#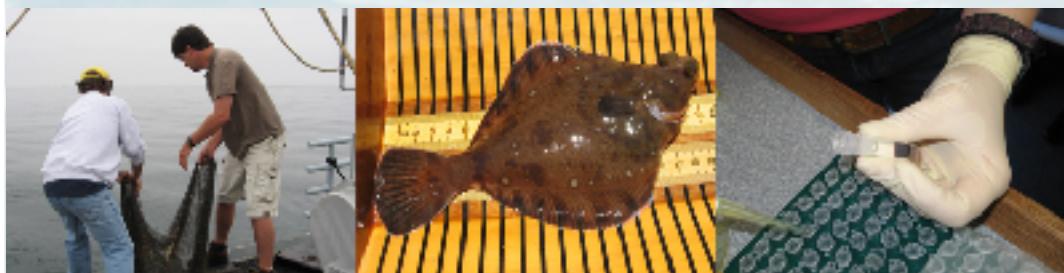 Sources and Effects of Endocrine Disruptors and Other Contaminants of Emerging Concern in the Southern California Bight Coastal Ecosystem

Steven M. Bay

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Daniel Schlenk

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Joseph R. Gully

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Southern California Coastal Water Research Project

Sources and Effects of Endocrine Disruptors and other Contaminants of Emerging Concern in the Southern California Bight Coastal Ecosystem

Steven M. Bay[1], Doris E. Vidal-Dorsch[1], Daniel Schlenk[2], Kevin M. Kelley[3], Michael E. Baker[4], Keith A. Maruya[1], and Joseph R. Gully[5]

[1]*Southern California Coastal Water Research Project, Costa Mesa, CA*

[2]*University of California, Riverside, Riverside, CA*

[3] *California State University Long Beach, Long Beach, CA*

[4] *University of California, San Diego, La Jolla, CA*

[5] *Sanitation Districts of Los Angeles County, Whittier, CA*

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EXECUTIVE SUMMARY

Thousands of chemicals are in daily use for which little is known about their fate and effects on aquatic life. These compounds include pharmaceuticals and personal care products (PPCPs), current use pesticides (CUPs), natural and synthetic hormones, and industrial and commercial compounds (ICCs). Collectively known as contaminants of emerging concern (CECs), many of these compounds are discharged into coastal waters from point and nonpoint sources and have the potential to cause adverse biological effects. There is little information to assess the ecological impacts of CECs, partly because environmental monitoring programs usually focus on priority pollutants such as trace metals, chlorinated pesticides, PCBs, and petroleum hydrocarbons that historically contaminated coastal waters. Concern over the environmental impacts of CECs has increased in recent years as a result of studies showing their common occurrence in waste discharges and receiving waters, and instances of biological effects such as endocrine disruption on fish and wildlife associated with some CECs. Over a billion gallons of treated municipal wastewater are discharged into southern California coastal waters every day. These discharges represent a potentially significant source of CEC exposure for marine life. However, only limited information is available regarding the types, concentrations, and fate of CECs discharged to the Southern California Bight (SCB) from treated wastewater discharges and their potential for ecological impacts.

This 2006 Coastal Effects Study was a collaborative effort among SCCWRP, major southern California municipal wastewater agencies, and universities. This study was designed to investigate the impacts of CECs from ocean wastewater discharges on fish in the SCB. Samples of effluent, ocean water, sediment, and fish were collected from multiple locations and analyzed to address six key questions:
- What types of CECs are discharged into the SCB from municipal wastewater outfalls?
- Are SCB marine life exposed to CECs from municipal wastewater discharges?
- Is there evidence of endocrine disruption or other physiological effects in SCB fish?
- Are effects on fish related to historical contaminants or current municipal wastewater discharges?
- Are specific chemicals responsible for the effects?
- Are the physiological effects adversely impacting fish populations or communities?

Effects analyses focused on the hornyhead turbot (*Pleuronichthys verticalis*), a species of flatfish associated with bottom sediments throughout the SCB. Four sites located near the largest ocean discharges of municipal wastewater were studied, in addition to a reference site. Effluent, water, sediment, and fish tissue samples were analyzed for a diverse suite of CECs, including PPCPs, hormones, and ICCs. Fish biological analyses included molecular indicators (e.g., gene expression and hormones), measures of reproductive condition, sexual maturity, and abundance. Trawl caught fish monitoring data were used to assess the health of fish communities near the outfalls as well as the reference area.

What types of CECs are discharged into the SCB from municipal wastewater outfalls?
Low concentrations of many PPCPs and ICCs were frequently measured in the effluent samples. The most frequently detected PPCPs included analgesics (e.g., naproxen), antibiotics (e.g., sulfamethoxazole), antimicrobials (e.g., triclosan), antidepressants, antiepileptics, and cholesterol medications. Frequently detected ICCs included bisphenol A (plastic component), octylphenol (surfactant), and TCPP (flame retardant). The median concentrations of the detected compounds were below available toxicity thresholds.

Are SCB marine life exposed to CECs from municipal wastewater discharges?
Some CECs were detected in sediment and water samples near the outfall sites, indicating the potential for exposure to fish from direct contact or via their diet. Seawater CEC concentrations were extremely low (less than one part per trillion) and well below concentrations expected to produce toxic effects. Hornyhead turbot livers contained compounds such as PBDEs (flame retardant) and nonylphenol (surfactant), confirming exposure to some CECs. Elevated concentrations of legacy contaminants such as DDTs and PCBs were also present in sediment and livers from most sites, indicating that marine life are exposed to multiple types of contaminants in the SCB.

Is there evidence of endocrine disruption or other physiological effects in SCB fish?
Hormone analyses suggested the presence of several types of physiological effects in the fish. These included reduced plasma cortisol levels (evidence of an impaired stress response), relatively high levels of plasma estradiol in male fish, and reduced plasma thyroxine (thyroid hormone). Male fish also contained low levels of plasma vitellogenin (egg yolk protein). However, there was no evidence of fish feminization, impaired reproductive function, or poor fish condition associated with these molecular changes. It was not possible to determine if these responses are evidence of endocrine disruption, because cause was not definitively determined. Some responses may seem unusual, but they may be normal aspects of hornyhead turbot physiology. Additional studies of the baseline physiological characteristics of this species are needed to determine if these responses represent abnormal or contaminant-related responses.

Are effects on fish related to historical or current municipal wastewater discharges?
Most of the physiological responses in hornyhead turbot were found at all sites, and could not be directly associated with effluent discharges. Two responses did show an apparent association with the outfall sites: concentrations of the thyroid hormone (thyroxine) were lower at all discharge sites relative to the reference, and estradiol concentrations were lower at the Los Angeles, Palos Verdes, and Orange County outfall sites. Further study is needed to confirm these results and determine the cause.

Are specific chemicals responsible for the effects?
No specific associations between individual chemicals and physiological effects can be made from this study. The types of responses observed in hornyhead turbot have been linked to chemical exposure in other studies, but limitations in the chemical analyses of fish tissue in our study restricted our ability to distinguish chemical-specific relationships. These responses may have been caused by legacy contaminants or other environmental factors, in addition to current wastewater discharge constituents. .

Are the physiological effects adversely impacting fish populations?
The physiological responses found in this study did not appear to be associated with adverse impacts on fish reproduction or populations. Fish from discharge and reference sites had similar reproductive cycles. Analysis of long-term monitoring data also showed that hornyhead turbot populations were stable (or increasing) and that fish community composition near the outfall discharges was typical of that expected in uncontaminated reference areas.

This collaborative study represents one of the most comprehensive investigations to date of CECs and their effects in coastal waters of the US. The data generated provide a valuable foundation for guiding and interpreting future studies in southern California. The lack of observed adverse effects on fish reproduction and populations indicates little, if any, current impact on fish in offshore waters, but more research is needed to understand the potential threat posed by these compounds. The prevalence and persistence of CECs in municipal wastewater also suggests that further study of biological effects is warranted in habitats with a greater potential for CEC exposure, such as effluent-dominated streams and estuaries.

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BACKGROUND

Three decades of monitoring by southern California water quality agencies have provided much information regarding legacy priority pollutants such as DDT, PCBs, mercury and lead. In contrast, little is known about the sources, fate and effects of thousands of other chemicals in current use. Some of these are newly developed compounds, many of which are designed to affect biological systems, have a widespread use, and are chronically discharged into aquatic habitats (Alvarez-Cohen and Sedlak 2003, Chen et al. 2006, Snyder 2008). These so-called "contaminants of emerging concern" (CECs) can be classified into four major categories: pharmaceuticals and personal care products (PPCPs); current use pesticides (CUPs); natural and synthetic hormones; and industrial and commercial chemicals (ICCs). Examples of CECs are: oxybenzone (UV light blocker), fipronil (insecticide), ethinylestradiol (synthetic estrogen in birth control pills), and polybrominated diphenyl ethers (PBDEs; flame retardants).

Most CECs have not been extensively studied for occurrence and effects, often due to the lack of available analytical methods, yet they may represent a risk to aquatic life after being released to the environment (Petrovic et al. 2004, Brooks et al. 2006, Snyder 2008). For example, CUPs such as pyrethroids have been identified as the cause of sediment and receiving water toxicity throughout California (Holmes et al. 2008, Anderson et al. 2010, Lao et al. 2010). Some CECs disrupt the endocrine systems of animals by interfering with the action of hormones involved with reproduction or growth. Research in regions outside southern California suggests that environmental concentrations of some CECs may be sufficient to produce endocrine disruption in fish living in coastal waters (Rule et al. 2006, Deng et al. 2007, Alvarez et al. 2009, Björkblom et al. 2009, Iwanowicz et al. 2009).

Recent studies have detected multiple CECs in treated municipal wastewater effluents discharged into coastal waters of the Southern California Bight (SCB) and nearby sediments (Tilton et al. 2002, Sapozhnikova et al. 2004, Schlenk et al. 2005). These studies have also reported the presence of indicators of endocrine disruption in local fish, such as vitellogenin (an egg yolk protein) production in male flatfish. However, the cause and significance of these effects are not known because the previous studies were limited in scope. As a result, there is insufficient information available to characterize the nature and magnitude of endocrine disruption in southern California fish. Additional information is also needed to determine whether endocrine responses are adversely impacting fish health, and if current CEC inputs from municipal wastewater discharges are responsible. Without such information, water quality management agencies cannot make informed decisions regarding the need to monitor and regulate CECs.

This project was designed to address data gaps regarding CECs in southern California coastal waters. The goal of the project was to help answer the following questions:
- What types of CECs are discharged into the SCB from municipal wastewater outfalls?
- Are SCB marine life exposed to CECs from municipal wastewater discharges?
- Is there evidence of endocrine disruption or other physiological effects in SCB fish?
- Are effects on fish related to historical or current municipal wastewater discharges?
- Are specific chemicals responsible for the effects?
- Are the physiological effects adversely impacting fish populations or community structure?

In order to address these questions, one of the largest and most comprehensive studies in the nation on CECs and their effects on coastal fish was conducted. This study, known as the 2006 Coastal Effects Study, was a collaborative effort coordinated by SCCWRP and a Steering Committee comprised of scientists from southern California's four largest municipal wastewater treatment agencies and major universities (Table 1). Sampling and analyses were conducted by the Steering Committee agencies and some of the nation's leading chemical and pathology laboratories.

Table 1. Steering Committee and partner laboratories involved in the 2006 Coastal Effects Study.

Member	Organization	Sponsor	Sampling	Analysis
Steering Committee				
Steven Bay, Dr. Doris Vidal-Dorsch Dr. Keith Maruya	Southern California Coastal Water Research Project	X	X	X
Joe Gully	Los Angeles County Sanitation Districts	X	X	X
Dr. Jeffrey Armstrong	Orange County Sanitation District	X	X	
Curtis Cash	City of Los Angeles	X	X	
Dr. Timothy Stebbins	City of San Diego	X	X	
Scott Johnson	Aquatic Bioassay and Consulting Lab		X	
Dr. Daniel Schlenk	University of California Riverside		X	X
Dr. Kevin Kelley	California State University Long Beach	X	X	X
Dr. Michael Baker Dr. Gary Hardiman	University of California San Diego			X
Laboratory				
	Southern Nevada Water Authority			X
	Mississippi State Chemistry Lab			X
	Experimental Pathology Laboratories			X

METHODS

Study Design

The 2006 Coastal Effects Study was designed to build upon recent research in southern California which found physiological changes suggestive of endocrine disruption in the hornyhead turbot (*Pleuronichthys verticalis*), a common species of flatfish that lives on soft bottom sediments along the coast of the SCB (Cooper 1994). Flatfish such as hornyhead turbot are good choices for environmental monitoring because they live in contact with the sediment, have high site fidelity, feed on sediment-dwelling animals, and are monitored locally for tissue contamination, thus providing a model organism for studying exposure to environmental contaminants in specific areas.

A comprehensive and integrated chemical and biological investigation was conducted, which focused on the four largest ocean discharges of municipal wastewater to the SCB (Figure 1). A total of over one billion gallons per day of treated wastewater is discharged by these outfalls, which are located at depths ranging from approximately 60-100 meters. Samples of final effluent from each of the treatment plants, as well as near-bottom seawater, sediment, and hornyhead turbot from the study areas were collected to characterize the fate of a diverse suite of CECs.

Each of the effluent types and associated sampling sites represented a different combination of effluent treatment and historical contamination, providing an opportunity to examine the relative impacts of current CEC inputs and legacy contamination on the fish. For example, effluents discharged at the Santa Monica Bay (LA) and Palos Verdes (PV) locations received 100% secondary treatment, and the sediments of these areas contained relatively high legacy contamination from DDT and PCBs. The Orange County (OC) site received effluent with partial secondary treatment and contained little legacy sediment contamination. The San Diego (SD) site received advanced primary treated effluent and also had little legacy sediment contamination. A fifth sampling area was located near Dana Point (DP); this area was distant from large wastewater discharges and served as a reference site. The DP area has been used as a reference in previous SCCWRP studies.

Three different sampling designs were used to address the study questions. The first sampling design consisted of collection of quarterly samples of wastewater effluent and near bottom water from each of the five sites. Chemical analysis of these samples was used to describe the occurrence and water exposure concentrations of CECs. Samples were collected between May 2006 and February 2007.

The second sampling design was intended to document spatial patterns of sediment contamination, fish exposure, and biological effects relative to wastewater discharges. Samples of sediment and fish were collected from the five study sites (LA, PV, OC, DP, and SD) during a single sampling event between the end of May and the beginning of June 2006. The sediment sample was a composite of surface sediment from three separate grabs. Fifty hornyhead turbot were collected by otter trawl from each site and dissected onboard ship (Figure 2) to obtain samples for chemical analysis (liver) and biological analysis (blood, liver, gonad). The liver samples were composited by gender prior to chemical analysis, resulting in two samples per site. All biological analyses were conducted on individual fish samples.

