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Integrated coastal effects study: Synthesis of findings

By

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Integrated coastal effects study: Synthesis of findings

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Abstract

Concern over the environmental impacts of contaminants of emerging concern (CECs) has increased in recent years as a result of studies showing their occurrence in waste discharges and receiving waters, and instances of fish endocrine disruption associated with some CECs. 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 study investigated the impacts of CECs from ocean wastewater discharges on SCB fish. Samples of effluent from the four major municipal wastewater treatment plants were collected. In addition, seawater, sediment, and hornyhead turbot (*Pleuronichthys verticalis*) from the effluent discharge areas and a reference station were also sampled and analyzed for multiple chemical and biological indicators. Low concentrations of many pharmaceutical, personal care products and industrial and commercial compounds were frequently measured in the effluent samples. Some CECs were detected in sediment and seawater collected near the outfall sites, indicating the potential for fish exposure. Seawater CECs were detected at concentrations lower than one part per trillion. Fish livers contained certain types of CECs confirming exposure. Fish plasma hormone analyses suggested the presence of physiological effects including reduced cortisol levels, relatively high levels of male estradiol, and reduced thyroxine. Male fish plasma also contained low levels of vitellogenin. Most fish responses were found at all sites, and could not be directly associated with effluent discharges. However, concentrations of thyroxine were lower at all discharge sites relative to the reference, and estradiol concentrations were lower at three of the four outfall sites. The physiological responses found in this study did not appear to be associated with adverse impacts on fish reproduction or populations. Overall, fish from discharge and reference sites had similar reproductive cycles. Analysis of long-term monitoring data showed that hornyhead turbot populations were stable (or increasing) and that the fish community composition near the outfall discharges was typical of that expected in reference areas.

Introduction

Three decades of monitoring by southern California water quality agencies have provided a great deal of 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"

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(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 compounds (ICCs).

The occurrence and effects of most CECs have not been extensively studied, perhaps 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 a cause of sediment toxicity throughout California (Bay *et al.* 2011a, Schiff *et al.* 2011). Some CECs disrupt the endocrine systems of animals by interfering with the action of hormones involved with reproduction or growth (Forrester *et al.* 2003, Hoger *et al.* 2006, Evrard *et al.* 2010). 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, Alvarez *et al.* 2009, Björkblom *et al.* 2009, Iwanowicz *et al.* 2009).

Recent studies have detected multiple CECs in sediments collected near wastewater effluents discharges in coastal waters of the Southern California Bight (SCB) (Sapozhnikova *et al.* 2004, Schlenk *et al.* 2005). Previous studies have also reported the presence of indicators of endocrine disruption in local fish, such as the production of vitellogenin (an egg yolk protein) in male flatfish (Roy *et al.* 2003, Schlenk *et al.* 2005, Rempel *et al.* 2006, Deng *et al.* 2007). A TIE evaluation of WWTP sediment extracts failed to identify causative agents (Schlenk *et al.* 2005). The significance of these results are uncertain because the previous studies were limited in scope and interpretation is confounded by co-occurring legacy contaminants (e.g., DDTs, PCBs) that may produce similar effects. As a result, the nature and magnitude of endocrine disruption in southern California fish is not known. Additional information is 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, the 2006 CEC Coastal Effects Study, 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?

Sample collection, analysis, and data interpretation was a collaborative effort among SCCWRP, southern California's four largest municipal wastewater treatment agencies, and major universities. This paper provides a synthesis of the key findings of the study and is intended to complement other papers in this series that describe the results in greater detail.

Methods

Study Design

This 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 . Flatfish such as hornyhead turbot are used 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 (Cooper 1994, 1996).

A comprehensive and integrated chemical/ 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 (Lyon *et al.* 2006). Samples of final effluent from each of the treatment plants, as well as near-bottom seawater, sediment, and hornyhead turbot from the

