Characterization Studies of a Thyroid Endocrine-disrupted Condition in Wild Fishes of San Francisco Bay

Final Report

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Characterization Studies of a Thyroid Endocrine-disrupted Condition in Wild Fishes of San Francisco Bay

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October 2010

FINAL REPORT

CHARACTERIZATION STUDIES OF A THYROID ENDOCRINE-DISRUPTED CONDITION IN WILD FISHES OF SAN FRANCISCO BAY

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EXECUTIVE SUMMARY

It is well documented that many California coastal environments are contaminated by human-derived chemicals, particularly in locations adjacent to developed and industrialized urban centers, such as in San Francisco Bay. However, it is not well understood to what extent existing contaminants, many with continuing inflows into the environment, are impacting the biota. This kind of information is needed to improve ecological-based management of California's coastal and estuarine environments. New information on contaminant effects can facilitate strategic planning, mitigation plans, and allocation of resources regarding environmental management.

Aimed at addressing the lack of information, this project continued a process of characterizing a thyroid endocrine-disrupted condition exisiting in wild fish residing in San Francisco Bay, which was identified in a prior RMP-based project (SFEI Project #701). Objectives in this study included elucidation of underlying mechanisms generating this condition, possible environmental causes, as well as its potential consequences physiologically (i.e.,possible connection to growth).

The results from this study provided additional lines of evidence indicating that the thyroid endocrine system function is significantly, sometimes substantially, disrupted in fish residing in different locations in San Francisco Bay and, importantly, that the effects are directly related to exposure to specific classes of environmental contaminants. Specific components of the thyroid endocrine system are altered, leading to altered thyroid hormone levels, which may be transduced into changes in growth regulatory systems.

Specific Findings of the Study

1. The thyroid endocrine system exhibits significant alterations in association with residence of the fish in different San Francisco Bay locations. The primary circulating thyroid hormone, thyroxine (T4), is consistently lower in fish from certain locations (e.g., Oakland Inner Harbor) as compared with fish from other locations (e.g., Redwood City). The active thyroid hormone, triidothyronine (T3), which is generated by deiodinase enzymes in peripheral tissues (outside

of the thyroid gland) and which circulates in smaller quantities, also exhibits significant location-associated changes that do not necessarily follow T4. Alterations in T3/T4 ratios indicate that some locations may be associated with increased or decreased peripheral deiodinase activity, which may constitute an important form of thyroid endocrine disruption that occurs outside of the thyroid gland. Other changes, such as reduced total thyroid hormones (T4+T3 levels), indicate that there may be effects at the level of the thyroid gland in which hormone production is impacted.

2. As a result of the above findings, a field-based experimental approach was taken, in which fish were tested for the ability of their thyroid to produce T4. In these experiments, thyroid-disrupted fish from Oakland Inner Harbor were compared with reference fish at Redwood City that have normal thyroid function. Over two different years of study, it was consistently found that one of the fish species (shiner perch) was significantly impaired in its thyroidal ability to produce T4, pointing to the thyroid gland as a target of the environmental effect (e.g., by an endocrine-disrupting chemical, or EDC). In a second species (Pacific staghorn sculpin), this type of impairment was not evident, yet another functional component of the thyroid endocrine system appeared to be impacted in this species (next paragraph).

3. An assay was established to measure hepatic 5'-deiodinase activity directly, and used to assess whether thyroid endocrine system disruptions may be related to changes in this important enzyme system that operates outside of the thyroid gland (e.g., in target tissues such as the liver). In both species, this system could be activated hormonally, and such responses were not altered in fish according to location. However, in the sculpin residing in Oakland Inner Harbor, hepatic 5'-deiodinase activity was significantly depressed, which was correlated with reduced T3/T4 ratios. Thus, in the sculpin, environmental effects on peripheral deiodinase activity may be driving thyroid disruptions leading to relatively reduced T3 levels.

4. Additional evaluation of hepatic deiodinase activity in the fish indicated that it can be altered in association with specific environments, and that it importantly influences thyroid endocrine status, most notably the relative concentrations of T3 in the blood circulation. T3/T4 ratio was highly significantly correlated with hepatic 5'-deiodinase activity, indicating that measurement of T3 and T4 in plasma (and calculation of T3/T4) serves as an effective

indicator of peripheral deiodinase status in fish. It was also determined that hepatic 5'-deiodinase activity was significantly related to chlordane exposures in fish, in accordanc with earlier findings that thyroid hormones, and especially T3/T4 ratio, are positively correlated with tissue chlordane concentrations. There was also an overall positive relationship between deiodinase and PCB exposures, further pointing to both classes of chemicals as thyroid-disruptive agents, likely acting through impacts on peripheral deiodinases.

5. Abnormal thyroid endocrine status is known to impact growth and developmental processes in all vertebrate animals, including fish. The results of this study indicate that the principal growth regulator, IGF-I, also exhibits environment-related alterations, including reduced levels in thyroid-disrupted fish from Oakland Inner Harbor. Reductions in IGF-I were significantly correlated with tissue exposures to PCBs and chlordanes (similar to thyroid hormones). In addition, in one of the species (sculpin), thyroid endocrine status was significantly related to IGF-I levels, indicating a functional link to the growth system.

Conclusions and Management Implications

The findings of this study demonstrate that wild fish residing in different inshore estuarine environments of the San Francisco Bay area are exhibiting significant alterations in their thyroid endocrine systems. This has now been observed over four different years of study. It is also notable that two different fish species representing distinct life histories exhibited a number of similar alterations at the same study sites, although some differences are also evident.

This work confirms that current-day exposures to contaminant chemicals are significantly related to endocrine disruption in wild fishes. Alterations in thyroid endocrine system components were strongly related to exposure to PCBs and chlorinated pesticides (chlordanes). Sublethal effects of contaminant chemicals, particularly through endocrine disruption mechanisms, are likely to lead to a number of impairments and reduced physiological `performance in wild life.

The scientific approaches undertaken through this work demonstrate the strong potential of new screening methodologies, such as endocrine or biomarker measurements, to assess environmental/water quality effects in fish and wildlife. Strengthening our understanding of the links between different types of environmental contaminants, endocrine disruption effects, and associated phenotypic impacts, are critical in continuing to validate these approaches and ultimately in establishing effective thresholds for different types of contaminants.

GENERAL INTRODUCTION

The San Francisco Bay Area represents an intensive interface between an estuarine environment and a substantial human population and its varied industrial and other activities. The San Francisco Bay appears on the Section 303(d) list of impaired water bodies, as required by the Clean Water Act (www.waterboards.ca.gov/sanfranciscobay/TMDL/303dlist.htm). The principal pollutants listed include trace elements (Hg and Se), pesticides (dieldrin, chlordane, and DDTs), additional chlorinated compounds (e.g., PCBs, dioxin and furan compounds), PAHs, and others. Data collected through the Regional Monitoring Program for Water Quality in the San Francisco Estuary (RMP) since 1997 and in other studies, indicate that contaminant concentrations in the San Francisco Bay ecosystem are sufficiently elevated to cause concern for adverse effects on biota (e.g., Urquhart and Regalado, 1991; Fairey et al., 1997; Schwartzback et al., 1997; Davis et al., 1999, 2004, 2006; Gunther et al., 1999; Schwartzbach and Adelsbach, 2002; Greenfield et al., 2005; Conner et al., 2007; Davis et al., 2007; Pulse of the Estuary, 2008. Contaminants such as PCBs and pesticides accumulate to high concentrations at the top of the food web. Fish concentrations of several classes of contaminants are consistently well above thresholds of concern for health. For example, shiner perch, white croaker, and white sturgeon all typically exhibit total PCB (sum of congeners) concentrations above 100 ng/g ww, and sometimes well above 200 ng/g ww, as compared with a screening value of 10 ng/g ww (PCB TMDL of the San Francisco Bay Regional Water Quality Control Board). As average sediment PCB concentrations are between 5.7-8.7 ppb in recent years (Pulse of the Estuary, 2008), substantial bioaccumulation into fish tissues is indicated.

Despite the widely documented chemical contamination in San Francisco Bay, the effects of such contaminants on wildlife are not well understood. In the scientific literature, an increasing number of industrial, domestic and pharmaceutical chemicals are being found to significantly alter endocrine systems ("environmental endocrine disruption"). Such chemicals, classified as endocrine-disrupting compounds (EDCs), can therefore lead to disruptions in the regulation of physiology, leading to impaired homeostasis, adaptation, and ultimately health and survival (Mattheissen and Law, 2002; Matthiessen, 2003; Hotchkiss et al., 2008;

Wingfield, 2008). These issues have raised substantial concerns internationally, let alone regionally.

The endocrine system encompasses a wide variety of hormones, from steroids to peptides to others, and there are typically several organizational levels and components that comprise each endocrine "axis". Organizational levels may include hypothalamic (brain), pituitary, peripheral endocrine organ, target organs, and the respective set of molecules responsible for signal transduction at the cellular level. Therefore, many potential targets for disruption in endocrine systems exist. For a particular chemical to have an endocrine-disruptive effect (i.e., be classified as an EDC), it must directly or indirectly perturb an endocrine axis. This may occur by altering one or more specific components (e.g., expression of a receptor, steroidogenic enzyme activity, or competitive binding against a natural hormone) which leads to dysfunction of the whole axis. Thus, upon measurement of an endocrine disruption effect, which may initially entail measurements of blood plasma concentrations of a particular hormone, there will be several potential underlying targets implied for EDC actions. The initial hormone measurerment(s) may be a first step in the process of identifying endocrine disruption in an animal, which may then be followed by work to define how an EDC is acting (underlying mechanisms) and what kind of chemical(s) may be responsible.

In our prior work to screen for different types of endocrine disruption in San Francisco Bay fish, it became evident that the thyroid endocrine system was impacted in association with exposure of fish to PCBs and chlorinated pesticides (Kelley et al., 2009; Brar et al., 2010). The overall objective of the present study was to further characterize this form of endocrine disruption, in terms of evaluating its underlying causes and effects, in the same fish species in which the original work was done, shiner surfperch (*Cymatogaster aggregata*) and Pacific staghorn sculpin (*Leptocottus armatus*) (**Figure 1**).

Figure 1. Shiner surfperch (*Cymatogaster aggregata*; upper panel) and Pacific staghorn sculpin (*Leptocottus armatus*; lower panel) are indigenous species inhabiting inshore locations within San Francisco Bay.

INTRODUCTION –Thyroid Endocrine Disruption Study

The thyroid endocrine system has been increasingly identified as a target of EDCs in mammals, birds, and fish (Brucker-Davis, 1998; Khan et al., 2002; Ishihara et al., 2003; Crofton, 2008; Boas et al., 2006; Blanton and Specker, 2007). Since alterations in thyroid hormones can have important physiological and developmental effects in all vertebrates, abnormal function or disruption of the thyroid system presents a serious threat to health and survival. Published research is beginning to demonstrate that certain kinds of environmental contaminants derived from human activities (as described further below) can alter thyroid systems in wildlife (Brouwer et al., 1998; Brucker-Davis, 1998; Boas et al., 2006; Zoeller et al., 2007; Soldin et al., 2008; Brar et al., 2010).

The thyroid endocrine system is present throughout the vertebrates and it is vital for physiological homeostasis. Among the myriad of vertebrate hormones, thyroid hormones are distinct in that they exert their effects on virtually all tissues, with target receptors found in all cell types (Boas et al., 2006; Eales, 2006; Norris, 2007). In higher vertebrates, metabolism, growth, and development all depend upon normal thyroid hormone levels and regulation (Brouwer et al., 1998; Eales, 2006; Norris, 2007). In lower vertebrates, such as amphibians and fishes, thyroid hormones are known to play important roles as metamorphosis-inducing hormones (Power et al., 2001; Yamano, 2005; Shaio and Wang, 2006; Blanton and Specker, 2007). Thyroid hormones are produced upon activation of the neuroendocrine “hypothalamo-pituitary-thyroid” (HPT) axis, as illustrated in **Figure 2**.

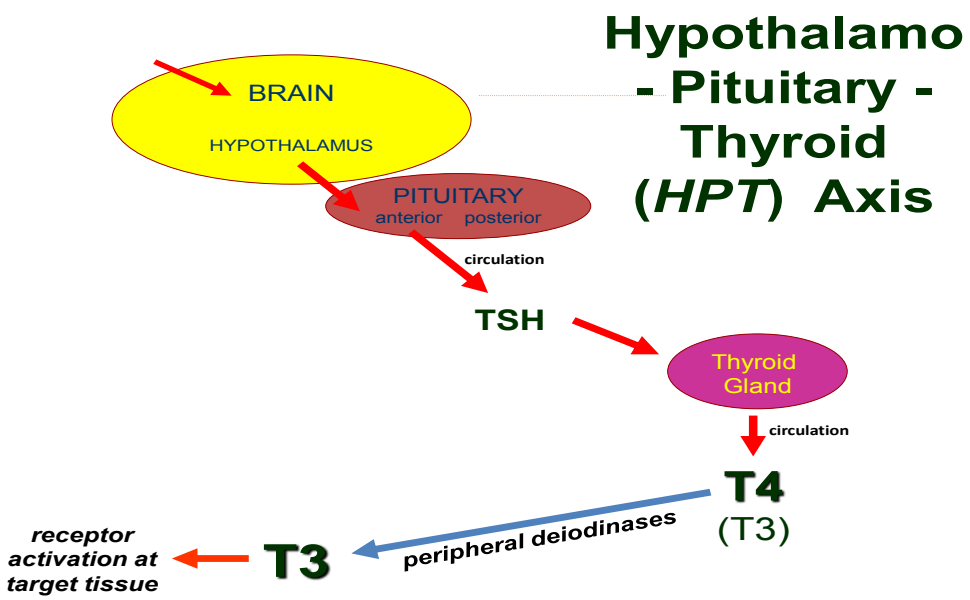
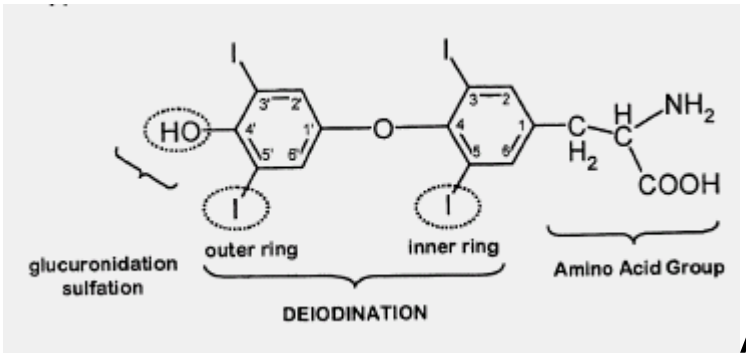


Figure 2. Chart illustrating the hypothalamo-pituitary-thyroid (HPT) axis in vertebrate animals including fish. Description of the organization of the HPT axis and its components is given in the text.

There are two principal thyroid hormones (**Figure 3**), thyroxine (**T4**; 3,5,3',5'-tetraiodo-L-thyronine) and triiodothyronine (**T3**; 3,5,3'-triiodo-L-thyroinine), which are produced and secreted into the bloodstream upon activation of the HPT axis. Under hypothalamic (brain) control, the pituitary secretes thyroid-stimulating hormone (TSH) into the blood circulation, which then proceeds to the thyroid gland where it activates the synthesis and release of T4 and T3. Unlike most other endocrine glands, the thyroid stores large supplies of hormone precursor, thyroglobulin (TG), in an extracellular ("colloid") lumen inside thyroid follicles (Griffin & Ojeda, 2004; Norris, 2007). As part of the synthetic process, the TG is iodinated. Follicular cells trap inorganic iodide (I^-) and convert it into iodine (I^o) using peroxide enzymes, and the I^o then rapidly substitutes one or two hydrogens on the tyrosyl rings of TG's tyrosine residues. This generates many diiodotyrosines (DIT) and some monoiodotyrosines (MIT) within the TG protein. Oxidative coupling of DIT with DIT, or DIT with MIT, generates T4 or T3 side-groups, respectively. In addition to stimulating TG gene expression, pituitary TSH also activates thyroid follicular cells to endocytose colloid lumen (containing iodinated TG), which is followed by fusion of the internalized vesicles with lysosomes and a resulting

hydrolysis of TG and release of T4 and T3. Both thyroid hormones then enter the blood circulation.

Although both thyroid hormones are produced, T4 represents 95% of the thyroid hormone output in fish, and it is typically present in higher quantities than T3 in the blood circulation. However, it is T3 that binds to thyroid hormone receptors with highest affinity and it is the more potent hormone (Gershengorn et al., 1979; Norris, 2007; Blanton and Specker, 2007). The higher concentrations of T4 in the circulation are generally considered to serve as a pool of prohormone that can be converted into T3 by target tissues (Griffin & Ojeda, 2004; Picard-Aitken et al., 2007; Zoeller et al., 2007). Conversion from T4 to T3 is accomplished through action of two iodothyronine deiodinases, type-1 (D1) or type-2 (D2), which remove the outer ring iodine (5' position, **Figure 3**). These 5'-deiodinases are primarily responsible for regulating the concentrations of T3 outside of the thyroid gland (Adams et al., 2000; Plohman et al., 2002; Shepherdley et al., 2002; Griffin & Ojeda, 2004; Norris, 2007). A third deiodinase (D3) may also act to prevent T3 formation by removing the inner ring iodine (at the 5-position) and generating the inactive form, reverse T3 (rT3). In the present study, the following thyroid endocrine parameters were measured in different tests or experiments, enabling a multi-component evaluation of thyroid endocrine status in the fish: T4 concentration, T3 concentration, total thyroid hormone (total TH) concentration, T3/T4 ratio, and hepatic 5'-deiodinase activity.



