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Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay

I. Hepatic contamination and mixed-function oxidase (MFO) activity during the reproductive season

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Abstract

Concentrations of neutral organic contaminants and activities of microsomal P-450 mixed-function oxidase (MFO) were measured in the livers of the starry flounder *Platichthys stellatus* (Pallas), collected from more- and less-contaminated sites in San Francisco Bay, during the 1984–1985 reproductive season. Starry flounder collected at the Berkeley (Bk) site, located in the more urbanized central portion of San Francisco Bay, had greater liver concentrations of polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAH) (fluorescence equivalents of pyrene) than those collected at a site in northern San Pablo Bay (SP), where urban development is less intense and more distant. Hepatic aryl hydrocarbon hydroxylase (AHH) activity, a particular MFO, in males and in gonadally immature females fluctuated significantly from September 1984 through April 1985 with the Bk population exhibiting significantly greater activities during this period. Site differences were especially notable during the time of spawning (January–March), as AHH activities of starry flounder from SP declined and those from Bk remained elevated. These site differences appear to be due to P-450 isozymes (e.g. P-450E) whose activities are inducible by some PCBs and PAHs, as males and gonadally immature females from the two sites were not different in their hepatic AHH activities when assayed in the presence of an inhibitor of P-450E, 7,8-benzoflavone (7,8-BF). Greatly reduced hepatic AHH activities in females coincided with the onset of vitellogenesis; however, a comparison of female starry flounder bearing yolky eggs from the two sites during several successive reproductive seasons indicated significantly greater AHH activity in those caught at Bk than those from SP. There was a linear relationship between hepatic AHH activity and its inhibition by 7,8-BF in all *P. stellatus* assayed and more than 98% of individuals caught in San Francisco Bay had hepatic AHH activites that were suppressed by 7,8-BF. Therefore, widespread induction of hepatic microsomal P-450 by PAH-type compounds in San Francisco Bay is indicated and, further, P-450 induction is apparent in female starry flounder in a portion of San Francisco Bay during gametogenesis and the time of spawning.

Introduction

Whether continual exposure of fishes to the complex contaminant mixtures in urban coastal waters has an effect on reproduction is not well understood. Two studies in the Baltic Sea indicated that reduced viable hatch is associated with residues of chlorinated hydrocarbons < 300 ng g^{-1} in the gonads of the Baltic flounder *Platichthys flesus* (Von Westernhagen et al. 1981) and the herring *Clupea harengus* (Hansen et al. 1985). Recent studies of inducible cytochrome P-450 monoxygenases indicate that there may be two mechanisms of toxic action resulting from MFO catalytic activity, e.g. aryl hydrocarbon hydroxlase (AHH) activity. First, the *in-vivo* metabolism of polynuclear aromatic hydrocarbons (PAH) may result in the binding of PAH metabolites to proteins and DNA (Varanasi and Gmur 1980, Varanasi et al. 1981, 1982), and such chemical lesions may lead to reproductive or developmental damage (Mattison 1980). Second, there is increasing evidence that exposure to xenobiotic compounds may alter steroidogenesis (Freeman et al. 1982, Truscott et al. 1983) and that steroids are substrates for contaminant-induced P-450 isozymes (Klotz et al. 1983, 1984, Forlin and Haux 1985). If contaminants in coastal ecosystems arising from urban activity affect the reproductive or developmental success of fishes, then there is reason to suspect that populations may be altered and, ultimately, that recreational and commercial fisheries may decline.

Environmental induction of MFO activity by PAH-type inducers is apparently widespread in coastal fish populations of North America. Using 7,8-BF, a competitive inhibitor of the catalytic activity of cytochrome

P-450E in aryl hydrocarbon hydroxylation (Klotz et al. 1983), as a probe, 100% of scup captured in the vicinity of Woods Hole, Massachusetts, USA, (Stegeman and Binder 1979) and 85% of winter flounder captured near Mount Desert Island, Maine (Foureman et al. 1983) exhibited inhibition of hepatic microsomal AHH activity with the probe. Whether naturally occurring inducers from plants, e.g. indoles and flavanoids (Wattenberg et al. 1976, Pantuck et al. 1979) may have contributed to these inductions is not known, but the ubiquitous occurrence of PCB and PAH in coastal areas has been established.

In this study, relationships between chlorinated hydrocarbon tissue residues, hepatic MFO activity and several measures of gamete viability and reproductive success were investigated in *Platichthys stellatus* from a major urbanized bay-estuarine system. In the first paper, we report on the tissue concentrations of chlorinated and polynuclear aromatic hydrocarbons and compare hepatic MFO activity during the reproductive season between the more contaminated central bay site, Berkeley (Bk), and a less contaminated site in northern San Pablo Bay (SP). Some data are also presented on *P. stellatus* captured in Oakland Outer Harbor, a contaminated central bay site not far from Bk (Fig. 1). In the subsequent paper (Spies and Rice 1988), we report on the relationships between reproductive success, contaminant concentrations in tissues and hepatic MFO activity in *P. stellatus* from San Francisco Bay. The second paper also reports differences in reproductive success, MFO activity and contaminant concentrations between fish captured at Bk and SP.