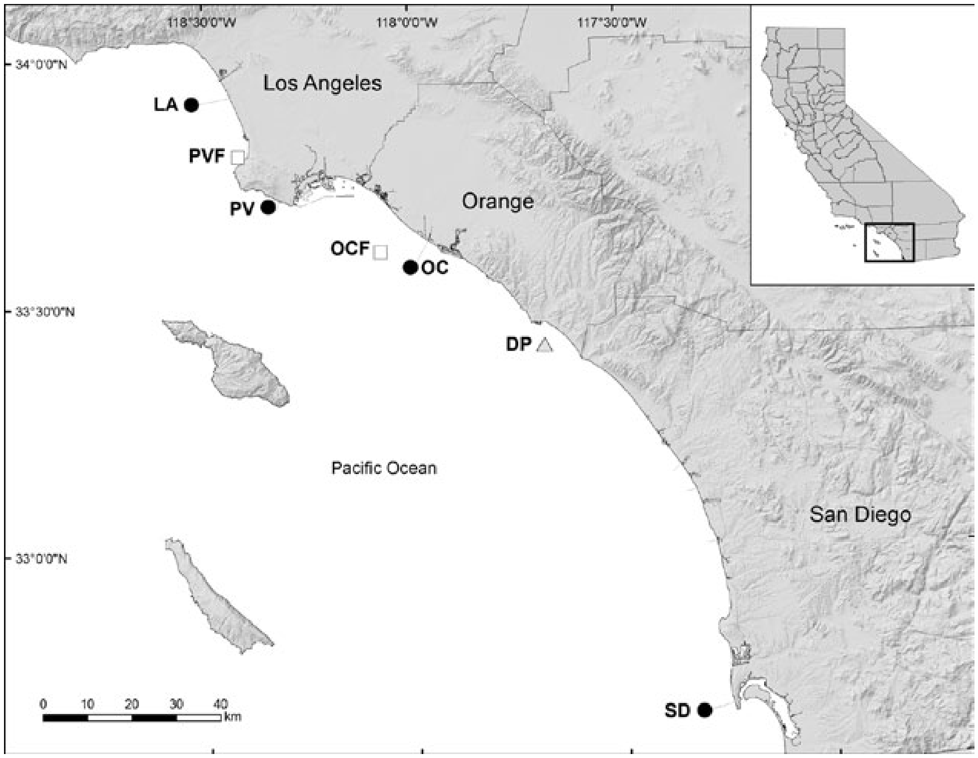

Figure 1. Project study sites. Four sites were located near large discharges of municipal wastewater (LA, PV, OC, and SD). In addition, samples were collected from one reference station (DP) and two far field stations (PVF and OCF).

study areas were collected to characterize the fate of a diverse suite of CECs. The effluents used have been described by Vidal-Dorsch *et al.* (2011a).

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 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).

Chemical and Biological Analyses

A diverse suite of CECs and legacy contaminants was measured in effluent, seawater, sediment, and fish liver. A complete description of these measurements can be found in (Bay *et al.* 2011b). 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 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 currently not 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.

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) 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 sampled 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). Further descriptions of the methods used for chemical and biological analysis are described elsewhere (Brar *et al.* 2010; Forsgren *et al.* 2011; Vidal-Dorsch *et al.* 2011a,b).

Data Analyses

Summary statistics and analysis of variance were used to analyze the data. 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; $p < 0.05$). One-half of the detection limit was substituted for constituent concentrations reported by the laboratory as not detected. Station averages of differential 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.

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.

Results and Discussion

This study represents the most comprehensive investigation to date of CECs and their effects in coastal offshore waters. Thousands of chemical and biological measurements were made during this study, the detailed results can be found in related publications (Bay *et al.* 2011b, Forsgren *et al.* 2011, Vidal-Dorsch *et al.* 2011a, Maruya *et al.* 2011). The key findings are described in this document and are organized with respect to the project questions. The data created by this project are available in a relational database (www.sccwrp.org).

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

Diverse types of CECs were 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. Of the 31 PPCPs measured, 11 were detected in every sample analyzed, regardless of treatment level. Five ICCs and one of the hormones were also detected in 100% of the effluent samples.

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 and ICCs varied significantly among effluent types (Figure 2), 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

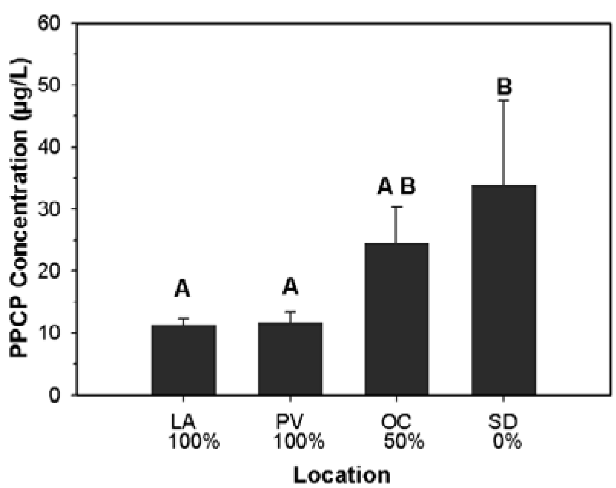

Figure 2. Average total concentration of pharmaceutical and personal care products (+ standard error) in quarterly wastewater effluent samples. The percentage of secondary treatment is shown for each effluent type at the time of the study. Bars with the same letter are not statistically different from each other.

with longer treatment plant residence times (Drewes *et al.* 2008, USEPA 2010). 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.

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

The results indicated 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 1). Only a small proportion of the target analytes were detected in seawater and the concentrations were 400-1,000 times lower than those 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. The ANOVA analysis of seawater samples 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 and little research has been done to determine the effects of mixtures of CECs in environmental samples.

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 nonylphenol (surfactant), and the LA, PV and OC sediments contained PBDEs (flame retardants). Livers of hornyhead turbot from all the sites also contained PBDEs and nonylphenol (Figure 3). Some chemicals that were not detected in the sediment were found in the livers of fish from the same site, such as PCBs (SD and DP stations) and diazepam (all stations; (Kwon *et al.* 2009)). This finding highlights the fact that even if a contaminant is not

Table 1. Effluent and seawater occurrence and median concentrations (µg/L) for chemicals detected in seawater samples.