A

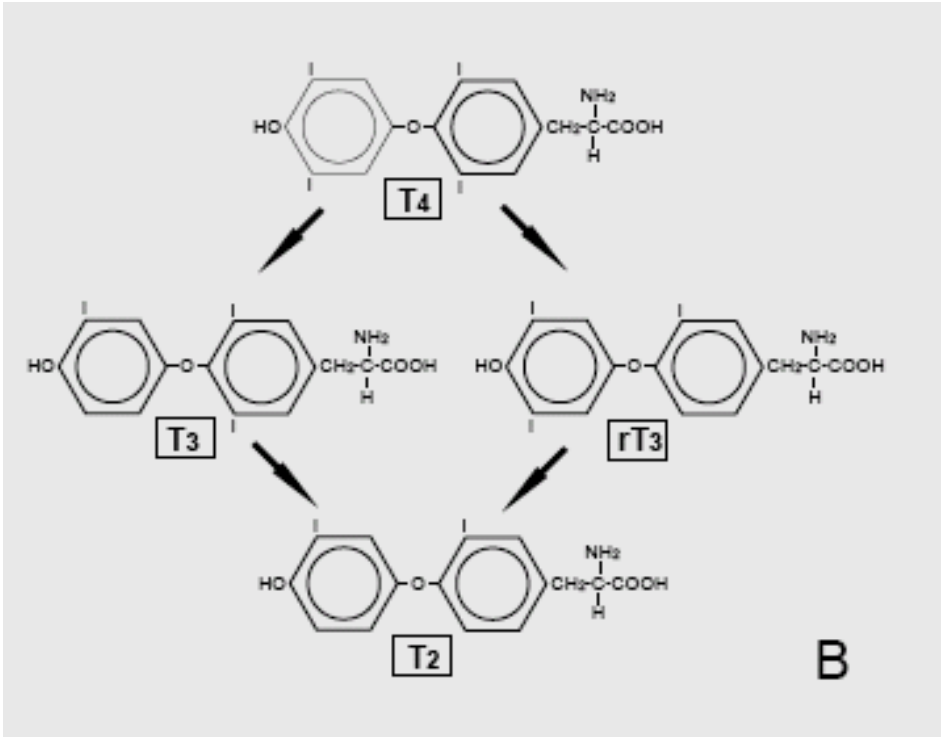


Figure 3. Thyroid hormones and thyronine deiodinases. Panel A shows the molecular structure of thyroxine (T4); removal of the 5' (outer ring) iodine generates T3. Panel B shows the possible deiodinase conversions: type-1 (D1) and type-2 (D2) deiodinases, present in a wide variety of tissues, remove the 5' iodine, generating active T3 hormone. Inner ring deiodinase (D3) may also serve to inactivate the thyroid hormone signal by generating rT3. The doubly deiodinated form (T2) is also inactive.

As in mammals, thyroid hormones are essential for early development in fishes, including larval-juvenile transitions and the induction of metamorphosis in flatfish (Miwa and Inui, 1987; Inui et al., 1989; Yamano and Miwa, 1998; Power et al., 2001; Yamano, 2005; Shaio and Wang, 2006; Schnitzler et al., 2008; Klaren et al., 2008). Thyroid hormones are also deposited into the yolk of fish eggs, and are used during subsequent embryonic development

(Kobuke et al., 2987; Leatherland, 1989; Power et al., 2001; Yamano, 2005; Schnitzler et al., 2008). In juvenile and adult life, thyroid hormones are necessary (permissive) for normal somatic growth and they have a number of effects on growth endocrine genes, such as pituitary growth hormone (GH), GH receptors, and hepatic IGF-I synthesis (e.g., Power et al., 2001; Plohman et al., 2002; Rousseau et al., 2002; Schmid et al., 2003). However, there is no evidence for metabolic actions of thyroid hormones in fish comparable to those defined in mammals (van Ginniken et al., 2007).

EDC effects on the HPT axis are increasingly being documented in laboratory tests and experiments (Brouwer et al., 1998; Khan et al., 2002; Brown et al., 2004; Boas et al., 2006; LeRoy et al., 2006; Crofton, 2008; Soldin et al., 2008). Given the multi-level and -component neuroendocrine HPT axis, there are a large number of potential target genes or proteins that may be altered by an EDC(s). Such effects could range from agonistic to antagonistic actions on target tissue thyroid hormone receptors, to alterations in thyroid hormone synthetic pathways within the thyroid, to deiodinase functions in peripheral tissues, or carrying proteins in the blood (Griffin & Ojeda, 2004; Boas et al., 2006; Yang et al., 2006; Gauger et al., 2007). PCBs, dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD), chlorinated pesticides, polybrominated diphenyl ethers (PBDEs), and metals such as mercury, have all been implicated as thyroid-disruptive agents when tested experimentally in animals (Adams et al., 2000; Brown et al., 2004; Boas et al., 2006; Darnerud et al., 2001; Hallgren et al., 2001; Zhou et al., 2001; Hallgren et al., 2002; Khan et al., 2002; LeRoy et al., 2006; Soldin et al., 2008). However, current knowledge is limited mostly to laboratory testing of a small subset of chemicals that can be present in human-impacted environments.

In our prior work on shiner perch and Pacific staghorn sculpin, we documented significant alterations in circulating T4 and T3 concentrations associated with different field sites tested in the San Francisco Bay. These findings were recently published (Brar et al., 2010). However, further studies were needed that would more closely characterize the underlying mechanisms responsible for the endocrine disrupted condition in the fish. In addition, it was of interest to evaluate the possible relationship between altered thyroid status and changes in the regulation growth.

The first objective of this project was to screen different San Francisco Bay sites for the incidence and magnitude of thyroid disruption in fish, based upon plasma measures of T3 and T4. Given that our former project (Kelley et al., 2009; SFEI project #701) had completed T4 measurements on fish sampled in 2007 from 11 different locations, and that interpretation of such results would be facilitated by adding data on T3 concentrations, we reported the T3 data generated under the current project in the Final Report of Project #701. That final report also addressed the relationships between contaminant exposures and thyroid endocrine parameters, and a paper was recently published by Brar et al. (2010) describing the overall findings.

Based upon the above findings, study locations in San Francisco Bay were chosen for a second phase of research. In addition, groups of archived samples were evaluated for new parameters, in order to take advantage of the multiple endocrine and contaminant measures already taken on these individuals. Therefore, in both experimental strategies, fish groups with significantly altered thyroid hormone status (or fish expected to exhibit thyroid disruption at a particular field location) were compared with normal fish from reference locations. In the field-based studies, fish were evaluated for the capability of their thyroid to synthesize thyroid hormones and the degree to which peripheral deiodinase activity was altered, both of which were implicated by our earlier findings (Brar et al., 2010). In addition, the changes in these HPT components were evaluated for their relationships to contaminant exposures, while thyroid status was evaluated for its relationship to the growth regulator, insulin-like growth factor-I (IGF-I; Kelley et al., 2002; 2006). The results of these studies provided novel insight into the mechanisms by which thyroid disruption is occurring in San Francisco Bay fish.

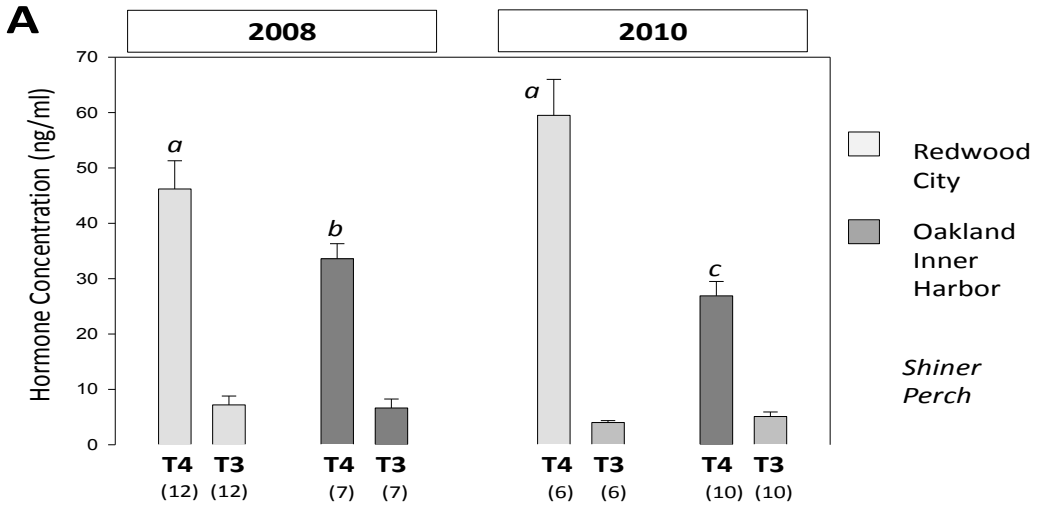
RESULTS and DISCUSSION

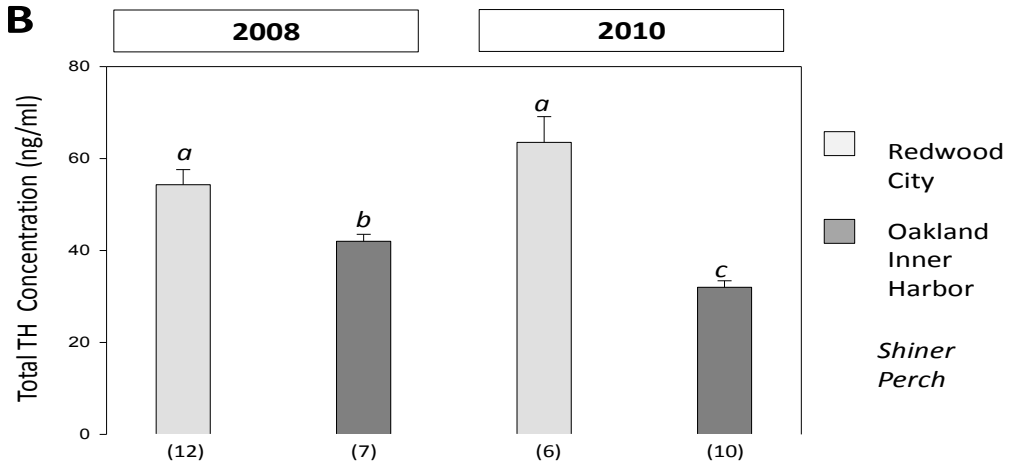
As stated above, we determined that thyroid endocrine status was significantly disrupted in San Francisco Bay fish in a spatially distinct manner. As part of these findings, it was established that fish from Redwood City had plasma concentrations of T4 and T3 that were comparable to levels in reference fish with very low contaminant exposures sampled from a remote location on the western side of Catalina Island. As a result of these findings, which were consistent over two years of study, we chose the Redwood City location an "in-bay" reference site in which functionality of the thyroid endocrine system appears to be normal. Other published works (Khan et al., 2002; LeRoy et al., 2006) and the results of the present study also support these conclusions.

In contrast, fish sampled from Oakland Inner Harbor have consistently exhibited significantly altered thyroid endocrine status in association with high contaminant exposures. One of the more notable findings reported in Brar et al. (2010) was that tissue concentrations of selected congeners of PCBs were significantly correlated with thyroid endocrine parameters. While T4 levels were consistently depressed when tissue PCB concentrations were elevated ($R = -0.40$ to -0.65, $p=0.05$ to 0.001), T3 concentrations were consistently increased with increasing exposures to PCBs ($R = 0.41$ to 0.67, $p=0.05$ to 0.0001). These findings pointed to two possible effects on the thyroid endocrine system: ***1)*** reduced thyroid gland synthetic capability (production of T4) and ***2)*** possible interference in the peripheral conversion of T4 into T3 (i.e., effects on 5'-deiodinase activity). In the work reported herein, we set out to characterize the extent to which one or both of these potential defects in the HPT axis were operative in thyroid-disrupted fish.

In two additional years of field-based studies, we consistently observed that shiner perch had significantly reduced plasma T4 concentrations when sampled from Oakland Inner Harbor as compared with Redwood City (**Figure 4A**), in agreement with earlier studies (Brar et al., 2010). Total plasma TH (T4 + T3 concentrations) exhibited the same spatial pattern (**Fig. 4B**), indicative of reduced thyroid output of T4. The T3/T4 ratio indicated relatively higher plasma T3 concentrations in Oakland perch both years, which was more pronounced ($p<0.01$) in the 2010 study (**Fig. 4C**). While increased T3/T4 ratio may serve as an indicator

of potentially enhanced peripheral 5'-deiodinase activity, the enhanced ratios in the Oakland perch appeared to be due mostly to reduced T4 concentrations (reduced thyroid production?). In accordance, T3 concentrations were not different in fish from either location (**Fig. 4A**)





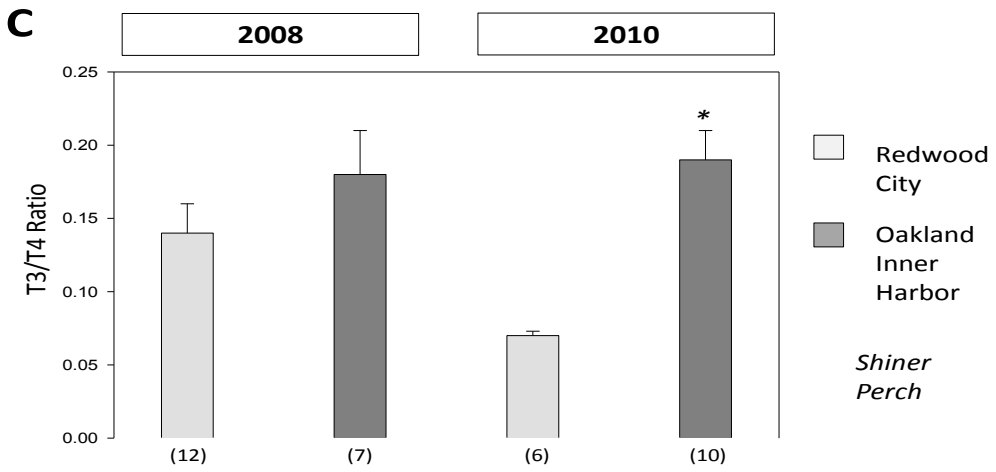
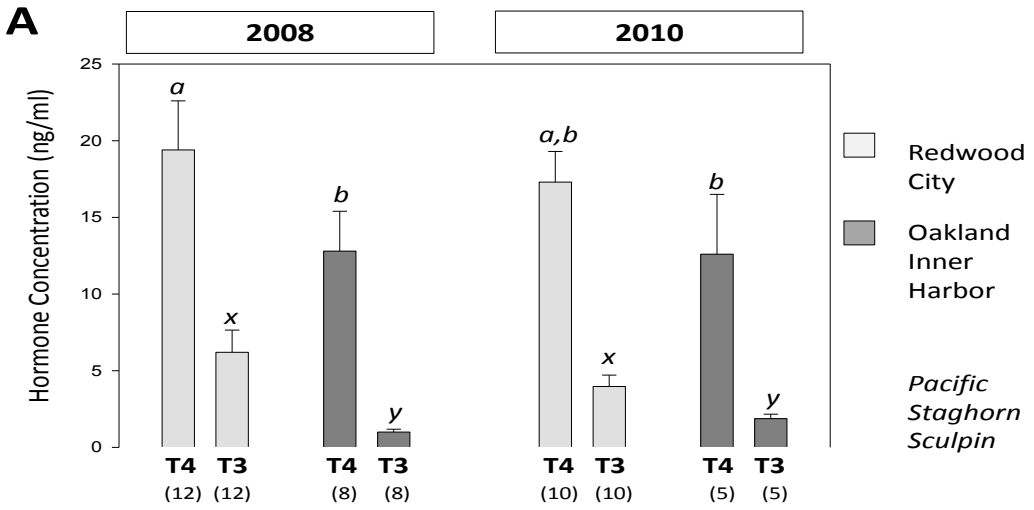


Figure 4. Plasma concentrations of T4 and T3 (panel **A**), total TH concentrations (panel **B**), and T3/T4 ratio (panel **C**) in shiner perch sampled from Redwood City (light bars) and Oakland Inner Harbor (dark bars) in two years of study. Mean ± SEM values are given, with number of samples (n) shown in parentheses under each bar. ^{a,b,c}Superscripts denote significantly different values ($p<0.05$); *asterisk in panel C denotes significant difference as compared with group from Redwood City in 2010 ($p<0.01$).