Materials and methods

Site and species selection

The two study sites within San Francisco Bay differ in their concentrations of organic contaminants. The concentrations of total polynuclear aromatic hydrocarbons (PAHs) in sediments of Bk were reported to be $4\,600 \pm 1\,800$ ng g^{-1} (dry wt); comparable values from the San Pablo Bay site were $2\,600 \pm 1\,300$ ng g^{-1} (Spies et al. 1985). Concentrations of total polychlorinated biphenyls (PCBs) between the central San Francisco Bay and San Pablo Bay also appear to be different, as total PCB in sediments from Oakland have been reported to be 61 ± 12 ng g^{-1} and those from SP, 9.3 ± 2.3 ng g^{-1} (dry wt) (NOAA 1987). Somewhat similar differences between sediments from these two areas in total PCBs were also reported by Chapman et al. (1986).

Platichthys stellatus (Pallas) is one of the most common and widespread flatfish in the Bay (Smith and Kato 1979). Previous study has established that this species actively transforms BaP to precarcinogens and that exposure to known inducers of AHH activity increased production of forms of BaP that bind to DNA *in vitro* (Varanasi and Gmur 1980). This species reproduces once a year. Adult females always carry a clutch of previtellogenic oocytes, that gives rise to a clutch of yolky eggs in summer (Orcutt 1950). The yolky eggs pass group-synchronously through the remaining maturation stages, as defined by Yamamoto (1956). Spawning occurs from December through February.

Collection and dissection

Platichthys stellatus were collected with 5 and 7 m otter trawls towed for 20 min in depths from 2.5 to 7 m at the stations indicated in Fig. 1. These stations are located on extensive subtidal mud flats that slope gently toward the deeper main channels. We sampled each site monthly from August 1984 through April 1985 to determine if differences exist between these sites in seasonal hepatic AHH activities, with and without the 7,8-BF probe. Data on mature fish from collections in the previous two reproductive seasons (1982–1983, 1983–1984) were used to supplement data collected in 1984–1985. In 1984–1985 flounder, less than 22.5 cm standard length were not retained. When more than ten flounder were captured at a station we retained more of the larger, sexually mature fish. Although females grow to larger sizes than males (Orcutt 1950), each collection included several males. Despite extensive trawling, no *P. stellatus* were captured at Bk in January and March 1985. Previous analyses indicated that size and hepatic AHH activity were unrelated in this species (Spies et al. 1985). Also, handling stress has been shown not to have a measurable effect on hepatic AHH activity in salmon (Colloidi et al. 1984). Captured fish were maintained on the vessels in flowing bay water until transported to the recirculating marine aquaria at Lawrence Livermore National Laboratory (LLNL). Fish were sacrificed on the day following capture. Solvent-rinsed tools were used to remove the livers and the gonads, which were weighed, and aliquots of each were put aside for subsequent analyses. For each fish, the gonadosomatic index (GSI) was calculated as [gonad wt/(body wt-gonad wt)] × 1000.

Hepatic MFO activity

Slices < 5 mm thick were removed from the posterior liver for the *in-vitro* assay of microsomal enzyme activity. These were immediately frozen on dry ice and transferred within an hour to a freezer maintained at −76 °C. One or more liver slices were later used to prepare a microsomal pellet. These microsomes were assayed for AHH activity using the method of Nebert and Gelboin (1968) as described previously (Spies et al. 1982). A portion of the hepatic microsomes from each fish in the 1984–1985 reproductive season was reassayed with 10^{-4} *M* 7,8-BF. This concentration was determined to cause maximal suppression of AHH activity; 25 °C was determined to be a more optimal assay temperature than either 18° or 37 °C. At 25 °C, reaction kinetics were linear for 10 min. An Aminco Bowman

