

Reproductive Impairment in a Fish Inhabiting a Contaminated Coastal Environment off Southern California

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ABSTRACT

White croaker (Genyonemus lineatus), collected from a highly contaminated site in San Pedro Bay and from a reference site 80 km away (Dana Point), were induced to spawn in the laboratory. Forty-one per cent of San Pedro Bay females and 54% of Dana Point females spawned. Examination of the ovaries of non-spawning females revealed that spawning was imminent in the remainder of Dana Point fish but only in 16% of the San Pedro Bay fish. The remainder of the San Pedro Bay fish (43%) contained only immature, yolky oocytes. No croakers containing more than 3.8 ppm ovarian total DDT could be induced to spawn whereas 36% of a contemporaneous San Pedro Bay sample had ovarian total DDT residues in excess of 4 ppm. This suggests that the inability to induce spawning in white croaker may be associated with an ovarian total DDT threshold of about 4 ppm. These data, coupled with observed decreases in fecundity (32%), fertility (14%), and early oocyte loss (30%) relative to reference fish, could partially explain the population declines observed for many southern California fishes since the 1940s.

INTRODUCTION

Several of the major sport and commercial fish stocks in the coastal waters off southern California declined substantially over the last 40 years (Bell,

1971; Oliphant, 1979). Coastal waters near Los Angeles have received significant inputs of chlorinated hydrocarbon (CHC) contaminants, notably DDT and PCBs. An estimated 2400 metric tons (t) of DDT wastes were discharged or dumped into San Pedro Bay between 1947 and the early 1970s; approximately 40 t of DDT and 50 t of PCB were discharged from 1971 to 1983 (Schafer, 1984; Chartrand *et al.*, 1985; Brown *et al.*, 1986). Because the decline in fishery stocks was temporarily related to CHC discharges, it has been suggested that exposure to these contaminants contributed to reduced fecundity and/or larval survival of sportfishes such as white sea bass (*Atractoscion nobilis*) and kelp bass (*Paralabrax clathratus*) (Young, 1963; Vojkovich & Reed, 1983).

Large numbers of white croaker (Sciaenidae: *Genyonemus lineatus*), a species related to the white sea bass, live close to the sediment-water interface in degraded habitats near Los Angeles sewage discharges where food is abundant (Love *et al.*, 1984). White croaker was the principal sportfish landed by fishermen on piers in the Los Angeles area in the early 1980s (Puffer *et al.*, 1982). Over 700 000 pounds were landed commercially at Los Angeles area ports in 1984 (NMFS, 1985). Human consumption warnings were posted by California Department of Health Services in early 1985 at several popular sportfishing locations near Los Angeles because of high CHC levels in white croaker muscle (Gossett *et al.*, 1982; California Department of Fish and Game, 1987).

Female white croaker are batch spawners, spawning 18–24 times during the season (November to April). Peak spawning occurs in February and March (Love *et al.*, 1984). During autumn, hepatic DDT and PCB stores are mobilized into the maturing gonads. Peak gonadal CHC concentrations occur in October with mean total DDT and PCB concentrations of 4.9 ppm and 1.1 ppm wet weight, respectively (Cross & Hose, 1986). Because high frequencies of preovulatory atresia were observed in fishes from San Pedro Bay which also contain high CHC residues (Cross & Hose, 1988), the relationship between reproductive impairment in white croaker and environmental exposure to chlorinated hydrocarbons was investigated.

MATERIALS AND METHODS

Fish were collected by hook-and-line from San Pedro Bay and the reference area, Dana Point. Only females with total lengths > 19 cm (the length at which 100% of the population is sexually mature (Love *et al.*, 1984)) were returned to the laboratory. Following a one-week acclimation period, 79 females (28 from Dana Point and 51 from San Pedro Bay) were injected with

1 IU/g body weight human chorionic gonadotrophin (HCG). Forty hours later, eggs were manually stripped and fertilized with pooled milt ($n > 3$) from the respective sites. Variability in fertilization success in marine fish is not influenced by sperm characteristics, provided they are motile (Spies & Rice, 1988). Fertilization success was estimated 12 h later as the percentage of eggs exhibiting a visible perivitelline space. Six Dana Point fish which released only immature hydrated oocytes (identified by the presence of multiple oil globules) were not used for fertilization success determinations. Average weights of San Pedro Bay croaker were not significantly different from those of Dana Point fish. Ovaries of females that did not spawn were catheterized and 200 oocytes were staged microscopically (Wallace & Selman, 1981; Hunter & Macewicz, 1985).