Pacific staghorn sculpin exhibited similar differences in plasma T4 concentrations and total TH concentrations as those observed in the perch (**Figure 5**). Total TH concentrations were significantly reduced in the sculpin from Oakland Inner Harbor as compared with the Redwood City sculpin in both years (**Fig. 5B**). The same pattern was observed in plasma T4 concentrations in the 2008 study (**Fig. 5A**); in 2010, however, T4 was not significantly different, but did tend to be reduced in the Oakland fish. In contrast to shiner perch, the sculpin exhibited reduced T3 concentrations in Oakland in both years (**Fig. 5A**) and this was reflected in T3/T4 ratios, which were significantly lower in the Oakland fish in both years (**Fig. 5C**). Therefore, in addition to reduced thyroid hormones overall (as in shiner perch), peripheral 5'-deiodinase activity may also be reduced in sculpin residing at the Oakland Inner Harbor location.


A
2008
2010
Hormone Concentration (ng/ml)
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x
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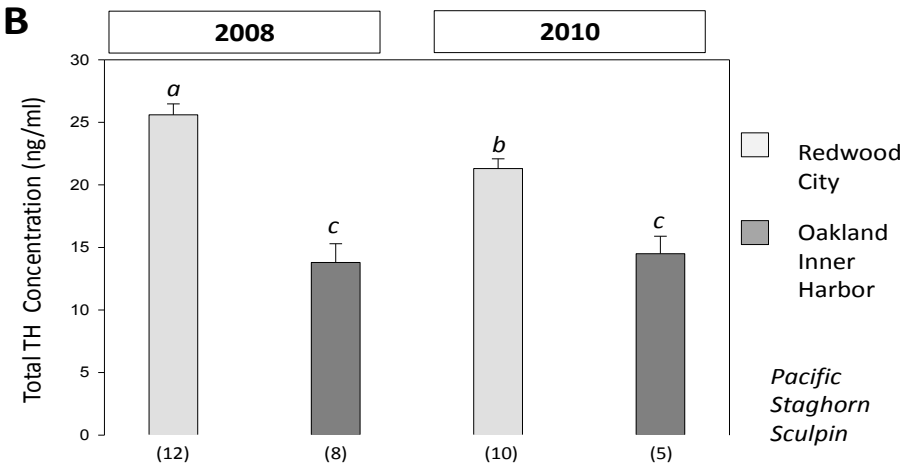
Redwood City
Oakland Inner Harbor
Pacific Staghorn Sculpin


B
2008
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Total TH Concentration (ng/ml)
30
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Redwood City
Oakland Inner Harbor
Pacific Staghorn Sculpin

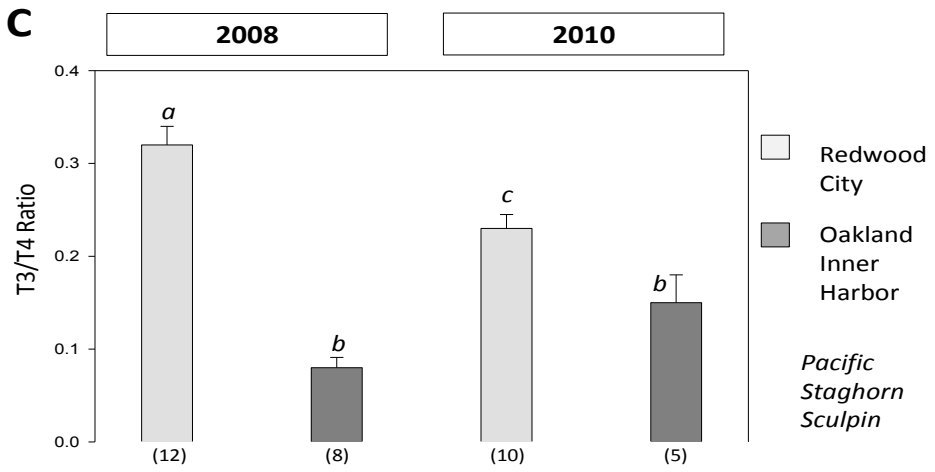


Figure 5. Plasma concentrations of T4 and T3 (panel **A**), total TH concentrations (panel **B**), and T3/T4 ratio (panel **C**) in Pacific staghorn sculpin sampled from Redwood City (light bars) and Oakland Inner Harbor (dark bars) in two years of study. Mean ± SEM values are given, with number of samples (n) shown in parentheses under each bar. ^{a,b,c}Superscripts or ^{x,y}superscripts denote significantly different values ($p<0.05$).

Given the putative disruption of thyroid hormone synthetic output (reduced T4 and total TH) in Oakland Inner Harbor fish, it was of interest to test whether the thyroid gland of impacted fish could exhibit an ability to synthesize and release T4. To test this ability, fish thyroid output was challenged by treating with the pituitary hormone, TSH (see Introduction). The action of TSH is highly specific, in activating the production and release of T4, which occurs specifically within thyroid glandular follicles (Inui et al., 1989; Blanton and Specker, 2007; Norris, 2007). As discussed earlier, the principal thyroid hormone produced (>95%) by the fish thyroid is T4, whereas T3 is produced peripherally as a result of 5'deiodinase conversion of T4 into T3, which occurs in target tissues including the liver.

In experiments undertaken in 2008, shiner perch collected from the Redwood City location were injected with TSH at a dose of 0.5 µg/g body weight, and exhibited a robust, 9.5-fold increase in plasma T4 concentrations after 2 hr as compared with saline-injected controls

(p<0.001; **Figure 6A**). This response in T4 was associated with a 4-fold increase in plasma T3 concentrations in the same animals (p<0.01; **Fig. 6B**). In contrast, when shiner perch from Oakland Inner Harbor were identically tested, their response to TSH was absent, both with respect to T4 as well as T3 concentrations. In an identical experimental design carried out in 2010, the results were highly consistent. While TSH injections elicited a 7.5-fold increase in plasma T4 in fish from Redwood City (p<0.001 vs. saline-injected controls), the response was substantially diminished in the perch from Oakland Inner Harbor (**Figure 7A**). The response in plasma T3 concentration was also similar to that observed in the 2008 experiments, with a 4-fold increase in TSH-treated fish from Redwood City (p<0.05) but no response in the fish from Oakland Inner Harbor (**Figure 7B**). Therefore, in shiner perch, the ability of the thyroid gland to respond to TSH was significantly impaired in the thyroid-disrupted fish from Oakland Inner Harbor, an effect observed in two separate years of study.

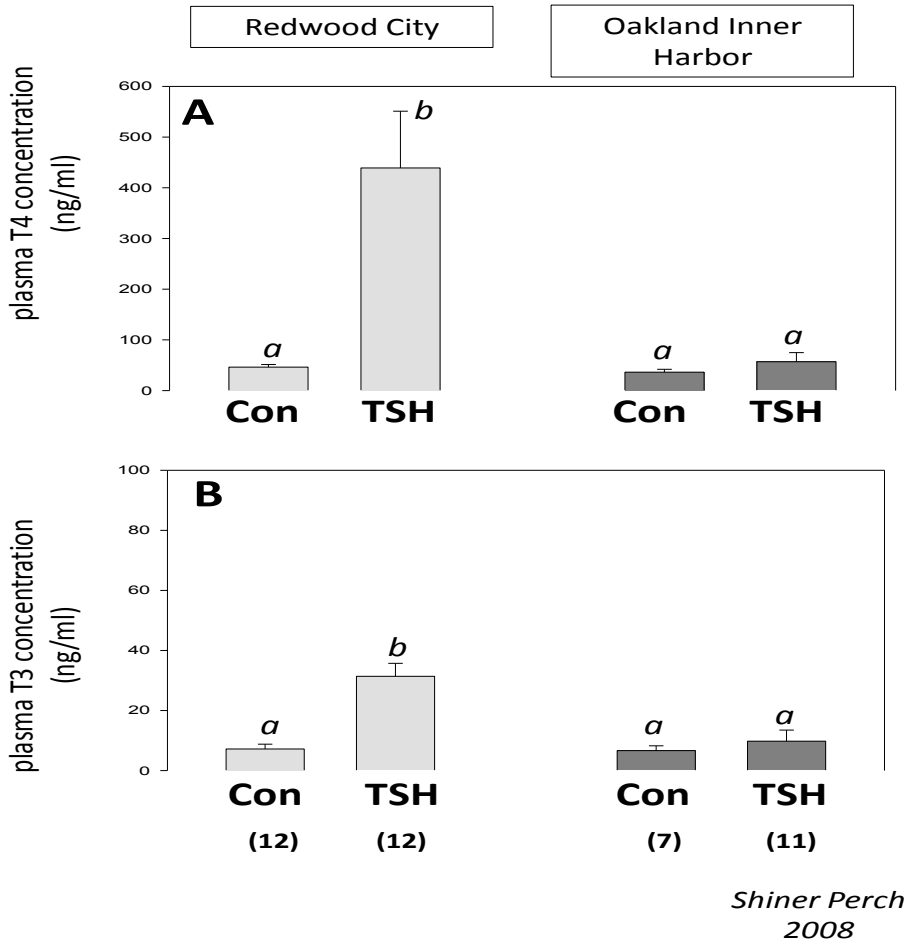


Figure 6. Plasma concentrations of T4 (panel **A**) and T3 (panel **B**) in shiner perch injected with saline (control) or pituitary TSH. Fish groups from Redwood City (light bars) and Oakland Inner

Harbor (dark bars) in 2008 were compared for their responsiveness to TSH. Mean ± SEM values are given, with number of samples (n) shown in parentheses under the graphs. [a,b]Superscripts denote significantly different values ($p<0.05$).

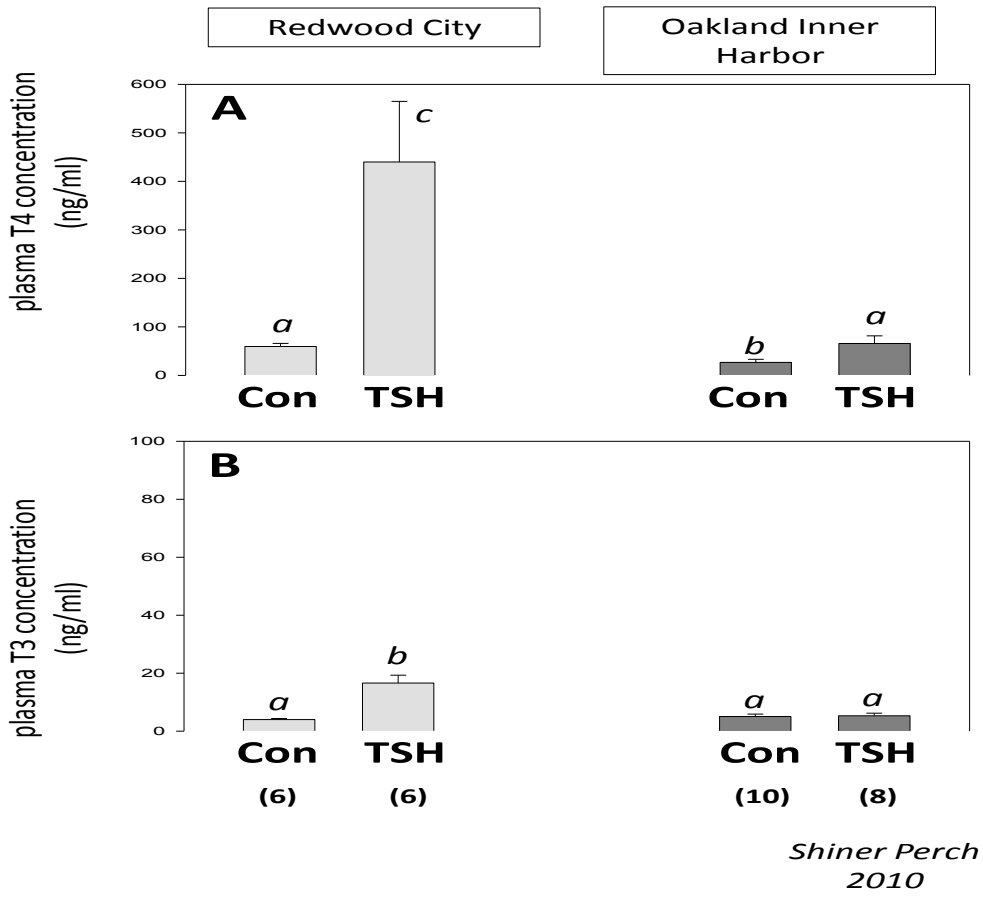


Figure 7. Plasma concentrations of T4 (panel **A**) and T3 (panel **B**) in shiner perch injected with saline (control) or pituitary TSH. Fish groups from Redwood City (light bars) and Oakland Inner Harbor (dark bars) in 2010 were compared for their responsiveness to TSH. Mean ± SEM values are given, with number of samples (n) shown in parentheses under the graphs. [a,b,c]Superscripts denote significantly different values ($p<0.05$).

Pacific staghorn sculpin did not show the same impaired thyroid responsiveness. Although both species exhibited reduced T4 concentrations in association with Oakland, the sculpin from both locations responded significantly to TSH treatments, exhibiting between 3- to 6-fold increases in T4 ($p<0.01$) in both the 2008 (**Figure 8**) and 2010 (**Figure 9**) studies. Plasma T3 also exhibited increased concentrations in response to TSH in both years (**Figures 8B** and **9B**), consistent with the increasing T4 concentrations in the same fish. Therefore, the nature of the thyroid disruption between the two species appears to be distinct, with shiner

perch exhibiting an impairment in thyroid synthetic responsivenss that is not existent in the Pacific staghorn sculpin.

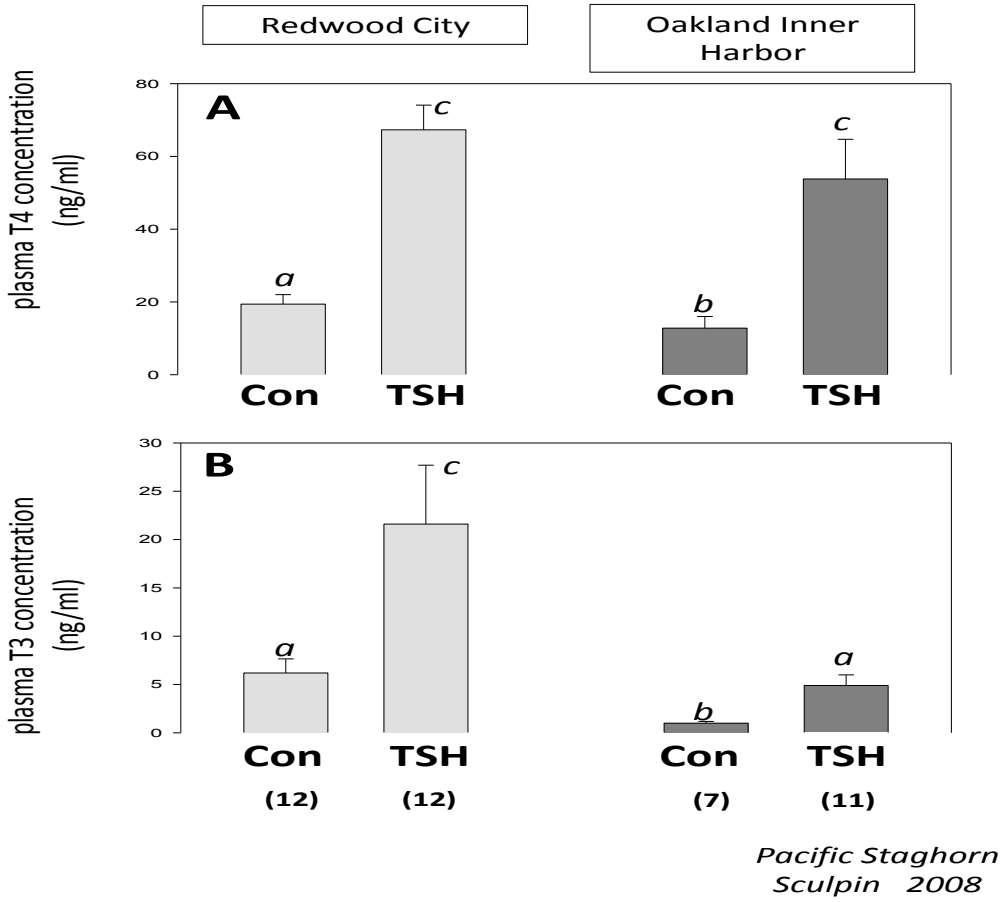


Figure 8. Plasma concentrations of T4 (panel **A**) and T3 (panel **B**) in Pacific staghorn sculpin injected with saline (control) or pituitary TSH. Fish groups from Redwood City (light bars) and Oakland Inner Harbor (dark bars) in 2008 were compared for their responsiveness to TSH. Mean ± SEM values are given, with number of samples (n) shown in parentheses under the graphs. [a,b,c]Superscripts denote significantly different values ($p<0.05$).