The third sampling design was intended to document temporal and small-scale spatial variations in biological indicators over the fish's reproductive cycle. This sampling was focused on three areas that represented a wide range of expected contaminant exposure: Palos Verdes (PV), Orange County (OC), and Dana Point (DP). Small-scale spatial variation was investigated by collecting fish from two far field sites used in monitoring programs as a local reference for Palos Verdes (PVF) and Orange County (OCF). Thirty hornyhead turbot were collected from each site at quarterly intervals between May 2006 and February 2007 (samples from the first quarter were the same as those collected for the spatial study design).
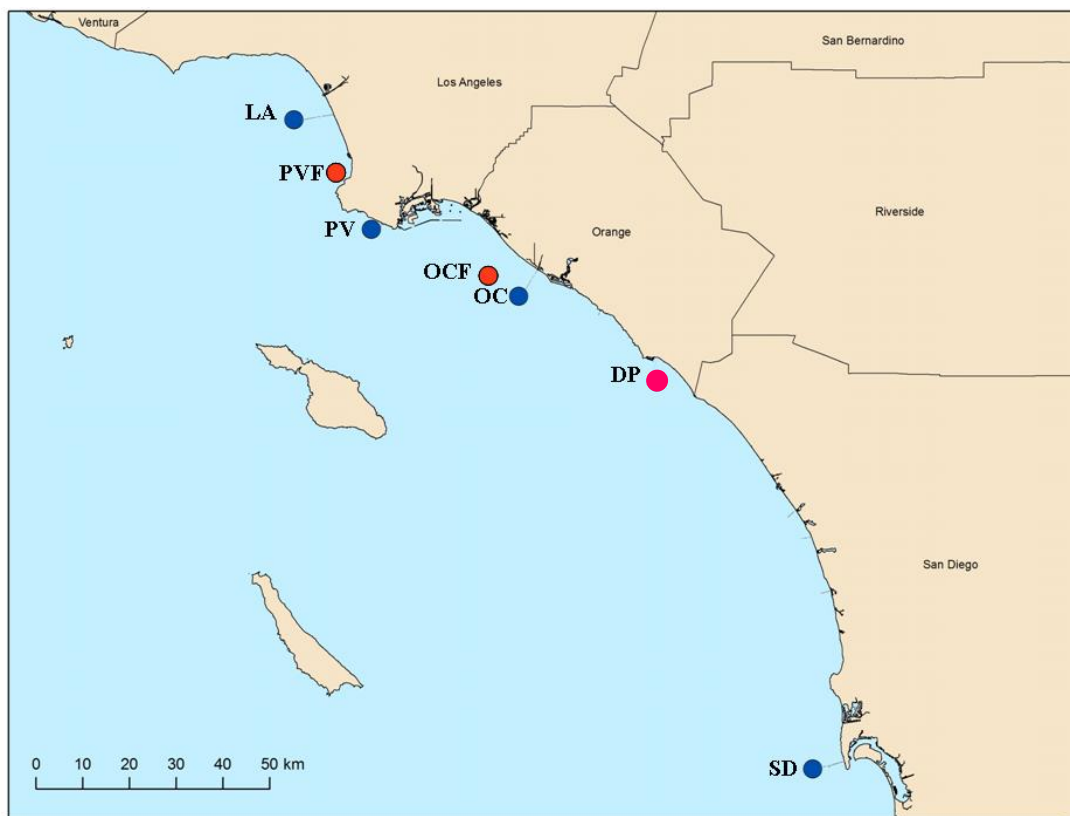
Figure 1. Project study sites. Four sites were located near large discharges of municipal wastewater (LA, PV, OC, and SD). In addition, samples were collected from one reference station (DP) and two far field stations (PVF and OCF).

Figure 2. Hornyhead turbot on sediment (A), blood sampling (B) and liver removal (C).

Analyses

A diverse suite of CECs and legacy contaminants was measured in effluent, seawater, sediment, and fish liver (Table 2). The CEC analytes included PPCPs, CUPs, natural and synthetic hormones, and ICCs. Not all analytes were measured in all types of samples due to limitations in sample size, analytical methods, and also because of low probability of occurrence in a given matrix. Legacy contaminants (PCBs and chlorinated pesticides) were only measured in sediment and liver samples as the effluents are not currently a significant source of these compounds.

Multiple biological indicators representing different levels of response (e.g., molecular to population) were measured in the fish. The parameters were selected in order to link highly sensitive molecular responses to more ecologically relevant measures such as reproduction and survival (Figure 3).

Blood plasma samples were analyzed by specific radioimmunoassay or enzyme immunoassay to determine the concentrations of hormones involved in reproduction (estradiol and 11-ketotestosterone (11-KT)), development (thyroxine), and stress response (cortisol). The concentration of vitellogenin (VTG), a protein involved in egg yolk production, was also measured in plasma by ELISA (enzyme-linked immunosorbent assay). Reproductive hormones and VTG were measured in all fish, while thyroxine and cortisol were only measured in samples from the spatial study component.

One half of the gonad from each fish was preserved for histological analysis of sex, maturity state, and the presence of abnormalities in sexual development (e.g., presence of eggs in male gonad). The remaining gonad was weighed and then preserved for gene expression analysis. The gonadal somatic index (GSI), a measure of reproductive status, was calculated using one of the gonads. The 1/2 GSI was calculated as the ratio of the right gonad weight divided by the total body weight of the fish.

Overall fish condition was described in terms of the condition factor (CF: total body weight divided by the standard length) and liver somatic index (LSI: liver weight divided by the total body weight).
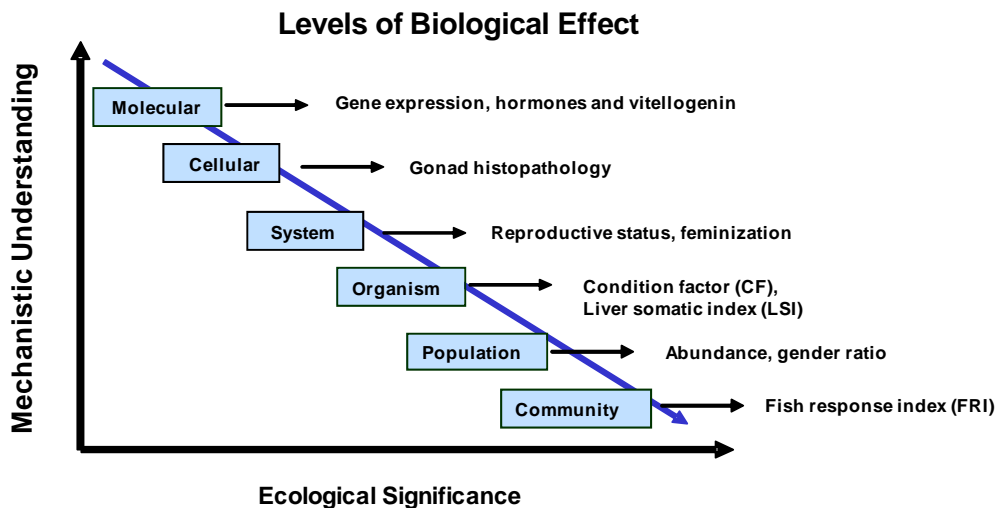
Figure 3. Levels of biological response and corresponding indicators examined in this study. Each level has different characteristics of sensitivity, interpretation, and ecological relevance.

Table 2. Chemical analytes included in study by matrix.

Chemical Name	Effluent	Seawater	Sediment	Tissue
CUPs				
Atrazine	Yes	Yes	Yes	Yes
Barban	No	No	Yes	Yes
Chlorpyrifos	No	No	Yes	Yes
Diazinon	Yes	Yes	Yes	Yes
Diuron	No	No	Yes	Yes
Lindane	Yes	Yes	Yes	Yes
Methoxychlor	Yes	Yes	Yes	Yes
Metolachlor	Yes	Yes	Yes	Yes
Propanil	No	No	Yes	Yes
Pyrethroid pesticides	No	No	Yes	Yes
Hormones				
17α-ethynylestradiol (EE2)	Yes	Yes	Yes	Yes
17ß-estradiol (E2)	Yes	Yes	Yes	No
Estrone (E1)	Yes	Yes	Yes	No
Progesterone	Yes	Yes	No	No
Testosterone	Yes	Yes	No	No
ICCs				
Benzophenone	Yes	Yes	No	No
Bis(2-Ethlyhexyl)adipate	No	No	Yes	No
Bis(2-Ethylhexyl)phthalate	No	No	Yes	No
Bisphenol A	Yes	Yes	No	No
Butyl benzyl phthalate	No	No	Yes	No
Butylated hydroxytoluene	Yes	Yes	No	No
Diethylphthalate	No	No	Yes	No
Dimethylphthalate	No	No	Yes	No
Di-n-butylphthalate	No	No	Yes	No
Di-n-octylphthalate	No	No	Yes	No
Dioctyl phthalate	Yes	Yes	No	No
Hydroxyanisole	Yes	Yes	No	No
N,N-diethyl-meta-toluamide	Yes	Yes	No	No
Nonylphenol	Yes	Yes	Yes	Yes
Octachlorostyrene	Yes	Yes	No	No
Octylphenol	Yes	Yes	No	No
PBDE-100	No	No	Yes	Yes
PBDE-153	No	No	Yes	Yes
PBDE-154	No	No	Yes	Yes
PBDE-47	No	No	Yes	Yes
PBDE-99	No	No	Yes	Yes
TCEP	Yes	Yes	No	No
TCPP	Yes	Yes	No	No
Legacy Compounds				
Aldrin	No	No	Yes	Yes
Chlordane	No	Yes	Yes	Yes

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Table 2. Continued.

Chemical Name	Effluent	Seawater	Sediment	Tissue
Legacy Compounds (continued)				
DDD	No	Yes	Yes	Yes
DDE	No	Yes	Yes	Yes
DDMU	No	No	Yes	Yes
DDT	No	Yes	Yes	Yes
Dieldrin	No	No	Yes	Yes
Endrin	No	No	Yes	Yes
Heptachlor	No	Yes	Yes	Yes
Heptachlor epoxide	No	Yes	Yes	Yes
Nonachlor	No	Yes	Yes	Yes
Oxychlordane	No	No	Yes	Yes
PCB Congeners	No	Yes	Yes	Yes
Toxaphene	No	No	Yes	Yes
PPCPs				
Acetaminophen	Yes	Yes	No	No
Atenolol	Yes	Yes	No	No
Atorvastatin	Yes	Yes	No	No
Carbamazepine	Yes	Yes	Yes	Yes
Diazepam	Yes	Yes	Yes	Yes
Diclofenac	Yes	Yes	No	No
Dilantin	Yes	Yes	No	No
Enalapril	Yes	Yes	No	No
Erythromycin	Yes	Yes	No	No
Fluoxetine	Yes	Yes	No	No
Galaxolide	Yes	Yes	No	No
Gemfibrozil	Yes	Yes	No	No
Hydrocodone	Yes	Yes	No	No
Ibuprofen	Yes	Yes	No	No
Iopromide	Yes	Yes	No	No
Linuron	Yes	Yes	Yes	Yes
Meprobamate	Yes	Yes	No	No
Musk Ketone	Yes	Yes	No	No
Naproxen	Yes	Yes	No	No
Norfluoxetine	Yes	Yes	No	No
O-Hydroxy atorvastatin	Yes	Yes	No	No
Oxybenzone	Yes	Yes	Yes	Yes
p-Hydroxy atorvastatin	Yes	Yes	No	No
Risperidone	Yes	Yes	No	No
Simvastatin	Yes	Yes	Yes	Yes
Simvastatin hydroxy acid	Yes	Yes	No	No
Sulfamethoxazole	Yes	Yes	No	No
Tonalide	Yes	Yes	No	No
Traseolide	Yes	Yes	No	No
Triclosan	Yes	Yes	Yes	Yes
Trimethoprim	Yes	Yes	No	No
Vinclozolin	Yes	Yes	No	No

Long-term annual monitoring data for fish abundance at the discharge sites were examined in order to assess impacts on hornyhead turbot populations and demersal fish community structure. Similar data for the DP site were compiled from regional monitoring studies conducted in 1998 to 2008. The population data were summarized by decade and analyzed for significant changes using one-way analysis of variance (ANOVA). The monitoring data were also used to calculate the fish response index (FRI), a measure of demersal fish community impact associated with pollution stress. The FRI was calculated using species abundance data and pollution tolerance scores to determine whether or not the species composition was similar to that characteristic of reference conditions.

Gene expression analyses were conducted on a subset of male turbot liver and gonad samples collected from each site during the spatial study. These analyses were intended to explore the utility of newly developed gene expression analysis tools in monitoring applications. Samples were selected for analysis to represent a range of variation in hormone and VTG concentrations.

Two types of gene expression analysis methods were used. The first method was a custom gene microarray containing probes for 39 genes involved in physiological processes such as reproduction, growth, development, stress response, contaminant response and detoxification, response to infection, and hormone activity. Two liver samples from each of five sites were analyzed using the microarray. The microarray measured relative RNA concentrations for each of the genes in terms of probe signal intensity. Differential gene expression (fold induction) was calculated for each sample as the ratio (log_2 transformed) of the sample signal intensity to that of a reference site fish from DP.

In the second method, gene expression was measured for a subset of genes using quantitative polymerase chain reaction (qPCR) techniques. Liver and gonad tissue RNA from 10 fish per station were analyzed. The liver samples were analyzed for four genes: vitellogenin (VTG), estrogen receptors alpha and beta (ER_A and ER_B), and cythochrome p450 family 1, subfamily A (CYP1A). A different suite of genes were analyzed in gonad tissue: hydroxysteroid 11 beta dehydrogenase type-2 (11-HSD-2), hydroxysteroid 17 beta dehydrogenase type-1 (17-HSD-1), steroidogenic acute regulator (StAR) and cythochrome p450 type A, family 19 (CYP19). Differential gene expression ratios were also calculated using the qPCR data. Fold induction was calculated relative to the pooled RNA of five fish from the Dana Point sample set.

Chemical measurements in effluent and seawater samples were summed by chemical group (e.g., hormones, PPCPs, ICCs), averaged by station, and analyzed using one-way analysis of variance (ANOVA; $p < 0.05$). Station averages of differential gene expression (qPCR data), as well as plasma concentrations of hormones and VTG, were also compared using ANOVA. Samples with significant differences by ANOVA were subsequently analyzed using a Tukey test, to identify differences among specific sites.

