (SL) ≤ 23 cm, but these differences were not statistically significant.
- Male HT had higher E2 concentration at a SL ≤20 cm but the difference was not statistically significant.
- There was no particular trend in the measured T levels between HT males with testis-ova and those without.

- The Spearman correlation showed that VTG and E2 were significant and positively correlated for female and male ES.
- The Spearman correlation showed that the relationship between T and E2 is positive and significant for male ES.
- The Spearman correlation showed that the relationship between IGF-1 and cortisol is positive and significant for male ES.
- There was a positive Spearman correlation between VTG and gonad maturity in female or male ES.
- There was no particular trend in the measured T levels between ES males with testis-ova and those without.

Spearman correlation results for plasma analyses relationships

Protein Relationship	Female	**Male**
Hornyhead turbot		
E2 (pg/µg protein) & VTG (ng/µg protein)	*Significant*	*Significant*
E2 (pg/µg protein) & T (ng/ml)	A. Significant	Not Significant
E2 (pg/µg protein) & Cortisol (ng/ml)	Not Significant	Not Significant
E2 (pg/µg protein) & Standard Length	Not Significant	Not Significant
VTG (ng/µg protein) & Cortisol (ng/ml)	B. Significant	Not Significant
IGF-1 (ng/ml) & Cortisol (ng/ml)	Not Significant	Not Significant
T (ng/ml) & Cortisol (ng/ml)	Not Significant	Not Significant
T (ng/ml) & VTG (ng/µg protein)	Not Significant	Not Significant
E2 (pg/µg protein) & Gonadal Maturity	Not Significant	Not Significant
English sole		
E2 (pg/µg protein) & VTG (ng/µg protein)	C. Significant	D. Significant
E2 (pg/µg protein) & T (ng/ml)	Not Significant	*Significant*
E2 (pg/µg protein) & Cortisol (ng/ml)	Not Significant	Not Significant
E2 (pg/µg protein) & Standard Length	Not Significant	Not Significant
VTG (ng/µg protein) & Cortisol (ng/ml)	Not Significant	Not Significant
IGF-1 (ng/ml) & Cortisol (ng/ml)	Not Significant	E. Significant
T (ng/ml) & Cortisol (ng/ml)	Not Significant	Not Significant
T (ng/ml) & VTG (ng/µg protein)	Not Significant	Not Significant
E2 (pg/µg protein) & Gonadal Maturity	F. Significant	Not Significant

Statistics of significant (≤ 0.05) Spearman correlations for plasma protein analyses in male and female fish.

Protein Relationship	**Female**			**Male**		
Hornyhead turbot	**N**	**r**	**p**	**N**	**r**	**p**
E2 (pg/µg protein) & VTG (ng/µg protein)	33	0.483	0.004	26	0.442	0.024
E2 (pg/µg protein) & T (ng/ml)	34	0.440	0.001			
VTG (ng/µg protein) & Cortisol (ng/ml)	33	0.664	0.000			

English sole						
E2 (pg/µg protein) & VTG (ng/µg protein)	19	0.607	0.005	19	0.607	0.005
E2 (pg/µg protein) & T (ng/ml)				16	0.479	0.058
IGF-1 (ng/ml) & Cortisol (ng/ml)				18	0.655	0.003
E2 (pg/µg protein) & Gonadal Maturity	32	0.434	0.013			

HISTOLOGY AND PATHOLOGY ANALYSES

Gonad Histopathology

- Gonadal pathological incidences in HT were found at 12 stations.
- Pathological incidences in ES gonads were found at 18 stations.
- There were three main types of pathology identified in females: oocyte (eggs) atresia increased of endoparasitism, and protozoa present in the ovary.
- There were seven main types of pathologies identified in males: oocytes in testis, hemorrhage, interstitial fibrosis, macrophage aggregates, mineralization, and degeneration of the testis.

Female Atresia

- There were six stations (out of 33 sampled) were female HT and ES showed oocyte (eggs) atresia.
- There was no particular geographic trend among stations were females presented oocyte atresia.

Male Testis-Ova

- There were six stations (out of 33) where sampled males had testis-ova (oocytes in testis) for hornyhead (HT) and English sole (ES). These stations are located near outfalls between Santa Monica Bay and the Santa Ana River.
- 11 males were impacted, six ES, and five HT.

SEDIMENT CHEMISTRY

Female Atresia and Sediment Chemical Concentrations

- Oocyte atresia in HT or ES females was not related to high concentrations of total PCBs, chromium, DDTs or chlordane concentrations in sediment.

Male Testis-Ova and Sediment Chemical Concentrations

- Male testis-ova were present in stations with high levels of PCBs, DDTs and total chlordane.
- No particular pattern was observed between chromium and testis-ova.

Vitellogenin, Estradiol and Sediment Chemical Concentrations

- There was no relationship between VTG plasma concentrations and total PCBs, chromium, DDTs and chlordane in sediment for either ES or HT.
- There was no relationship between E2 in plasma and total PCBs, chromium, DDTs and chlordane in sediment for either ES or HT.

Students supported:

UC Riverside

Graduate Students
Wendy Hwang (moved to another laboratory Oct 2003)
Ola Bawardi (Graduated with MS December 2005)
Mary Ann Irwin (aka Mary Ann Rempel)- (2003-2004)

Undergraduate Students
Hector De Hero
Mai Thi
Ola Bawardi as undergraduate 2002-2003

UC Berkeley
Edward P. Kolodziej (PhD in 2004) (2002-2004)

UC Davis
Amanda Palumbo (PhD candidate 2002-2004)

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W. Hwang, Y. Sapozhnikova, M. Kelly, D. Montagne, J. Armstrong, A. Palumbo, R. Tjeerdema, D. Schlenk (2003) Evaluation of Marine Sediments from the Southern California Bight for Estrogenic Activity Using *in vitro* and *in vivo* Assays. Poster presentation at the 12th International Symposium on Pollution Responses in Marine Organisms (poster presentation).

W. Hwang, C. Seruto, L.A Roy, J.Armstrong, K.Sakamoto, K.Kahara, S.Reddy, Y. Sapoqhnikova, B. Brownawell, D.Schlenk (2003) Assessment of demersal flatfish species for exposure and adverse effects resulting from exposure to environmental estrogens off the coast of Orange county, CA, USA 12th International Symposium on the Pollutant Responses in Marine Organisms (Poster Session).

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