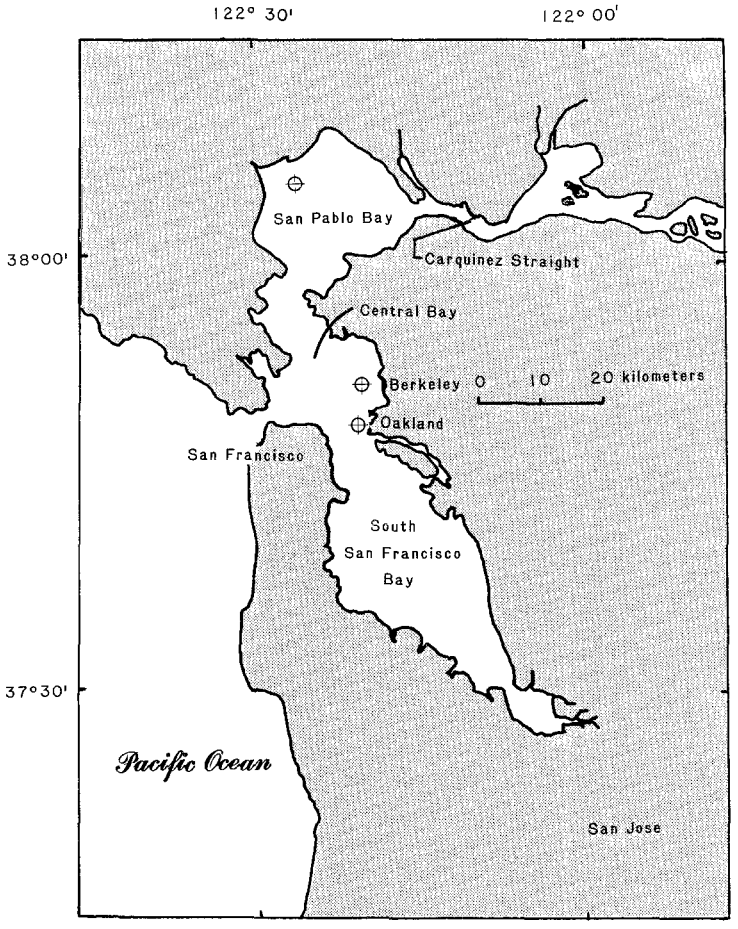

Fig. 1. Location of sampling stations in San Francisco Bay. The two main study sites were Berkeley (more contaminated) and San Pablo Bay (less contaminated); some data from Oakland Outer Harbor, a contaminated central bay site, are also presented in this study

spectrofluorometer was used to quantify the 3-OH benzo (a)pyrene metabolite. The spectrofluorometer was calibrated with quinine sulfate standards. In addition, a standard of microsomes from 3-methylcholanthrene-induced mice was assayed with each batch of microsomes as a control for assay conditions. Fluorescence values of assays were corrected according to the quinine sulfate standard. Based on duplicate and triplicate assays from seven fish, the mean coefficient of variation was 14.6% for AHH activity, and 2% for the mean percent change in AHH activity with the addition of the AHH inhibitor, 7,8-BF. Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin (BSA) as the standard. Hepatic AHH specific activities, are reported as pmol 3-OH-benzo(a)pyrene min^{-1} mg^{-1} protein.

The effects of freezing whole-liver tissue on subsequently determined hepatic AHH activity was assessed for *Platichthys stellatus* by comparing activities of fresh liver from two males with liver samples from the same individuals frozen for 5, 19 and 40 d and assayed as outlined above. Mean AHH activities of frozen samples were within 15% of mean values of freshly prepared microsomes, with no obvious pattern of either increased or decreased activities with time whether the thawed tissues were assayed the same day or whether microsomes were refrozen in a buffer (0.05 *M* phosphate in 30% glycerol) and later assayed. This variation was nearly the same as the coefficient of variation for replicate determinations, 14.6%.

The effects of freezing whole-liver tissue on P-450 monoxygenase activities and UDP glucuronyl transferase were investigated for *Salmo gairdneri* by Forlin and Anderson (1985). Little loss of activity was found when small slices of tissue were preserved for up to 3 d in liquid nitrogen and subsequently assayed; however, at dry-ice temperature (–75 °C) a loss of 72 to 85% of monoxygenase activity occurred in 24 h. This is in contrast to our results, where there was no detectable loss of AHH activity in frozen liver tissue maintained at –76 °C for up to 40 d. We attribute this to intraspecific differences in the susceptibility of P-450 enzymes to degradation by freezing at –75° to –76 °C. It would therefore appear appropriate to test each new species for susceptibility to degradation of P-450 under various conditions of freezing when undertaking field studies that necessitate preservation of whole tissue before microsomal fractions can be prepared.

Neutral organic contaminants

Residues of chlorinated hydrocarbons in liver were determined using methods similar to those of Ozretich and Schroeder (1986). Briefly, tissues were macerated in pre-

Table 1. *Platichthys stellatus.* Concentrations ($\mu g\ g^{-1}$ lipid ± SD) of neutral organic contaminants in livers of fish collected from two sites in San Francisco Bay in October 1984. These concentrations can be converted to wet wt basis ($\mu g\ g^{-1}$) by multiplying by mean lipid contents (112 mg g^{-1} for BK, 140 mg g^{-1} for SP) and dividing by 1000. bd: below detection

Site (*n*)	HCB[a]	Total phthalates[b]	DDTs				PCBs (Aroclors)				PAH (total)[c]
			DDD	DDT	DDE	Σ	1242	1254	1260	Σ	
Berkeley (10)	bd 0.19	$10.7\times10^4\pm 16.0\times10^4$	3.0± 1.1	1.3± 1.6	2.8± 0.21	7.1± 4.1	0.57± 0.80	18.0± 9.1	41.8± 23.5	60.3± 32.1	2.6
San Pablo Bay (15)	bd 0.35	$10.3\times10^4\pm 9.4\times10^4$	2.3± 0.83	2.3± 1.8	1.5± 0.46	6.2± 2.5	1.09± 1.3	6.2± 2.2	11.7± 12.9	19.0± 13.4	0.14
P for site differences	–[d]	0.93	0.10	0.13	0.03	0.53	0.26	0.0001	0.0004	0.0002	–[e]