RESULTS

Thousands of chemical and biological measurements were made during this study, and the results are described in detail in scientific journal publications in preparation. The key findings are described in this document and are organized with respect to the project questions. A summary of the chemical analyses is provided in Appendix A and a database is available that contains the results of the biological measurements.

What Types of CECs are Discharged into the SCB from Municipal Wastewater Outfalls?

Diverse types of CECs are discharged into the SCB from municipal wastewater outfalls. Most of the target PPCP, ICC, and hormone analytes were frequently detected in effluent samples from each of the four wastewater treatment facilities (Figure 4). Of the 31 PPCPs measured, 11 were detected in every sample analyzed, regardless of treatment level or effluent type. The most frequently detected PPCPs included analgesics (e.g., naproxen), antibiotics (e.g., sulfamethoxazole), antimicrobials (e.g., triclosan), antidepressants, antiepileptics, and cholesterol medications. Five ICCs and one of the hormones were also detected in 100% of the effluent samples. The synthetic estrogen ethinylestradiol was not detected in any of the effluent samples analyzed. Frequently detected ICCs included bisphenol A (plastic component), octylphenol (surfactant), and TCPP (flame retardant). The CUPs were detected at the lowest frequency, with none of the compounds detected in more than 63% of the samples.

Percent Detection in Effluent
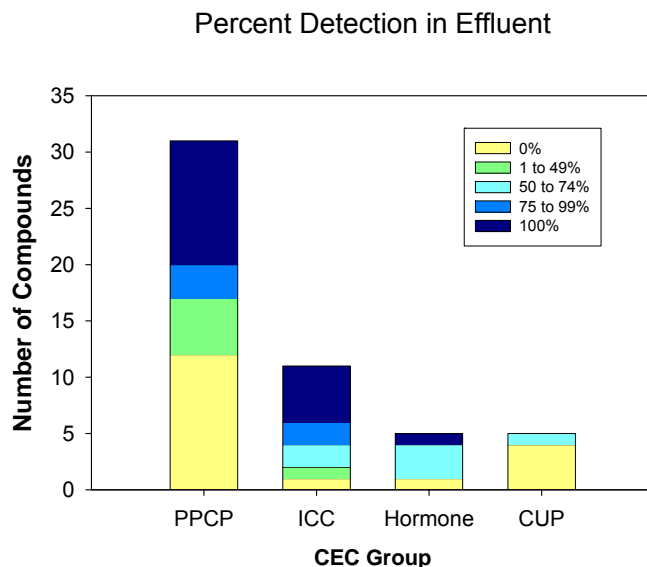
Figure 4. Detection frequency of CECs, by group, in quarterly samples of wastewater effluent. Colors represent different categories of percent detection ranging from 0% (never detected) to 100% (always detected). The height of the bar indicates the number of chemicals in each occurrence category for pharmaceuticals and personal care products (PPCP), industrial and commercial compounds (ICC), hormones and current use pesticides (CUP).

Effluent CEC concentrations were low, with values less than five parts per billion (µg/L). Median concentrations for detected compounds were lower than available toxicity thresholds (Fent et al. 2006). Concentrations of individual constituents were variable among effluent types in some cases, and showed no consistent trend between sampling times or effluent types. However, the total concentration of PPCPs varied significantly among effluent types (Figure 5), with the lowest concentrations in the effluents that

received full secondary treatment (LA, PV). This trend is consistent with the results of other studies that show greater removal of CECs with longer treatment plant residence times. Influent CEC concentrations were not measured in this study; trends in influent CEC concentrations could therefore reflect differences in waste input characteristics among geographic regions. Similar average concentrations among effluent types were measured for other compound groups such as hormones, ICCs, and CUPs.

Some of the compounds ubiquitously found in southern California effluent samples have also been found in effluent by multiple studies throughout the US (Glassmeyer et al. 2005). Examples include the analgesics ibuprofen and naproxen, the antibiotic sulfamethoxazole, the cholesterol inhibitor gemfibrozil, and the surfactant degradation product nonylphenol. Median concentrations for most of the detected CECs were similar to those reported for other US effluents (Glassmeyer et al. 2007). However, concentrations of gemfibrozil, naproxen, atenolol, ibuprofen and sulfamethoxazole in our study were higher than concentrations typically found in other studies (Drewes et al. 2002, Gross et al. 2004, Glassmeyer et al. 2005, Thomas and Foster 2005, Zuccato et al. 2005, Gagne et al. 2006, Glassmeyer et al. 2007, Palmer et al. 2008, Spongberg and Witter 2008).
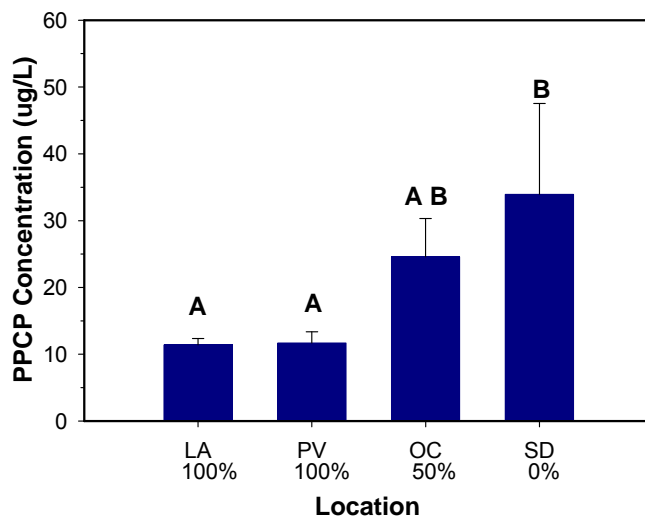
Figure 5. Average total concentration of pharmaceutical and personal care products (+ standard error) in quarterly wastewater effluent samples. The percentage of secondary treatment is shown for each effluent type at the time of the study. Bars with the same letter are not statistically different from each other.

Are SCB Marine Life Exposed to CECs from Municipal Wastewater Discharges?

The results indicate that fish are likely exposed to CECs from effluent discharges through multiple pathways. Some PPCP and ICC compounds were detected in seawater samples collected near the ocean floor at the fish sampling locations (Table 3). Only a small proportion of the target analytes were detected in seawater and the concentrations were 400-1,000 times lower than present in the effluent, which is consistent with the expected dilution of the effluent upon discharge. These seawater concentrations (usually less than one part per trillion or ng/L) were generally near the analytical detection limits for the compounds. ANOVA analysis of analytes detected in the seawater showed that there were no statistically significant differences ($p > 0.05$) between concentrations found in the reference area, and those found in the areas near the outfall discharges.

The concentrations found in this study were far below those expected to produce short-term toxic effects For example, the USEPA seawater aquatic life water toxicity threshold for nonylphenol is 1.7 µg/L (Brooke and Thursby 2005), and the maximum concentration found in the seawater samples was 0.23 µg/L. However, evaluation of potential chronic effects for CECs is uncertain because aquatic life toxicity thresholds have only been developed for a few of these compounds.

Table 3. Effluent and seawater median concentrations (µg/L) for chemicals detected in seawater samples. Seawater percent occurrence values also shown.

Chemical Group or Use	Chemical Name	Effluent Median	Seawater Median	Occurrence %
Beta-blocker	Atenolol	2.20	0.0004	90
Cholesterol Regulator	Gemfibrozil	3.25	0.0009	90
Analgesic	Naproxen	2.30	0.0007	75
Antibiotic	Sulfamethoxazole	0.92	0.0005	70
Antibiotic	Trimethoprim	0.62	0.0007	60
Antidepressant	Meprobamate	0.35	ND[1]	50
Analgesic	Diclofenac	0.13	ND	40
ICC	Butylated hydroxytoluene	0.29	ND	40
Antimicrobial	Triclosan	0.79	ND	40
ICC	Nonylphenol	1.42	ND	35
Analgesic	Ibuprofen	1.45	ND	30
Antiepileptic	Carbamazepine	0.27	ND	25
Cholesterol Regulator	Atorvastatin	0.11	ND	15
ICC	Benzophenone	0.42	ND	15
Hormone	Estrone	0.04	ND	10
ICC	Octylphenol	0.69	ND	10
ICC	TCPP	1.10	ND	10

[1] Median was not calculated because of low frequency of detection.

Sediment contamination is a likely pathway of fish exposure to some CECs. Sediment samples from all locations, including the DP reference site, contained triclosan (antimicrobial), and the LA, PV and OC sediments contained PBDEs (flame retardants) and nonylphenol (surfactant). Livers of hornyhead turbot from all the sites also contained PBDEs and nonylphenol (Figure 6). Some chemicals that were not detected in the sediment were found in the livers of fish at the SD and DP stations (e.g., PCBs). This finding highlights the fact that even if a contaminant is not found at detectable levels in sediment or seawater, the contaminant may still be present in the environment and able to be accumulated by organisms in that area. Only a partial suite of PPCPs were analyzed in the liver and sediment samples, so no conclusion can be made regarding the accumulation of other PPCPs in fish.
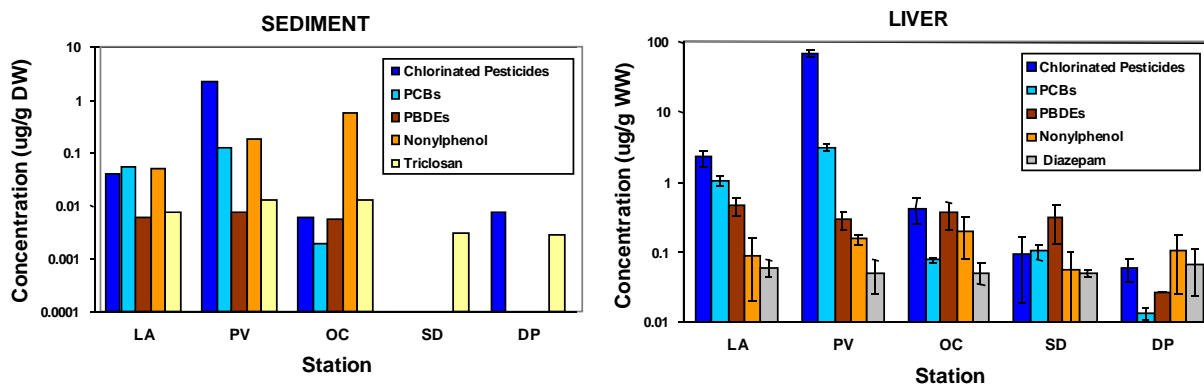
Figure 6. Concentrations of selected legacy contaminants and CECs in sediment and liver tissue. Chlorinated pesticides= sum of aldrin, chlordane, DDD, DDE, DDT, DDMU, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, methoxychlor, oxychlordane, nonachlor, and toxaphene; PCBs= sum of 28 PCB congeners; PBDEs= sum of five polybrominated diphenyl ether congeners. Diazepam was not detected in sediment samples.

The sediment and tissue data also illustrate that hornyhead turbot were exposed to multiple legacy contaminants at concentrations that ranged over 200-fold (e.g., PCB and chlorinated pesticides). This pattern of exposure is the result of historical discharges that produced widespread contamination in the SCB. Since chlorinated pesticides and PCB compounds have the potential to cause endocrine disruption and other effects often associated with some CECs, these legacy contaminants must be considered when evaluating the biological effects of CECs in the SCB. Sediment and tissue levels of legacy contaminants for the DP site were consistently among the lowest concentrations measured, indicating the suitability of this station as a low contamination reference in this study.

Is There Evidence of Endocrine Disruption or Other Physiological Effects in SCB Fish?

This study detected several molecular-level responses associated with physiological changes in hornyhead turbot. The relationship of these changes to contaminant exposure and endocrine disruption cannot be established without further study. However, no adverse impacts on fish condition or reproduction were found.

Hormones and Vitellogenin

Variations in some molecular indicators in blood plasma that were potentially abnormal were observed. Yet, in most cases these responses were found at all sites, (including the reference area) and could not be directly associated with the effluent discharges. These widespread responses included the frequent detection of low levels of vitellogenin (VTG) in male fish (Figure 7), and high concentrations of estradiol in male fish relative to females (Figure 8).
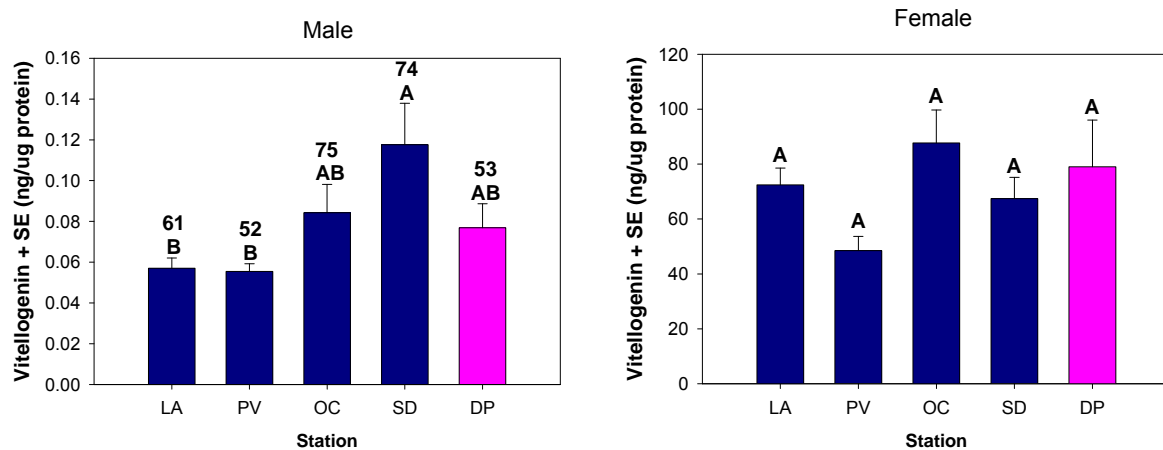
Figure 7. Average vitellogenin concentrations in male and female hornyhead turbot. Bars with same letter are not statistically different from each other. The numbers on top of the bars indicate the percent of males with detectable VTG. Samples collected in May-June 2006.

More than half of the male hornyhead turbot sampled contained detectable concentrations of VTG in plasma. Male VTG concentrations were generally 1,000-fold lower than females and were unlikely to disrupt reproduction, but they may be indicative of exposure of the fish to estrogens in the environment. Male fish from the SD site had significantly higher concentrations of VTG relative to PV and LA fish, while no differences in female VTG were present. Male DP hornyhead turbot also contained similar concentrations of VTG, suggesting that this response was not associated with current outfall discharges.