Chemical Group or Use	Chemical Name	Effluent		Seawater	
		Median	Occurrence (%)	Median	Occurrence (%)
Beta-blocker	Atenolol	2.20	100	0.0004	90
Cholesterol Regulator	Gemfibrozil	3.25	100	0.0009	90
Analgesic	Naproxen	2.30	100	0.0007	75
Antibiotic	Sulfamethoxazole	0.92	100	0.0005	70
Antibiotic	Trimethoprim	0.62	100	0.0007	60
Antidepressant	Meprobamate	0.35	100	ND[1]	50
Analgesic	Diclofenac	0.13	100	ND	40
ICC	Butylated hydroxytoluene	0.29	100	ND	40
Antimicrobial	Triclosan	0.79	100	ND	40
ICC	Nonylphenol	1.42	94	ND	35
Analgesic	Ibuprofen	1.45	94	ND	30
Antiepileptic	Carbamazepine	0.27	100	ND	25
Cholesterol Regulator	Atorvastatin	0.11	100	ND	15
ICC	Benzophenone	0.42	100	ND	15
Hormone	Estrone	0.04	100	ND	10
ICC	Octylphenol	0.69	100	ND	10
ICC	TCPP	1.10	100	ND	10

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

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.

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

Potentially abnormal variations were observed in some blood plasma indicators. Yet, in most cases these responses were found at all sites including the reference area, and could not be directly associated with effluent discharges. These widespread responses included the frequent detection of low levels of vitellogenin (VTG) in male fish (Figure 4), and high concentrations of estradiol in male fish relative to females (Figure 5).

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

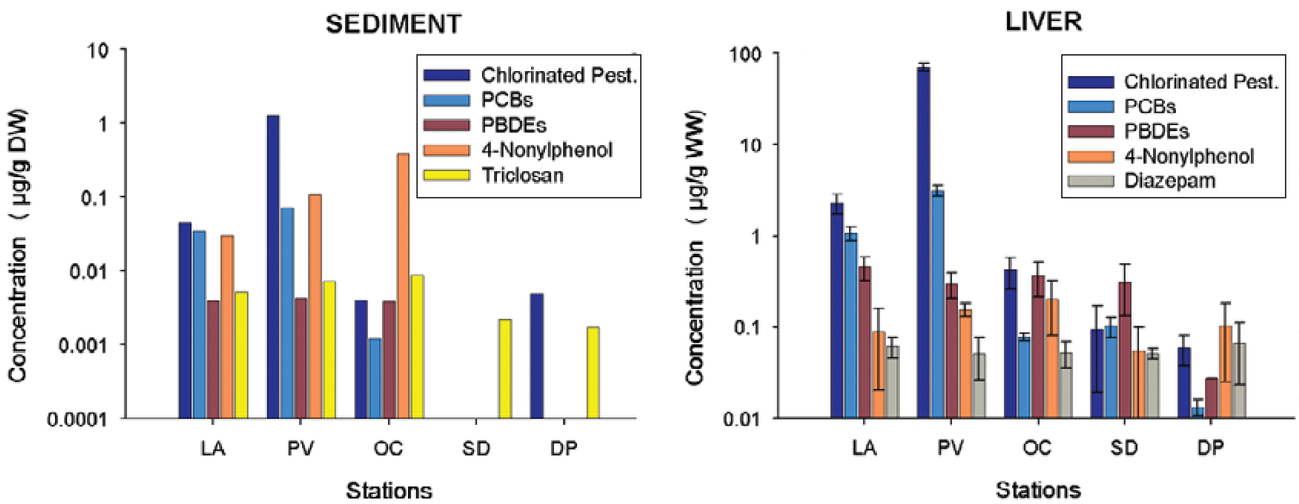

Figure 3. Concentrations of selected legacy contaminants and CECs in sediment and liver tissue. Chlorinated pesticides= sum of aldrin, chlordane, DDD, DDE, DDT, DDMU, dieldrin, endrin, heptachlor, heptachlor epoxide, lindane, methoxychlor, oxychlordane, nonachlor, and toxaphene; PCBs= sum of 28 PCB congeners; PBDEs= sum of five polybrominated diphenyl ether congeners. Diazepam was not detected in sediment samples.

to disrupt reproduction, but they may be indicative of fish exposure to estrogens in the environment. Male fish from the SD site had significantly higher concentrations of VTG relative to PV and LA fish, but 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 were lower than that in females for most fish species; yet male hornyhead turbot estradiol concentrations were similar or greater than those of females (Figure 5). Elevated estradiol in males did not appear to be associated with outfall discharges, as a similar pattern was detected at the DP reference site. Measurements of estradiol in other species of southern California flatfish do not show this unusual pattern (Hagstrom 2008), although flatfish species from other areas 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

