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

II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory*

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Abstract

Gonadally mature *Platichthys stellatus* (Pallas) were captured at two localities in San Francisco Bay in 1983–1985 and were induced to spawn in the laboratory; they were evaluated for relationships between several measures of survival through successive early life-history stages, chlorinated hydrocarbon concentrations in maternal liver and spawned eggs, and maternal hepatic mixed-function oxidase (MFO) activity. The effect of laboratory holding on hepatic aryl hydrocarbon hydroxylase (AHH), a particular MFO activity, and concentrations of chlorinated hydrocarbons were also evaluated. Significant negative correlations were found between maternal hepatic AHH activity at the time of spawning and percent viable (floating) eggs, fertilization success, and embryological success. Embryological success was also negatively correlated with concentrations of polychlorinated biphenyls (PCBs) in eggs. Laboratory holding for 45 d, about twice the mean time to spawning, resulted in no significant changes in chlorinated hydrocarbon concentrations, but significant decreases in liver concentrations of phthalate esters and hepatic AHH activity. Females captured at the more urbanized central bay site, Berkeley (Bk), had a lesser proportion of floating eggs, poorer fertilization success, and higher hepatic AHH activities than those captured at a site in northern San Pablo Bay (SP). These results indicate the potential for a serious effect of lipophilic neutral organic contaminants on reproduction of an important estuarine flatfish species. Several mechanisms of toxic action are suggested to account for the observed effects, including the binding of toxic metabolites of contaminants to macromolecules and the alteration of sex steroids in females with contaminant-induced P-450 isozyme(s).

Introduction

Dilute concentrations of lipophilic xenobiotic contaminants in coastal waters near urban areas accumulate to high concentrations in fishes unless they are rapidly metabolized. Some xenobiotic compounds induce their own metabolism or that of related compounds. For example benzo(a)pyrene exhibits substrate-induced metabolism (Kurelec et al. 1979, Varanasi and Gmur 1980) and is not substantially accumulated in unaltered form, but PCBs induce the same apparent isozyme(s) (Melancon et al. 1981, Addison et al. 1982, Lech et al. 1982), their oxidation is not as readily catalyzed, and they are consequently accumulated (McDermott-Ehrlich et al. 1978, Pizza and O'Connor 1983, Bruggeman et al. 1984). Some products of the initial oxidation of xenobiotic compounds are excreted as conjugated products of further detoxification reactions (Melancon and Lech 1978, Varanasi and Gmur 1981, Gmur and Varanasi 1982); however, other metabolites [e.g. the 7,8-diol-9,10-epoxide of benzo(a)pyrene] covalently bind to DNA and proteins in the liver and gonads of pleuronectid flatfish (Varanasi et al. 1981, 1982). Such chemical lesions may lead to a variety of cellular disorders, including those with teratogenic manifestations (see review by Conney 1982). Furthermore, the substrates oxidized by the major isozyme induced by polynuclear aromatic hydrocarbon (PAH)-type compounds in fish, P-450E, include testosterone (Klotz et al. 1983, 1984), thereby indicating that xenobiotic compounds may influence hormonal regulation in fish, as has been indicated in higher vertebrates (see review by Parkinson and Safe 1981). Thus, the induction of microsomal oxidases by xenobiotic compounds may potentially lead to reproductive effects in fish through at least two general types of mechanisms.

Induction of hepatic microsomal mixed-function oxidases, often several-fold above that in unexposed fish, appears to be common in coastal marine environments of North America, as fish collected in widely ranging localities on the Atlantic and Pacific coasts have shown char-

* Some preliminary findings included in this study were presented at the Third International Symposium on Responses of Marine Organisms to Pollutants (Spies et al. 1985a)

acteristics of induction by PAH-type compounds (Stegeman and Binder 1979, Spies et al. 1982, Foureman et al. 1983, Little et al. 1984).

The possible reproductive consequences of increased microsomal mixed-function oxidase from contaminant exposure in populations of feral fishes has not been investigated previously. The goal of this study was to determine if *Platichthys stellatus* collected from localities in San Francisco Bay with differing degrees of contamination and spawned under controlled laboratory conditions showed such relationships. In the first paper of this series (Spies et al. 1988) it was found that *P. stellatus* from a contaminated site in San Francisco Bay, Berkeley (Bk), had significantly greater concentrations of PCB and maintained greater hepatic aryl hydrocarbon hydroxylase (AHH) activity during the reproductive season than those from a less contaminated site in northern San Pablo Bay (SP). The latter population exhibited decreasing hepatic AHH activity during the time of spawning compared to Bk. Furthermore, the site differences were due to activities of isozyme(s), e.g. P-450E, responsible for aryl hydroxylation and whose activity is inhibited by 7,8-benzoflavone (Klotz et al. 1983). Results presented here indicate that in laboratory-spawned females, hepatic AHH activity is inversely correlated with developmental success and that PCB content of eggs is inversely correlated with embryological success. Further site differences in maternal AHH activity at the time of spawning and two measures of developmental success were found for fish captured at Bk and SP.