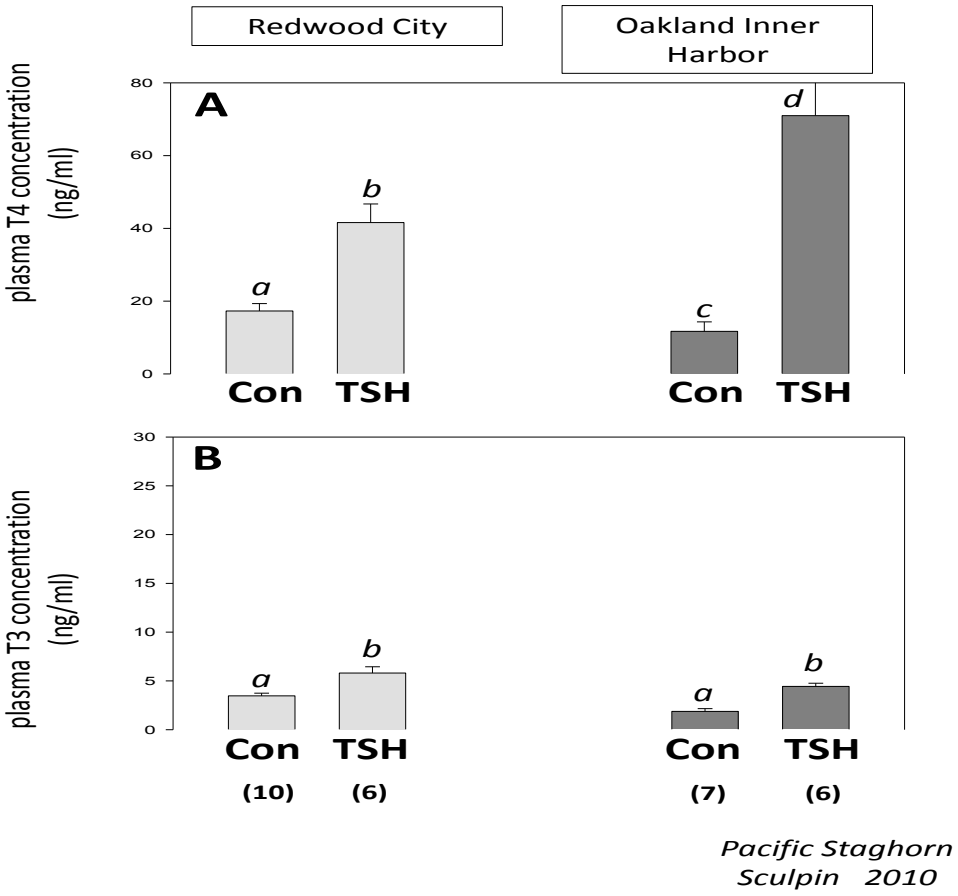


Figure 9. Plasma concentrations of T4 (panel **A**) and T3 (panel **B**) in Pacific staghorn sculpin injected with saline (control) or pituitary TSH. Fish groups from Redwood City (light bars) and Oakland Inner Harbor (dark bars) in 2010 were compared for their responsiveness to TSH. Mean ± SEM values are given, with number of samples (n) shown in parentheses under the graphs. [a,b,c,d]Superscripts denote significantly different values ($p<0.05$).

In 2010, additional locations in the south Bay were screened for potential thyroid disruptive effects in fish, including Guadalupe River and Coyote Creek. These sites were chosen for the potential that fish from this area may experience higher exposures to environmental mercury. In the event that thyroid disruption was evident based upon T4 and T3 measurements, tissue samples were saved from these animals for potential measurement of methyl-mercury by our collaborators (www.IIRMES.org). It was only possible to collect Pacific staghorn sculpin from these sites (likely due to reduced salinity avoided by shiner perch); furthermore, only 10 scuplin could be collected from the Guadalupe River, and so the TSH-injection experiments were not carried out for this group. As shown in **Figure 10**, sculpin from both Guadalupe River and Coyote Creek exhibited plasma T4 and T3

concentrations comparable to those seen in sculpin from reference locations (e.g., Redwood City, Catalina Island). Furthermore, sculpin from Coyote Creek exhibited a thyroid response to TSH challenge similar to that in sculpin from Redwood City. Therefore, we did not observe any anomalies in sculpin from the south Bay and at present do not plan to pursue measurements for mercury in these fish. In addition, we made some preliminary measurements of T4 and T3 in another estuarine fish, the longjaw mudsucker (*Gillichthys mirabilis*), that were captured both in the Guadalupe River and in Oakland Inner Harbor (**Figure 10**). While the total number of samples was small, the data suggest that this species has comparatively low T4 concentrations, particularly when sampled from the Guadalupe River, although further work is needed to determine this.

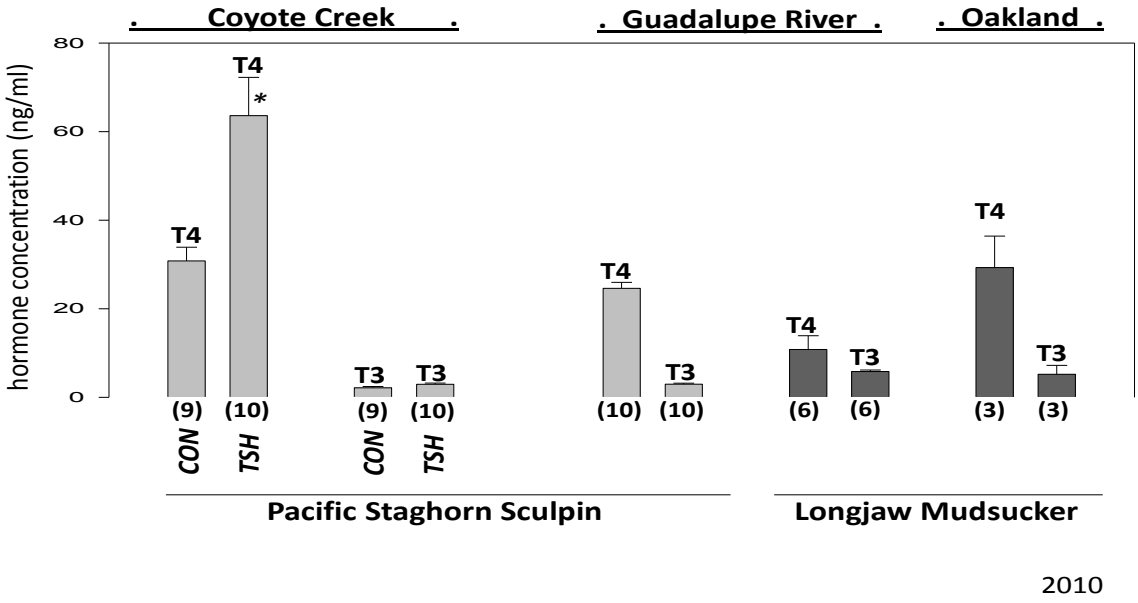
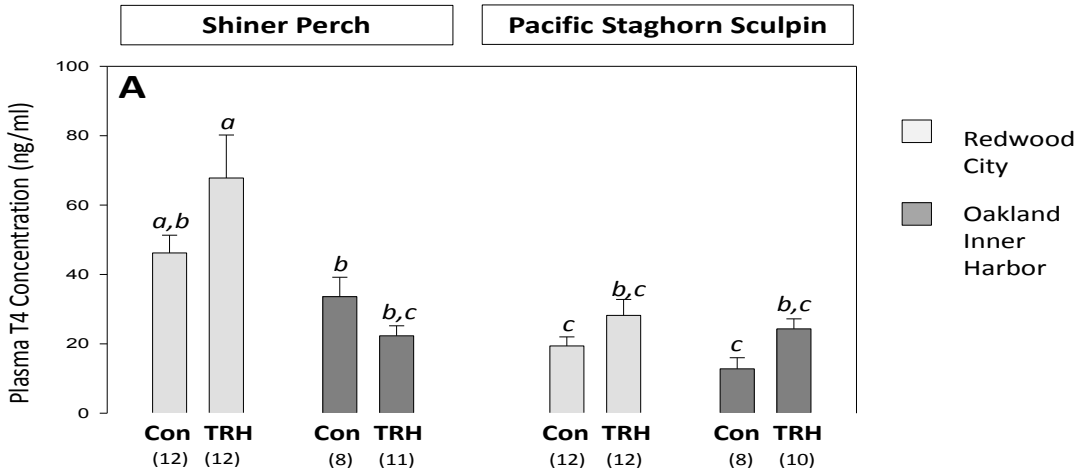


Figure 10. Plasma concentrations of T4 and T3 in Pacific staghorn sculpin sampled at Coyote Creek and Guadalupe River. Scuplin from Coyote Creek were injected with saline (CON) or pituitary TSH. A second fish species, the longjaw mudsucker (*Gillichthys mirabilis*; dark bars), was collected from Guadalupe River and Oakland Inner Harbor and measured for plasma T4 and T3 concentration. Mean ± SEM values are given, with number of samples (n) shown in parentheses under each bar.

Although in fishes the hypothalamic peptide, TRH (thyrotropin-releasing hormone), is not known to play a role in regulating pituitary TSH release, as in most other vertebrates, studies indicate that TRH peptide has another action in the HPT axis, in activating peripheral 5'-

deiodinase activity to increase T3 concentrations (Blanton and Specker, 2007; Zoeller et al., 2007). Given the observed differences in T3/T4 ratio in shiner perch and Pacific staghorn sculpin (**Figs. 4C** & **5C**), differences in deiodinase activity were suggested. Therefore, an additional set of experiments was carried out in order to test whether TRH peptide exerted an effect on T4 and/or T3 levels in the fish, and whether any response to TRH may be altered in association with the impaired HPT axis in fish from the Oakland Inner Harbor location. Treatment of either species from either location with 2 µg/g TRH did not significantly alter plasma T4 concentrations, but it did significantly increase T3 concentrations by >2-fold (**Figure 11**), in accordance with reported TRH actions on deiodinase. Even when T3 levels were relatively low (sculpin in Oakland), TRH still elicited a 2-fold increase in T3 ($p<0.05$; **Fig. 11B**). Therefore, the effect of TRH was consistent in fish from both the Redwood City and Oakland Harbor locations, and indicated that this component of the HPT axis was not altered in the thyroid-disrupted fish.



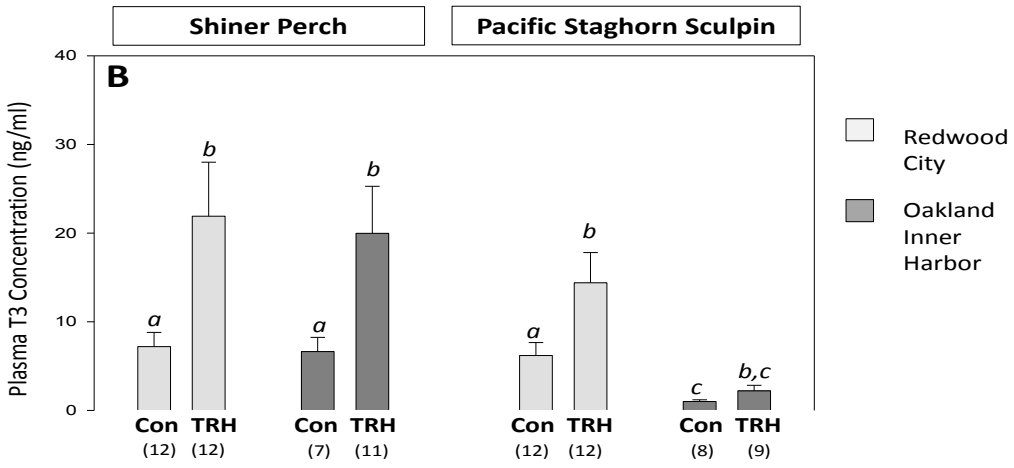


Figure 11. Plasma concentrations of T4 (panel A) and T3 (panel B) in shiner perch and Pacific staghorn sculpin injected with saline (control) or TRH peptide. Fish groups from Redwood City (light bars) and Oakland Inner Harbor (dark bars) were compared for their responsiveness to TSH peptide. Mean ± SEM values are given, with number of samples (n) shown in parentheses under each bar. [a,b,c,d]Superscripts denote significantly different values ($p<0.05$).

In the shiner perch sampled in the field in 2010 (but not in 2008), the T3/T4 ratio was 2.8-fold greater in fish from Oakland Inner Harbor as compared with Redwood City (**Figure 4C**). While this may suggest an increase in peripheral 5'-deiodinase activity (yielding a greater proportion of T3 in the blood plasma), it was also noted that the Oakland fish had corresponding decreases in plasma T4 and total TH concentrations (**Figs. 4A & 4B**, respectively). Therefore, reduced thyroid output was implicated as a primary underlying mechanism causing this effect. In Pacific staghorn sculpin, on the other hand, T3/T4 ratio exhibited an opposite response in fish from Oakland Inner Harbor in both years: reduced T3/T4 ratio (**Fig. 5C**). This suggests that peripheral 5'-deiodinase activity may be altered (reduced?) in sculpin from the Oakland Inner Harbor, and it was demonstrated above that the thyroid was not impaired in its synthetic responsiveness. It was therefore of interest to determine whether differences in 5'-deiodinase activity existed between these groups, which may account for the differences in T3/T4 ratio and identify whether this component of the HPT axis is involved in the thyroid endocrine disruption. In addition, it was of interest to ascertain whether deiodinase activity may be altered in association with other San Francisco Bay locations.

Based on procedures described by Leatherland et al. (1990) and Leatherland and Farbridge (1992), an assay to measure hepatic 5'-deiodinase was established and validated in 2009 for use in these studies (further described in Methodology section). In the fish collected in 2010 from Redwood City and Oakland Inner Harbor, liver samples were tested for 5'-deiodinase activity (**Figure 12**). In shiner perch, there was no significant difference hepatic 5'-deiodinase activity between the two groups, in accordance with the earlier data indicating that perch from Oakland had reduced thyroid synthetic output but no alteration in conversion of T4 into T3. In Pacific staghorn sculpin, on the other hand, hepatic 5'-deiodinase activity was significantly depressed in fish from the Oakland Inner Harbor as compared with Redwood City ($p<0.01$); this result is consistent with the reduced T3/T4 ratio observed at this location, and suggests an environmental factor(s) affects peripheral 5'-deiodinase activity.

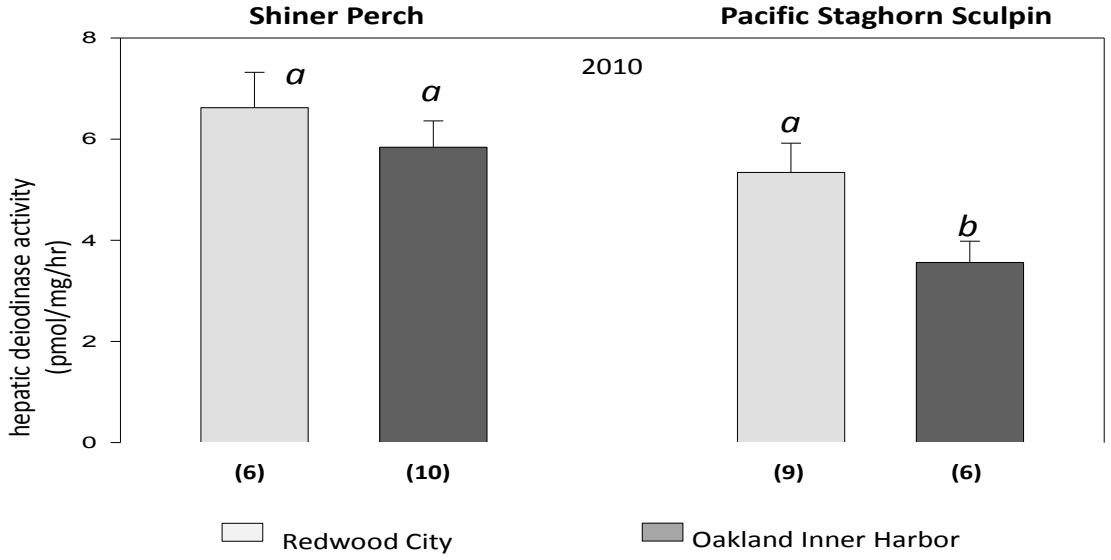


Figure 12. Hepatic 5'-deiodinase activity in shiner perch (left side) and Pacific staghorn sculpin (right side) sampled from Redwood City (light bars) and Oakland Inner Harbor (dark bars). Mean ± SEM values are given, with number of samples (n) shown in parentheses under each bar. [a,b]Superscripts denote significantly different values ($p<0.05$).

Given the establishment of the 5'-deiodinase assay for these studies and the availability of some liver samples remaining from shiner perch sampled in 2007 (Kelley et al., 2009), it was of interest to determine whether differences in deiodinase activity could be detected in groups that exhibited differences in T4, T3 and T3/T4 ratio. From these experiments, it was determined that hepatic deiodinase activity was not significantly altered in shiner perch from Oakland Inner Harbor, San Pablo Bay or Richmond Lauritzen Channel (DDT superfund site), as compared with perch sampled from the "reference" locations, Catalina Island and Redwood City (**Figure 13**), in agreement with the 2010 data shown in Figure 12. In contrast, shiner perch sampled in San Leandro Bay exhibited >2.5-fold higher hepatic 5'-deiodinase activity, which was significantly greater than that in the San Pablo Bay group ($p<0.01$) and which tended to be higher than in all other groups. This finding is notable, since T3/T4 ratio was >2-fold higher in perch from San Leandro Bay as compared with San Pablo Bay and Oakland Inner Harbor (Brar et al., 2010).