Unexpectedly high concentrations of plasma estradiol in male hornyhead turbot were observed at all study sites. Estradiol concentration in males is lower than that in females for most fish species; yet male hornyhead turbot estradiol concentrations were similar or greater than those of females (Figure 8). Measurements of estradiol in other species of southern California flatfish do not show this unusual pattern. Elevated estradiol in males did not appear to be associated with outfall discharges, as a similar pattern was detected at the DP reference site. Studies of other flatfish species have shown a similar pattern of relative estradiol concentration (Scott et al. 2007). This pattern may represent either a widespread response to environmental factors or be a normal characteristic of the species.

Statistically significant differences in estradiol concentrations among stations were observed. Estradiol concentrations in fish (either males or females) from LA, PV, and OC were approximately half of those in fish from SD and DP (Figure 8). This trend may represent a response to historical outfall discharges, as fish from LA, PV, and OC also have higher concentrations of legacy chlorinated hydrocarbon contaminants in their tissues that can have antiestrogenic effects. These differences could also reflect varying sexual maturity states among the fish at the time of sampling.

Figure 8. Average estradiol concentrations for male and female hornyhead turbot. Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

No consistent pattern in the concentration of 11-KT, the principal form of testosterone in fishes, was present among the outfall sites (Figure 9). This hormone regulates reproduction and growth in fish. As expected, males had significantly higher concentrations of the androgen as compared with females. While the concentration of 11-KT varied approximately three-fold among stations in both sexes, a significant difference was only present in PV females. The observed variation in 11-KT concentrations may represent differences in reproductive stages of the fish among sites. It is unlikely that legacy contamination is responsible for the variations in 11-KT since the hormone concentrations do not correspond to trends in fish tissue chlorinated hydrocarbon exposure among the sites.
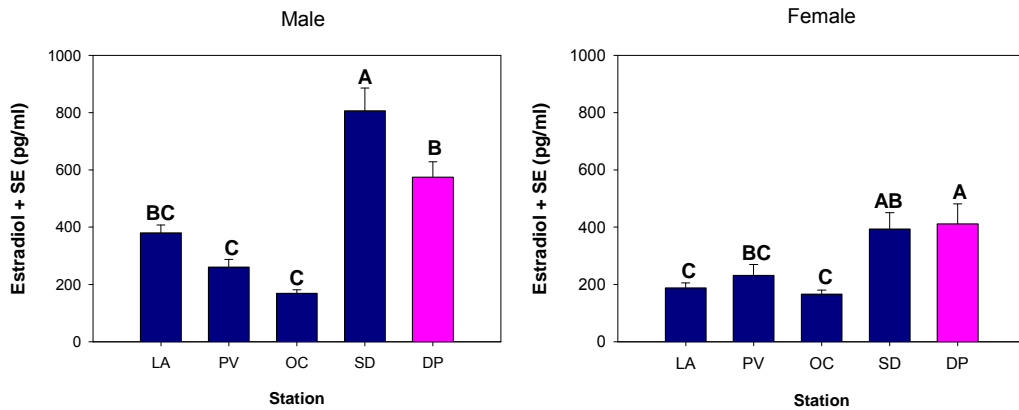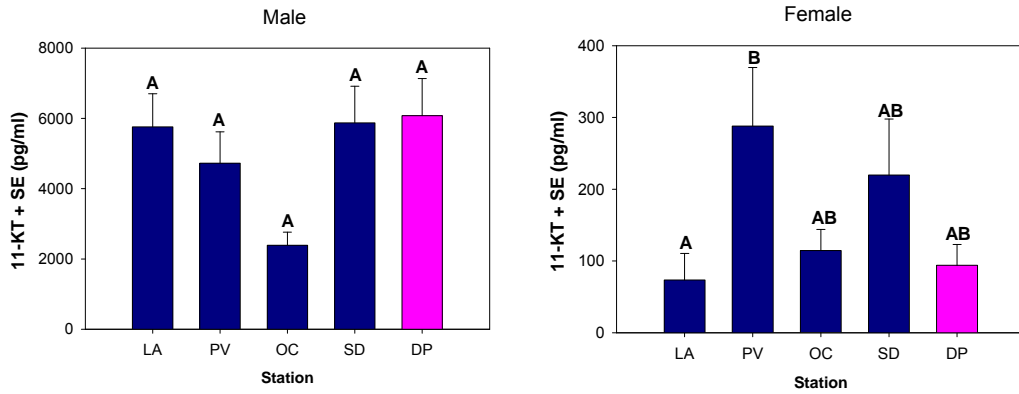

Figure 9. Average 11-keto testosterone concentrations for male and female hornyhead turbot. Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

Evidence of a region-wide inhibition in the stress response system of hornyhead turbot was observed in this study. The hormone cortisol is normally produced in response to stress, such as that resulting from the fish capture and handling methods used in this study. Cortisol concentrations in hornyhead turbot from all sites were less than half the concentration observed in other fish species subjected to a similar degree of stress (Figure 10). These results may be indicative of chronic stress, which is known to diminish the ability of organisms to produce cortisol in response to stress, or could be due to contaminant impacts on the cortisol-producing endocrine tissue (interrenal) as reported in the scientific literature (Kubokawa et al. 1999, Evrard et al. 2010).

The average concentration of thyroxine (a thyroid hormone) was reduced in fish from each of the outfall sites relative to the reference site (Figure 10), particularly in the PV site where the greatest legacy contamination exists. Thyroid hormones have important roles in regulating growth, early development, and metabolism. Reduced levels of thyroid hormones could lead to impairment of physiological functions essential to the well-being and survival of the organism. This is the first report of thyroxine concentrations in hornyhead turbot; it is unknown whether this pattern is present at other times of the year or in other southern California species. Recent studies in San Francisco Bay have also observed reduced thyroxine levels in two indigenous fish species from locations with increased contaminant exposure (Brar et al. 2010).
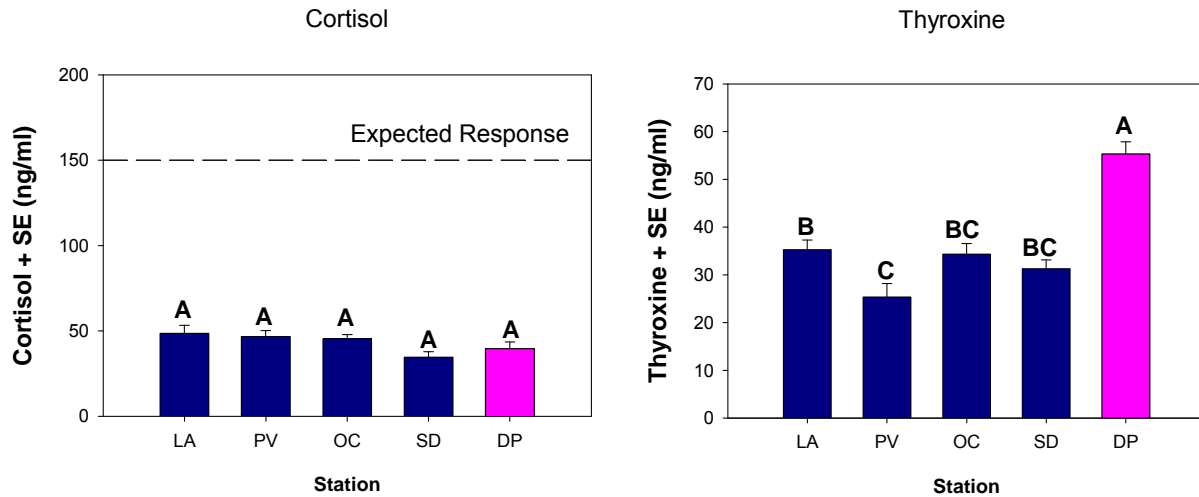
Figure 10. Cortisol and thyroxine average concentrations for hornyhead turbot (combined data for males and females). Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

Gonad Histopathology and Feminization

No evidence of feminization or abnormal sexual differentiation was observed in this study. Histological analysis of the gonads found no instances of feminization (e.g., presence of developing eggs in male gonad) out of 373 male fish examined in both the spatial and temporal components of this study.

Atresia (oocyte degeration) was observed in females from all stations. The incidence of atresia was low (20% or less) at all sites and did not show any apparent relationship with effluent discharge or legacy contamination. The presence and severity of the atresia condition seemed to correspond to the fish reproductive stage.

Wide variations in male to female sex ratios were observed, but there was no consistent trend or significant difference related to site or time of year. The variations in sex ratios were likely due to normal factors such as sampling variability and sex-specific aggregation behavior.

Reproductive Cycle

Analysis of quarterly fish collections at selected sites were used to compare temporal changes in hormones and gonad condition as an indicator of subtle effects on reproduction among sites. Female fish tended to be sexually mature (larger gonads) during May to August sampling periods, as indicated by high values for the gonad somatic index (1/2 GSI, Figure 11). This trend was confirmed by histological evaluation of the maturity state of developing eggs in the gonad (Figure 11). The reproductive cycle of males was similar to the females, in general. Females from the OCF (farfield site for OC discharge area) did not exhibit this general reproductive cycle. There was little variation in the GSI or gonad maturity of OCF females throughout the year.
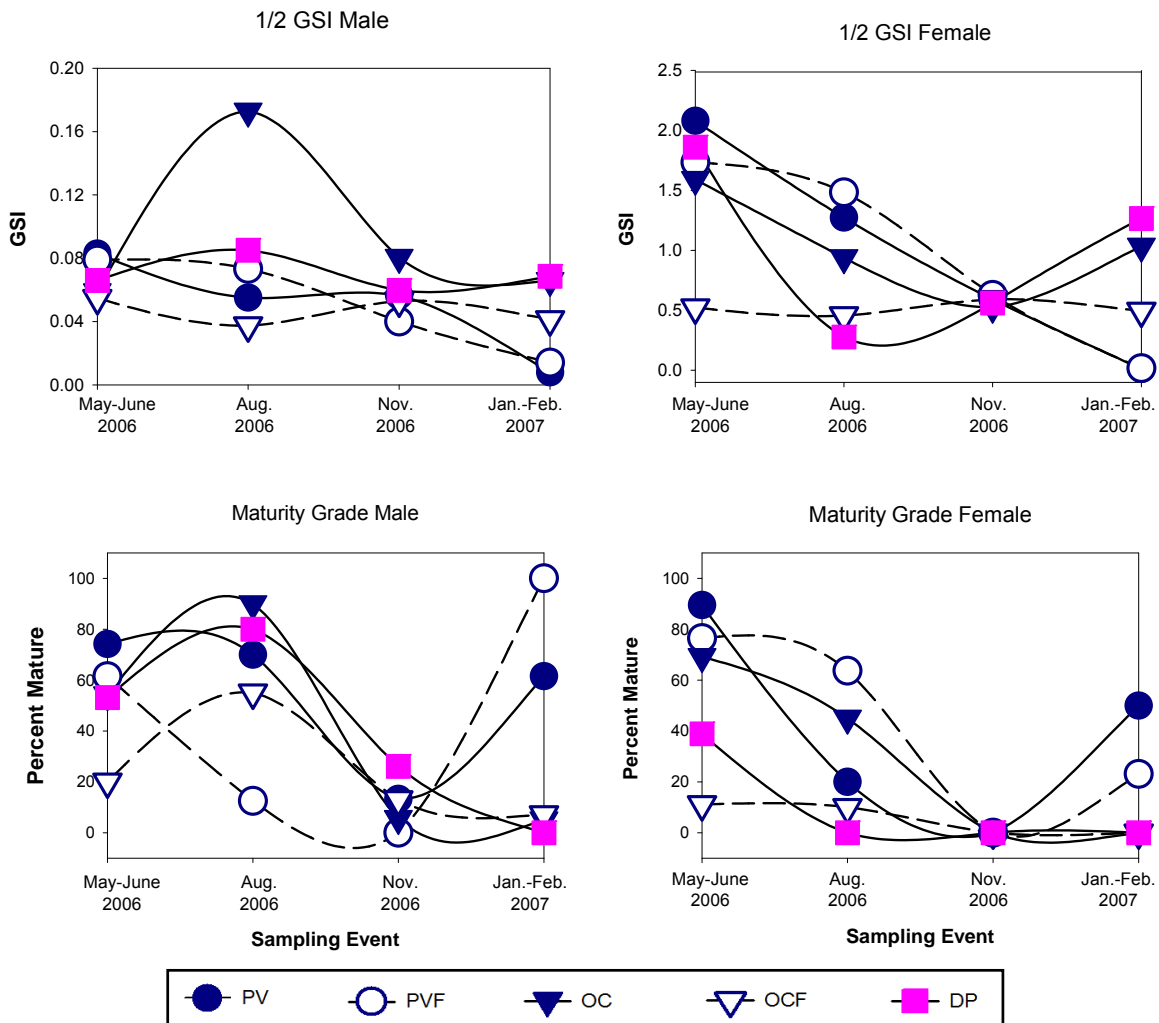
Figure 11. Temporal trends in gonad somatic index (1/2 GSI) and the percentage of mature males and females.

17

Male and female plasma vitellogenin concentrations also varied temporally (Figure 12). VTG variation generally corresponded to variations in GSI and maturity state, especially in females. Little temporal variation in female VTG concentrations was observed at OCF, a finding consistent the GSI and maturity state data, and perhaps evidence of an impaired reproductive cycle at this station.
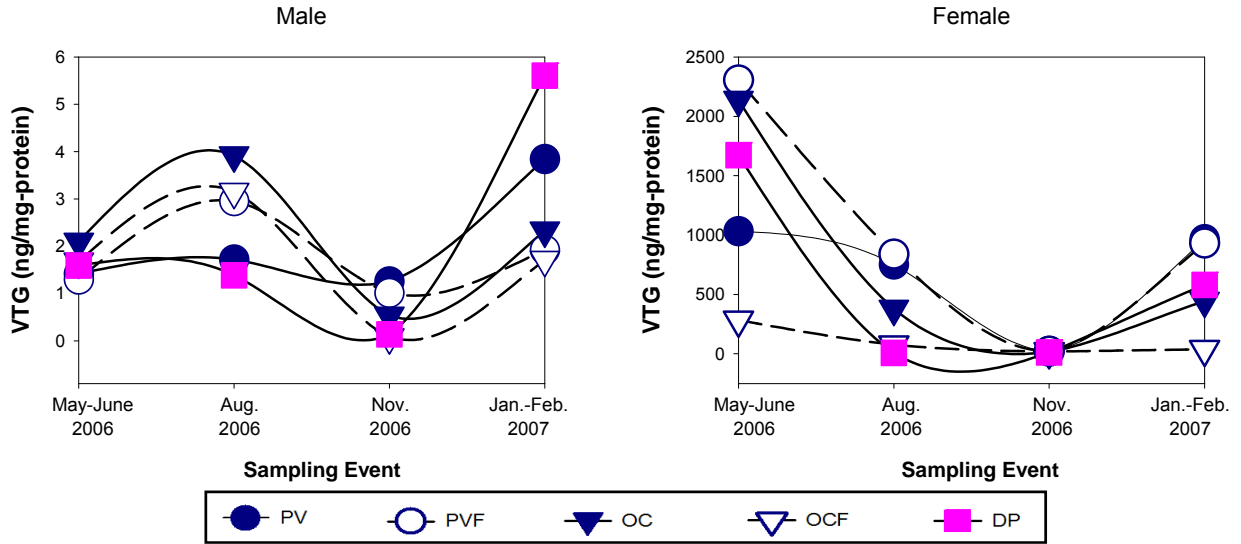
Figure 12. Temporal trends in vitellogenin for male and female hornyhead turbot.