[a] Hexachlorobenzene
[b] Includes sums of dimethyl, diethyl, dibutyl, benzybutyl, dioctyl and bis-(2-ethylhexyl) phthalates. Concentrations "bd" were arbitrarily assigned values of 0 in order to compare totals at the two sites
[c] Each sample made up of combined extracts of livers of 20 fish collected in Aug. and Sep. 1984; values in $\mu g\ g^{-1}$, not lipid-normalized
[d] Means, statistical tests and SD not applicable for range of values including "bd"
[e] Statistical tests not applicable for single values from each site

cleaned beakers, water was removed by addition of precombusted (600 °C) anhydrous Na_2SO_4, acetonitrile (uv grade) and an internal standard of bromoveritrol and 4,4′-dibromobiphenyl was added, and the mixture was homogenized by a high-speed tissue macerator (Polytron) to produce the first extract. The first extract was decanted after settling, and the mixture was extracted twice more, with the final extract clarfied by centrifugation. The extracts were combined, and made up to 100 ml, a subsample was removed to gravimetrically determine extracted lipids, and the remaining extract was cooled overnight to 4 °C. A second sub-sample was removed for isolation, identification, and quantification of aromatic compounds of interest (PCB, PAH, DDT and phthalate esters). Interfering saturated compounds (e.g. alkanes), remaining lipids and fatty acids were removed by passing the extract through disposable solid-phase chromatography columns (Baker) with C_{18} and NH_2 solid-phase absorbents. The concentrated extract was analyzed by gas chromatography (Hewlett-Packard, 5880) using a ^{65}Ni electron-capture detector and a 0.25 mm i.d., 30 m fused-silica capillary column internally coated with cross-linked methyl silicone. Chlorinated hydrocarbons of interest were analyzed based on retention times and response factors of authentic external standards. Values of analytical blanks were subtracted, and final concentrations were corrected for recovery of internal standards. PCBs were quantified based on unique peaks in Aroclor 1242, 1254, and 1260 standards. Use of these methods resulted in 70% of recoveries being within 50 to 90%. Analysis of split samples produced values within 10% of the mean for 80% of the chlorinated compounds analyzed. Analyses of liver extracts for PAH present in the sediments of San Francisco Bay revealed that only rarely would PAHs be present above the detection limits.

To obtain a relative estimate of the concentrations of PAH in the liver of *Platichthys stellatus* from the study sites, the liver extracts that had been analyzed for chlorinated hydrocarbons were combined for 20 fish from each site in August and September 1984 and analyzed by high-performance liquid-chromatography with fluorescence detection (Environmental Protection Agency Method 610). The total fluorescence of the extract was normalized to the fluorescence of a pyrene standard to estimate total PAH content of livers. The detected PAHs were presumed to be mainly metabolized forms present in the livers.

Results

Neutral organic contaminants in livers of *Platichthys stellatus*

The results of the analysis of *Platichthys stellatus* collected during October 1984 from Bk ($n=10$) and SP ($n=15$) for neutral organic contaminants are presented in Table 1. Lipid-normalized concentrations of hexachlorobenzene, total phthalates, DDD, DDT, ΣDDTs and Aroclor 1242 were either similar in their ranges or had mean values that were not significantly different between sites. In contrast, flounder from Bk had significantly greater concentrations of DDE, Aroclors 1254, 1260 and ΣPCBs. Total PAH concentration, although based on a single composite sample, was nearly 20 times greater (not lipid-normalized) in Bk-captured fish than those from SP. To determine if there were factors other than site that might explain the variance in these data, we carried out several regressions and analyses of variance (SAS 1985). The mean extractable lipid contents of livers of flounder were not significantly different between sites (Bk, 112 ± 58 and SP, 140 ± 28 mg^{-1}) (ANOVA, $P=0.18$) or sexes ($P=0.62$). There were no differences in sexes in the log-transformed and lipid-normalized concentrations of hexachlorobenzene, HCB ($P=0.62$), ΣPCB ($P=0.36$) or ΣDDT ($P=0.36$). Likewise, there were no relationships between standard length and log-transformed, lipid-normalized concentrations of HCB ($P=0.87$), ΣPCB ($P=0.84$) or ΣDDT ($P=$