Among the individuals in which T4, T3, T3/T4 ratio and deiodinase activity were all measured, a highly significant positive correlation between deiodinase activity and T3/T4 ratio was observed($R=0.94$; **Table 1**). Consistent with this finding was an inverse correlation between T4 and deiodinase and a non-significant trend of increased T3 with increasing deiodinase. Thus, T3/T4 ratio appears to have value as an indicator of peripheral deiodinase activity. However, the lack of altered hepatic deiodinase activity in the Redwood City perch (**Figs. 12** & **13**), despite altered T3/T4 ratios, suggests that other factors beyond deiodinase may play a role in altering T3/T4 ratio in this species, such as reduced thyroidal T4 output as proposed earlier. Use of T3/T4 ratio as an indicator of deiodinase activity can be improved by additionally evaluating total TH, which provides a control for assessing the effects of thyroidal output in influencing T3/T4 values.

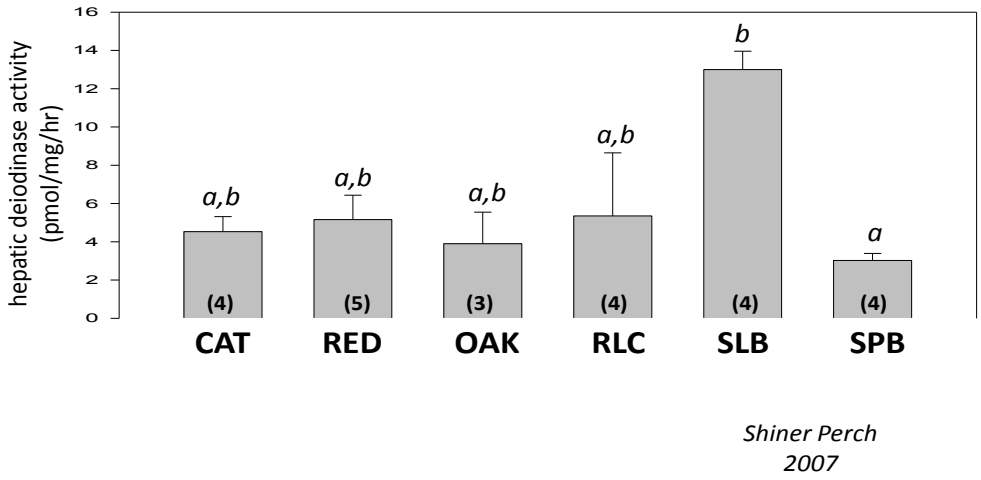


Figure 13. Hepatic 5'-deiodinase activity in shiner perch sampled in 2007 from Catalina Island (CAT), Redwood City (RED), Oakland Inner Harbor (OAK), Richmond Lauritzen Channel (RLC), San Leandro Bay (SLB), and San Pablo Bay (SPB). Mean ± SEM values are given, with number of samples (n) shown in parentheses inside each bar. [a,b]Superscripts denote significantly different values ($p<0.05$).

Table 1. Relationships between thyroid endocrine parameters among shiner perch sampled in 2007 (Brar et al., 2010). Plasma concentrations of T4 and T3, T3/T4 ratio, and hepatic deiodinase activity were evaluated using Pearson correlation tests. Correlation coefficients (R) followed by their significance values (p) are given in each cell; significant values are in bold font. For correlations among T4, T3 and T3/T4 ratio, n=28; for correlations with deiodinase activity, n=9.

	T4	T3	T3/T4
T3	-0.15, p=0.46	--	--
T3/T4	**-0.67**, p=0.0001	**0.55**, p=0.003	--
Deiodinase	**-0.69**, p=0.041	0.55, p=0.13	**0.94**, p=0.0001

The shiner perch from the 2007 study that were measured for hepatic 5'-deiodinase activity and measured for concentrations of pesticides, PAHs, and PCBs (n=9) made it possible to evaluate the relationships existing between deiodinase activity and exposure of the animals to the different contaminants. As reported in Brar et al. (2010), chlordanes and DDT/DDT metabolites exhibited significant correlations with thyroid endocrine parameters in shiner perch; the chlordanes, in particular, exhibited strongly positive correlations with T3/T4 ratio (R=0.62-0.75). In the present work, there were no relationships detected between hepatic 5'-deiodinase activity and DDT or its metabolites (**Table 2**). However, significant positive correlations between gamma chlordane (R=0.61) and cis-nonachlor (R=0.69) were identified. In addition, alpha chlordane and trans-nonachlor showed consistent, non-significant trends. Therefore, data on T3/T4 ratio (Brar et al., 2010) and the present data on hepatic deiodinase activity, both implicate chlordanes as EDCs in the San Francisco Bay environment that may lead to altered peripheral deiodinase activity.

Hepatic 5'-deiodinase activity was also significantly correlated with exposures to PCBs (Table 2). Among the PCB congeners that were previously found to be correlated with T3/T4 ratio (R=0.40-0.50; Brar et al., 2010), PCB 097 was significantly correlated with deiodinase activity (R=0.60, $p<0.01$) while the others (PCBs 99, 101, 105, 110, and 118) exhibited non-significant positive relationships with deiodinase (**Table 2**). In the present study, PCB 087 emerged as a congener that was strongly related to hepatic deiodinase activity (R=0.82, $p<0.01$; **Table 2**); this congener exhibited a non-significant positive relationship with T3/T4 ratio (R=0.33, p=0.07) and was negatively correlated with T4 (R= -0.46, $p<0.01$; Brar et al., 2010). These data support the conclusion that PCBs are related to thyroid endocrine disruption in shiner perch, and point to altered hepatic deiodinase as a possible mechanism of action.

Finally, a significant negative correlation was observed between hepatic 5'-deiodinase activity and phenanthrene (**Table 2**). This PAH was previously found to be correlated with T3, but not T4 or T3/T4 ratio (Brar et al., 2010). This finding points to a possible link between environmental hydrocarbons and thyroid endocrine functions.

Unfortunately, it was not possible to evaluate hepatic 5'-deiodinase in the Pacific staghorn sculpin sampled in 2007, due to a lack of liver samples remaining from the study. Given the strong relationships already established between thyroid endocrine parameters and chlordanes and PCBs in this species (Brar et al., 2010), it would appear likely that findings consistent with those in shiner perch would be demonstrated.

Table 2: Relationships between hepatic 5'-deiodinase activity and concentrations of pesticides, polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in shiner perch sampled in 2007. Correlation coefficients (R) followed by their significance values (p) are given in each cell (n=9). R and p-values below are for chemicals previously shown to exhibit significant correlation with T4, T3 or T3/T4 ratio; *asterisk indicates PCB congeners that exhibited significant correlation with T3/T4 ratio (Brar et al., 2010). †Daggar symbol identifies dioxin-like PCB congeners. Full list of contaminant chemicals measured are provided in Appendix B.

	5'-deiodinase			**5'-deiodinase**
PESTICIDES:	**R, p-value**		**PCBs:**	**R, p-value**
gamma chlordane	**0.61**, p=0.048		028	0.20, p=0.61
alpha chlordane	0.56, p=0.115		031	0.50, p=0.18
trans-nonachlor	0.35, p=0.36		044	0.54, p=0.14
cis-nonachlor	**0.69**, p=0.039		049	0.45, p=0.23
4,4-DDE	-0.29, p=0.45		087	**0.82**, p=0.007
2,4-DDD	-0.31, p=0.41		097*	**0.60**, p=0.009
4,4-DDD	-0.28, p=0.46		099*	0.43, p=0.25
4,4-DDT	-0.31, p=0.41		101*	0.40, p=0.28
			105*†	0.47, p=0.21
PAHs:			110*	0.36, p=.033
Biphenyl	-0.19, p=0.62		118*	0.34, p=0.38
Acenaphthene	-0.15, p=0.69		167†	0.54, p=0.13
Fluorine	-0.36, p=0.33			
Phenanthrene	**-0.40**, p=0.029			

It has been long recognized that somatic growth in vertebrates is dependent upon normal thyroid status, but it is not clear the degree to which thyroid hormones may be related to the growth endocrine system in fish (Eales, 2006; Norris, 2007). In vertebrates, the principal growth regulator is insulin-like growth factor-I (IGF-I), which is secreted in reponse to activation through the hypothalamus and pituitary (Kelley et al., 2002, 2006). In studies of laboratory reared fish, IGF-I levels are significantly correlated with growth rate (Beckman et al, 2004a; Dyer et al., 2004a; Davis and Peterson, 2006; Peters, 2009). Although in the present field-based studies, growth rate was not evaluated, it was possible to measure concentrations of IGF-I, and then to relate these levels to thyroid endocrine parameters as well as contaminant exposures.

Taking advantage of the large number of measurements that had been completed in the fish sampled in 2007 (hepatic contaminants, other endocrine paramaters), measurement of IGF-I concentrations in these individual was pursued. Given the small blood volumes obtainable from both shiner perch and Pacific staghorn sculpin and the principal emphasis on measuring thyroid hormones, there was a limited quantity of plasma remaining for IGF-I measurements. However, subsets of samples were collected that contained enough remaining plasma volume for IGF-I analysis was from a majority of the San Francisco Bay study; a larger number of samples were recovered from shiner perch, that have slightly greater blood volumes than sculpin. Separate discussions of the results from each species follow:

Shiner Perch. As shown in **Figure 14**, IGF-I concentrations in shiner perch exhibited significant differences among study locations; notably, IGF-I levels were significantly reduced in fish sampled from Oakland Inner Harbor and the San Francisco Waterfront as compared with several other locations.

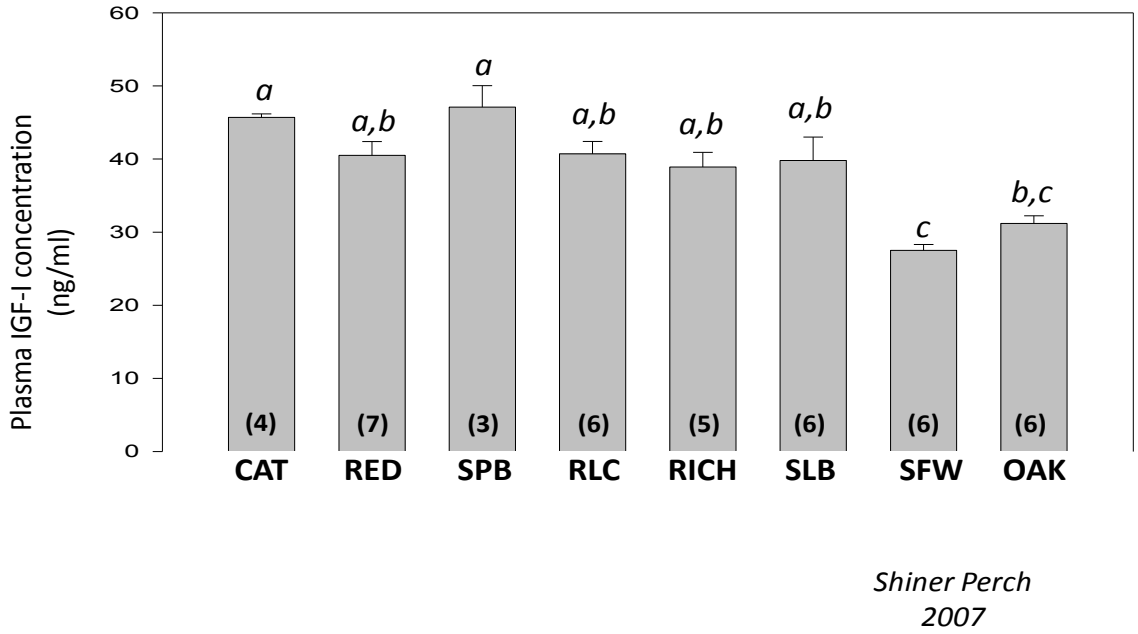


Figure 14. Plasma concentrations of IGF-I in shiner perch sampled in 2007 from Catalina Island (CAT), Redwood City (RED), San Pablo Bay (SPB), Richmond Lauritzen Channel (RLC), Richmond Harbor (RICH), San Leandro Bay (SLB), San Francisco Waterfront (SFW), and Oakland Inner Harbor (OAK). Mean ± SEM values are given, with number of samples (n) shown in parentheses inside each bar. ^{a,b,c}Superscripts denote significantly different values ($p<0.05$).

The pattern of differences in IGF-I across the San Francisco Bay sites did not parallel the observed differences in thyroid endocrine parameters (Brar et al., 2010), although both endocrine systems exhibited reduced hormone levels in the Oakland groups relative to several other locations. In order to ascertain whether any significant relationships existed between IGF-I and thyroid endocrine status, correlation analyses were carried out among shiner perch individuals in which both IGF-I and thyroid hormones were evaluated. The results of these analyses indicated that IGF-I was not correlated to thyroid endocrine parameters in this species (**Table 3**), suggesting that the thyroid endocrine system and growth regulatory system are not interacting, at least under the circumstances of the study's experimental design. Other components of the growth system, such as IGF-binding proteins (IGFBPs) which regulate the bioactivity of IGF-I (Kelley et al., 2006), may conceivably

mediate interactions between the thyroid system and growth, and they deserve future analysis.

Table 3. Relationships between IGF-I and thyroid endocrine parameters among shiner perch sampled in 2007. Plasma concentrations of IGF-I, T4, and T3, and T3/T4 ratio were evaluated using Pearson correlation analyses. Correlation coefficients (R) followed by their significance values (p) are given in each cell; significant values are in bold font. Analyses were carried out using 43 individuals for which all measurements (IGF-I, T4, T3, T3/T4 ratio) were undertaken.

	T4	T3	T3/T4
T3	0.14, p=0.37	--	--
T3/T4	**-0.64**, p=0.00001	**0.56**, p=0.0003	--
IGF-I	0.08, p=0.63	-0.14, p=0.39	-0.09, p=0.60

Despite the lack of detection of IGF-I and thyroid system interaction, the significantly reduced levels of the growth factor in fish from some San Francisco Bay sites indicated potential environmental effects on the growth system in shiner perch. Eleven of the fish measured for IGF-I were also measured for hepatic contaminants, and therefore it was possible to evaluate the relationships between exposure to specific environmental chemicals and levels of IGF-I. As shown in **Table 4**, three out of the four chlordanes measured showed non-significant negative relationships with IGF-I concentrations, while alpha-chlordane was significantly correlated with IGF-I (R= -0.70; p=0.01; **Figure 15**). Only one PAH, fluoranthene, was significantly correlated with IGF-I (R= -0.62, $p<0.05$), whereas several PCB congeners exhibited strong negative correlations with IGF-I concentrations (**Table 4**; **Fig. 14B**). Therefore, these findings indicate that exposure of shiner perch to certain contaminant chemicals, PCBs in particular, is associated with impacts on the growth endocrine system. Notably, these are the same types of contaminants that lead to thyroid disruption.

Table 4: Relationships between concentrations of the growth regulator, IGF-I, and concentrations of pesticides, polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) measured in liver in shiner perch. Correlation coefficients (R) followed by their significance values (p) are given in each cell (n=11 individuals measured for IGF-I as well as hepatic contaminants). Some values are included in which p>0.05 and p<0.15, in order to illustrate potential relationships; bold font indicates p<0.05. †Daggar symbol indicates dioxin-like PCB congener. Full list of contaminant chemicals measured are provided in Appendix B.