Materials and methods

Most gonadally mature *Platichthys stellatus* (Pallas) were trawled from either near Berkeley (Bk) or at a site in northern San Pablo Bay (SP) (see Fig. 1 of preceding paper in this issue). *P. stellatus* spawn in the winter months in central California (Orcutt 1950), and we collected fish for this study mainly in January and February from 1984 and 1985. A small number of additional fish came from near Richmond or the Oakland Outer Harbor; a few fish were also collected late in December 1983. Fish were transported to our laboratory and acclimated for several days to our seawater system, which is maintained at 11° to 13 °C and at a salinity of 29 to 30‰. The holding aquaria measured 58 × 58 × 47 cm (158.1 liters), and two to three females were placed in each aquarium. Males were generally maintained separately.

One to three days after capture, gonadally mature females were started on a course of carp pituitary-extract injections (1 mg kg^{-1} d^{-1}, i.m.) to induce spawning. Based on histological examination of ovaries of fish captured during 1985 in San Francisco Bay, a majority of females are in Maturation Stage VII late in the reproductive season (Yamamoto 1956). This is the tertiary yolk stage, and the nuclei at this stage are still close to the center of the developing oocytes. Some movement of nuclei to peripheral positions and germinal vesicle breakdown was observed as early as 10 d after the start of pituitary injections. Hydration and ovulation appeared to follow shortly thereafter, but the total time from the start of injections to hydration varied substantially between females. Each female spawned between two and five times. Females that had not spawned within 43 d were eliminated from analyses of relationships between hepatic AHH activity and reproductive success because of the positive relationship observed between numbers of days of injections and hepatic AHH activity after 47 d (see "Results – Relationship of pituitary injections to hepatic AHH activity and fertilization success").

In order to allow for the possibility that various effects of contaminant exposure might find expression at specific developmental stages (see Rosenthal and Alderdice 1976), we adopted measures that indicated survival through separate early life-history stages. Thus, where N = total no of eggs spawned, V = no. of eggs that float (viable eggs), F = no. of fertilized eggs, H = no. of eggs that hatched, and L = total no. of normal larvae, we define the following measures of survival through early life-history stages: (1) % floating eggs = $(V/N)*100$, (2) % fertilization success = $(F/V)*100$, (3) % embryological success = $(H/F)*100$, and (4) % normal larvae = $(L/H)*100$. Sources of variability for these measures in more than 100 spawnings have been assessed and a standard protocol adopted for spawning and evaluation of developmental success (Spies et al. 1985 b).

A major source of variability in egg survival is related to the sequence of eggs in spawning: eggs spawned (= stripped) first do not develop as well as those spawned last, and a regular progression of egg quality in the early part of a spawning can be seen as aliquots are taken during the course of a stripping. This phenomenon is a function of egg position within the ovarian lumen after ovulation, and may be related to the time between ovulation and stripping. Although we cannot precisely control this source of variability due to differences between females, we have incorporated two procedures into our protocol that minimize its effect. Firstly, the first 2 to 3 aliquots (30 ml each) of spawned eggs are not used in estimating reproductive success. Secondly, the second and subsequent spawnings of each female are done at 48 h intervals to standardize as much as possible the time between ovulation and stripping. Since the sources of between-spawn variability are unknown, there was no basis for evaluating or controlling its effect on the outcome of these experiments; we therefore simply averaged the reproductive success measures for all spawnings of each female. Males contribute less than 1% to the variability in fertilization success provided they have motile sperm; therefore, the main effects of contaminants on egg survival could be best determined by evaluating the spawning females. With these features in the protocol, between-female variance for the reproductive success measures from 123 spawnings of 43 females was as follows: 45.3% for floating eggs, 48.2% for fertilization success, 35.7% for embryological success and 16.1% for percent normal larvae. We

therefore concluded that substantial differences in reproductive success measures could be detected between females with the following procedure.

(1) Females that were noticeably hydrated were watched closely and stripped once they had freely flowing eggs. Subsequent spawnings were carried out at 48 h intervals.

(2) Eggs were collected in a series of 30 ml aliquots. The number of aliquots varied between 2 and 8, with 5 to 6 being the usual number.

(3) From each aliquot, two 10 ml portions of eggs were removed to evaluate reproductive success. The remainder of eggs (the first two or three aliquots were excluded) were combined, and 10 ml were taken to produce a pooled aliquot which was independently evaluated for the reproductive success measures. The remainder of the pooled eggs was sampled, and this sample was later analyzed for the concentrations of chlorinated hydrocarbons.