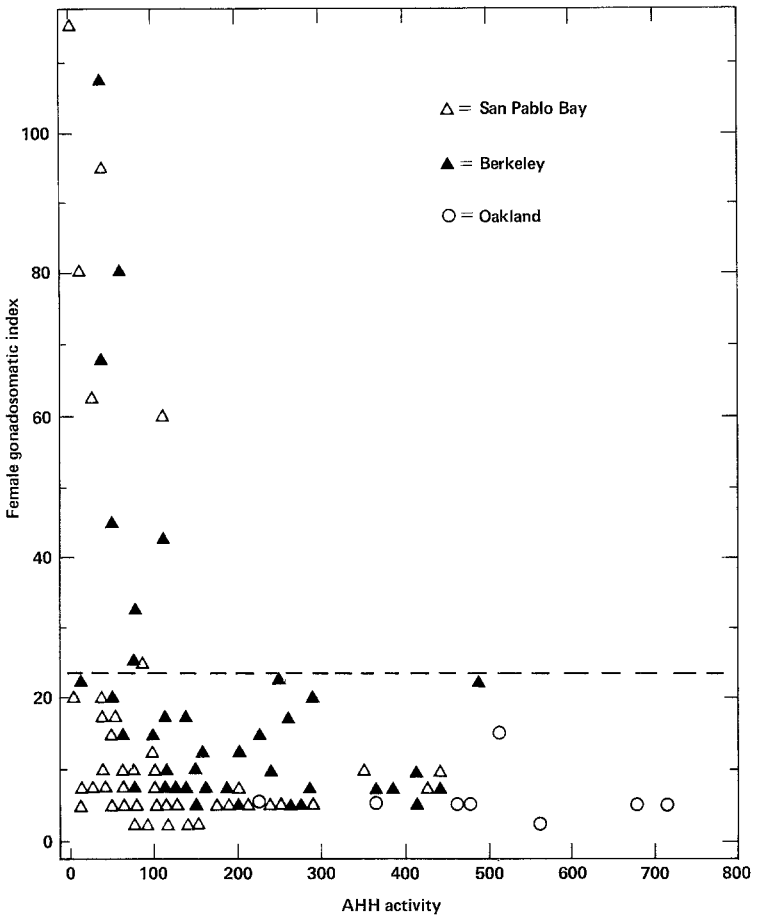

Fig. 2. *Platichthys stellatus.* Relationship between hepatic aryl hydrocarbon hydroxylase (AHH) activity [pmol 3-OH benzo(a)pyrene mg^{-1} protein min^{-1}] and female gonadosomatic index (GSI). Dashed line separates females bearing yolky eggs (GSI > 22) from those bearing only non-yolky eggs (GSI < 22). Based on collections from San Francisco Bay, 1982–1985

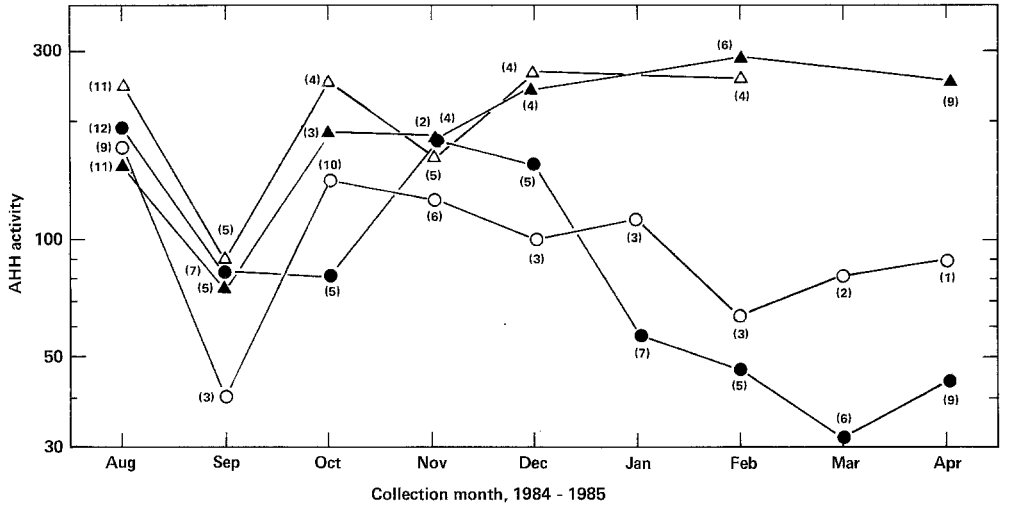

Fig. 3. *Platichthys stellatus.* Hepatic AHH activity by sex in monthly collections from Berkeley and San Pablo Bay during 1984–1985 reproductive seasons; units as in Fig. 2. Females with yolky eggs are not included. Triangles are Berkeley collections; circles, San Pablo Bay collections. Open symbols are males; filled symbols, females. Number of fish forming each mean are shown in parentheses

0.84). However, the log-transformed, lipid-normalized concentrations of ΣDDT and ΣPCB were strongly correlated in these fish ($P = 0.02$).

Hepatic AHH activity during reproductive season

All females from our 1982–1985 collections with a gonadosomatic index (GSI) > 22 had hepatic AHH specific activities below 200 U [pmol 3-OH benzo(a)pyrene min^{-1} mg^{-1} protein], while males and less mature females had a greater range of maximum activities (Fig. 2). Since a GSI of 22 is the lower boundary for females with yolky eggs (Spies et al. 1986), the onset of yolk production in this species coincides with greatly reduced hepatic AHH activity. In Fig. 3, the mean hepatic AHH specific activities of males and females with GSI < 22 are shown for BK and