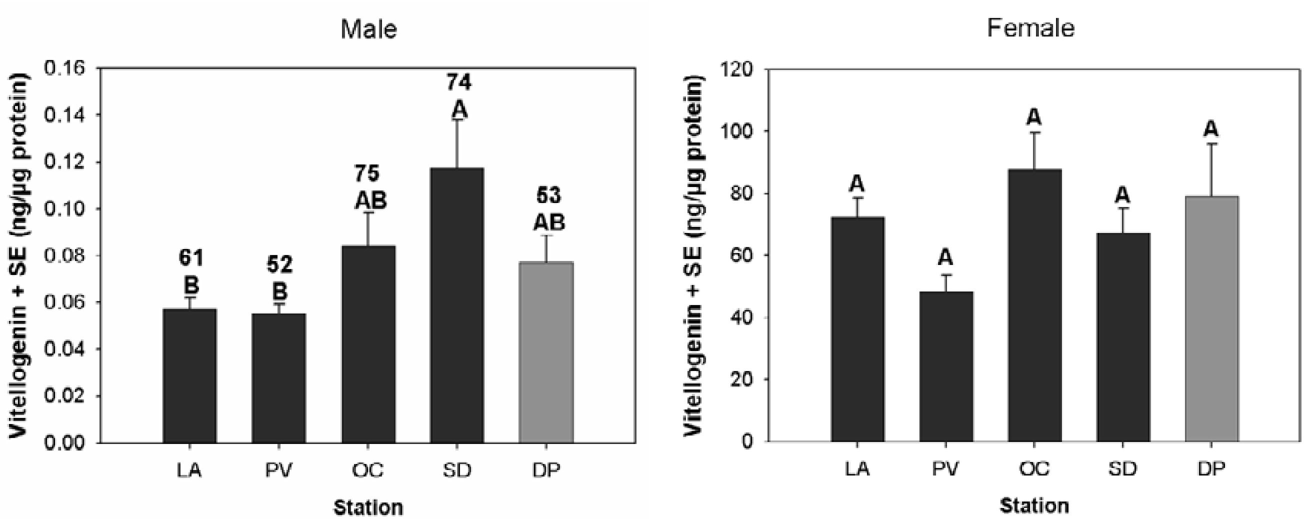

Figure 4. Average vitellogenin concentrations in male and female hornyhead turbot. Bars with same letter are not statistically different from each other. The numbers on top of the bars indicate the percent of males with detectable VTG. Samples collected in May-June 2006.

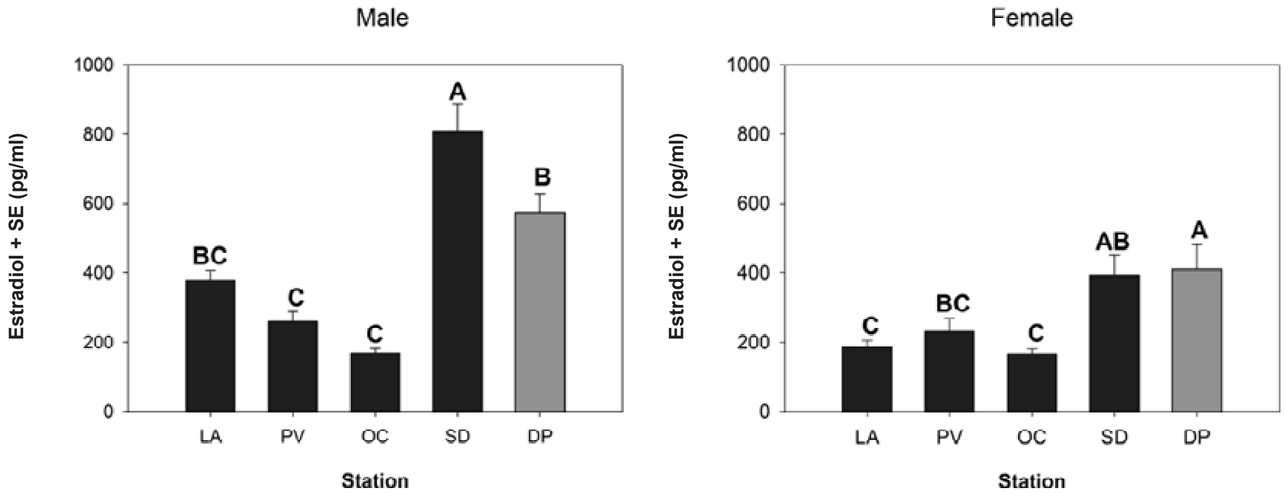

Figure 5. Average estradiol concentrations for male and female hornyhead turbot. Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

half of those in fish from SD and DP (Figure 5). 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, which can have antiestrogenic effects.

No consistent pattern in the concentration of 11-KT, the principal form of testosterone in fishes, was present among the outfall sites (Figure 6). 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.

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 7). 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