To ensure that site-specific differences in fecundity were not due to variations in the timing of oocyte maturation, numbers of early oocytes (which do not undergo cyclic atresia) were compared. Histological sections of ovaries were prepared from six fish from each site collected in October, the onset of the reproductive season (Love *et al.*, 1984). This time point was chosen to reduce the substantial variability in ovarian CHC concentrations after spawning commenced (Cross & Hose, 1986). Numbers of viable and atretic early oocytes (oogonia, primordial oocytes plus primary oocytes not yet entering primary growth) (Gulyas & Mattison, 1979; Mattison, 1980) were counted in five high power ($400\times$ magnification) fields. Because of individual differences in reproductive state, numbers of early oocytes were standardized to 25 total oocytes per field. Standardized early oocyte counts were compared using a Mann-Whitney test.

Total DDT and PCB concentrations were measured individually in the livers and ovaries of females spawning between November 1985 and January 1986 (Gossett *et al.*, 1982). The ovaries consisted primarily of stromal tissue containing unspawned immature oocytes and occasionally, unspawned maturing oocytes. Samples (1 to 5 g) were homogenized in a 20 ml aliquot of pesticide-quality acetone, filtered and re-extracted into *n*-hexane. The hexane fraction was prepared by cleaning on activated Florisil (750°C for 4 h). Analysis for CHC was performed using a Tracor MT220 gas chromatograph equipped with an electron capture detector and a $1.8\text{ m} \times 2\text{ mm}$ i.d. glass column packed with 1.5% OV17 + 1.95% QF1 on 80 to 100 mesh Gaschrom Q. Column temperature was 200°C with a nitrogen flow of 20 ml/min. Total DDT measurements were comprised of approximately 85% DDE isomers, a lesser percentage of DDT isomers and trace amounts of DDD isomers. Total PCB measurements usually represented Aroclor 1254 equivalents; rarely trace amounts of Aroclor 1242 equivalents were detected.

RESULTS

Forty-one per cent of San Pedro Bay females (21 individuals) and 54% of Dana Point females (15 individuals) were induced to spawn; the difference is not significant (Chi-square, $p > 0.25$). Females from San Pedro Bay produced a mean of 67 400 eggs, significantly less than the mean of 104 500 for Dana Point fish (Mann-Whitney U-test, $p > 0.01$) (Fig. 1). Based on regressions of the number of eggs spawned against body weight, the predicted number of eggs spawned by an average size female (182 g) from San Pedro Bay was $75\,000 \pm 14\,900$ ($\bar{Y} \pm SE$) compared to $111\,900 \pm 9100$ for the same size female from Dana Point. Batch fecundity estimates are the only useful fecundity measurement for batch spawners such as white croaker (Hunter *et al.*, 1985).

Fertilization success was also significantly lower among San Pedro Bay fish ($\bar{X} = 80\%$) compared to fish from Dana Point ($\bar{X} = 93\%$) (Mann-Whitney U-test, $p < 0.05$). All of the non-spawning Dana Point fish ($n = 13$) contained hydrated oocytes, indicating that oocytes were maturing and spawning was imminent (Hunter *et al.*, 1985). Of the non-spawning San Pedro Bay fish, eight (16%) contained hydrated oocytes and 22 (43%) had only yolky oocytes which were unresponsive to HCG induction.

Livers of San Pedro Bay croakers averaged 1.5 ppm total DDT wet weight and 1.4 ppm total PCB wet weight (Table 1) with hepatic CHC concentrations at least an order of magnitude lower in Dana Point fish.

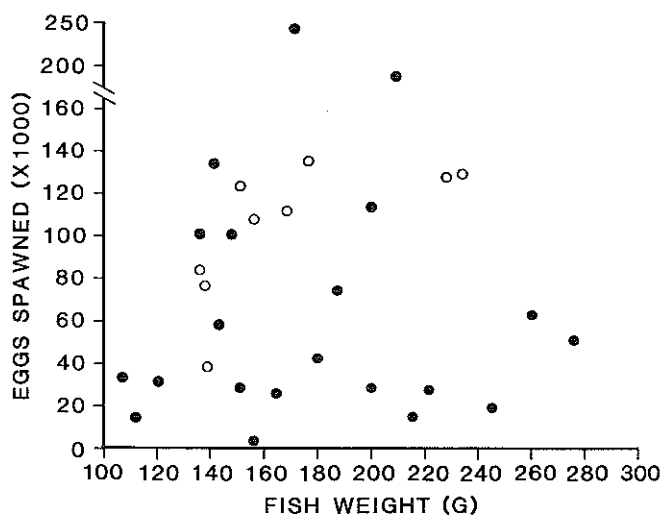


Fig. 1. Relationship between fecundity and fish weight in white croaker induced to spawn with HCG. ● = San Pedro Bay, ○ = Dana Point.