PESTICIDES	**IGF-I**		**PCBs**	**IGF-I**
gamma chlordane	-0.59, p=0.058		**049**	**-0.65**, p=0.031
alpha chlordane	**-0.70**, p=0.013		**052**	**-0.68**, p=0.021
trans-nonachlor	-0.54, p=0.088		066	-0.53, p=0.091
cis-nonachlor	-0.43, p=0.18		**070**	**-0.62**, p=0.043
			074	**-0.65**, p=0.029
			095	**-0.72**, p=0.014
			099	-0.58, p=0.063
			101	-0.53, p=0.091
			110	**-0.83**, p=0.001
PAHs	**IGF-I**		118†	-0.52, p=0.10
fluoranthene	**-0.62**, p=0.043		138	-0.60, p=0.053
			141	**-0.74**, p=0.009
			149	**-0.71**, p=0.014
			151	**-0.67**, p=0.024
			156†	-0.59, p=0.073
			158	**-0.62**, p=0.044
			167†	**-0.81**, p=0.0025
			170	**-0.63**, p=0.037
			177	-0.59, p=0.059
			183	-0.54, p=0.084
			187	**-0.61**, p=0.047
			200	-0.56, p=0.075
			total PCB s	**-0.61**, p=0.044

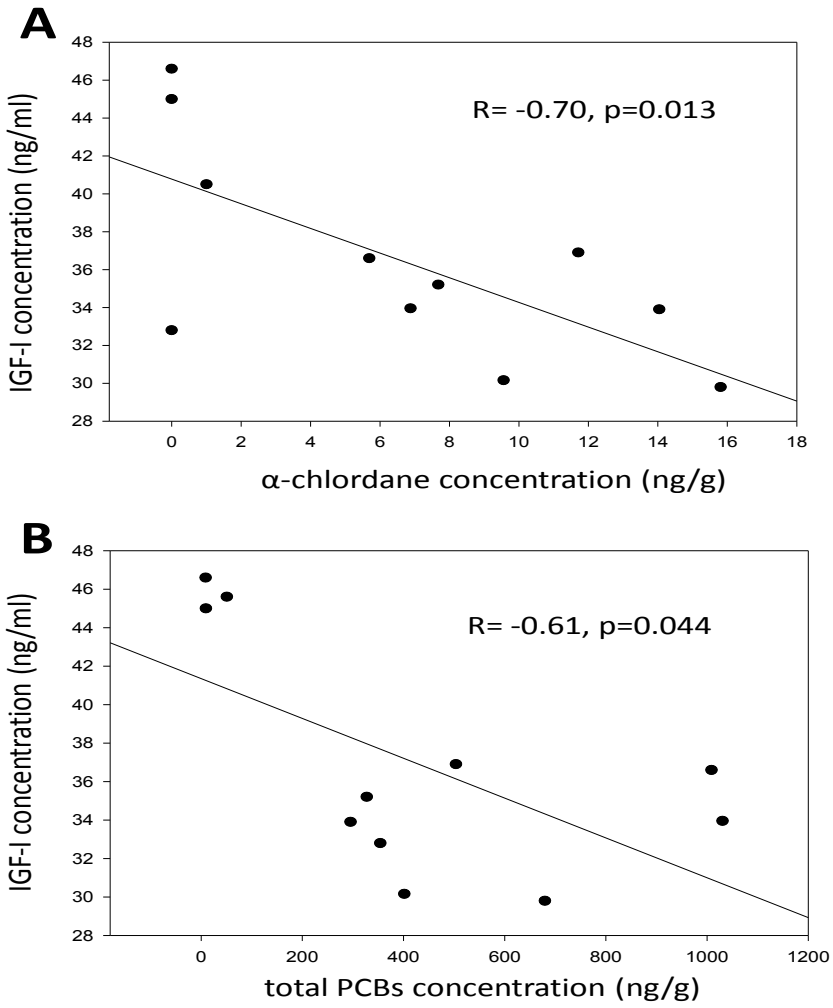


Figure 15: Correlations between plasma concentrations of the growth regulator, IGF-I, and hepatic concentrations of α-chlordane (panel **A**) and total PCB (panel **B**) in shiner perch. Correlation coefficients (R) and p-values are given for each correlation above, with data on additional contaminants provided in Table 4. Eleven shiner perch were measured for IGF-I as well as hepatic contaminants.

Pacific staghorn sculpin. The number of samples with adequate volumes to allow IGF-I measurements were more limited in the sculpin; however, the total number (N=27) was of interest for use in correlation analyses with thyroid endocrine parameters. Only two of these sculpin samples were individuals that also were measured for hepatic contaminants; therefore, the IGF-I measures in this species could not be evaluated for their relationship to contaminant exposures, as was done in the shiner perch. Despite the small sample sizes per location in San Francisco Bay, some location associated differences in IGF-I concentrations in sculpin appeared to be evident (**Figure 16**). Significantly lower values in sculpin from the San Pablo Bay site were observed, as compared with several other locations, while intermediate levels were seen in fish from Oakland Inner Harbor and Hunter's Point.

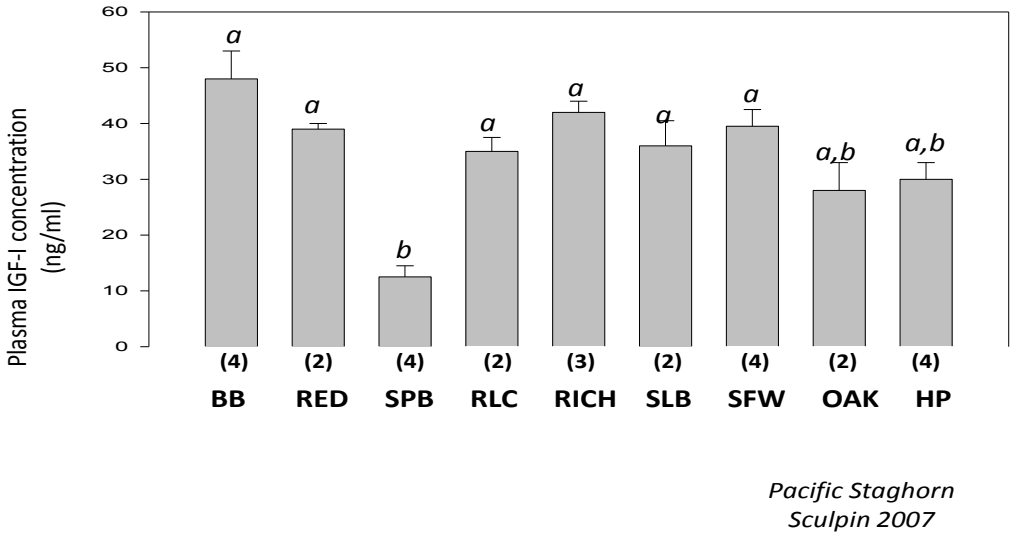
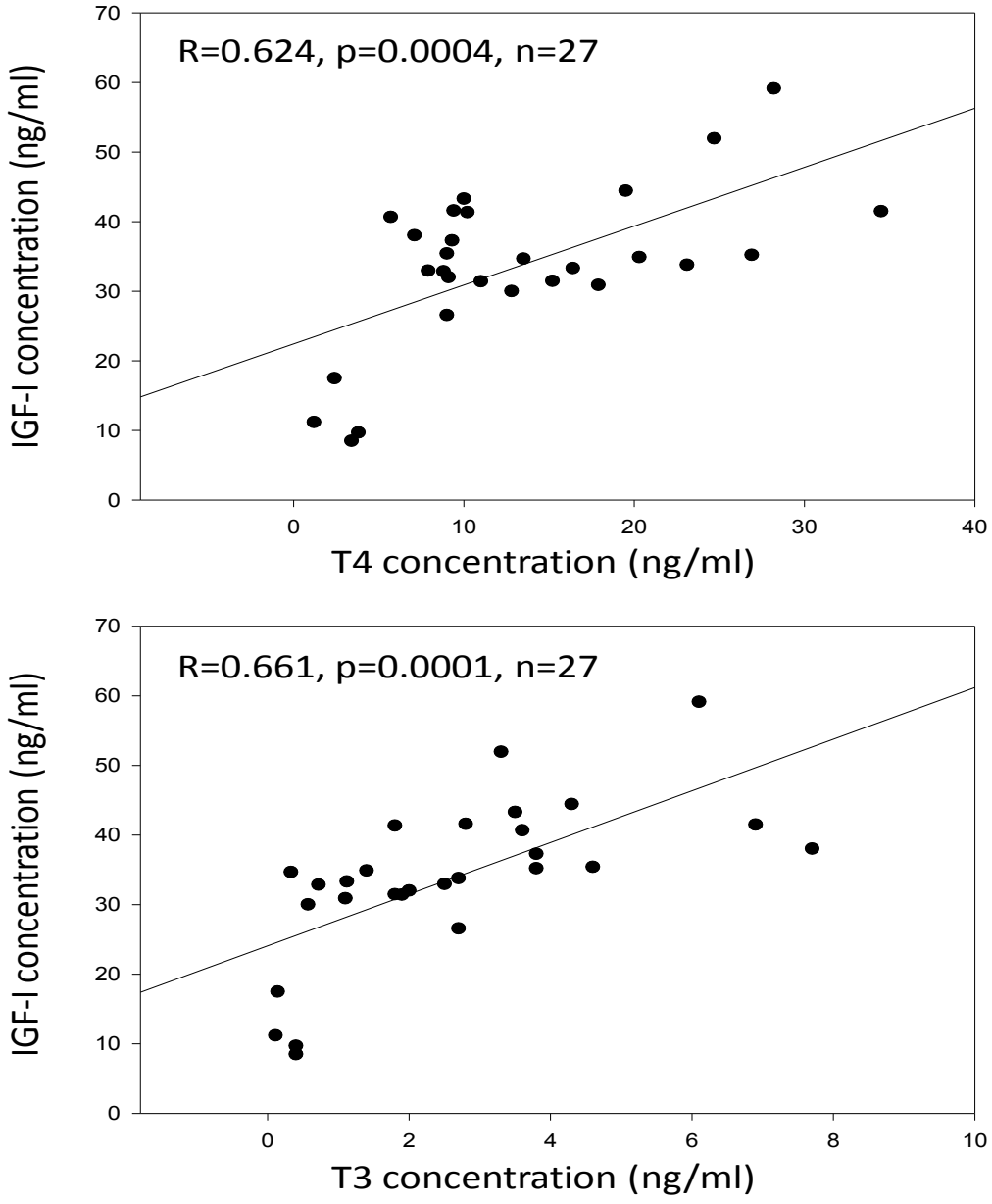


Figure 16. Plasma concentrations of IGF-I in Pacific staghorn sculpin sampled in 2007 from Bodega Bay (BB), Redwood City (RED), San Pablo Bay (SPB), Richmond Lauritzen Channel (RLC), Richmond Harbor (RICH), San Leandro Bay (SLB), San Francisco Waterfront (SFW), Oakland Inner Harbor (OAK), and Hunter's Point (HP). Mean ± SEM values are given, with number of samples (n) shown in parentheses inside each bar. ^{a,b}Superscripts denote significantly different values ($p<0.05$).

Correlation analyses demonstrated significant positive relationships between IGF-I and thyroid hormones in Pacific staghorn sculpin (**Figure 17**), in contrast to the lack of detectable relationships in shiner perch. This difference between the species is not readily explainable, but it is noteworthy that plasma concentrations of T4 and T3 are comparatively lower in

Pacific staghorn sculpin as compared with shiner perch when sampled at the same locations and times; additionally, contaminant exposures are generally greater in the sculpin (Brar et al., 2010). This raises the possibility that additional perturbation of the thyroid hormones in sculpin (from already reduced levels) may be more readily transduced into effects on the growth endocrine system. An additional explanation for the difference in interaction of thyroid hormones and IGF-I between the two species may relate to developmental differences, since the sculpin evaluated in these studies are immature juveniles, a stage typically characterized by activated growth and which may therefore be more sensitive to hormonal disruptions.



Pacific staghorn sculpin 2007

Figure 17: Correlations between plasma concentrations of the growth regulator, IGF-I, and T4 (panel **A**) and T3 (panel **B**) in Pacific staghorn sculpin.

Concluding Remarks

Our studies and several prior studies (Fairey et al., 1997; Davis et al., 2004, 2007; Oros et al., 2005, 2007; Hunt et al., 2008) have consistently demonstrated that shiner surfperch, Pacific staghorn sculpin, and other indigenous fish of San Francisco Bay are contaminated with a variety of environmental contaminant chemicals, which include several suspected EDCs. However, it has not been understood to what extent these contaminant exposures may be related to maladaptive alterations in resident fish. This study provided a "follow-up" characterization of a thyroid endocrine-disrupted condition discovered in wild fish residing in San Francisco Bay.

The present study demonstrated that one of the key mechanisms underlying this endocrine-disrupted condition was impairment in the hormone synthetic capacity of the thyroid. In addition, it was also evident that there were alterations in hepatic 5'deiodinase activity. These effects were associated with reduced thyroid hormone levels in the fish, as well as alterations in the relative concentrations of T3, the more active thyroid hormone. Importantly, it was possible to correlate changes in the endocrine system to contaminant exposures, which identified certain types of contaminants (including α-chlordane and certain PCB congeners) as strongly related to specific effects, such as activation of 5'-deiodinase and alterations in T3/T4 ratio. Published studies indicate that PCBs interfere with deiodinase activity in fish (Adams et al., 2000; Brown et al., 2004; Coimbra et al., 2005; Picard-Aitken et al., 2007). The role of chlordanes, however, in altering deiodinase has not been previously reported in the literature.

Of the PCBs that are correlated with thyroid endocrine parameters, five were co-planar "dioxin-like" PCBs (105, 118, 156, 157, 167; Brar et al., 2010) which presumably would strongly activate AhR-mediated responses (Safe et al., 1985; van den Berg et al., 1998; Burgin et al., 2001; Gauger et al., 2007). While these PCBs are implicated in the thyroid disruption by this study, it is also notable that a large number of non-coplanar PCBs were also significantly correlated with thyroid parameters, in both species. In experimental studies, both co-planar and non-co-planar PCBs have been implicated in thyroid disruption (e.g.,

Adams et al., 2000; LeRoy et al., 2006; Gauger et al., 2007), suggesting both AhR- and non-AhR-mediated mechanisms. Recent studies in mammals indicate that AhR-mediated pathways may alter expression of genes in thyroid follicular cells that are involved in thyroid hormone production (Pocar et al., 2006).

As described in the Introdution, the thyroid endocrine system is essential for normal growth and development in all vertebrates. In fish, thyroid hormones regulate metamorphic processes, including larval-to-juvenile transformations, the parr-to-smolt transformation in salmonids, and flatfish metamorphosis (Power et al., 2001; Brown et al., 2004; Yamano, 2005; Boas et al., 2006; Blanton and Specker, 2007; Morgado et al., 2007). In adult fish, thyroid hormones are importance in the regulation of growth, nutrient utilization, and reproduction. Their exact mode(s) of action are not completely understood, even in mammals. Fish grow faster and are healthier when thyroid hormones are higher (Gomez et al., 1997; Power et al., 2001; Plohman et al., 2002; Yamano, 2005), and thyroid hormones appear to be regulators of hepatic IGF-I expression in fish (Schmid et al., 2003; Reinecke, 2006). Therefore, disruption of the thyroid system would be likely to have important physiological consequences relating to growth and its regulation.

In Pacific staghorn sculpin, thyroid endocrine status was significantly related to a principal regulator of growth, IGF-I. This points to an important connection between thyroid endocrine status and growth in fish, and therefore concern that environmental thyroid disruption has the possibility of impacting growth rate in fish. Importantly, this finding was derived from wild fish in the real setting of San Francisco Bay locations with varying contaminant presence. Future experimental studies need to build upon these findings, wherein the thyroid endocrine system is experimentally “disrupted” (e.g., use of goitrogens to inhibit thyroid hormone production) to varying degrees, and growth parameters are measured (skeletal and muscular growth rates, tissue protein expression, IGF-I, regulatory IGF-binding proteins, thyroid hormones, and other biomarkers) in order to obtain the specific data required to calibrate an accurate model. Through this approach, it would be possible to extrapolite endocrine and other biomarkers, measured in field-sampled fish, to effects on growth, enhancing the translation to physiological performance relevant to population-level impacts. This would be a step forward indeveloping stronger scientific approaches for ecosystem-based management.

METHODOLOGY

Fish Collection and Sampling Sites

The fish studied and evaluated in this work were caught and sampled within and outside of San Francisco Bay in August 2007, August 2008, and August 2010. The locations within the San Francisco Bay area at which fish were sampled are shown in map illustrated in **Figure 18**. The latitudes and longitudes for the study sites are provided in the **Appendix Table A**. Shiner surfperch ranged from 8-11 cm in length, all of mature length and age (http://hmsc.oregonstate.edu/projects/msap/). Pacific staghorn sculpin ranged from 7-11 cm in length, all young-of-the-year and immature (http://hmsc.oregonstate.edu/projects/msap/). All fish were captured using an otter trawl pulled behind a small craft and held in aerated aquaria until they were delivered to an on-shore location where they could be sampled. The time of net capture to tissue sampling was recorded for each individual fish. Each fish was measured for standard length followed by tissue collection. Blood was collected from the caudal vein using a heparinized syringe with a 22g needle, centrifuged for 3 min at 3000 rpm to pellet the cells, and plasma was removed. Plasma samples were stored frozen in dry ice until transport to the laboratory, where they were stored at -80˚C until endocrine assays were carried out (described below). Livers were removed whole, weighed to the nearest 0.1 g, placed into clean, labeled foil envelopes, and frozen on dry ice until transport to the laboratory, where they were stored at -80˚C until chemical analyses and/or 5'-deiodinase activity assays were carried out (described below). In the field-based experiments, fish were placed into tanks containing aerated natural seawater, after injection with saline (control) or hormone (TSH or TRH), and then held for a 2 hr period. Afterward, fish were sampled for blood and tissues as described above. TSH and TRH were obtained from Sigma Chemical Company (St. Louis, MO).