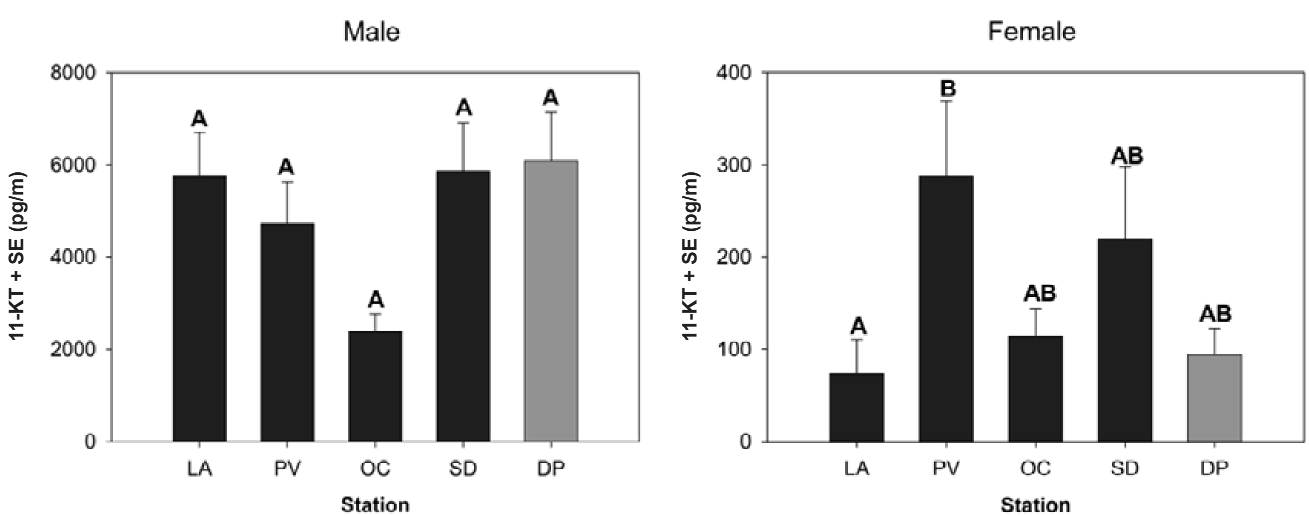

Figure 6. Average 11-ketotestosterone (11-KT) concentrations for male and female hornyhead turbot. Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

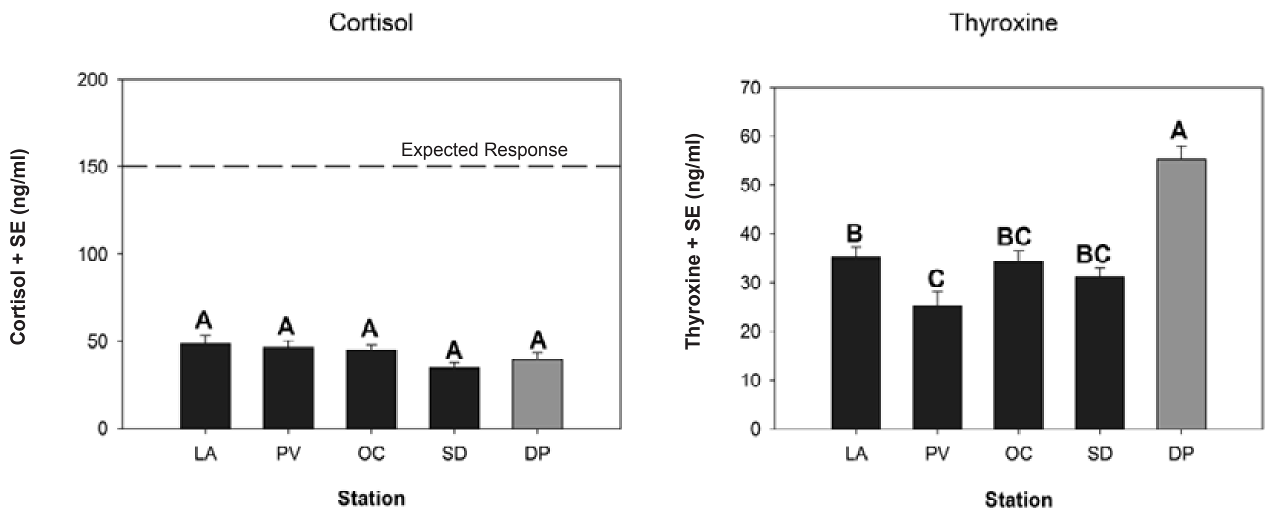

Figure 7. Cortisol and thyroxine average concentrations for hornyhead turbot (combined data for males and females). Bars with same letter are not statistically different from each other. Samples collected in May-June 2006.

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 was reduced in fish from each of the outfall sites relative to the reference site (Figure 7), particularly in the PV site where the greatest legacy contamination exists. Recent studies in San Francisco Bay have also observed reduced thyroxine levels in two species of fish from contaminated locations and associated the changes with PCB bioaccumulation (Brar *et al.* 2010). 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.

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 degeneration) 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 discharges or legacy contamination. The presence and severity of atresia seemed to correspond to the fish reproductive stage, as a normal feature of the reproductive cycle.

Wide variations in male to female sex ratios were observed, but there was no consistent trend 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 the May to August sampling periods, as indicated by high values for the gonad somatic index. This trend was confirmed by histological evaluation of the maturity state of developing eggs in the gonad (Figure 8). The reproductive cycle of males was similar to the females, in general. Females from 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.

Male and female plasma vitellogenin concentrations also varied temporally. 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.