TABLE 1

Hepatic and Ovarian Contaminant Concentrations in White Croaker from San Pedro Bay and the Less Contaminated Reference Site, Dana Point (Values are in ppm wet weight)

Organ	San Pedro Bay		Dana Point	
	Total DDT $\bar{X} + SD(n)$	Total PCB $\bar{X} + SD(n)$	Total DDT $\bar{X} + SD(n)$	Total PCB $\bar{X} + SD(n)$
Liver	1.52 + 0.77 (19) ^a	1.35 + 1.34 (19) ^a	0.17 + 0.07 (8)	0.03 + 0.06 (8)
Ovary	2.10 + 0.85 (19) ^a	1.67 + 1.02 (19) ^a	0.31 + 0.18 (8)	0.16 + 0.08 (8)

^a Significantly higher than Dana Point, $p < 0.001$.

Mean total DDT and PCB concentrations in the ovaries of San Pedro Bay croakers were 2.1 ppm and 1.7 ppm, respectively. Ovarian DDT concentrations were not correlated with liver measurements of San Pedro Bay fish, although ovarian and hepatic PCB concentrations were significantly correlated ($r = 0.88$, $p < 0.05$). CHC measurements of San Pedro Bay fish spawning during January–March were compared to those measured in the general population of fishes caught at the same time. Ovaries of spawning fishes contained an average of only 1.9 ppm total DDT ($SD = 0.7$, $n = 15$), significantly less than the mean of 4.4 ppm ($SD = 3.5$, $n = 14$) measured in the ovaries of the general population (Mann-Whitney U-test, $p < 0.05$) (Fig. 2). Thirty-five per cent of the fish from the general population had ovarian total DDT concentrations greater than 3.3 ppm (the upper 95% confidence limit of DDT concentrations in spawners); 43% had levels greater than 3.0 ppm. The latter figure is identical to the percentage of fish which were unresponsive to hormone induction. None of the fish spawned in this study ($n = 19$, tissues of two fish were unavailable for CHC analysis) had ovarian DDT concentrations greater than 3.8 ppm. Distributions of ovarian total PCB were similar between the spawning group ($\bar{X} = 1.5$ ppm, $SD = 1.0$, $n = 15$) and the general population ($\bar{X} = 1.8$ ppm, $SD = 1.1$, $n = 14$).

Ovarian sections of San Pedro Bay fish contained fewer early oocytes than did Dana Point samples (Mann-Whitney U-test, $p < 0.01$). Atresia was rarely observed in early oocytes of reference fishes (2.1% of all those examined) while 15.0% of the early oocytes from San Pedro Bay fish were atretic (Chi-square, $p < 0.01$). Mean numbers of viable early oocytes were 2.6 ($SD = 0.5$) and 1.3 ($SD = 0.8$) for Dana Point and San Pedro Bay, respectively. Because the finite nature of early oocytes has not been established for fish as it has for mammals (Hunter *et al.*, 1985), the consequences of the observed atresia are unclear. Nevertheless, manifestations of early oocyte destruction might include premature cessation of reproduction or decreased fecundity in older individuals (Mattison *et al.*,

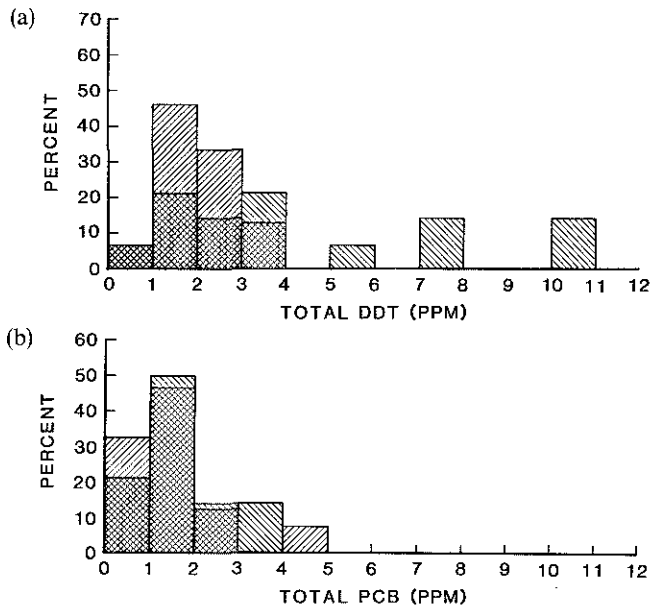


Fig. 2. Distributions of (a) ovarian total DDT (b) ovarian total PCB in San Pedro Bay white croaker spawned in the laboratory (▨) compared to the general population of females trawled (▩) in January through March.

