EFFECTS OF BENZO(A)PYRENE ON THE EARLY DEVELOPMENT OF CALIFORNIA GRUNION, *LEURESTHES TENUIS* (PISCES, ATHERINIDAE)

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ABSTRACT

Benzo(a)pyrene (BaP), which is carcinogenic and mutagenic in mammals, exists worldwide in the marine environment. Sources of this polycyclic aromatic hydrocarbon include oil spills, industrial effluents, and atmospheric fallout. This study is the first to examine the effects of BaP on the embryonic development of a teleost, the California grunion. Gametes were stripped from spawning adults, and eggs were artificially fertilized. The fertilized eggs were then incubated for up to 14 days with initial concentrations of BaP ranging from 0 to 869 ppb. Steady-state tissue levels of BaP ranged from 0.46 to 19.92 ppm, which represented bioaccumulation factors of 146-437 times the steady-state BaP concentrations in seawater. When compared with controls, embryos exposed to initial BaP levels of 24 ppb or greater showed decreased hatchings, reduced notochord lengths, and increased morphological abnormalities. These results suggest that exposure of grunion embryos to BaP in contaminated areas may lead to their decreased survival.

Benzo(a)pyrene (BaP) is one oil constituent commonly found in marine sediments and organisms (ZoBell 1971; Neff 1979). This polycyclic aromatic hydrocarbon is introduced into the ocean via oil spillage, offshore drilling leaks, industrial effluents, runoff of asphalt roads, creosoted pilings, and atmospheric fallout (Andelman and Suess 1970; Dunn 1976; Puffer et al. 1979). Because BaP exhibits toxic, mutagenic, and carcinogenic properties in mammals, one might infer that it could also exert detrimental effects on fish populations (Heidelberger 1975; Miller 1978). Such effects could lead directly to a decrease in a valuable food source and pose a public health problem in the consumption of contaminated seafoods (Dunn and Fee 1979).

Awareness of significant BaP contamination in the marine biota has led to research on adult stages (Lee et al. 1972; Puffer et al. 1979) and, more recently, the sensitive embryonic-larval stages of fish (Hose et al. 1981, 1982). Grunion are particularly suitable for such a study because their embryonic development is well documented and they are easily reared in captivity (David 1939; Ehrlich and Farris 1971). Furthermore, grunion spawn on sandy beaches where developing eggs remain in the sand until the tide uncovers, agitates, and stimulates the eggs to hatch (Walker 1952). During this time, the developing eggs may be exposed to BaP. Therefore, we have undertaken this study to examine the effects of BaP on the early life history of California grunion, *Leuresthes tenuis*.

MATERIALS AND METHODS

Decontaminated seawater (sterilized, free of detectable BaP and particulate matter) was obtained by exposure of Los Angeles Harbor water to direct sunlight for 1 wk. Photooxidation by sunlight resulted in the breakdown of contaminating BaP to noncarcinogenic byproducts such as phenols and quinones (National Academy of Sciences 1972). Seawater exposed to sunlight was filtered through Whatman No. 5 filters to remove large, particulate matter and then sterilized by ultraviolet light. Water was maintained at a salinity of 31-32 ‰, a pH of 7.7-7.9, and a temperature of 20.0°-21.5°C.

BaP was dissolved in acetone, mixed with decontaminated seawater, and stirred for 24 h. The added concentrations of BaP in seawater were 5, 10, 100, 500, 1,000, and 5,000 ppb. The final concentration of acetone in the control and BaP-treated groups did not exceed 0.04%. Spawning California grunion were collected at Redondo Beach, Calif. Gametes were stripped and artificially fertilized in BaP-free, decon-

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Manuscript accepted November 1982.
FISHERY BULLETIN: VOL. 81, NO. 3, 1983.

taminated seawater. One hour after fertilization, three replicates of 25-35 eggs for each treatment level were placed in glass incubation jars (12.8 × 5.4 cm) which were wrapped with black tape and aerated with Pasteur pipettes connected to air pumps. Each jar contained 100 ml of decontaminated seawater to which various levels of BaP had been added as described above. In all cases, sand was excluded as an incubation medium. Dissolved BaP concentrations were measured when the California grunion eggs were introduced into the glass jars and on alternate days until day 15, using fluorescence spectroscopy (365 nm excitation, 405 nm emission) (Felton et al. 1982). Seawater samples (2 or 4 ml) were analyzed by Aminco-Bowman spectrophotofluorometer sensitive to 2 ng BaP.