(4) To evaluate percent floating eggs in an aliquot, 10 ml of eggs were placed in a graduated, glass, 40 ml centrifuge tube; 30 ml of sea water were added, the contents were stirred, and after standing for 15 to 30 min the volume ratios of floating and sinking eggs (V/N) were determined.

(5) A male which had been determined to have sperm that remained motile for 2 min after stripping was used to fertilize the eggs.

(6) To evaluate fertilization success in an aliquot, 10 ml of eggs were placed in a wetted, glass, 400 ml beaker, two drops of sperm were added, the contents were vigorously stirred, 300 ml of seawater were added, and the beaker was placed in a seawater table for 20 to 30 min. Floating eggs were then transferred to clean seawater in a second 400 ml breaker with a piece of Nitex screen. Fertilization success (F/V) was determined at the 4 to 8 blastomere stage after 3 to 4 h at 11° to 13 °C.

(7) To determine embryological success and percentage normal larvae, 150 to 200 eggs that had been evaluated for fertilization success were transferred to a 500 ml glass beaker provided with a 2 to 3 ml min^{-1} flow of seawater and a screened outflow to retain the eggs. Usually only the pooled aliquot from each spawning was evaluated. Dead eggs and embryos were removed daily and preserved in 10% neutral formalin. After 80 to 100 h, the remaining sample was preserved and H/F and L/H were later determined.

(8) In nearly all cases, the pooled aliquot was used to provide a single determination per spawning of each reproductive-success measure. The measures of percent floating eggs and percent fertilization success in the serial aliquots were used in the above-mentioned analysis of sources of variance.

(9) Females had between two and five spawnings, with most females being sacrificed after three spawnings. As mentioned earlier in this section, the mean values of each measure for all spawnings were used to derive one series of reproductive success values for each female.

The procedures for determining concentrations of chlorinated hydrocarbons and hepatic microsomal P-450 activities in *Platichthys stellatus* from San Francisco Bay are described in the preceding study (Spies et al. 1988).

The effects of the laboratory environment on the concentrations of chlorinated hydrocarbons and hepatic AHH activity in *Platichthys stellatus* were determined experimentally. Twenty subadult *P. stellatus* were collected in San Pablo Bay on 19 January 1984 [standard length = 25 ± 2 (SD) cm]. Ten fish were sacrificed after collection and ten were held in our seawater system for 45 d, occasionally feeding them frozen shrimp, *Crangon franciscorum*, from the site of collection. The remained ten fish were sacrificed at the end of the experiments and the concentrations of chlorinated hydrocarbons and the AHH activity were determined for the liver tissue.

Data for this study was analyzed with a standard statistical package (SAS 1985).

Results

Effect of laboratory holding on concentrations of chlorinated hydrocarbons and AHH activity in livers

After 45 d in our recirculating seawater system, there was a significantly lower mean hepatic AHH activity in *Platichthys stellatus* compared to that in fish at the start of the experiment (Table 1). During 1985, hepatic AHH activity declined during this period in fish from SP (Spies et al. 1988), although in this case the decline was somewhat

Table 1. *Platichthys stellatus.* Comparison of hepatic AHH activity [pmol 3-OH benzo(a)pyrene min^{-1} mg^{-1} protein] of fish from San Pablo Bay with those held in laboratory for 45 d

	Day 0	Day 45
Mean	92	27
n	10	9
95% CL[a]	(66, 12)	(13, 56)

[a] Values are from log-detransformed data, so confidence interval (CL) is asymmetric

Table 2. *Platichthys stellatus.* Effect of laboratory holding for 45 d on concentrations of organic contaminants in livers ($\bar{x} \pm$ SD, $\mu g\ g^{-1}$ lipid)

Day No.	n	Σ Phthalates[a]	HCB[b]	Σ DDT	Σ PCB[c]
0	10	$182 \times 10^3 \pm 180 \times 10^3$	0.028 ± 0.03	17 ± 6	110 ± 77
45	9	$10 \times 10^3 \pm 12 \times 10^3$	0.05 ± 0.03	29 ± 18	170 ± 160
P[d]		0.01	0.17	0.14	0.57

[a] Includes dimethyl, diethyl, dibutyl, benzylbutyl, dioctyl, and bis(2-ethylhexyl) phthalate esters
[b] Hexachlorobenzene
[c] Aroclors 1242 + 1254 + 1260
[d] Probability that mean concentrations are different