1980). The data in Fig. 1 suggest a size-related decrease in the fecundity of croakers from San Pedro Bay. Three-quarters of fish weighing 200 g or more spawned under 70 000 eggs, approximately 50% of the average number spawned by similarly-sized reference fish.

DISCUSSION

Our results indicate that the reproductive success of an important sport and commercial fish is impaired in a highly contaminated area off southern California. Contaminant-related reproductive impairment in a benthic flatfish, the starry flounder (*Platichthys stellatus*), was recently reported from San Francisco Bay (Spies & Rice, 1988). Although the authors of that study mentioned that some of the flounder failed to spawn following their hormone induction regimen, chemical analyses of those fish were not conducted. Data from white croaker suggest that ovarian total DDT concentrations above a threshold of 4 ppm prevent spawning in white croakers, a figure similar to the previously reported LC_{50} for salmon eggs, 2.9 ppm (Burdick *et al.*, 1964; 1972). Other researchers have observed DDT-dependent larval mortality in rainbow trout at ovarian DDT concentrations

of 7.1 ppm (Hopkins *et al.*, 1969), winter flounder (*Pseudopleuronectes americanus*) between 2.0 and 4.6 ppm DDT in eggs (Smith & Cole, 1973), and in spotted sea trout (*Cynoscion nebulosus*) at 8.0 ppm DDT in eggs (Butler *et al.*, 1972). In contrast, a recent study by Hansen *et al.* (1985) found that viable hatch of Baltic herring (*Clupea harengus*) was reduced at ovary DDE concentrations > 18 ppb. With the exception of a single study by the same group (von Westernhagen *et al.*, 1981), ovarian PCB burdens in excess of those measured in croakers from the contaminated site appear necessary to elicit reproductive impairment or larval mortality (Hogan & Brauhn, 1975; DeFoe *et al.*, 1978; Sivarajah *et al.*, 1978; Monod, 1985). It is possible that the congener-specific method for PCB quantification (Schwartz *et al.*, 1987) will yield more precise correlations between PCB body burdens and reproductive effects.

It is likely that DDT is not solely responsible for observed reproductive effects since many other contaminants (such as polycyclic aromatic hydrocarbons and metals) found at high concentrations in San Pedro Bay water and fishes (Brown *et al.*, 1986; Malins *et al.*, 1986) are generally acknowledged to produce reproductive toxicity (Horning & Neiheisel, 1979; Mattison *et al.*, 1980; Hose *et al.*, 1981). Contaminant interactions through induction of bioactivating enzyme systems, synergism or differential localization within subcellular compartments probably modulate the toxicity of CHC (Freeman & Idler, 1975; Brown *et al.*, 1982). The mechanisms involved in reproductive toxicity are not completely understood but are thought to include modulation of hormone levels essential for oocyte maturation and ovulation, toxicity to developing gametes or nutritive cells, and generalized stress responses (Saxena & Garg, 1978; Truscott *et al.*, 1983; Spies & Rice, 1988). Effects similar to those described here (destruction of early oocytes, preovulatory atresia, decreased fertility and fecundity) were induced following laboratory exposure to polycyclic aromatic hydrocarbons in mammals (Mattison *et al.*, 1980) and to certain CHC in fishes (Buckler *et al.*, 1981; Nagler *et al.*, 1986).

Survival and normal development of fish embryos and larvae are dependent not only on gametic body burdens of contaminants but on ambient water contamination as well. Like many commercially important fishes, white croaker release positively-buoyant, pelagic eggs which develop in upper surface waters. Surface waters from San Pedro Bay are highly toxic to pelagic fish eggs and larvae and the toxicity is related to total CHC (DDT plus PCB) concentrations (Cross *et al.*, 1987). Because pelagic eggs can travel considerable distances before larvae settle, eggs from uncontaminated areas could be carried into degraded environments. Estimation of local white croaker abundance is thus impossible using reproductive measures of resident fishes like those presented here; instead, the population declines

predicted from this study would be spread over a greater area and possibly amplified due to contamination of surface waters.

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