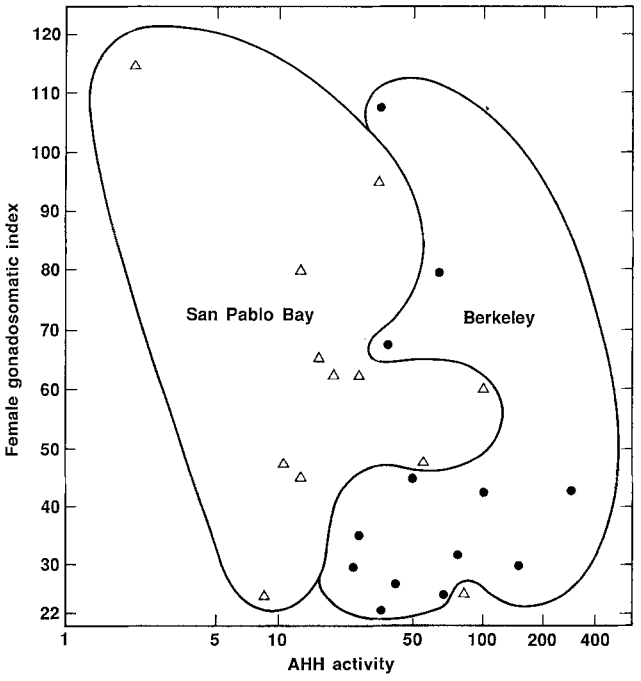

Fig. 4. *Platichthys stellatus.* Relationship between hepatic AHH activity and gonadosomatic index in females bearing yolky eggs caught at Berkeley and San Pablo Bay from August through March, 1982–1985; units as in Fig. 2

Table 2. *Platichthys stellatus.* Results of analysis of variance of hepatic AHH activity in monthly collections from San Francisco Bay during 1984–1985 reproductive season

	P
Variable	
Sex	0.888
Site	0.0001
Month	0.0001
Interactions	
Sex × Site	0.340
Sex × Month	0.360
Site × Month	0.0009
Sex × Site × Month	0.236

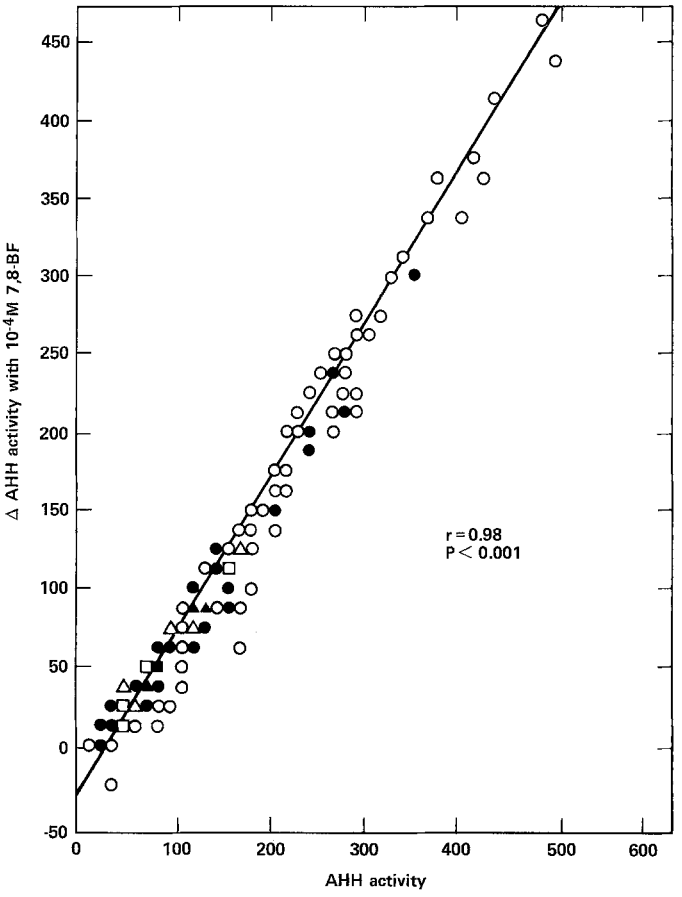

Fig. 5. *Platichthys stellatus.* Relationship between hepatic AHH activity and change in hepatic AHH activity with 10^{-4} *M* benzoflavone (7,8-BF) for all individuals collected in 1984–1985 reproductive season; units as in Fig. 2. ○ = 1 observation, ● = 2, △ = 3, ▲ = 4, □ = 5, ■ = 6 observations

SP collections during the 1984–1985 reproductive season. An analysis of variance of these data indicated that there were significant differences between sites and months, but not between sexes (Table 2). The significant interaction between sites and months is a result of the divergence in AHH activity after November 1984. Although the analysis of variance indicated that there were no significant differences in AHH activity due to sex, the females collected from SP between January and March had consistently lower mean activities than did males.

Since there were only 12 females with yolky eggs collected in 1984–1985, the site comparison of gonadally-mature females (GSI > 22) is based on all those collected from August through March, 1982–1985 (Fig. 4). Most of these females were collected in October, December, January, and February. Analysis of these data shows that there was not a significant relationship between GSI and AHH activity (ANOVA, $P > 0.05$); however, the mean AHH activity of Bk females, 59 U, was significantly higher than that of SP females, 21 U ($P < 0.0001$). Thus, for both sexes, the more-contaminated site, Bk, had individuals with greater hepatic AHH activity during the reproductive season than those from the SP site. For males and gonadally immature females, the site differences were greater from December 1984 through March 1985.