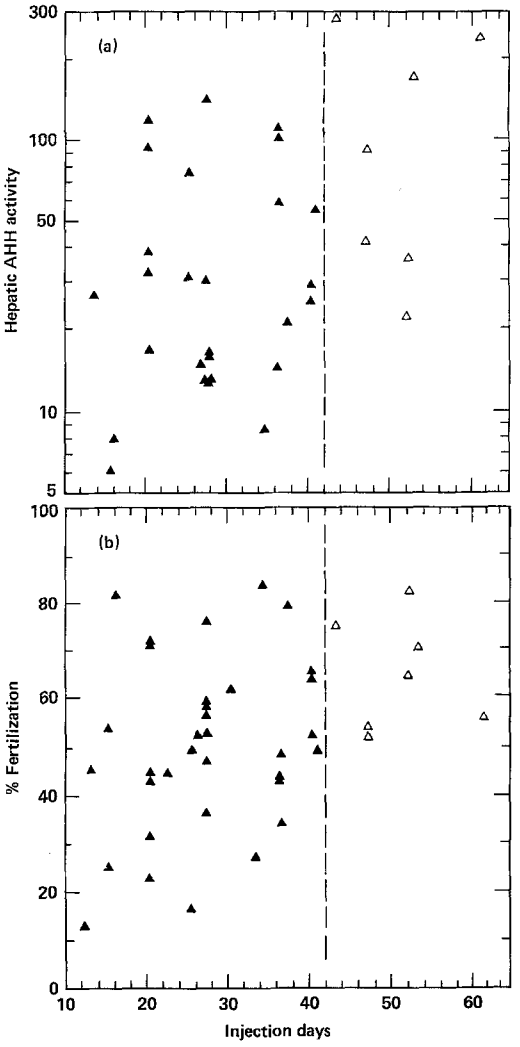

Fig. 1. *Platichthys stellatus.* Relationship between number of days of carp-pituitary injections and (a) hepatic AHH activity in spawning females and (b) mean percent fertilization of eggs spawned. There were no significant relationships between variables using data to left of dashed lines (▲), but adding data to right of lines (△) produced significant relationships

greater for fish in the laboratory. We also attribute this decline to our seawater source, the Bodega Bay Marine Laboratory, located more than 75 km from the nearest major urban coastal area, San Francisco Bay. In contrast to the declining AHH activities, hepatic concentrations of chlorinated hydrocarbons did not change significantly (Table 2). This is consistent with the known long half-lives of chlorinated hydrocarbons in fish (e.g. of PCB in trout, Niimi and Oliver 1983). Concentrations of phthalate esters declined very dramatically during this period in the laboratory (Table 2), indicating more rapid elimination rates than for PCBs.

Relationship of pituitary injections to hepatic AHH activity and fertilization success

We examined the possibility that injections of carp-pituitary extract may have had an effect on hepatic AHH activity or fertilization success in spawning females. Numbers of days of pituitary injections, at a dose rate of 1 mg pituitary kg^{-1} d^{-1}, were compared with both parameters (Fig. 1). Both independent variables were regressed in a step-wise fashion against number of days of injections (Table 3). After 47 d there were significant positive relationships between number of days of injections, hepatic AHH activity, and number of days of injections and fertilization success. With lesser number of days there were no relationships evident. We therefore did not include data for the small number of females that spawned after 43 d of pituitary injections in the comparisons of reproductive success measurements with hepatic AHH activity made in this paper. Since the start of pituitary injections occurred soon after capture from San Francisco Bay, days of laboratory holding were very similar to days of pituitary injections.

Table 3. *Platichthys stellatus.* Regression values for number of days of pituitary injections, hepatic AHH activity, and fertilization success in spawning females. Regressions were done using log-normal transformations of hepatic AHH activities and arcsine transformations of % fertilization. *n:* no. of spawning females; *r:* correlation coefficient, rho; *P* is for null hypothesis, H_0: slope = 0

Injection Day No.	Hepatic AHH activity			% fertilization		
	n	*r*	*P*	*n*	*r*	*P*
37	23	0.21	0.33	29	0.10	0.60
40	24	0.19	0.61	30	0.20	0.61
43	27	0.21	0.34	34	0.25	0.23
47	28	0.33	0.09	35	0.30	0.12
52	30	0.38	0.03	37	0.28	0.13
61	34	0.46	0.006	41	0.38	0.018

Relationships between reproductive success of spawning females, hepatic AHH activity and tissue concentrations of chlorinated hydrocarbons

We tested first for correlations between reproductive success measures and evidence of xenobiotic compound exposure in all females captured in San Francisco Bay and spawned in the laboratory. Increasing hepatic AHH activity was significantly correlated with poorer survival of spawned eggs, as evidenced by decreasing proportions of floating eggs, decreasing fertilization success, and decreasing embryological success in females with higher AHH activities (Fig. 2). Percent floating eggs and fertilization success were very highly significantly and negatively correlated, and embryological success was significantly negatively correlated with hepatic AHH activity of spawning females. In addition to these four measures, which indicate survival through separate successive early life-history stages, two more commonly employed measures, hatching success and viable hatch, which integrate survival from spawning to hatching and from spawning to development of normal larvae, respectively, were also very highly