In males, variation in 11-KT concentrations corresponded to the other measures of the male reproductive cycle (e.g., GSI, maturity state), with elevated levels in May-June at all sites. In females, the androgen was present at very low levels and did not correspond with other measures of the female reproductive cycle (as expected for females). In contrast to 11-KT, the concentrations of estradiol showed little similarity to other measures of the reproductive cycle. Estradiol was high in both sexes, at levels expected in reproductively active females, and it did not show consistent seasonal differences. These results cannot be associated with exposure of hornyhead turbot to contaminants, as they were evident at both discharge and reference sites. The duration and sampling frequency for the assessment of reproduction cycles was limited (e.g., one year and four sampling events), and may not have been sufficient to detect subtle changes in reproductive cycles between sites.
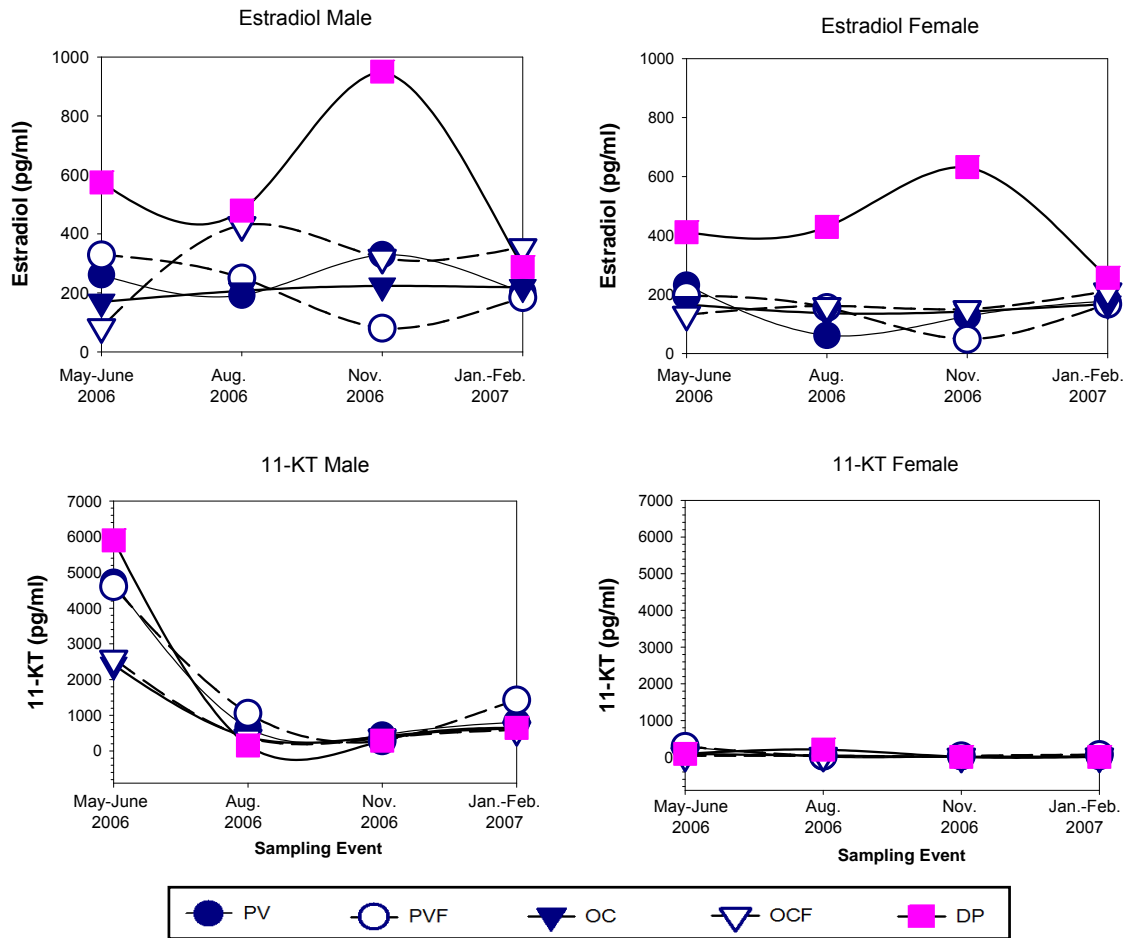
Figure 13. Temporal trends in estradiol and 11-keto testosterone (11-KT) in male and female hornyhead turbot.

Fish Condition

Overall measures of fish condition, the condition factor (CF) and liver somatic index (LSI), generally corresponded with the reproductive cycle of hornyhead turbot. Variations among sites in specific parameters were observed, however. Fish from OC, OCF, and DP (males) had the highest CF during the period of higher reproductive activity, while PV and PVF showed different trends (Figure 14). Relative liver size (LSI) varied among sites, with the highest LSI values in PV fish at all time periods. Elevated LSI values at PV may be associated with increased exposure to chlorinated hydrocarbons, which has been associated with liver enlargement in fish (Gunawickrama et al. 2008).
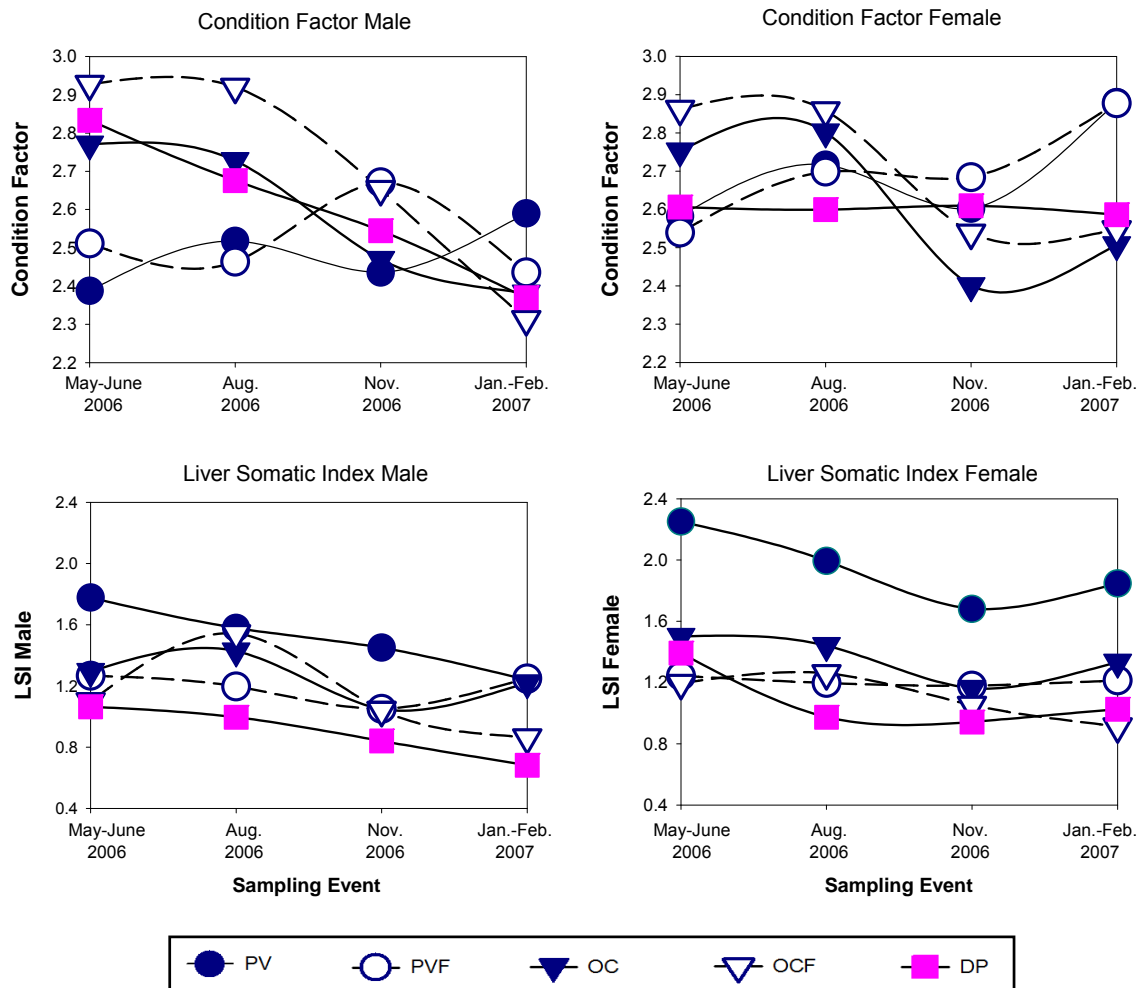
Figure 14. Temporal trends for condition factor and liver somatic index (LSI) in hornyhead turbot.

Gene Expression

The microarray analysis results indicated that gene expression in male hornyhead turbot from the outfall areas was generally similar to that of the reference area fish (Figure 15). The fold induction value for most of the 39 genes was less than 1.6 ($\log_2$ basis), relative to the fish from DP (DP03) used as a benchmark. Differential expression was observed for several genes, such as cytochrome P450 1A and glutathione S-transferase. Substantial variation in gene expression between the two individuals analyzed from each station was present. In some cases, such as for the vitellogenin genes, variation between the two DP fish was greater than that observed between stations. These results are only exploratory because of the small number of samples analyzed, and may only represent the condition of the fish used in this analysis rather than the population at a given area.
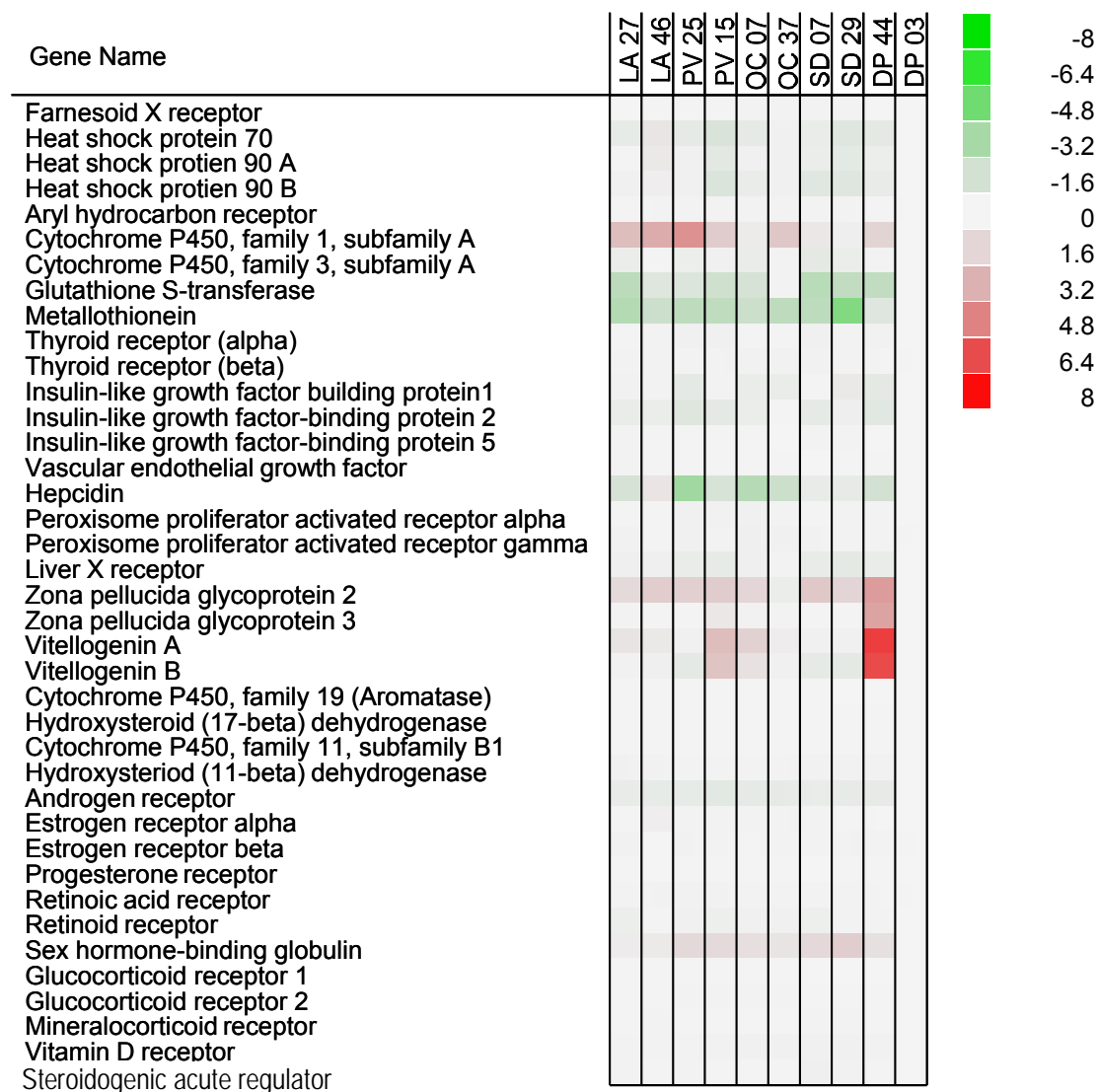
Figure 15. Microarray analysis of gene expression in male hornyhead turbot. The values shown represent relative expression log2 ratios. Red shading indicates the relative degree of upregulation when compared to a reference fish (DP 03). Green shading indicates down regulation. The numbers in the legend represent log2 ratios which correspond to each color.

Table 4. Functions of genes included on the hornyhead turbot microarray. Some genes may be associated with additional functions not listed in this table.