Inhibition of hepatic AHH activity by 7,8-benzoflavone

Further insight into site differences is provided by comparison of AHH catalytic activity of liver microsomes from individual fish with and without 10^{-4} *M* 7,8-BF. For all starry flounder we have assayed from San Francisco Bay there is a highly significant ($P < 0.001$) relationship between the extent of inhibition and the original activity (Fig. 5). So, in San Francisco Bay, hepatic microsomal P-450 isozymes sensitive to inhibition by 7,8-BF are responsible for the occurrence of high AHH activity in *Platich-*

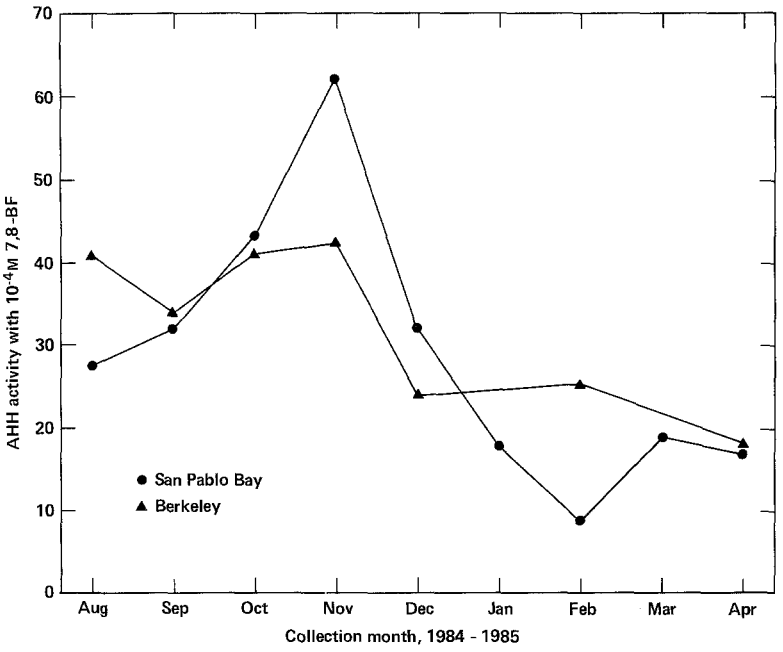

Fig. 6. *Platichthys stellatus.* Hepatic AHH activity not inhibited by 7,8-benzoflavone in males and in females not bearing yolky eggs, collected monthly at San Pablo Bay and Berkeley, 1984–1985; units as in Fig. 2

thys stellatus. In Fig. 6, hepatic AHH activity with 7,8-BF in the assay medium is compared for livers of male and gonadally immature females collected at the two sites. Interestingly, fish from the sites are not different when compared on this basis ($P = 0.99$), but there are significant differences between collection months ($P < 0.001$), with a prominent activity maximum in November 1984.

Hepatic AHH activity and chlorinated hydrocarbons

The possible relationship between hepatic AHH activity and chlorinated hydrocarbon concentrations was investigated in the field collections of October 1984. Since females bearing vitellogenic eggs have relatively low hepatic AHH activities, we investigated these relationships in males. There were no significant relationships found in this group between log-transformed hepatic AHH activity and log-transformed lipid-normalized concentrations of HCB ($P = 0.97$), ΣDDT ($P = 0.27$) and ΣPCB ($P = 0.27$). There were too few females with a GSI > 22 to analyze for such possible relationships.

Discussion

A general pattern has been found between two sites in San Francisco Bay with differing amounts of PCB and PAH, where *Platichthys stellatus* captured in the more contaminated site (Bk) maintain high hepatic AHH activity during the reproductive season, and fish captured in the less contaminated site (SP) exhibit reduced activities. This pattern is consistent with previously reported results. For example, a study of seasonal changes of AHH activity in *Tautogolabrus adspersus* that compared wild, captive oil-exposed and captive unexposed fish found that in both males and females from the wild population hepatic AHH activity declined during gonadal maturation from specific activities of greater than 200 to less than 50 U (Walton et al. 1983). The low activity was maintained through the short summer spawning season. In the laboratory, both males and females exhibited a very similar pattern, except that both sexes exposed to oil had slightly, but significantly higher hepatic AHH activity than captive controls during spawning. For several weeks after spawning, control individuals had slight, but significantly higher levels of hepatic AHH activity. Thus, in a controlled laboratory study it has been demonstrated that hepatic AHH activity is not suppressed to the same basal level during spawning in oil-exposed fish as in controls. A seasonal study of the salmonid *Corengus alba* in pristine lakes of northern Finland revealed a general depression of hepatic AHH activity before spawning in November, with females having slightly but significantly lower activities at the time of spawning. The 7-ethoxycoumarin o-deethylase activity exhibited a somewhat similar pattern. Mean minimal hepatic AHH activities were 2 pmol 3-OH-benzo(a)pyrene min^{-1} mg^{-1} protein or less in spawning females (Lindström-Seppä 1985). This general pattern, except for the somewhat earlier spawning and much higher hepatic AHH activities, has been observed here for *P. stellatus* in San Pablo Bay.