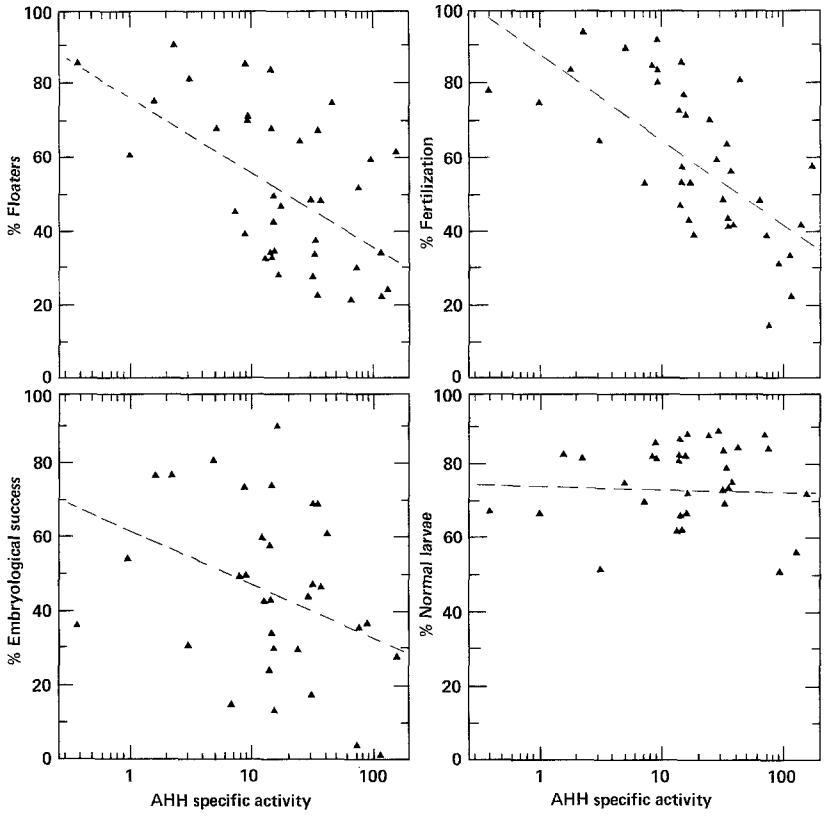

Fig. 2. *Platichthys stellatus.* Relationships between hepatic AHH activity and several measures of reproductive success for females collected in San Francisco Bay and spawned in laboratory

Table 4. *Platichthys stellatus.* Correlations between reproductive success measures, hepatic AHH activity, and contaminant concentrations for females collected in San Francisco Bay and spawned in laboratory. Correlations with AHH activity are based on females that spawned with less than 43 d of pituitary injections; correlations with PCB measures were arcsine-transformed before regression. Hatching success: proportion of spawned eggs that hatched; Viable hatch: proportion of eggs that became straight and normal larvae. ΣPCB: Aroclors 1242 + 1254 + 1260. Other details as in Table 3

	Successive stages of survival				Integrated survival	
	% floating	Fertilization success	Embryological success	Normal larvae	Hatching success	Viable hatch
Log AHH						
r	−0.56	−0.66	−0.36	−0.07	−0.58	−0.54
P	0.0003***	0.0001***	0.033*	0.68	0.001***	0.001***
Log ΣPCB in eggs						
r	−0.035	−0.051	−0.59	−0.18	−0.26	−0.28
P	0.85	0.78	0.001***	0.37	0.17	0.142

* Significant, *** very highly significant

significantly and negatively correlated with hepatic AHH activity of spawning females (Table 4).

There was also a very highly significant correlation between reduced embryological success and concentrations of ΣPCBs in eggs (Fig. 3, Table 4). There were no other correlations between reproductive success measures and any of the chemical residues, ΣDDT, ΣPCB, hexachlorbenzene or Σphthalate esters, measured in spawned eggs or in the maternal liver. The correlation between PCB content of eggs and embryological success was apparently not a result of egg-lipid content, as PCB concentrations normalized to lipid content of eggs were also correlated to embryological success (Fig. 3). Hatching success and viable hatch were not correlated with PCB content of eggs (Table 4).

Differences in reproductive success and hepatic AHH activity in females captured at two locations in San Francisco Bay

Significant differences in several parameters were evident between females captured at Bk and SP. These differences are consistent with the relationships described above, differences in hepatic AHH activity observed previously (Spies et al. 1988), and the variance associated with re-