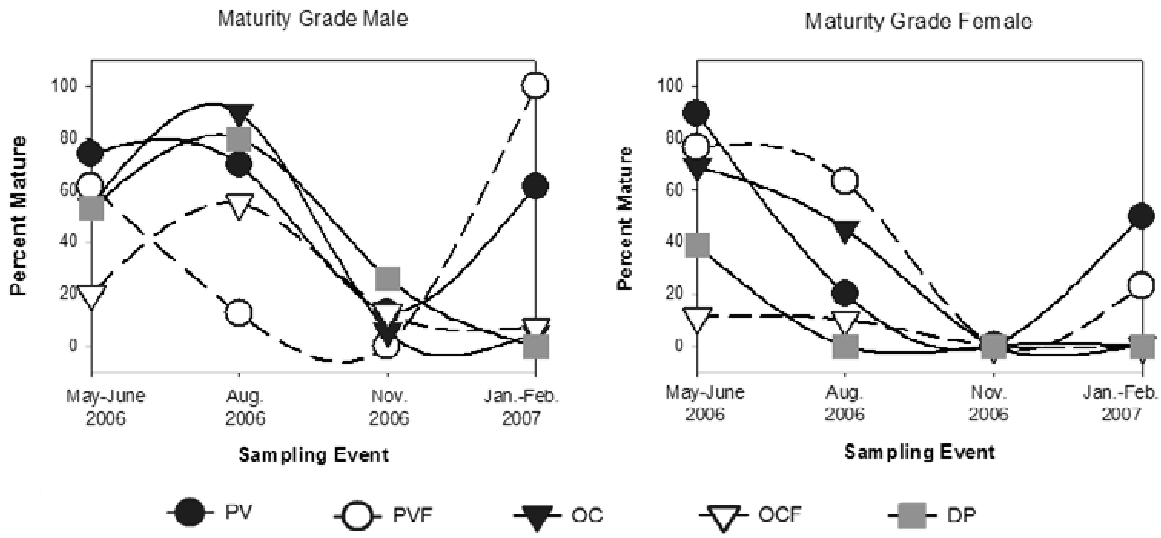

Figure 8. Temporal trends of the percentage of mature males and females.

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 (Figure 9). 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. In addition, 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.

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 did not. Relative liver size (LSI) varied among sites, with the highest LSI values in PV fish at all time periods (Figure 10). 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).

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 comparable estradiol levels in males and females were found at both 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 3). An alternative explanation for the cortisol, VTG, and estradiol results 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 thyroxine and estradiol. Hornyhead turbot thyroxine concentrations in plasma at all four discharge sites were less than in fish from DP. Fish