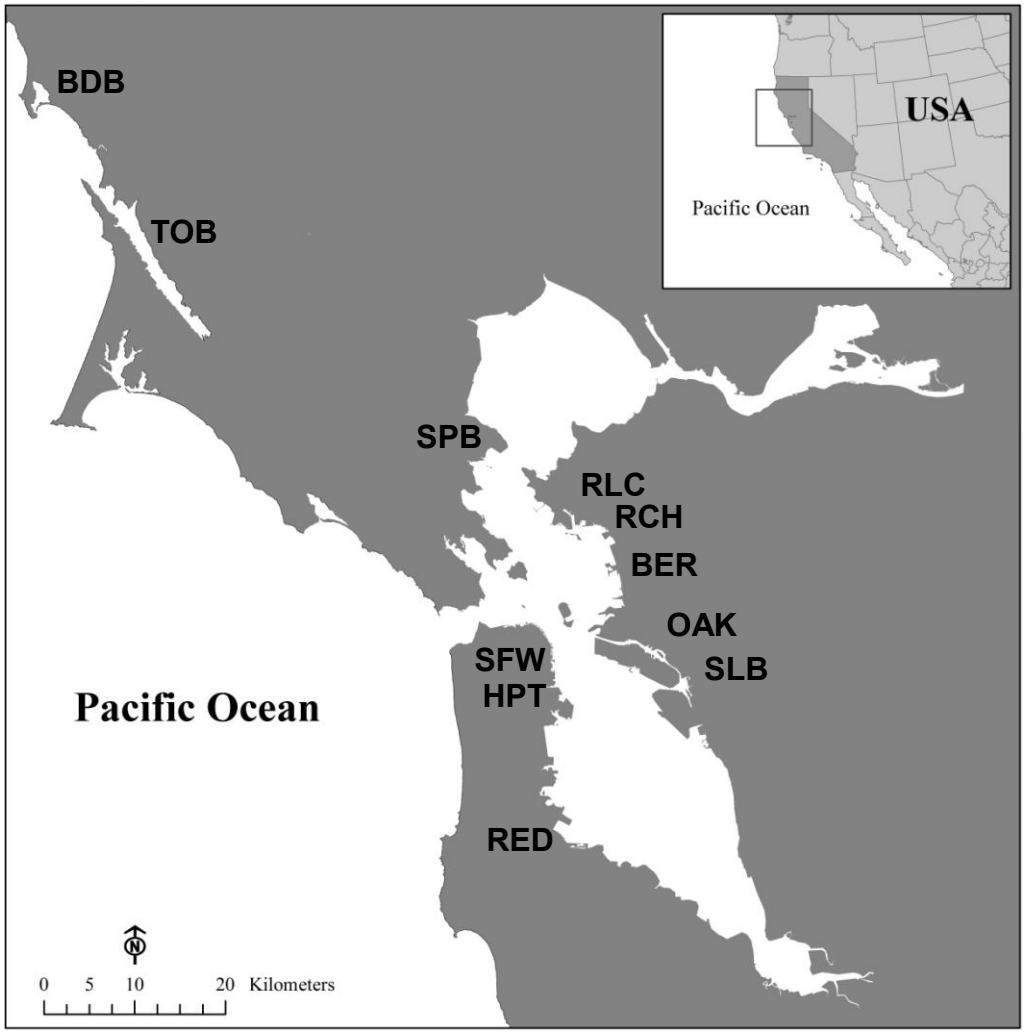


Figure 18. San Francisco Bay study sites in the endocrine disruption study. BDB=Bodega Bay; RED=Redwood City; HPT=Hunter's Point in San Francisco; SFW=San Francisco waterfront; SPB=San Pablo Bay; RLC=Richmond Lauritzen Channel; RICH=Richmond waterfront/marina; OAK=Oakland Inner Harbor; SLB=San Leandro Bay. Fish were also sampled at a remote location on Catalina Island. Latitudes and longitudes are provided in Appendix Table A.

Radioimmunoassay (RIA) method for measurement of IGF-I. Plasma concentrations of the growth regulator, IGF-I, were measured by specific RIA using commercially available standards and specific antisera from Diagnostic Systems Laboratory (Webster, TX), GroPep, Ltd. (Adelaide, Australia), and Santa Cruz Biotech (Santa Cruz, CA). These assays utilize competition between ^{125}I-labeled IGF-I and endogenous antigen (hormone) for a limited number of specific antibody binding sites. Separation of free and antibody-bound antigen was achieved using a double antibody precipitation, and ^{125}I-labeled complexes were counted (cpm) in a Perkin-Elmer Cobra II gamma counter (Packard Instruments Co., Boston MA). Standard curves, in which increasing concentrations of unlabeled hormone standards are added to the ^{125}I-labeled antigen, were utilized to calculate concentrations of hormones in the samples from the cpm of antibody-bound radiolabeled hormone (using SigmaPlot v.11 software; SYSTAT Software Inc. Chicago, IL). Concentrations of hormones are reported in ng/ml. All assays were previously validated and have and inter-assay variabilities of 7-12% (n=5-8 assays) (Kelley et al., 2001; Rodgers et al., 2003; Galima, 2004; Reyes, 2006; Petschauer, 2007).

Enzyme immunoassay (EIA) procedures for thyroid hormones. Plasma T4 and T3 concentrations were measured by specific EIA using commercially available reagents from Immunometrics, Ltd. (London, UK). Frozen plasma aliquots were thawed, vortexed, and centrifuged for 30 sec at 3000 rpm in order to pellet (remove) any remaining blood cells. In both EIAs, fluorescein-labeled hormone (T4 or T3) were used to compete with endogenous hormone for binding to the antiserum, followed by the addition of anti-fluorescein antiserum conjugated to magnetic particles. The resulting complexes were then magnetically sedimented using a magnetic plate apparatus, the supernatant was decanted, and a color reaction was carried out using substrate solution for alkaline phophatase (AP) enzymatic activity (anti-T4 and anti-T3 antisera are AP-conjugated). Absorption at 550 nm was then read on a microplate spectrophotomer (Molecular Devices, Sunnyvale, CA), in which intensity is inversely correlated with the concentration of hormone in the sample or standard. Standard curves for T4 or T3 (concentrations between 10-250 ng/ml) were used to calculate ng/ml concentrations from the unknown samples using SigmaPlot v.11 software (SYSTAT Software Inc.). The EIAs were previously validated for fish plasma, and exhibit intra-assay and inter-assay coefficients of variation of 2.9% and 4.1%, respectively (Brar, 2009; Brar et al., 2010).

Hepatic deiodinase assay. Hepatic 5'-deiodinase activity was measured using a modification of the assay originally described by Leatherland et al. (1990) and Leatherland and Farbridge (1992). Sub-samples of liver (~50 mg) from each field-collected liver sample were placed into 1.2 ml ice-cold phosphate buffer (0.1 M, pH 7.2), followed by homogenization using a Tissuemiser™ (Fisher Scientific) set at 10,000 rpm for 3 sec. One-half (0.5) ml of the homogenate was immediately transferred to assay tubes and incubated at 12ºC for 20 min in phosphate buffer with final concentrations of 10 mM dithiothreitol and 50 mM T4 (both obtained from Sigma Chemical Co.). Another 0.5 ml of the homogenate was placed into tubes, centrifuged at 14,000 rpm for 3 min, and their supernatants removed and immediately frozen at -80º C (used to measure endogenous T3 present prior to incubation). After 20 min, the incubated tubes were centrifuged and their supernatants were saved as above (used to measure T3 generated by 5'-deiodinase). Aliquots of the reminaing homogenate were used to determine protein concentration using the BCA™ protein assay kit (Thermo Scientific, Inc., Rockford, IL). T3 concentrations were measured by the EIA described above. T3 concentration in the non-incubated tubes was subtracted from the T3 concentration in the incubated tubes, to obtain values specifically for generated T3. F calculated values were expressed as fmol T3 generated / mg liver / hr (fmol/mg/hr).

Hepatic tissue extractions and contaminant analyses. Livers were measured for pesticides, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) using an institutional core facility (www.IIRMES.org). The lists of analytes measured and QA/QC procedures are provided in **Appendix Table B**. Liver samples were extracted for organic constituents using a pressurized fluid microwave extraction method. Samples were thawed to 25˚C in a solvent-rinsed Teflon pressurized extraction vessel, with the addition of 25 ml methylene chloride. Vessels were then sealed and randomly placed on a microwave carousel apparatus (MARS 5 microwave, XP-1500 *plus* vessels, CEM Corporation; Matthews, NC), and heated at 100 °C for 30 min. After cooling to 25˚C, the solvent was then decanted into collection flasks over sodium sulfate to separate any water from the extract and repeated for a total of 3 extractions. Extracts were evaporated to less than 1 ml using a Rotavapor® apparatus (Büchi Laboratory Equipment, Inc., Flawil, Switzerland), followed by addition of 4 ml n-hexane and evaporation again to 500 µl to eliminate any residual methylene chloride.

This was followed by column cleaning of the extract, with the eluate subsequently evaporated to 1 ml and transferred into a 2 ml GC/MS vial.

Contaminant concentrations in each sample were then measured by gas chromatography/ mass spectrometry (GC/MS) using an Agilent 6890N GC system with a DB-5 60 m gas chromatography column coupled to an Agilent 5973 Mass Selective Detector (Agilent Technologies, Santa Clara, CA). The concentration for each compound measured was calculated as ng compound per g wet weight (ng/g ww). A total of fifty fish were selected for hepatic contaminant analyses, as described in the Results.

Statistical analyses. All statistical tests and graphical representations were conducted using SigmaPlot v.11 software (SYSTAT, Inc., Chicago, IL). Mean ± SE values for the different parameters measured in this study (e.g., ng/ml plasma hormone concentration, ng/g ww tissue contaminant concentration) were calculated for each study location or experimental group and analyzed using one-way analysis of variance (ANOVA) followed by ad hoc pairwise multiple comparisons tests. In some of the datasets, values were first log transformed to obtain normality, followed by ANOVA and the Holm-Sidek ad hoc test. Relationships between parameters (e.g., hormone concentration vs. hepatic 5'-deiodinse) were evaluated using Pearson's correlation analysis to obtain correlation coefficients (R). In all statistical analyses, differences between groups or correlations were considered significant when $p < 0.05$.

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APPENDIX

Table A

Locations of Fish Sampling Trawls used in study.
Latitude and longitude of each trawl were determined by GIS.

Location	Latitude	Longitude
Oakland Inner Harbor		
(OAK)	37.786	122.255
Redwood city		
(RED)	37.517	122.207
San Francisco Waterfront		
(SFW)	37.777	122.388
Hunter's Point		
(HP)	37.724	122.360
San Pablo Bay		
(SPB)	37.960	122.476
San Leandro Bay		
(SLB)	37.753	122.218
Richmond Harbor		
(RICH)	37.910	122.351
Richmond Lauritzen Channel		
(RLC)	37.920	122.368
Bodega Bay		
(BB)	38.330	123.055
Tomales Bay		
(TB)	38.140	122.877
Santa Catalina Island		
(CAT)	33.385	118.475

Table B. Compounds Measured in Liver of Shiner Surfperch and Pacific Staghorn Sculpin by GC/MS. Minimum Detection Limit (MDL), Accuracy RL LCL UCL and EPA Method provided for each analyte.

Parameter	Group	MDL	MDL_Units	AccRange	RL	LCL	UCL	Method
Aroclor 1016	Aroclor PCBs	10	Ng/g	65 - 135%	20	65	135	EPA 8270Cm
Aroclor 1221	Aroclor PCBs	10	Ng/g	65 - 135%	20	65	135	EPA 8270Cm
Aroclor 1232	Aroclor PCBs	10	ng/g	65 - 135%	20	65	135	EPA 8270Cm
Aroclor 1242	Aroclor PCBs	10	ng/g	65 - 135%	20	65	135	EPA 8270Cm
Aroclor 1248	Aroclor PCBs	10	ng/g	65 - 135%	20	65	135	EPA 8270Cm
Aroclor 1254	Aroclor PCBs	10	ng/g	65 - 135%	20	65	135	EPA 8270Cm
Aroclor 1260	Aroclor PCBs	10	ng/g	65 - 135%	20	65	135	EPA 8270Cm
(PCB030)	Chlorinated Pesticides		ng/g	66 - 112%		66	112	EPA 8270Cm
(PCB112)	Chlorinated Pesticides		ng/g	59 - 118%		59	118	EPA 8270Cm
(PCB198)	Chlorinated Pesticides		ng/g	36 - 131%		36	131	EPA 8270Cm
(TCMX)	Chlorinated Pesticides		ng/g	63 - 117%		63	117	EPA 8270Cm
2,4'-DDD	Chlorinated Pesticides	1	ng/g	50 - 135%	5	50	135	EPA 8270Cm
2,4'-DDE	Chlorinated Pesticides	1	ng/g	60 - 130%	5	60	130	EPA 8270Cm
2,4'-DDT	Chlorinated Pesticides	1	ng/g	40 - 135%	5	40	135	EPA 8270Cm
4,4'-DDD	Chlorinated Pesticides	1	ng/g	70 - 130%	5	70	130	EPA 8270Cm
4,4'-DDE	Chlorinated Pesticides	1	ng/g	65 - 130%	5	65	130	EPA 8270Cm
4,4'-DDT	Chlorinated Pesticides	1	ng/g	35 - 140%	5	35	140	EPA 8270Cm
Alachlor	Chlorinated Pesticides	2	ng/g	60 - 140%	10	60	140	EPA 8270Cm
Aldrin	Chlorinated Pesticides	1	ng/g	54 - 130%	5	54	130	EPA 8270Cm
BHC-alpha	Chlorinated Pesticides	1	ng/g	47 - 132%	5	47	132	EPA 8270Cm
BHC-beta	Chlorinated Pesticides	1	ng/g	47 - 134%	5	47	134	EPA 8270Cm
BHC-delta	Chlorinated Pesticides	1	ng/g	43 - 134%	5	43	134	EPA 8270Cm
BHC-gamma	Chlorinated Pesticides	1	ng/g	44 - 132%	5	44	132	EPA 8270Cm
Chlordane-alpha	Chlorinated Pesticides	1	ng/g	55 - 131%	5	55	131	EPA 8270Cm
Chlordane-gamma	Chlorinated Pesticides	1	ng/g	55 - 133%	5	55	133	EPA 8270Cm
cis-Nonachlor	Chlorinated Pesticides	1	ng/g	57 - 136%	5	57	136	EPA 8270Cm
DCPA (Dacthal)	Chlorinated Pesticides	5	ng/g	65 - 129%	10	65	129	EPA 8270Cm
Dicofol	Chlorinated Pesticides	1	ng/g	29 - 172%	5	29	172	EPA 8270Cm
Dieldrin	Chlorinated Pesticides	1	ng/g	47 - 150%	5	47	150	EPA 8270Cm
Endosulfan Sulfate	Chlorinated Pesticides	1	ng/g	26 - 155%	5	26	155	EPA 8270Cm
Endosulfan-I	Chlorinated Pesticides	1	ng/g	43 - 132%	5	43	132	EPA 8270Cm
Endosulfan-II	Chlorinated Pesticides	1	ng/g	35 - 145%	5	35	145	EPA 8270Cm
Endrin	Chlorinated Pesticides	1	ng/g	42 - 157%	5	42	157	EPA 8270Cm
Endrin Aldehyde	Chlorinated Pesticides	1	ng/g	0 - 95%	5	0	95	EPA 8270Cm
Endrin Ketone	Chlorinated Pesticides	1	ng/g	26 - 161%	5	26	161	EPA 8270Cm
Heptachlor	Chlorinated Pesticides	1	ng/g	51 - 142%	5	51	142	EPA 8270Cm
Heptachlor Epoxide	Chlorinated Pesticides	1	ng/g	62 - 128%	5	62	128	EPA 8270Cm
Kepone	Chlorinated Pesticides	1	ng/g	60 - 120%	5	60	120	EPA 8270Cm
Methoxychlor	Chlorinated Pesticides	1	ng/g	16 - 185%	5	16	185	EPA 8270Cm
Mirex	Chlorinated Pesticides	1	ng/g	31 - 138%	5	31	138	EPA 8270Cm
Oxychlordane	Chlorinated Pesticides	1	ng/g	59 - 126%	5	59	126	EPA 8270Cm
Perthane	Chlorinated Pesticides	5	ng/g	47 - 169%	10	47	169	EPA 8270Cm
Total Chlordane	Chlorinated Pesticides		ng/g					EPA 8270Cm