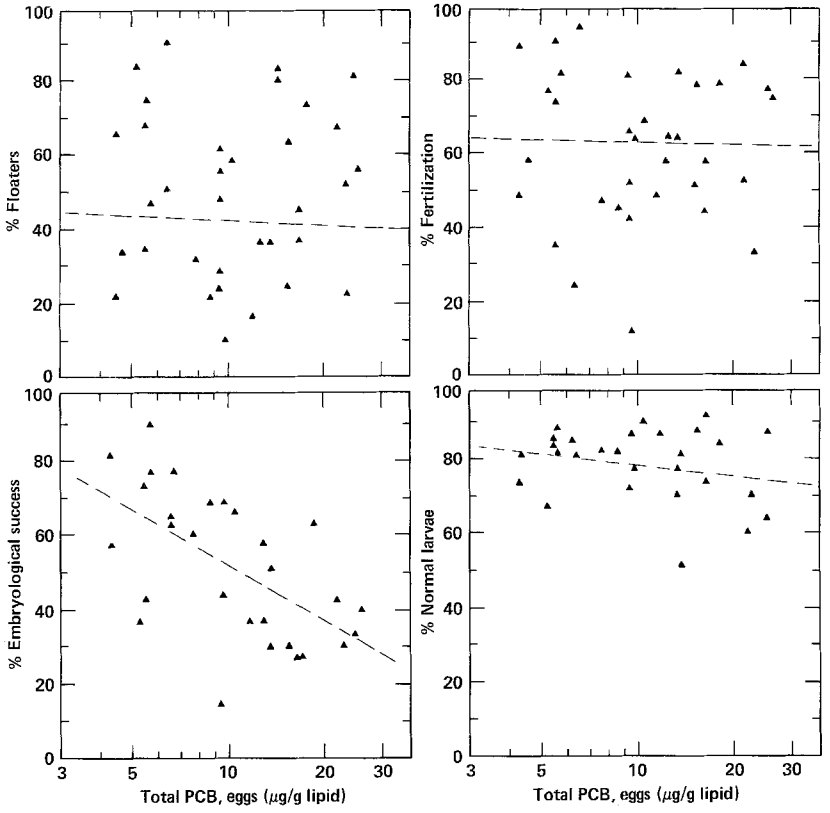

Fig. 3. *Platichthys stellatus.* Relationship between total PCB concentrations of spawned eggs and embryological success for female starry flounder captured in San Francisco Bay and spawned in the laboratory

Table 5. *Platichthys stellatus.* Hepatic AHH activity at spawning and mean time to spawning for gonadally mature females captured at two sites in San Francisco Bay. Data from Spies et al. (1988); include females with gonadosomatic index > 22

Site	AHH activity [pmol 3-OH benzo(a)pyrene min^{-1} mg^{-1}, $\bar{x} \pm SD$]		Mean time to spawn (d)
	Laboratory-spawned (1983–1985)	Field collections (1982–1985)	
San Pablo Bay (*n*)	7 (13)	21 (12)	23 (17)
Berkeley (*n*)	31 (14)	59 (13)	27 (18)
P for site differences	0.0003	0.0001	0.28

Table 6. *Platichthys stellatus.* Comparison of reproductive success (mean percent) of gonadally mature females captured at two sites in San Francisco Bay and induced to spawn in the laboratory. Data based on females that spawned with less than 43 d of pituitary injections; reproductive success measures were arcsine-transformed before being regressed

Site	Floating eggs	Fertilization success	Embryological success	Normal larvae
San Pablo Bay (*n*)	65.9 (19)	73.5 (19)	52.3 (17)	76.8 (17)
Berkeley (*n*)	43.1 (18)	51.2 (18)	41.8 (14)	74.9 (14)
P for site differences	0.001	0.001	0.24	0.66

productive success measures using our laboratory-spawning protocol (see "Materials and methods").

Hepatic AHH activities of spawning females that were captured at Bk were significantly higher than those captured at SP (Table 5). The hepatic AHH activities in spawning females are less than in gonadally mature females collected from the field earlier in gametogenesis. This is consistent with the extremely low AHH activities of spawning fish and a general suppression of MFO activity noted for other teleosts during vitellogenesis and spawning (Stegeman and Chevion 1979, Walton et al. 1983, Forlin et al. 1984, Lindström-Seppä 1985). Still, even at these very low activities, there were significant site differences evident that reflect site differences noted for other members of the population (Spies et al. 1988). Concentrations of chlorinated hydrocarbons in livers of *Platichthys stellatus* captured at Bk were significantly greater than those captured at SP during this study (Spies et al. 1988).

Reproductive success of females captured from Bk was consistently lower that those captured from SP (Table 6). These differences were highly significant for proportions of floating eggs and for fertilization success. Although mean embryological success was 10.5% less for females captured at Bk, this difference was not significant. Since between-female variance decreases and random error increases with each successive measure of reproduc-

tive success from spawning through hatching, more fish may have to be spawned to determine if differences exist between sites in embryological success or proportions of normal larvae.

It is interesting to note that the mean time to spawning in the laboratory for females captured at the two sites was very similar (Table 5), as one might expect if fish from these two sites were synchronous in their reproductive cycles.