Function	Gene Name
Bile biosynthesis/ transcription factor	Farnesoid X receptor
Cellular stress response	Heat shock protein 70
Cellular stress response	Heat shock protein 90 A
Cellular stress response	Heat shock protein 90 B
Contaminant response/ Detoxification	Aryl hydrocarbon receptor
Contaminant response/ Detoxification	Cytochrome P450, family 1, subfamily A
Contaminant response/ Detoxification	Cytochrome P450, family 3, subfamily A
Contaminant response/ Detoxification	Glutathione S-transferase
Contaminant response/ Detoxification	Metallothionein
Development/ mediates thyroid activities	Thyroid receptor (alpha)
Development/ mediates thyroid activities	Thyroid receptor (beta)
Development/Growth	Insulin-like growth factor building protein1
Development/Growth	Insulin-like growth factor-binding protein 2
Development/Growth	Insulin-like growth factor-binding protein 5
Development/Growth	Vascular endothelial growth factor
Infection response	Hepcidin
Lipid metabolism/ development/ transcription factor	Peroxisome proliferator activated receptor alpha
Lipid metabolism/ development/ transcription factor	Peroxisome proliferator activated receptor gamma
Lipid metabolism/ transcription factor	Liver X receptor
Reproduction/ egg: sperm binding	Zona pellucida glycoprotein 2
Reproduction/ egg: sperm binding	Zona pellucida glycoprotein 3
Reproduction/ egg: yolk protein	Vitellogenin A
Reproduction/ egg: yolk protein	Vitellogenin B
Reproduction/ Steroidogenesis	Cytochrome P450, family 19 (Aromatase)
Reproduction/ Steroidogenesis	Hydroxysteroid (17-beta) dehydrogenase type 1
Reproduction/ Steroidogenesis/ stress	Hydroxysteriod (11-beta) dehydrogenase type 2
Reproduction/ Steroidogenesis/ stress	Cytochrome P450, family 11, subfamily B1
Reproduction/ transcription factor	Androgen receptor
Reproduction/ transcription factor	Estrogen receptor alpha
Reproduction/ transcription factor	Estrogen receptor beta
Reproduction/ transcription factor	Progesterone receptor
Retinoid hormone transcription factor	Retinoic acid receptor
Retinoid hormone transcription factor	Retinoid receptor
Sex steroid hormone binding	Sex hormone-binding globulin
Steroid hormone transcription factor	Glucocorticoid receptor 1
Steroid hormone transcription factor	Glucocorticoid receptor 2
Steroid hormone transcription factor	Mineralocorticoid receptor
Steroid hormone transcription factor	Vitamin D receptor
Steroidogenesis/ other endocrine tissues	Steroidogenesis acute regulator

Most of the genes with the greatest variation in differential expression have functions that are associated with responses to contaminant transport, detoxification, infection response, and reproduction (Table 4). For example, increased expression of the cytochrome p450 1A gene was measured in many fish; production of this enzyme may be induced by exposure to PAHs and PCBs. We also observed differential gene expression in genes for two other proteins involved in contaminant detoxification and regulation, glutathione-S-transferase and metallothionein. These two genes were downregulated in many fish irrespective of site. Increased expression of two gene types associated with egg development and response to estrogens, vitellogenin and zona pellucida glycoprotein, was also observed in some fish from all sites.

The gene microarray results demonstrate the feasibility of using this technology to examine patterns of gene expression in monitoring species such as hornyhead turbot. This technique is useful for investigating patterns of response in multiple genes and for identifying genes for additional study. The results also showed that individuals from the same site can have very different gene expression levels, indicating that many more than two replicates per site are needed to determine whether trends among sites are present.

Quantitative measurements of hornyhead turbot gene expression were made using qPCR methods. Liver and gonad from 10 fish per station were analyzed and compared to a subset of fish from DP in order to calculate differential expression values. There were no statistically significant differences among sites in liver gene expression (Figure 16). The average gene expression for the DP fish was usually similar to that found in fish from other sites. Wide variation in expression of the gene for cytochrome P450 1A was observed among sites, with higher expression at LA and PV, areas where legacy contaminants were present at higher concentrations in sediment and fish liver (Figure 6). Like the microarray results, the qPCR data represent an exploratory analysis that may not be representative of all the fish collected at the corresponding site.
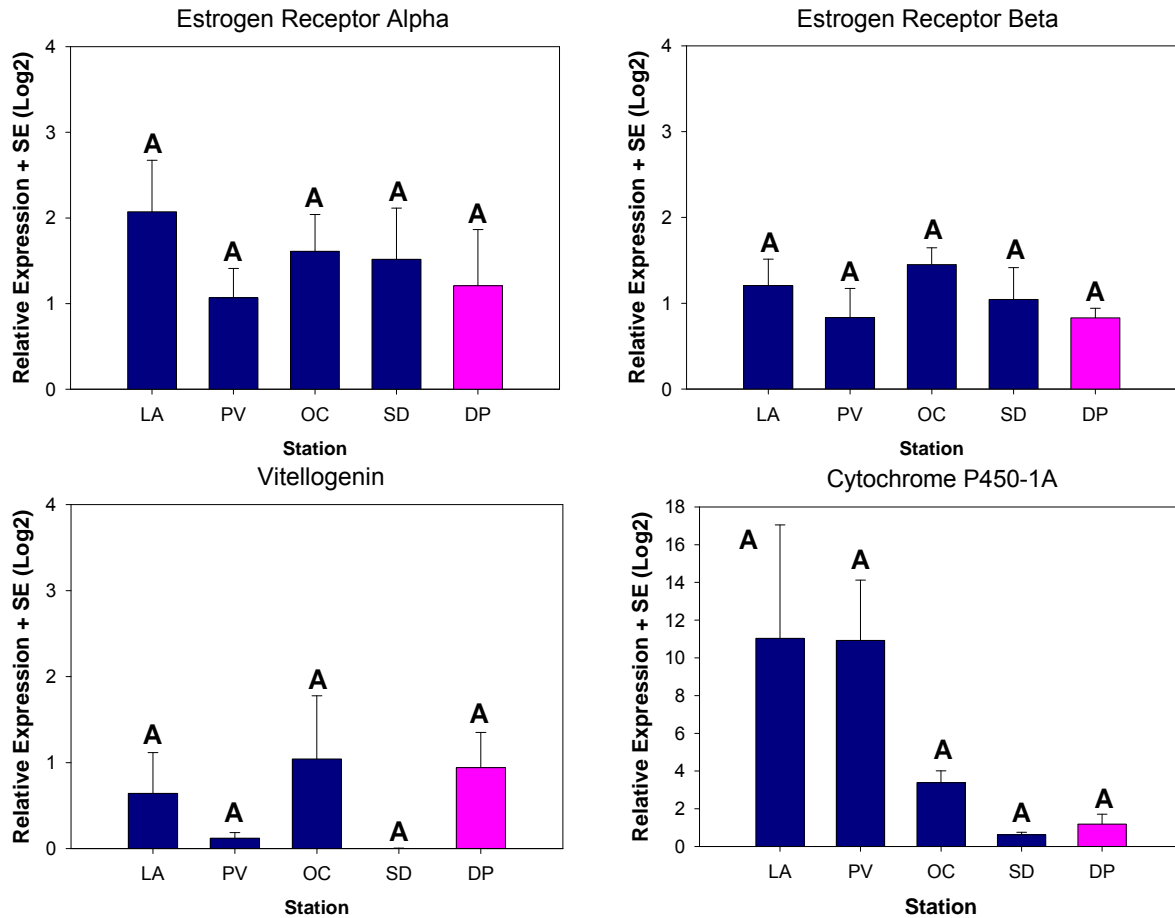
Figure 16. Differential expression (from qPCR analysis) of male hornyhead turbot liver genes. Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

Analysis of gonad tissue detected differences in relative expression among sites for three genes (Figure 17). Significant differences (p <0.05) in the expression of hydroxysteroid 11 beta dehydrogenase-2 (11-HSD-2), hydroxysteroid 17 beta dehydrogenase-1 (17-HSD-1), steroidogenic and acute regulator (StAR) were observed; in each case greater expression was observed in SD fish. Expression of aromatase (cythochrome p450, family 19) also showed a trend (not statistically significant) of increased expression in SD fish. Two fish from SD had aromatase expression levels that were orders of magnitude higher than the other fish from this station, which resulted in the high average expression level and variation for this station. Values for other SD fish were similar to those from the other sites. The consistency of pattern in gonad gene expression, with greater values at SD, suggests a difference in steroid hormone regulation or production at this site. Males from SD also contained elevated plasma estradiol concentrations relative to the other sites. However, insufficient data are available to determine whether these spatial differences reflect the influence of environmental factors or represent fish in a different phase of their reproductive cycle.
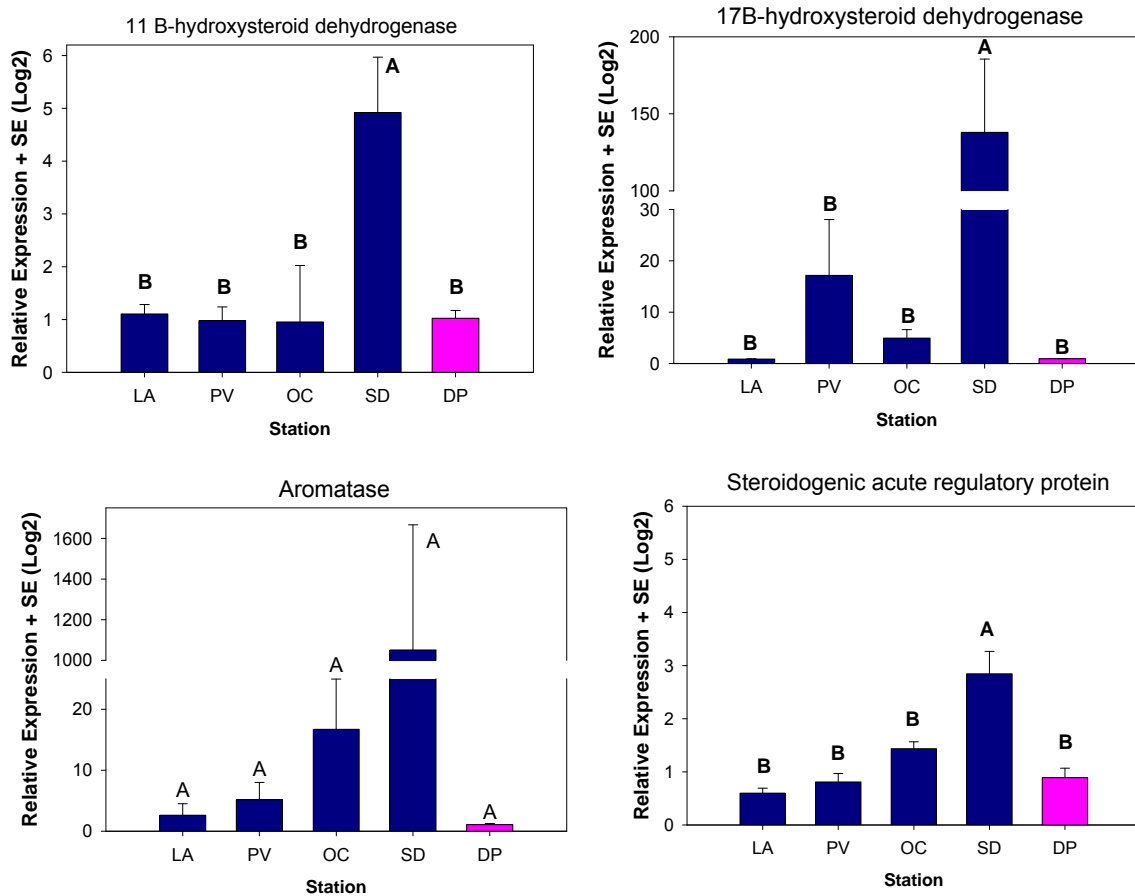
Figure 17. Differential expression (qPCR) of male hornyhead turbot gonad genes. Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

Are These Effects Associated with Either Historical or Current Municipal Wastewater Discharges?

The association of the molecular responses observed in this study with municipal wastewater discharge is uncertain for most parameters. Biological responses such as reduced cortisol response, VTG production in males, and high plasma estradiol in males were similar among the discharge and reference sites, indicating little relationship to the presence of effluent discharge or historical sediment contamination patterns. If these responses are due to chemical exposure, then this exposure must be widely distributed throughout the southern California Bight ecosystem and may have multiple sources. Bight-wide chemical exposure at low levels does occur, as shown by sediment and tissue analyses (Figure 6). An alternative explanation for the widespread cortisol, VTG, and estradiol responses is that they represent normal, but unusual, characteristics of hornyhead turbot. Additional analyses of hornyhead turbot from reference areas and laboratory studies are needed to determine the normal range of variation for the molecular indicators used in this study.

Two molecular indicators did show an apparent association with multiple municipal wastewater discharge sites: thyroid hormone (thyroxine) and estradiol. Hornyhead turbot thyroxine concentrations in plasma at all four discharge sites were less than in fish from DP. Fish thyroxine production is known to be reduced as a result of exposure to several types of contaminants that are more prevalent near outfall sites, such as PCBs and PBDEs. The thyroxine results need confirmation, as the results are based on a single collection event in May-June 2006. It is not known whether this reduction at outfall discharge sites persists over time or occurs at other locations. Reduced plasma estradiol concentrations were observed in fish from those sites with the highest concentrations of contaminants in the sediment: LA, PV and OC were also observed. Quarterly samples for LA and OC confirmed the trend for estradiol (Figure 13), suggesting this response may be related to contaminant exposure. Legacy contamination is a potential cause of the estradiol response, since the reduced concentrations were only present at discharge sites with substantial legacy contamination (LA, PV, OC), and not present at SD (lower legacy contamination).

Differences in male plasma estradiol concentrations and the expression of gonad genes involved with steroid hormone synthesis were present at only the SD outfall site. The association of these responses with municipal wastewater discharge is uncertain because similar responses were not observed at other discharge sites and there were no repeated measurements over time at SD. Additional samples of SD fish need to be analyzed to determine whether the plasma estradiol and gene expression results represent a site-specific response, as opposed to normal variations in the physiology of hornyhead turbot.

Are Specific Chemicals Responsible for the Effects?

No specific associations between individual chemicals and biological effects can be determined from this study. The responses observed for estradiol, cortisol, and thyroxine are not diagnostic for a single chemical type. The ability to evaluate chemical-specific associations in this study was limited because tissue chemical analyses were conducted on composites rather than on individual fish. Without chemical data on individuals, a robust statistical evaluation of possible cause-effect relationships cannot be conducted. Statistical associations with chemicals also need to be confirmed by controlled laboratory exposure studies as the statistical associations may be due to correlations with unmeasured chemicals or environmental factors. The mixture of exposure from legacy and current discharge also complicates determination of chemical linkages. Similar impacts on hormone concentration and gene expression can be caused by both legacy contaminants (e.g., DDTs, PCBs) and CECs (e.g., PBDEs, pharmaceuticals).