Total Detectable DDTs	Chlorinated Pesticides		ng/g					EPA 8270Cm
trans-Nonachlor	Chlorinated Pesticides	1	ng/g	54 - 131%	5	54	131	EPA 8270Cm
PCB001	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB002	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB003	PCB Congeners	1	ng/g	53 - 98%	5	53	98	EPA 8270Cm
PCB004	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB006	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB008	PCB Congeners	1	ng/g	60 - 151%	5	60	151	EPA 8270Cm
PCB009	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB016	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB018	PCB Congeners	1	ng/g	63 - 143%	5	63	143	EPA 8270Cm
PCB019	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB022	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB025	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB028	PCB Congeners	1	ng/g	59 - 145%	5	59	145	EPA 8270Cm
PCB031	PCB Congeners	1	ng/g	53 - 146%	5	53	146	EPA 8270Cm
PCB033	PCB Congeners	1	ng/g	61 - 149%	5	61	149	EPA 8270Cm
PCB037	PCB Congeners	1	ng/g	57 - 154%	5	57	154	EPA 8270Cm
PCB044	PCB Congeners	1	ng/g	61 - 141%	5	61	141	EPA 8270Cm
PCB049	PCB Congeners	1	ng/g	63 - 139%	5	63	139	EPA 8270Cm
PCB052	PCB Congeners	1	ng/g	68 - 137%	5	68	137	EPA 8270Cm
PCB056	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB056/060	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB065	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB066	PCB Congeners	1	ng/g	61 - 141%	5	61	141	EPA 8270Cm
PCB067	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB070	PCB Congeners	1	ng/g	60 - 144%	5	60	144	EPA 8270Cm
PCB071	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB074	PCB Congeners	1	ng/g	64 - 145%	5	64	145	EPA 8270Cm
PCB077	PCB Congeners	1	ng/g	61 - 147%	5	61	147	EPA 8270Cm
PCB081	PCB Congeners	1	ng/g	58 - 155%	5	58	155	EPA 8270Cm
PCB082	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB087	PCB Congeners	1	ng/g	70 - 128%	5	70	128	EPA 8270Cm
PCB095	PCB Congeners	1	ng/g	68 - 124%	5	68	124	EPA 8270Cm
PCB097	PCB Congeners	1	ng/g	66 - 127%	5	66	127	EPA 8270Cm
PCB099	PCB Congeners	1	ng/g	62 - 133%	5	62	133	EPA 8270Cm
PCB101	PCB Congeners	1	ng/g	67 - 129%	5	67	129	EPA 8270Cm
PCB105	PCB Congeners	1	ng/g	66 - 131%	5	66	131	EPA 8270Cm
PCB110	PCB Congeners	1	ng/g	67 - 128%	5	67	128	EPA 8270Cm
PCB114	PCB Congeners	1	ng/g	72 - 130%	5	72	130	EPA 8270Cm
PCB118	PCB Congeners	1	ng/g	67 - 132%	5	67	132	EPA 8270Cm
PCB119	PCB Congeners	1	ng/g	67 - 128%	5	67	128	EPA 8270Cm
PCB123	PCB Congeners	1	ng/g	66 - 134%	5	66	134	EPA 8270Cm
PCB126	PCB Congeners	1	ng/g	73 - 142%	5	73	142	EPA 8270Cm
PCB128	PCB Congeners	1	ng/g	75 - 123%	5	75	123	EPA 8270Cm
PCB128+167	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB132	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm

PCB138	PCB Congeners	1	ng/g	73 - 122%	5	73	122	EPA 8270Cm
PCB141	PCB Congeners	1	ng/g	74 - 117%	5	74	117	EPA 8270Cm
PCB146	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB147	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB149	PCB Congeners	1	ng/g	69 - 115%	5	69	115	EPA 8270Cm
PCB151	PCB Congeners	1	ng/g	72 - 118%	5	72	118	EPA 8270Cm
PCB153	PCB Congeners	1	ng/g	66 - 137%	5	66	137	EPA 8270Cm
PCB156	PCB Congeners	1	ng/g	79 - 121%	5	79	121	EPA 8270Cm
PCB157	PCB Congeners	1	ng/g	70 - 118%	5	70	118	EPA 8270Cm
PCB158	PCB Congeners	1	ng/g	72 - 120%	5	72	120	EPA 8270Cm
PCB167	PCB Congeners	1	ng/g	67 - 125%	5	67	125	EPA 8270Cm
PCB168	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB168+132	PCB Congeners	1	ng/g	45 - 124%	5	45	124	EPA 8270Cm
PCB169	PCB Congeners	1	ng/g	71 - 129%	5	71	129	EPA 8270Cm
PCB170	PCB Congeners	1	ng/g	65 - 120%	5	65	120	EPA 8270Cm
PCB173	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB174	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB177	PCB Congeners	1	ng/g	65 - 120%	5	65	120	EPA 8270Cm
PCB179	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB180	PCB Congeners	1	ng/g	65 - 123%	5	65	123	EPA 8270Cm
PCB183	PCB Congeners	1	ng/g	66 - 116%	5	66	116	EPA 8270Cm
PCB187	PCB Congeners	1	ng/g	65 - 115%	5	65	115	EPA 8270Cm
PCB189	PCB Congeners	1	ng/g	57 - 131%	5	57	131	EPA 8270Cm
PCB194	PCB Congeners	1	ng/g	46 - 129%	5	46	129	EPA 8270Cm
PCB195	PCB Congeners	1	ng/g	59 - 130%	5	59	130	EPA 8270Cm
PCB200	PCB Congeners	1	ng/g	55 - 112%	5	55	112	EPA 8270Cm
PCB201	PCB Congeners	1	ng/g	57 - 122%	5	57	122	EPA 8270Cm
PCB203	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB205	PCB Congeners	1	ng/g	60 - 125%	5	60	125	EPA 8270Cm
PCB206	PCB Congeners	1	ng/g	47 - 119%	5	47	119	EPA 8270Cm
PCB209	PCB Congeners	1	ng/g	38 - 122%	5	38	122	EPA 8270Cm
Total Detectable PCBs	PCB Congeners		ng/g					EPA 8270Cm
(d10-Acenaphthene)	Polynuclear Aromatic Hydrocarbons		ng/g	56 - 109%		56	109	EPA 8270Cm
(d10-Phenanthrene)	Polynuclear Aromatic Hydrocarbons		ng/g	63 - 114%		63	114	EPA 8270Cm
(d12-Chrysene)	Polynuclear Aromatic Hydrocarbons		ng/g	46 - 137%		46	137	EPA 8270Cm
(d12-Perylene)	Polynuclear Aromatic Hydrocarbons		ng/g	9 - 133%		9	133	EPA 8270Cm
(d8-Naphthalene)	Polynuclear Aromatic Hydrocarbons		ng/g	35 - 95%		35	95	EPA 8270Cm
1-Methylnaphthalene	Polynuclear Aromatic Hydrocarbons	1	ng/g	46 - 117%	5	46	117	EPA 8270Cm
1-Methylphenanthrene	Polynuclear Aromatic Hydrocarbons	1	ng/g	75 - 126%	5	75	126	EPA 8270Cm
2,3,5-Trimethylnaphthalene	Polynuclear Aromatic Hydrocarbons	1	ng/g	63 - 119%	5	63	119	EPA 8270Cm
2,6-Dimethylnaphthalene	Polynuclear Aromatic Hydrocarbons	1	ng/g	54 - 120%	5	54	120	EPA 8270Cm
2-Methylnaphthalene	Polynuclear Aromatic Hydrocarbons	1	ng/g	38 - 135%	5	38	135	EPA 8270Cm
2-Methylphenanthrene	Polynuclear Aromatic Hydrocarbons	1	ng/g	40 - 160%	5	40	160	EPA 8270Cm
Acenaphthene	Polynuclear Aromatic Hydrocarbons	1	ng/g	40 - 138%	5	40	138	EPA 8270Cm
Acenaphthylene	Polynuclear Aromatic Hydrocarbons	1	ng/g	25 - 128%	5	25	128	EPA 8270Cm
Anthracene	Polynuclear Aromatic Hydrocarbons	1	ng/g	45 - 121%	5	45	121	EPA 8270Cm
Benz[a]anthracene	Polynuclear Aromatic Hydrocarbons	1	ng/g	58 - 162%	5	58	162	EPA 8270Cm

Benzo[a]pyrene	Polynuclear Aromatic Hydrocarbons	1	ng/g	15 - 166%	5	15	166	EPA 8270Cm
Benzo[b]fluoranthene	Polynuclear Aromatic Hydrocarbons	1	ng/g	42 - 172%	5	42	172	EPA 8270Cm
Benzo[e]pyrene	Polynuclear Aromatic Hydrocarbons	1	ng/g	15 - 178%	5	15	178	EPA 8270Cm
Benzo[g,h,i]perylene	Polynuclear Aromatic Hydrocarbons	1	ng/g	0 - 193%	5	0	193	EPA 8270Cm
Benzo[k]fluoranthene	Polynuclear Aromatic Hydrocarbons	1	ng/g	31 - 169%	5	31	169	EPA 8270Cm
Biphenyl	Polynuclear Aromatic Hydrocarbons	1	ng/g	45 - 123%	5	45	123	EPA 8270Cm
Chrysene	Polynuclear Aromatic Hydrocarbons	1	ng/g	50 - 155%	5	50	155	EPA 8270Cm
Dibenz[a,h]anthracene	Polynuclear Aromatic Hydrocarbons	1	ng/g	13 - 190%	5	13	190	EPA 8270Cm
Dibenzothiophene	Polynuclear Aromatic Hydrocarbons	1	ng/g	61 - 127%	5	61	127	EPA 8270Cm
Fluoranthene	Polynuclear Aromatic Hydrocarbons	1	ng/g	59 - 143%	5	59	143	EPA 8270Cm
Fluorene	Polynuclear Aromatic Hydrocarbons	1	ng/g	55 - 131%	5	55	131	EPA 8270Cm
Indeno[1,2,3-c,d]pyrene	Polynuclear Aromatic Hydrocarbons	1	ng/g	5 - 198%	5	5	198	EPA 8270Cm
Naphthalene	Polynuclear Aromatic Hydrocarbons	1	ng/g	28 - 120%	5	28	120	EPA 8270Cm
Perylene	Polynuclear Aromatic Hydrocarbons	1	ng/g	5 - 166%	5	5	166	EPA 8270Cm
Phenanthrene	Polynuclear Aromatic Hydrocarbons	1	ng/g	60 - 135%	5	60	135	EPA 8270Cm
Pyrene	Polynuclear Aromatic Hydrocarbons	1	ng/g	42 - 166%	5	42	166	EPA 8270Cm
Total Detectable PAHs	Polynuclear Aromatic Hydrocarbons		ng/g					EPA 8270Cm

Quality Assurance / Quality Control (QA/AC) Procedure

Liver tissue extractions and GC/MS methods were subjected to QA/QC procedures according to EPA guidelines. Accuracy of GC/MS data was assessed by additional analyses of matrix spikes, surrogate spikes, certified reference materials, positive controls, and/or laboratory control materials on a minimum frequency of 1 per batch. We required that 95% of the target compounds greater than 10 times the minimum detectable limit (MDL) be within the specified acceptance limits. The acceptance ranges were determined from a minimum detection limit (MDL) study.

Precision of the data was determined by analysis of duplicate matrix spikes, blank spikes, and/or duplicate test sample analysis on a minimum frequency of 1 per batch. We required that for 95% of the compounds >10 times the MDL, the % Relative Percent Difference (%RPD) should be within the specified acceptance range (0-30%). The %RPD for the duplicate test sample analysis can be significantly affected by the homogeneity of the sample matrix within the sample container itself causing additional variability in the analytical results. In these cases, the QA/QC acceptance limits may be exceeded.

Samples were processed in batches for organic analysis. For our purposes, a batch is defined as a group of 20 or fewer samples of similar matrix, processed together under the same conditions and with the same reagents. Quality control samples are associated with each batch and are used to assess the validity of the sample analyses. The following quality control samples were analyzed with each batch: a procedural blank, replicates, matrix spikes, recovery surrogates, and blank spikes explained below. A typical batch size consisted of 10-15 samples.

Three types of standards were used during sample processing: recovery surrogates, standard spike solutions (for blank spikes and matrix spikes), and internal standards. Recovery surrogates were spiked into every extraction vessel to ensure extraction efficiency. For chlorinated pesticide and PCB analysis, the recovery surrogate solution contained the following four compounds: tetrachloro-m-xylene (TCMX), PCB 30, PCB 112 and PCB 198 in methylene chloride. For PAH analysis, the recovery surrogate solution contained the following 5 deuterated compounds: d10-Acenaphthene, d10-Phenanthrene, d12-Chrysene, d12-Perylene, d8-Naphthalene. A 100 µL aliquot was taken from a 10,000 ng/ml stock solution so that a total of 1000 ng of each compound in the recovery surrogate was added to each sample, to provide information regarding the extraction efficiency.

Overview of contaminant exposures in fish.

Our prior work measured substantial concentrations of contaminants in liver of fish residing in San Francisco Bay, including including polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and a variety of pesticides. A list of analytes measured is shown in the above table. It was also demonstrated measurement of individual livers was feasible, which enabled subsequent correlation analyses with endocrine parameters in the same fish (Brar et al., 2010).

Fish were sampled from locations that represented distinct environmental chemical signatures. For example, Richmond's Lauritzen Channel is a DDT-contaminated site, Oakland Inner Harbor is a heavily industrial site, while San Leandro Bay is a PCB-contaminated site (references cited in Methods section). On the other hand, Redwood City represented a site in which fish exhibited relatively lower contaminant exposures, while a site on Catalina Island had very low contaminant levels. Specific findings are provided in the Final Report of the Endocrine Disruption study (RMP; SFEI.org) and in Brar et al. (2010). Given that some of the archived plasma and liver samples from this former study were analyzed in the present study for additional endocrine parameters, a brief overview of the contaminant exposure data follow.

PCBs. In shiner surfperch, among 50 PCB congeners measured, 44 were detected, 26 were present in surfperch liver at concentrations >10 ng/g in one or more of the groups, while 16 were above 20 ng/g in one or more of the groups. Of the 12 dioxin-like co-planar PCBs (Appendix Table 1), only one (PCB 118) was present in concentrations above 20 ng/g in one or more of the groups, while three (PCBs 105, 126, and 156) were ≥10 ng/g in one or more of the groups. All detected PCB congeners were generally present at ≥2-fold higher concentrations in liver of fish from Oakland Inner Harbor and San Leandro Bay, as compared with fish from Redwood City and Richmond Lauritzen Channel, while PCB concentrations in the Catalina Island fish were very low overall (total PCBs were <20 ng/g) and most congeners were not detectable. In Pacific staghorn sculpin, PCBs tended to have relatively higher hepatic concentrations. Thirty-two of the detected PCB congeners were present in sculpin liver at concentrations >10 ng/g in one or more of the groups, while 25 were above 20 ng/g in one or more of the groups. As for the shiner perch, highest hepatic PCB congener concentrations measured in sculpin were for PCB 153 and PCB 138 (both present at >100 ng/g), followed by PCBs 101, 118 (dioxin-like), 180, 187, 149, 158 and 170, most of which were >40 ng/g in fish from all sites.

Pesticides. Richmond Lauritzen Channel, a DDT legacy site, was associated with very high liver concentrations of DDT and DDT metabolites in surfperch. Total DDTs were 7-12-fold higher in the fish from Richmond Lauritzen Channel as compared with all other groups ($p<0.001$). Metabolites 4,4 DDE and 4,4 DDD were present in particularly high concentrations (293.8±61.1 and 379.2±89.4 ng/g, respectively), which were >3.5-fold and >10-fold higher, respectively, as compared with that in all other groups ($p<0.05$). Concentrations of 2,4 DDT, 2,4 DDD and 4,4 DDT were mostly non-detectable in the other groups, but relatively high in the Richmond Lauritzen Channel shiner perch. A similar spatial pattern in hepatic DDT levels were observed in Pacific staghorn sculpin. However, sculpin at Richmond Lauritzen Channel had higher hepatic concentrations of 4.4-DDE (612.0 ± 83.6 ng/g) and 4,4-DDD (1072.9 ± 302.9 ng/g), 2.1-fold and 2.8-fold higher than the respective values in shiner surfperch ($p<0.05$). In addition, while 2,4-DDE was not detected in any shiner surfperch, it was detectable in sculpin from the Richmond Lauritzen Channel.

Gamma chlordane and alpha chlordane were widely detected in shiner perch, with highest concentrations in fish from San Leandro Bay ($p<0.05$); these pesticides were not detectable levels in fish from Catalina Island. Trans-nonachlor and cis-nonachlor were also widely detected and were present in liver of the San Leandro Bay surfperch in higher concentrations than in most other groups

($p<0.05$). In Pacific staghorn sculpin, hepatic concentrations of the chlordanes were more variable and showed no significant site-associated differences. While chlordane concentrations tended to be higher in sculpin as compared with shiner perch at most locations, sculpin from Richmond Lauritzen Channel had 2- to 4- times higher hepatic concentrations of the chlordanes than did shiner surfperch ($p<0.05$).

PAHs. Hepatic concentrations of a variety of PAHs were variable across the different groups of shiner surfperch and Pacific staghorn sculpin. In shiner surfperch, total PAHs were highest in fish from Richmond Lauritzen Channel, and lowest in fish from Catalina Island. In sculpin, the same location-associated differences were not evident; however, total PAHs were >3-fold higher as compared with corresponding shiner perch groups ($p<0.01$), except in San Leandro Bay. In most groups of either species, naphthalene constituted a major contributor (20-50%) of the total PAHs present. Other PAHs showing high hepatic concentrations in selected groups included acenaphthene, phenanthrene, anthracene, the methylnaphthalenes, and fluoranthrene.

As a result of this work, we were able to establish a database of fish that exhibited varying exposures to different types of contaminants. This, in addition to the ability to measure contaminants within individual livers, enabled correlation analyses to evaluate the potential relationships between parameters of endocrine physiology and exposure to the different types of contaminant chemicals.