Discussion

The data presented in this paper indicate that hepatic AHH activity of female *Platichthys stellatus* that are spawned in the laboratory is significantly related to survival of early life-history stages, particularly through fertilization. The differences between reproductive success of fish captured at the more contaminated site, Bk, and the less-contaminated site, SP, and the previously described differences in hepatic contaminant concentrations and AHH activity between fishes collected at these two sites and sacrificed soon after capture (Spies et al. 1988) strongly suggest that these relationships and site differences are due to xenobiotic compound exposure. The correlation between total PCB concentrations of eggs and embryological success in these fish (Table 4) provides further evidence that xenobiotic compounds accumulated in San Francisco Bay are having measurable effects on reproductive and developmental processes. We have examined the possibility that pituitary-extract injections differentially accelerated the processes leading to final oocyte maturation and hydration, causing some fish to spawn before they were physiologically competent. It might be supposed, for instance, that fish captured at Bk may have eventually lowered their hepatic AHH activity even further if they had spawned naturally. If that were true, one might expect to see a difference in the time to spawning between females captured at the two sites. However, there were no significant differences in time to spawning between fish captured at Bk and those captured at SP.

If the inverse relationship between hepatic AHH activity of spawning females and declining survival through early life-history stages has a contaminant etiology, there are several toxicological mechanisms that either individually or in combination may be responsible. First, PCB congeners may be directly toxic to eggs aside from their induction of cytochrome(s) P-450. There is evidence that toxicity of PCBs to chicken embryos is not causally related to P-450 induction (Rifkin and Muschick 1983). Thus, increased hepatic AHH activity and poor survival of gametes may be pleiotropic responses to PCB accumulation. While our data indicate that PCBs in eggs may be toxic during the period of development from fertilization to hatching, PCB content of eggs does not correlate with either proportion of floating eggs or fertilization success. Our data, therefore, are consistent with the possibility that at least two mechanisms of toxicity operate during gametogenesis and development: one related to AHH activity and manifesting its toxicity from ovulation of oocytes through hatching of embryos, and one relating to PCB content and manifesting its toxicity during embryological development.

A second mechanism of toxic action may be the increased production of toxic metabolites of contaminants with increasing hepatic AHH activity of female flounder. This mechanism could explain the toxicity of benzo(a)-pyrene (BaP) to early life-history stages of flathead sole (Hose et al. 1981). Since (1) 7,8-benzoflavone (7,8-BF) inhibits the toxic effect of BaP on mice oocytes (Shiromizu and Mattison 1984), (2) the differences in hepatic AHH activity between starry flounder from the two San Francisco Bay sites are due to the activity of isozyme(s) inhibited by 7,8-BF (Spies et al. 1988), (3) San Francisco Bay sediments contain several hundred $\mu g\,kg^{-1}$ of BaP and other potentially toxic polynuclear aromatic hydrocarbons (PAH) (Spies et al. 1988) that may also be accumulated and metabolized by fish, the relationships observed in this study may be a result of toxic metabolites of PAH or other contaminants. It appears, however, that in insects, metabolites of many pesticides are less toxic than parent compounds (Brattstein et al. 1986).

There is evidence from experiments with aflatoxin B_1-DNA adduct formation in *Salmo gairdneri* that would indicate contaminant adducts with DNA may have half-lives in livers of fish of more than 7 d (Shelton et al. 1986). Therefore, fish we spawn in the laboratory may still retain a substantial concentration of contaminant adducts formed in their habitat.

A third mechanism of toxic action may be the alteration of the amounts or kinds of sex steroids by contaminant-induced hepatic AHH activity. This would be a less direct manifestation of toxicity from contaminants or their metabolites, but this effect might be expressed more readily as steroids act at very low concentrations. For example, the peak plasma concentration of estradiol in *Leptocottus armatus* during oogenesis was only 6 $ng\,ml^{-1}$ (DeVlaming et al. 1984). Thus, if contaminant-induced isozyme(s) of microsomal P-450 metabolize estradiol, a several-fold increase of hepatic AHH activity might affect plasma concentrations of estradiol, overwhelming compensatory mechanisms. Such alterations during gonadal recrudescence in estradiol or at the time of final oocyte maturation in, for example, progestins, may affect gamete viability. The potential for an effect of PAH-type induction on estradiol concentrations in fish was indicated by increased amounts of radioactivity in the bile of *Salmo gairdneri* pretreated with β-naphthoflavone and injected with radioactive estradiol compared to fish that were not so pretreated (Forlin and Haux 1985). Further, testosterone biosynthesis and plasma concentrations of testosterone and 11-ketotestosterone are affected by exposure to PCB and petroleum (Freeman et al. 1982, Truscott et al. 1983). Since P-450 proteins catalyze all steroidogenic reactions from cholesterol to estradiol as well as steroid hydroxylation leading to clearance from the body, and

some P-450 activities are also regulated by steroids, possible contaminant effects are manifold.