The important role of legacy contaminants in some of these responses is suggested by the plasma estradiol and the liver P450 1A gene expression results. These two molecular indicators showed patterns of response associated with the LA, PV, and OC sites, where legacy sediment contamination is greatest. The production of P450 1A is often increased in fish exposed to PCBs and petroleum hydrocarbons (Katchamart et al. 2002, Hansson et al. 2006). Sediment at the SD site has little legacy sediment contamination.

Are the Biological Effects Adversely Impacting Fish Populations?

The biological responses observed in this study did not appear to be associated with reduced hornyhead turbot reproduction or survival. The gender ratio (relative proportion of male and female fish) of hornyhead turbot varied among sampling events and sites, but did not show a consistent trend indicative of altered sexual differentiation. In addition, no feminization of male fish was observed.

Lack of evidence for fish feminization is consistent with the results from the Bight 2003 regional survey. This survey examined 41 hornyhead turbot males from Santa Barbara to Orange County and initially found a seemingly high incidence (6%) of intersex (presence of eggs in male gonad). However, upon subsequent reanalysis of the tissue samples, all but two of the suspected intersex fish were the result of contaminated tissue samples.

Analysis of long-term monitoring data for the study sites indicated that hornyhead turbot populations are stable or increasing throughout the region (Figure 18). Statistically significant differences in the average abundance of hornyhead turbot were present at each of the discharge sites, but in all cases the abundance in the 2000's was greater than in previous decades. Analysis of long-term monitoring data from PV and OC shows annual variability in hornyhead turbot abundance. This variation appears to be related to variations in ocean temperature, with greater relative abundance of hornyhead turbot when coastal water temperature is lower (M.J. Allen, personal communication).
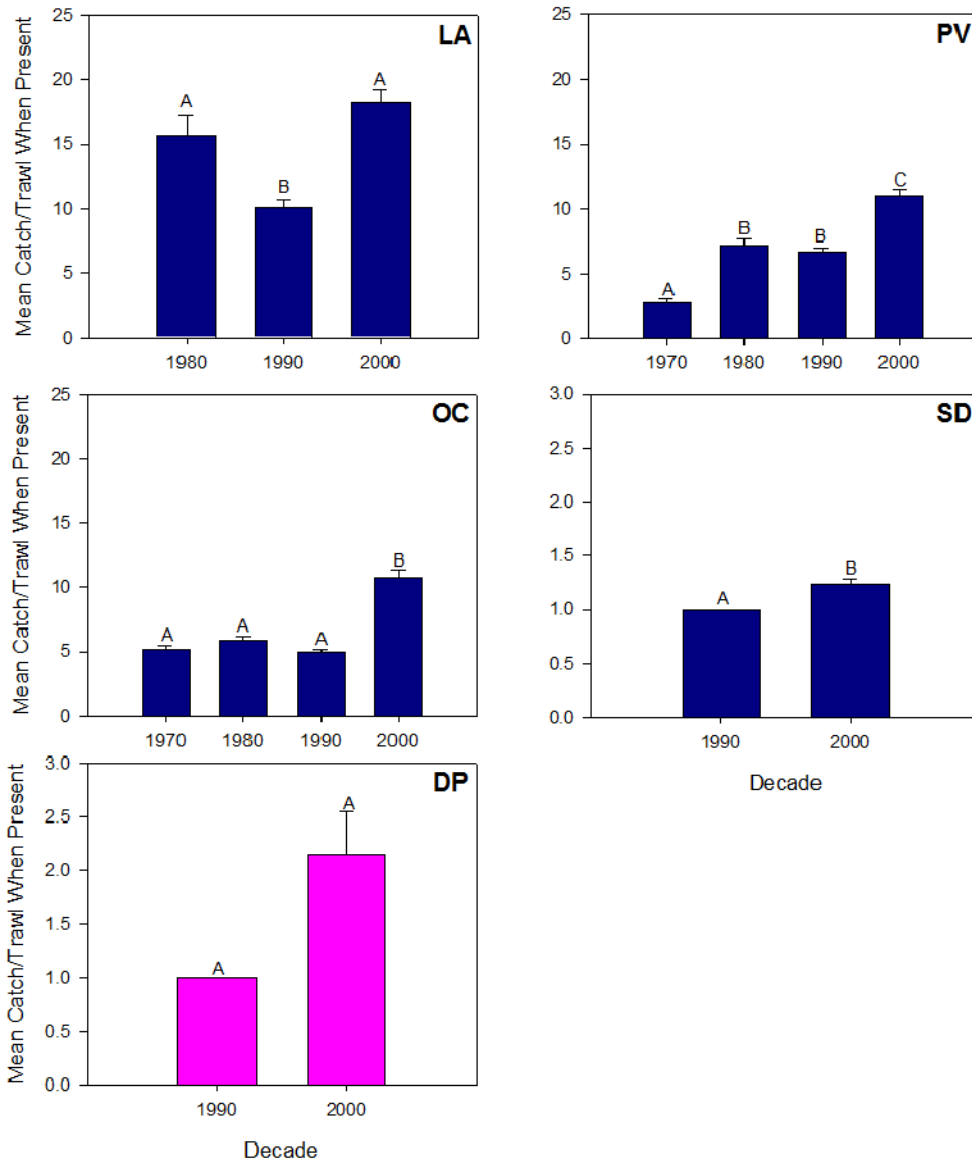
Figure 18. Average (+SE) abundance of hornyhead turbot over time. Data compiled from monitoring surveys, note difference in scales among plots.

Fish communities were also healthy at the study sites. The species composition and abundance of demersal (bottom-associated) fish measured in recent monitoring surveys was typical of that expected in unimpacted reference areas of the SCB (Figure 19). These results are consistent with Bight 2008 regional monitoring data, which indicate that the condition of offshore fish communities throughout the SCB is equivalent to that of reference areas.
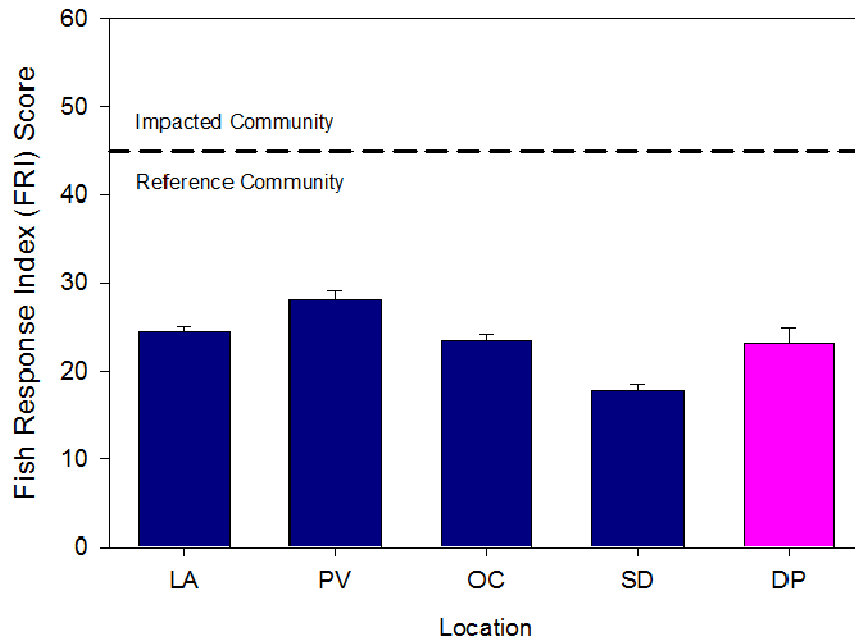
Figure 19. Fish community condition at the study sites, as indicated by the Fish Response Index (average + SE). Data compiled from 2003-2009 monitoring surveys.

SUMMARY AND RECOMMENDATIONS

This study represents the most comprehensive investigation to date of CECs and their effects in coastal offshore waters. Much has been learned regarding the occurrence, fate, and exposure of CECs in the SCB. This study has helped develop promising new assessment methods based on gene expression measurements and applied other molecular techniques on a region-wide scale. Such progress could not have been accomplished without the collaboration of local water quality management agencies and universities, and the existence of adaptive NPDES monitoring programs in the region that provide the flexibility to conduct special studies.

Several physiological changes have been detected that may be indicative of exposure to contaminants or endocrine disrupting compounds. However, more detailed chemical and molecular studies are needed before it can be concluded that endocrine disruption is occurring in hornyhead turbot. The biological significance of these changes appears to be low, as no clear impacts on reproductive condition or abundance of hornyhead turbot were observed. Substantial data gaps still remain that limit our ability to draw definitive conclusions regarding the impact of CECs in the southern California coastal waters and the need for management actions. The following types of research are needed to address these data gaps and improve the ability of water quality agencies to monitor and reduce the ecological risk associated with CECs:

Investigate additional species and habitats, especially those with the highest potential for exposure
Most of our knowledge regarding CEC exposure and effects is restricted to offshore coastal habitats and two species of flatfish. Similar studies are needed for other habitats (e.g., estuaries and rivers), types of contaminant discharges (e.g., nonpoint sources), and species to provide better context to determine constituents, areas, and responses of greatest concern. Particular emphasis should be placed on effluent-dominated water bodies.

Determine baseline physiological conditions for species and indicators used in monitoring
It is uncertain whether the molecular responses observed in this study represent contaminant exposure responses or normal physiological characteristics of hornyhead turbot. Additional studies of physiological parameters in fish from additional reference sites and over time is needed in order to establish normal baseline conditions.

Improve linkage between biology and chemistry data
Analyses of a wider range of CECs in sediments and tissues are needed to assess the fate and exposure of fish to PPCPs. Chemical analysis of individual fish is also needed to help establish whether the biological responses are related to CECs, legacy contaminants, or other factors.

Develop and refine molecular assessment tools
The measurements of hormones and histology used in the study provided sensitive and relevant measures of response for specific physiological systems. However, these measures may fail to detect impacts on other important processes (e.g., immune system) and provide little information regarding the mechanism or cause. Further development and refinement of molecular methods such as gene expression analysis for use in environmental studies is needed to provide a more comprehensive assessment of exposure and biological response. The preliminary application of gene microarray and qPCR techniques in this study demonstrates that such measurements are feasible and are likely to provide a more complete assessment of physiological response to environmental conditions. These molecular methods should also be applied in laboratory studies with specific contaminants of interest, thereby developing profiles of contaminant response that can be used to better assess the ecological risk of CEC exposure in receiving waters and possibly the cause of physiological changes.

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APPENDIX

Table A-1. Effluent median values for target analytes, reported as µg/ L, for each of the study sites. RL: reporting limit.

Analyte	RL	LA	PV	OC	SD
Current use pesticides					
alpha-Lindane	0.01	ND	ND	ND	ND
Atrazine	0.0003	ND	0.02	0.004	ND
beta-Lindane	0.01	ND	ND	ND	ND
delta-Lindane	0.01	ND	ND	ND	ND
Diazinon	0.01	ND	ND	ND	ND
Lindane	0.01	ND	ND	ND	ND
Methoxychlor	0.01	ND	ND	ND	ND
Metolachlor	0.01	ND	ND	ND	ND
Hormones					
Estradiol	0.0005	ND	0.01	0.004	0.002
Estrone	0.0002	0.05	0.03	0.05	0.02
Ethinylestradiol	0.001	ND	ND	ND	ND
Progesterone	0.001	0.001	ND	0.02	0.04
Testosterone	0.001	ND	0.01	0.02	0.08
Industrial/commercial compounds					
Benzophenone	0.025	0.1	0.09	1.03	0.82
Bisphenol A	0.05	0.33	1.3	0.5	0.32
Butylated hydroxytoluene	0.025	0.35	0.29	0.17	0.31
Butylbenzyl phthalate	0.05	ND	ND	0.58	0.96
Dioctyl phthalate	0.05	0.09	0.05	0.05	ND
Hydroxyanisole	0.025	0.15	0.1	0.15	0.2
N,N-diethyl-meta-toluamide	0.025	0.55	0.59	0.54	0.89
Nonylphenol	0.08	2.89	1.34	7.1	2.76
Octachlorostyrene	0.01	ND	ND	ND	ND
Octylphenol	0.025	0.29	0.41	1.24	1.03
TCEP	0.05	0.53	0.6	0.58	0.98
TCPP	0.05	0.85	2.01	0.69	1.14
Pharmaceuticals and personal care products					
Acetaminophen*	0.05	ND	ND	2.5	3.4
Atenolol	0.003	2.23	2.08	1.58	2.85
Atorvastatin	0.003	0.12	0.09	0.1	0.1
Carbamazepine	0.005	0.27	0.3	0.3	0.26
Diazepam	0.0003	0.004	0.005	0.002	0.01
Diclofenac	0.003	0.15	0.13	0.1	0.14
Dilantin	0.01	0.22	0.31	0.23	0.21
Enalapril	0.0003	0.01	ND	0.02	0.02
Erythromycin*	0.001	0.1	0.08	0.19	0.11

Table A-1. Continued.

Analyte	RL	LA	PV	OC	SD
Pharmaceuticals and personal care products					
Fluoxetine	0.001	0.04	0.02	0.02	0.01
Galaxolide	0.025	2.2	1.57	1.76	1
Gemfibrozil	0.0003	2.55	3.38	3.5	3.2
Hydrocodone*	0.001	0.01	0.02	0.02	0.03
Ibuprofen*	0.05	0.06	0.29	4.4	1.55
Iopromide*	0.001	0.11	0.14	0.11	0.003
Linuron	0.001	ND	ND	ND	ND
Meprobamate	0.003	0.31	0.34	0.36	0.49
Musk Ketone	0.25	ND	ND	ND	ND
Naproxen	0.005	0.19	0.46	4.39	13
Norfluoxetine	0.001	0.01	0.005	0.01	0.01
o-Hydroxy atorvastatin	0.005	0.12	0.05	0.09	0.11
Oxybenzone	0.005	0.02	0.02	0.29	0.78
p-Hydroxy atorvastatin	0.005	0.18	0.1	0.13	0.14
Risperidone	0.0003	0.002	ND	ND	ND
Simvastatin	0.0003	0.001	ND	ND	ND
Simvastatin hydroxy acid	0.0003	0.003	0.002	0.004	ND
Sulfamethoxazole	0.0003	0.74	0.65	0.92	1.4
Tonalide	0.025	0.3	0.19	0.24	0.1

ND= Not detected
* Semi-quantitative measurement

Table A-2. Seawater median values for target analytes, reported as µg/ L.