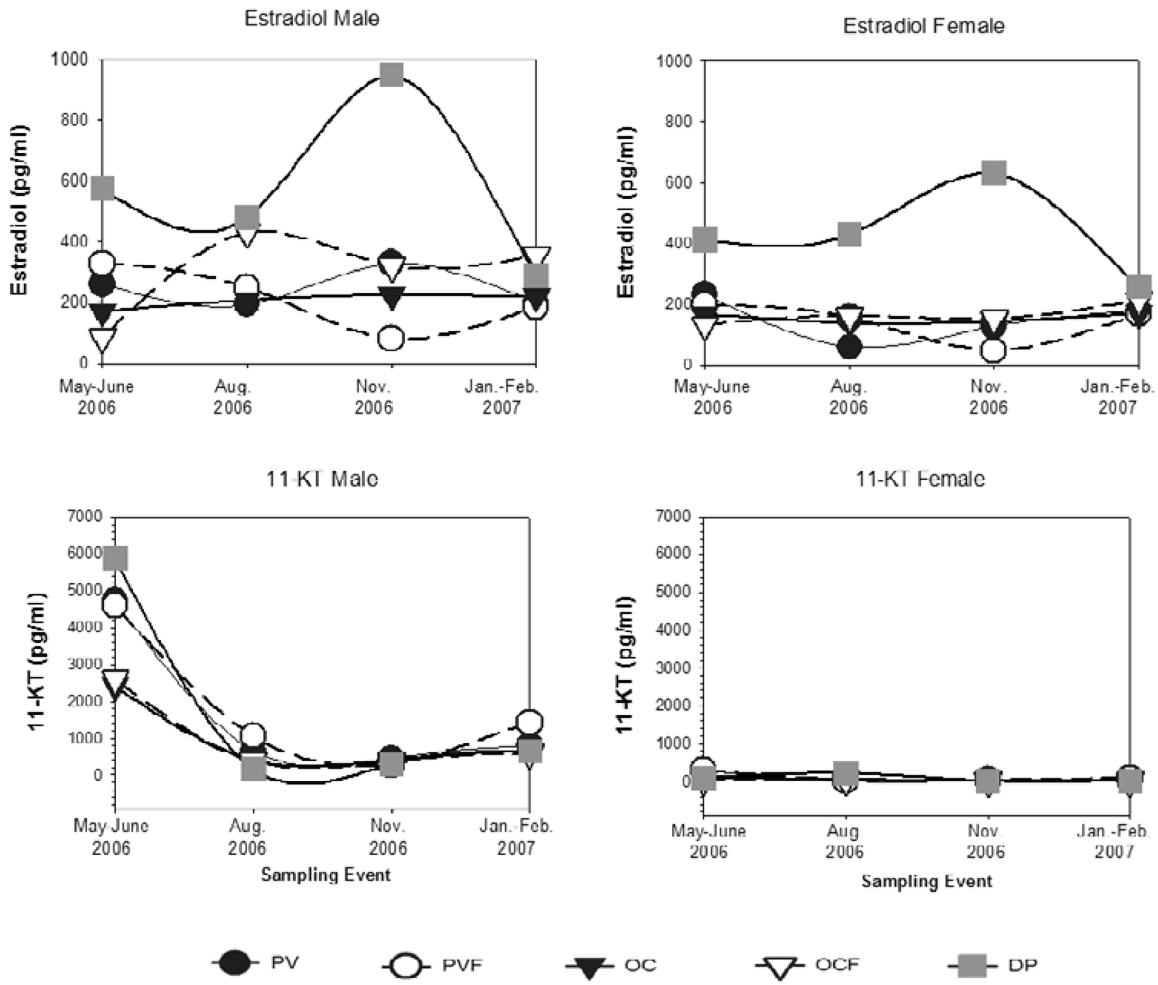

Figure 9. Average 11-ketotestosterone (11-KT) in male and female hornyhead turbot.

thyroid hormone 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 PBDE (Tomy *et al.* 2004, Iwanowicz *et al.* 2009). 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. Quarterly samples for LA and OC confirmed the trend for estradiol (Figure 9), 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 and higher quality effluents (LA, PV, OC), and not present at SD (lower legacy contamination). In contrast, increased male plasma estradiol concentrations were present at only the SD outfall site where both legacy contamination and level of treatment are relatively lower. The association of the SD estradiol response 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 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

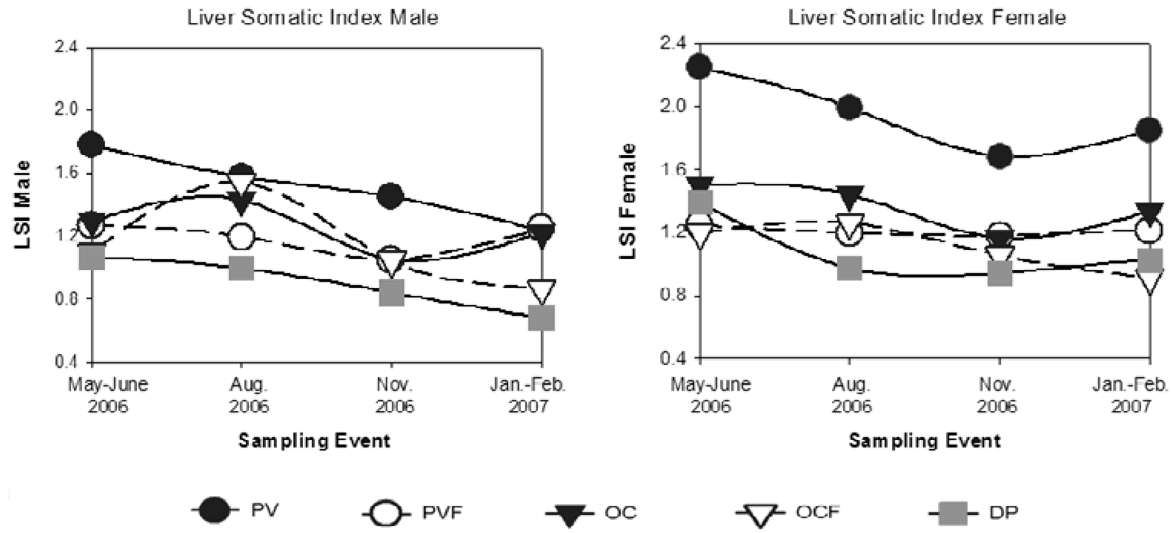

Figure 10. Temporal trends for liver somatic index (LSI) in hornyhead turbot.

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 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 results. This molecular indicator showed patterns of response associated with the LA, PV, and OC sites, where legacy sediment contamination and effluent quality is greatest.

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 revised results from the Bight 2003 regional survey. The 2003 study examined 42 hornyhead turbot males collected throughout the Southern California Bight. The findings of this study initially reported a seemingly high incidence (12%) of intersex (presence of developing eggs in male gonad), and noted that all five males with presumed intersex were collected near POTW discharges. In addition, intersex was also reported to be present in 8% of English sole (*Pleuronectes vetulus*). However, reanalysis of the tissue samples determined that the presence of oocytes in all but two of hornyhead turbot and all of the English sole classified as having intersex was the result of sample contamination introduced in the field or laboratory.

Analysis of long-term monitoring data for the study sites indicated that hornyhead turbot populations are stable or increasing throughout the region (Figure 11). 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, as the relative abundance of hornyhead turbot tends to increase when coastal water temperature is lower (M.J. Allen, personal communication).

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 12).