In the preceding study of hepatic AHH activity of *Platichthys stellatus* during the reproductive season in San Francisco Bay, the hepatic AHH activities of males and gonadally immature females diverged at the two sites as the time for spawning approached, with those from Berkeley exhibiting much higher activities (Spies et al. 1988). Gonadally mature females also exhibited significant differences in hepatic AHH activity during this period. Thus, the field data indicate that females from the more contaminated site, Bk, will spawn with elevated AHH activity, and the results presented here for gonadally mature females captured at Bk, held in the laboratory, and induced to spawn, are consistent with that prediction.

Chlorinated hydrocarbons have been shown to adversely affect reproduction in fish. For example, *Pseudopleuronectes americanus,* exposed to DDT and dieldrin in the laboratory before spawning produced eggs that exhibited decreasing fertilization associated with increasing dieldrin concentrations (Smith and Cole 1973). In the same paper, DDT concentrations in eggs, ranging from 0.5 to 5 ppm, were linearly and negatively correlated with survival 4 d after fertilization and at hatching. *Platichthys flesus* caught running ripe in the Baltic Sea and subsequently spawned exhibited reduced hatching success that appeared to be associated with concentrations of PCB greater than 120 ng g^{-1} in eggs (Von Westernhagen et al. 1981). Similarly, eggs of *Clupea harengus* retrieved from benthic algae exhibited reduced viable hatch that correlated partially with DDE and partially with PCB concentrations (Hansen et al. 1985). Reduced embryological success in *Platichthys stellatus* associated with increasing total PCB concentrations from 5 to 30 μg g lipid^{-1} ($\sim$50–200 μg kg^{-1} wet wt) is a similar, but much more significant correlation than the trend observed for its Baltic congener.

The correlations in *Platichthys flesus* were based on viable hatch, which integrates survival from spawning to swimming larvae. Viable hatch in *P. stellatus* was not significantly correlated with PCB concentrations in eggs, while the correlation of PCB egg content with embryological success, which measures survival from fertilization through hatching, was highly significantly correlated ($P = 0.001$). Thus, it appears that measures of survival through each successive early life-history stage are more sensitive to contaminant effects that may be expressed mainly during one stage than measures that integrate survival through many stages.

The threshold of effects in laboratory exposures to PCB have been much greater than indicated by the above field studies. For example, in eggs, concentrations of Aroclor 1254 up to 29 μg g^{-1} had no apparent effect on percentage of hatched eggs, and concentrations of up to 5.4 μg g^{-1} had no effect on survival of fry of *Cyprinodon variegatus* (Hansen et al. 1973). This disparity in apparent effects of PCB may indicate that synergistic effects between PCB and other chemicals occur in field-exposed fish, long-term exposures are significantly more toxic, or that undetected chemicals more embryo-toxic than PCB are being expressed with increasing concentrations of PCB in eggs.

The concentrations of chlorinated hydrocarbons associated with negative effects in this study and the studies of Baltic species indicate that reproductive problems may be associated with only moderate environmental concentrations of chlorinated hydrocarbons. Other species of fish in the Southern California Bight (McDermott-Ehrlich et al. 1978), Puget Sound (Malins et al. 1984) and New Bedford Harbor, Massachusetts (L. Bridges personal communication) would be expected to have higher concentrations of chlorinated hydrocarbons in their eggs. This raises two questions. Is the genus *Platichthys* particularly sensitive to chlorinated hydrocarbons? Is there a widespread problem with reproduction of estuarine fish populations near urban areas? More investigations of the effects of contaminants on reproductive success of feral fish are indicated.

The data presented in this and the preceding paper are consistent with a toxic effect of PCBs, and other PAH-type inducers on the survival of the early life-history stages of *Platichthys stellatus* living in San Francisco Bay. However, further biochemical studies of the concentrations and activities of induced P-450 isozymes, the reproductive success of naturally spawning fish, exposure of fish to PAH metabolites and other possible toxic compounds, and the nature of contaminant-endocrine interactions are required to understand more completely the mechanisms underlying this apparent problem.

Several questions remain to be answered in order to estimate the actual reproductive success of *Platichthys stellatus* populations in San Francisco Bay. Verification of the relationship between maternal hepatic AHH activity and the measures of reproductive success through hatching in naturally spawning fish would be very useful in this regard. Also, some indication of the degree that predation and exposure to contaminants in the waters of the Bay during development affect survival would also be required. Our first estimate is that the effects described are additive with other causes of mortality; however, such phenomena as density-dependent predation could alter this assumption. Finally, the degree to which immigration of larvae or young fish into central San Francisco Bay from less contaminated populations may compensate for the apparent decreases in reproductive success of indigenous *P. stellatus* needs to be assessed in any more comprehensive assessment of this potential problem.

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