Analyte	RL	LA	PV	OC	SD	DP
Current use pesticides						
alpha-Lindane	0.01	ND	ND	ND	ND	ND
Atrazine	0.003	ND	ND	ND	ND	ND
beta-Lindane	0.01	ND	ND	ND	ND	ND
delta-Lindane	0.01	ND	ND	ND	ND	ND
Diazinon	0.01	ND	ND	ND	ND	ND
Lindane	0.01	ND	ND	ND	ND	ND
Methoxychlor	0.01	ND	ND	ND	ND	ND
Metolachlor	0.01	ND	ND	ND	ND	ND
Hormones						
Estradiol	0.001	ND	ND	ND	ND	ND
Estrone	0.0002	ND	ND	0.0002	ND	ND
Ethinylestradiol	0.001	ND	ND	ND	ND	ND
Progesterone	0.001	ND	ND	ND	ND	ND
Testosterone	0.001	ND	ND	ND	ND	ND
Industrial/commercial compounds						
Benzophenone	0.025	ND	ND	ND	ND	ND
Bisphenol A	0.05	ND	ND	ND	ND	ND
Butylated hydroxytoluene	0.025	ND	ND	ND	ND	ND
Butylbenzyl phthalate	0.05	ND	ND	ND	ND	ND
Dioctyl phthalate	0.05	ND	ND	ND	ND	ND
Hydroxyanisole	0.025	ND	ND	ND	ND	ND
N,N-diethyl-meta-toluamide	0.025	ND	ND	ND	ND	ND
Nonylphenol	0.08	ND	ND	ND	ND	ND
Octachlorostyrene	0.01	ND	ND	ND	ND	ND
Octylphenol	0.025	ND	ND	ND	ND	ND
TCEP	0.05	ND	ND	ND	ND	ND
TCPP	0.05	ND	ND	ND	ND	ND
Pharmaceuticals and personal care products						
Acetaminophen*	0.05	ND	ND	ND	ND	ND
Atenolol	0.003	ND	ND	0.006	ND	ND
Atorvastatin	0.003	ND	ND	ND	ND	ND
Carbamazepine	0.005	ND	ND	ND	ND	ND
Diazepam	0.0003	ND	ND	ND	ND	ND
Diclofenac	0.003	ND	ND	ND	ND	ND
Dilantin	0.01	ND	ND	ND	ND	ND
Enalapril	0.0003	ND	ND	ND	ND	ND
Erythromycin*	0.002	ND	ND	ND	ND	ND
Fluoxetine	0.001	ND	ND	ND	ND	ND
Galaxolide	0.025	ND	ND	ND	ND	ND
Gemfibrozil	0.0003	0.002	0.0009	0.01	0.0005	0.0004

Table A-2. Continued.

Analyte	RL	LA	PV	OC	SD	DP
Pharmaceuticals and personal care products						
Hydrocodone	0.002	ND	ND	ND	ND	ND
Ibuprofen*	0.05	ND	ND	ND	ND	ND
Iopromide	0.002	ND	ND	ND	ND	ND
Linuron	0.001	ND	ND	ND	ND	ND
Meprobamate	0.003	ND	ND	ND	ND	ND
Musk Ketone	0.25	ND	ND	ND	ND	ND
Naproxen	0.005	ND	ND	0.008	ND	ND
Norfluoxetine	0.001	ND	ND	ND	ND	ND
o-Hydroxy atorvastatin	0.005	ND	ND	ND	ND	ND
Oxybenzone*	0.001	ND	0.003	0.003	ND	ND
p-Hydroxy atorvastatin	0.005	ND	ND	ND	ND	ND
Risperidone	0.0003	ND	ND	ND	ND	ND
Simvastatin	0.0003	ND	ND	ND	ND	ND
Simvastatin hydroxy acid	0.0003	ND	ND	ND	ND	ND
Sulfamethoxazole	0.0003	0.0007	0.0003	0.001	0.0007	0.0004
Tonalide	0.025	ND	ND	ND	ND	ND
Traseolide	0.025	ND	ND	ND	ND	ND
Triclosan	0.01	ND	ND	ND	ND	ND
Trimethoprim	0.0003	0.0005	ND	ND	ND	ND
Vinclozolin	0.01	ND	ND	ND	ND	ND

ND= Not detected
*Semi-quantitative measurement

Table A-3. Chemical concentrations in sediment samples by station. Data reported as µg/kg.

Analyte	RL	LA	PV	OC	SD	DP
Current use pesticides						
Atrazine	20	ND	ND	ND	ND	ND
Barban	10	ND	ND	ND	ND	ND
Bifenthrin	10	ND	ND	ND	ND	ND
Chlorpyrifos	10	ND	ND	ND	ND	ND
cis-Permethrin	10	ND	ND	ND	ND	ND
Cyfluthrin	10	ND	ND	ND	ND	ND
Cypermethrin	10	ND	ND	ND	ND	ND
Deltramethrin	10	ND	ND	ND	ND	ND
Diazinon	10	ND	ND	ND	ND	ND
Diuron	10	ND	ND	ND	ND	ND
Fenvalerate	10	ND	ND	ND	ND	ND
Flucythrinate	10	ND	ND	ND	ND	ND
Fluometuron	10	ND	ND	ND	ND	ND
Fluvalinate	10	ND	ND	ND	ND	ND
l-cyhalothrin	10	ND	ND	ND	ND	ND
Methoxychlor	2	ND	ND	ND	ND	ND
Metolachlor	50	ND	ND	ND	ND	ND
Propanil	10	ND	ND	ND	ND	ND
Tefluthrin	10	ND	ND	ND	ND	ND
trans-Permethrin	10	ND	ND	ND	ND	ND
Hormones						
17α-ethynylestradiol	4	ND	ND	ND	ND	ND
17β-estradiol	2	ND	ND	ND	ND	ND
Estrone	0.6	ND	ND	ND	ND	0.9
Industrial/commercial compounds						
4-nonylphenol	20	30	110	380	ND	ND
BDE047	1	25	28	22	ND	ND
BDE099	1	14	14	16	ND	ND
BDE100	1	ND	ND	ND	ND	ND
BDE153	1	ND	ND	ND	ND	ND
BDE154	1	ND	ND	ND	ND	ND
bis(2-Ethlyhexyl)adipate	10	ND	ND	ND	ND	ND
bis(2-Ethylhexyl)phthalate	10	121	231	471	29	5
Butyl benzyl phthalate	20	100	25	ND	ND	ND
Diethylphthalate	10	ND	ND	ND	11	ND
Dimethylphthalate	10	ND	ND	ND	ND	ND
di-n-butylphthalate	10	16	11	12	13	5
Di-n-octylphthalate	50	ND	ND	ND	ND	ND

Table A-3. Continued.

Analyte	RL	LA	PV	OC	SD	DP
Legacy Industrial/commercial compounds						
PCB008	1	ND	ND	12	ND	ND
PCB018	1	ND	13	ND	ND	ND
PCB028	1	ND	22	ND	ND	ND
PCB044	1	ND	39	ND	ND	ND
PCB049	1	ND	30	ND	ND	ND
PCB052	1	10	53	ND	ND	ND
PCB066	1	21	55	ND	ND	ND
PCB077	1	17	28	ND	ND	ND
PCB087	1	19	30	ND	ND	ND
PCB095	1	14	29	ND	ND	ND
PCB099	1	18	29	ND	ND	ND
PCB101	1	39	14	ND	ND	ND
PCB105	1	15	20	ND	ND	ND
PCB110	1	ND	ND	ND	ND	ND
PCB118	1	34	9.2	ND	ND	ND
PCB126	1	ND	ND	ND	ND	ND
PCB128	1	ND	ND	ND	ND	ND
PCB138	1	40	14	ND	ND	ND
PCB149	1	25	32	ND	ND	ND
PCB153	1	41	46	ND	ND	ND
PCB170	1	14	14	ND	ND	ND
PCB180	1	22	19	ND	ND	ND
PCB187	1	17	18	ND	ND	ND
PCB194	1	ND	ND	ND	ND	ND
PCB195	1	ND	ND	ND	ND	ND
PCB201	1	ND	ND	ND	ND	ND
PCB206	1	ND	ND	ND	ND	ND
PCB209	1	ND	ND	ND	ND	ND

Table A-3. Continued.

Legacy pesticides						
2,4'-DDD	2	ND	26	ND	ND	ND
2,4'-DDE	2	8	180	ND	ND	ND
2,4'-DDT	2	ND	ND	ND	ND	ND
4,4'-DDD	2	ND	92	ND	ND	ND
4,4'-DDE	2	ND	900	4	ND	5
4,4'-DDT	2	ND	50	ND	ND	ND
Aldrin	2	ND	ND	ND	ND	ND
alpha Chlordane	2	ND	ND	ND	ND	ND
cis-nonachlor	2	ND	ND	ND	ND	ND
DDMU	2	ND	ND	ND	ND	ND
Dieldrin	2	ND	ND	ND	ND	ND
Endrin	2	ND	ND	ND	ND	ND
gamma BHC	2	ND	ND	ND	ND	ND
Legacy pesticides						
gamma Chlordane	2	ND	ND	ND	ND	ND
Heptachlor	2	ND	ND	ND	ND	ND
Heptachlor epoxide	2	ND	ND	ND	ND	ND
Oxychlordane	2	ND	3	ND	ND	ND
t-Nonachlor	2	ND	ND	ND	ND	ND
Toxaphene	50	ND	ND	ND	ND	ND
Pharmaceuticals and personal care products						
Carbamazepine	0.1	0.1	ND	0.1	ND	ND
Diazepam	0.1	ND	ND	0.1	ND	ND
Linuron	10	ND	ND	ND	ND	ND
Oxybenzone	0.2	ND	ND	ND	ND	ND
Simvastatin	0.5	ND	ND	ND	ND	ND
Triclosan	0.4	5.1	7.3	8.6	2.1	2

ND= Not detected

Table A-4. Chemical concentrations in composite liver samples of female (F) and male (M) hornyhead turbot by station. Data reported as µg/kg.

Analyte	RL	LA F	LA M	PV F	PV M	OC F	OC M	SD F	SD M	DP F	DP M
Hormone											
17α-ethynylestradiol	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Current use pesticides											
Atrazine	20	ND	ND	ND	ND	ND	ND	ND	48	ND	ND
Barban	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorpyrifos	10	ND	ND	ND	ND	36	31	ND	ND	ND	ND
cis-Permethrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyfluthrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Deltramethrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diazinon	10	5	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fenvalerate	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Flucythrinate	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fluometuron	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fluvalinate	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
l-cyhalothrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methoxychlor	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor	50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Propanil	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tefluthrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
trans-Permethrin	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Industrial/commercial compounds											
4-nonylphenol	50	25	130	150	100	50	290	30	70	ND	150
BDE047	1	190	330	120	240	100	260	52	240	11	12
BDE099	1	67	120	31	51	59	150	48	150	10	8
BDE100	1	51	97	40	75	34	74	21	66	4	4
BDE153	1	7	16	5	6	7	18	6	12	1	1
BDE154	1	10	25	7	16	10	17	5	15	1	2
Legacy industrial/commercial compounds											
PCB008	1	4.4	6.8	9.8	17	4.6	5.2	13	6.4	1.7	ND
PCB018	1	1.3	3.4	20	27	ND	ND	ND	ND	ND	ND
PCB028	1	3.3	4.9	54	71	ND	ND	ND	ND	ND	ND
PCB044	1	2.8	3.2	26	40	1.1	ND	ND	ND	ND	ND
PCB049	1	6.6	19	100	140	1.4	1	ND	ND	1	ND
PCB052	1	10	22	140	210	1.7	1.4	ND	1	1.4	ND
PCB066	1	25	34	180	270	2.1	1.7	ND	ND	ND	ND

Analyte	RL	LA F	LA M	PV F	PV M	OC F	OC M	SD F	SD M	DP F	DP M
Legacy industrial/commercial compounds											
PCB077	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB087	1	11	9.4	71	97	ND	ND	ND	ND	ND	ND
PCB095	1	9.1	19	43	77	ND	1.7	ND	1.4	ND	ND
PCB099	1	43	63	160	200	4.9	6.6	10	5.7	1	1.1
PCB101	1	56	79	190	310	4.9	4.8	ND	4	1.8	ND
PCB105	1	29	39	95	140	ND	ND	ND	ND	ND	ND
PCB110	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB118	1	82	120	340	420	ND	ND	13	5.9	ND	1.3
PCB126	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB128	1	5	9.6	17	27	ND	ND	ND	ND	ND	ND
PCB138	1	160	170	350	360	10	13	36	8.6	3.6	3.5
PCB149	1	51	74	55	130	4.6	14	18	9.4	1	ND
PCB153	1	160	220	400	470	14	20	25	10	3.1	2.3
PCB170	1	30	50	66	86	3	4	ND	3.6	ND	ND
PCB180	1	74	100	180	220	5.1	5.8	13	5.8	1.4	1.3
PCB187	1	52	86	74	120	6	ND	ND	6.8	ND	1.1
PCB194	1	20	29	30	35	1.2	ND	ND	2.1	ND	ND
PCB195	1	6.4	16	18	21	ND	ND	ND	ND	ND	ND
PCB201	1	23	41	38	53	2.6	2.5	ND	3	ND	ND
PCB206	1	9.7	24	17	22	1.8	2.6	ND	2.8	ND	ND
PCB209	1	2.9	6.5	65	5.8	1.1	ND	ND	ND	ND	ND
Legacy pesticides											
2,4'-DDD	2	18	22	110	120	5	7	ND	5	ND	ND
2,4'-DDE	2	110	160	2700	4500	27	12	ND	5	ND	ND
2,4'-DDT	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4'-DDD	2	28	33	730	860	14	5	ND	7	3	ND
4,4'-DDE	2	1393	2393	52793	64693	453	173	12	113	22	73
4,4'-DDT	2	13	20	53	70	8	8	ND	13	5	ND
Aldrin	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
alpha Chlordane	2	ND	6.3	ND	6	2	3	ND	ND	ND	ND
cis-nonachlor	2	6	11	9	13	5	6	ND	6	ND	ND
DDMU	2	110	142	6500	7800	40	7	ND	ND	ND	ND
Dieldrin	2	6	ND	7	7	ND	5	ND	5	ND	ND
Endrin	2	ND	ND	ND	ND	ND	ND	ND	5	ND	ND
gamma BHC	2	ND	ND	2	ND	ND	ND	ND	ND	ND	ND
gamma Chlordane	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor epoxide	2	6	6	13	18	10	3	ND	4	ND	ND

Table A-4. Continued.

Analyte	RL	LA		PV		OC		SD		DP	
		F	M	F	M	F	M	F	M	F	M
Legacy pesticides											
Oxychlordane	2	5	6	24	31	ND	4	ND	ND	ND	ND
t-Nonachlor	2	3	45	93	100	9	18	ND	ND	ND	ND
Toxaphene	50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pharmaceuticals and personal care products											
Diazepam	8	45	76	26	76	35	69	44	58	23	110
Carbamazepine	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Linuron	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxybenzone	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Simvastatin	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Triclosan	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND= Not detected