The divergence of hepatic AHH activities during the reproductive season in the more- (Bk) and less- (SP) contaminated populations is consistent with the notion that more highly induced flounder do not lower the catalytic activity of 7,8-BF-inhibited isozymes of hepatic cytochrome P-450 to the same basal activities during the spawning season as do fish from the less-contaminated site. Thus, fish from Bk with greater liver concentrations of PCBs and PAHs appear destined to reproduce with greater hepatic AHH activity than those from San Pablo

Bay. Although month-by-month comparisons have not been made for gonadally mature females, site differences in hepatic AHH activity were evident within the aggregate of such females captured during several reproductive seasons. In the following paper, evidence is presented that gamete viability, zygote formation and embryological development decrease with increasing hepatic AHH activity of spawning females (Spies and Rice 1988). The low hepatic AHH activities of gonadally mature female flounder compared to males and gonadally immature females is consistent with known effects of estradiol on teleost hepatocytes: initiation of vitellogenin production (Sundararaj and Wain 1981, Selman and Wallace 1983, DeVlaming et al. 1984) and suppression of cytochrome P-450 content (Stegeman and Chevion 1979, Hansson and Gustafson 1981, Forlin et al. 1984).

The lack of a relationship between hepatic AHH activity and hepatic PCB concentrations is not unexpected. Although coplanar PCBs induce isozymes of the cytochrome P-450 complex (Lech et al. 1982), other inducers of this system, e.g. PAH, may be participating in induction, especially individual compounds that we have not quantified but which have lipid solubilities similar to inducing PCBs. Also, the possible role of natural products, such as flavanoids or indoles from plants, has not been investigated.

The high fluorescence of liver extracts and the lack of detectable concentrations of most PAH parent compounds when these extracts are analyzed by gas chromatography in Bk-captured fish indicate that aromatic hydrocarbons are effectively metabolized, as has been demonstrated previously for this species (Varanasi et al. 1979, Varanasi and Gmur 1980). The inhibition of hepatic AHH activity by 7,8-BF in greater than 95% of *Platichthys stellatus* that were captured indicates that nearly all individuals in San Francisco Bay are affected by PAH-type inducers. This compares to 85% of *Pseudopleuronectes americanus* in coastal Maine (Foureman et al. 1983), 66% of *Paralichthys lethostigma* captured near St. Augustine, Florida (Little et al. 1984), and 100% of *Stenotomus chrysops* captured near Woods Hole, Massachusetts (Stegeman and Binder 1979). The activities of Bk-captured fish rarely exceeded 400 pmol 3-OH benzo(a)pyrene min^{-1} mg^{-1} microsomal protein, while activities of 1 800 U were induced in *Platichthys stellatus* by injection of 5 mg benzo(a)pyrene kg^{-1} (Spies, unpublished data), so it appears that full induction has not occurred in the wild population from Berkeley. *P. stellatus* captured from the area of the Oakland Outer Harbor in central San Francisco Bay had hepatic activities up to 715 pmol 3-OH BaP min^{-1} mg^{-1} protein (Fig. 2). If, in fact, the divergence of AHH activities after December 1984 at the two main collection sites is a result of the relative lack of suppression of P-450 activity in the Bk population due to the effects of contaminants, and the measured PCBs are the most significant inducers, then the threshold for a differential pattern of response in hepatic AHH activity is between 60 mg g^{-1} lipid (Bk) and 19 mg g^{-1} lipid (SP) ΣPCB. Obviously, further study is required to test this suggestion, particularly of the possible role of PAH or other chlorinated aromatic hydrocarbons in induction and whether PAH, like ΣDDT, covaries with ΣPCB tissue concentrations.

Differences in hepatic AHH activity between the two sites in male and gonadally immature female flounder is due to the activity of cytochrome(s) P-450 that are inhibited by 7,8-BF. Since Klotz et al. (1983) isolated a hepatic cytochrome, P-450E, that is the major isozyme catalyzing hydroxylation of BaP in scup, it is suspected that the activity of a similar or identical cytochrome is responsible for these site differences.

Finally, while the habits of *Platichthys stellatus* in San Francisco Bay have not been carefully studied, it probably behaves similarly to its European congener *P. flesus,* living in confined habitats within the estuary most of the year (Hartley 1947, Wirjoatmodjo and Pitcher 1984). While we do not have independent confirmation that the populations sampled for this study had not been recently in other parts of the estuary or in the Pacific Ocean, the significant differences in the concentrations of DDE and PCBs in fish between Bk and SP mirror differences in concentrations of chemical contaminants found in the sediments of the two sampling areas and indicate that intermixture of populations had not been sufficient to obscure these trends.

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