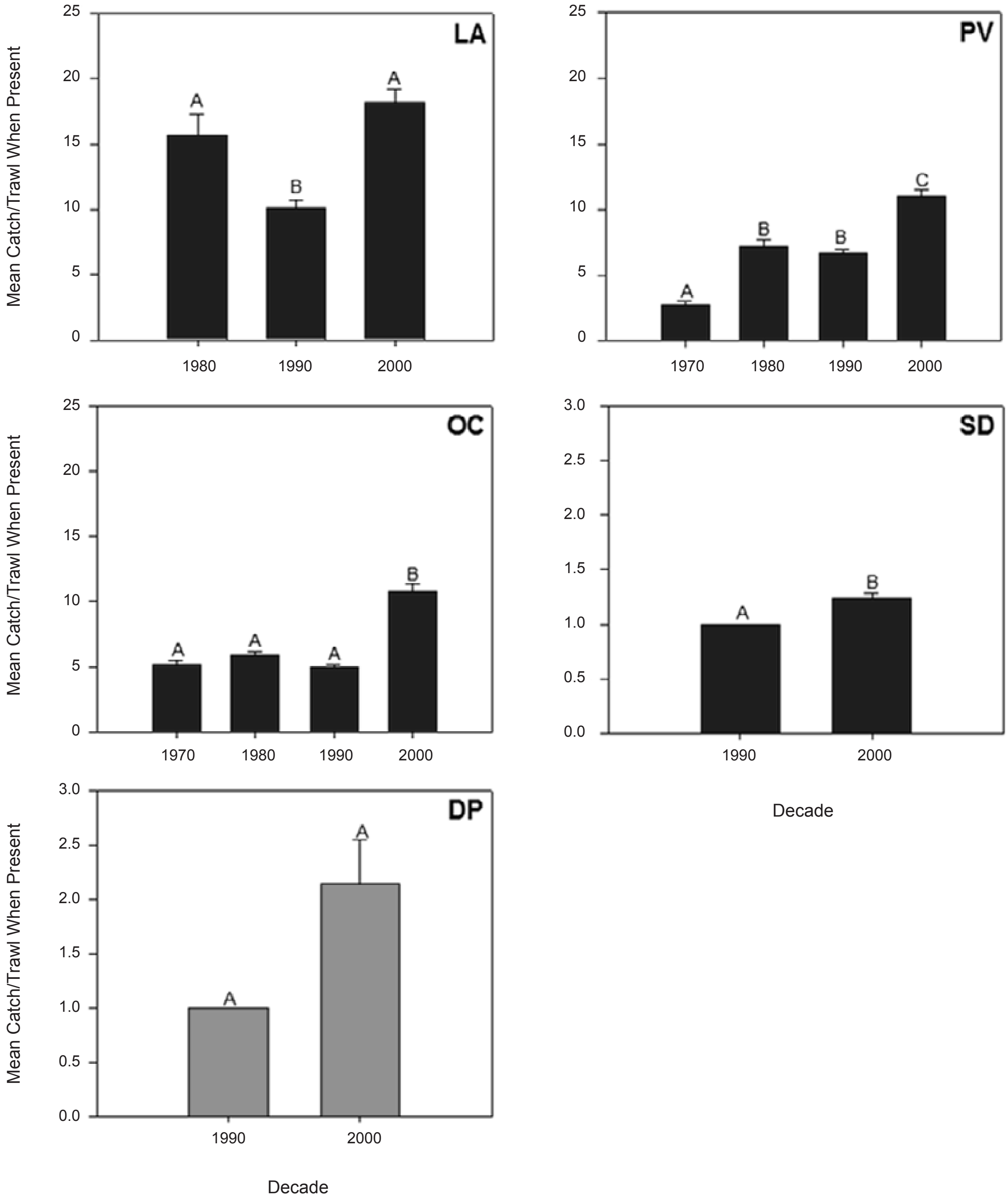

Figure 11. Average (+ standard error) abundance of hornyhead turbot over time. Data compiled from monitoring surveys (notice differences in the scales among plots).

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.

Most of our knowledge regarding CEC exposure and effects in southern California is limited to offshore coastal habitats and two species of flatfish. Similar studies are needed for other habitats with high potential for CEC exposure (e.g., estuaries and effluent-dominated rivers) and other species to provide better context to determine constituents, areas, and responses of greatest concern.

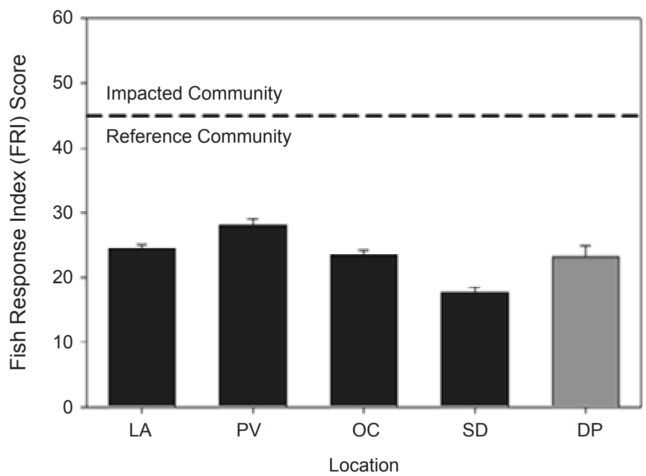

Figure 12. Fish community condition at the study sites, as indicated by the Fish Response Index (average + SE). Data compiled from 2003-2009 monitoring surveys.

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