Hatching and morphological abnormalities were observed and photographed at intervals over a 14-d period using a Wild M5 dissecting microscope and a Zeiss[5] photomicroscopy attachment. The significance of arc sin-transformed percentages of abnormalities was tested using analysis of variance followed by the Student-Newman-Keuls multiple range test (Sokal and Rohlf 1969). Notochord length of embryo and yolk-sac larvae was measured using a calibrated ocular micrometer. Notochord length was defined as the distance from the tip of the snout to the tip of the notochord before flexion and was always measured on the left side of the embryos and larvae. Deformed, circular-shaped embryos were measured from the posterior tip of the deformed notochord to the opposite side of the embryo, and this diameter was used to calculate the circumference which was considered to be equal to the notochord length. All values were recorded to the nearest 0.1 mm. Differences were tested using analyses of variance and the Student-Newman-Keuls test.

To measure accumulation of BaP by California grunion embryos, two additional series of incubation jars were prepared containing similar BaP concentrations and to which was added a small amount (6.7 nCi) of (α7, 10-^{14}C) benzo(a)pyrene (Amersham/Searle Corp., Arlington Heights, Ill.; 21.7 mCi/mmol, 99% chemical and radiochemical purity). On alternate days, three replicate samples of two eggs each were taken from the ^{14}C-BaP series to measure BaP accumulation using the method of Hose et al. (1981). Radioactivity was measured using a Beckman LS250 scintillation counter with an efficiency of 80% at 4°C. Total radioactivity was calculated using a series of solubilized embryos as the quenched standards.

[5]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

RESULTS

BaP Determinations

Added BaP levels of 5, 10, 100, 500, 1,000, and 5,000 ppb to seawater yielded initial BaP concentrations in the incubation jars of 4, 7, 24, 297, 361, and 869 ppb, respectively, when measured at time "0" when the California grunion eggs were introduced into the jars. Dissolved BaP levels declined thereafter with a half-life of 3.0±0.1 d ($\bar{x}$±SD) until steady-state levels of 24 ppb (361 ppb initial), 9 ppb (297 ppb initial), 5 ppb (24 ppb initial), 3 ppb (7 ppb initial), and 2 ppb (ppb initial) were reached within 4-10 d (Fig. 1). Stable BaP levels occurred most rapidly at lowest doses, while BaP concentrations in jars receiving the highest initial dose of 869 ppb decreased throughout the experimental period and did not achieve steady-state.

Accumulation of ^{14}C-BaP

The amount of BaP plus its metabolites in each egg corresponding to measured ^{14}C increased from day 1

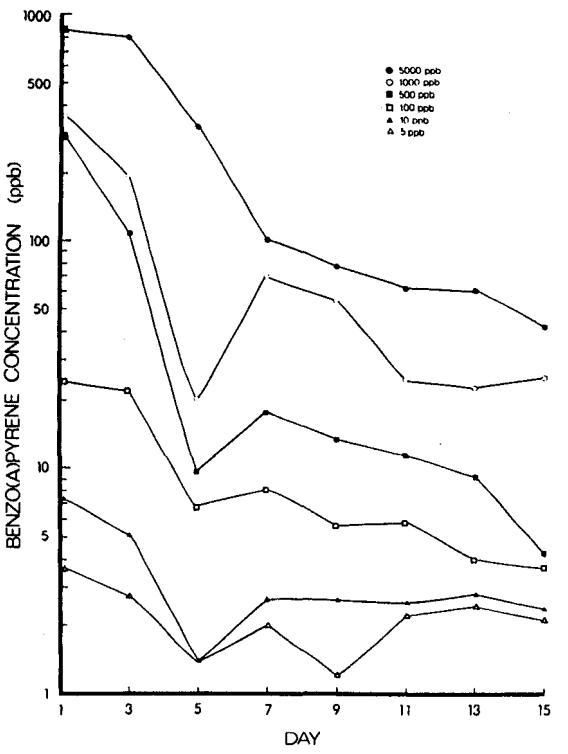


FIGURE 1.—Dissolved benzo(a)pyrene (BaP) concentrations following addition of 5-5,000 ppb BaP during the 14-d incubation period for the California grunion.

to day 3 in direct proportion to initial BaP concentrations and remained at steady-state levels thereafter for all groups except those exposed to an initial BaP dose of 297 ppb (Fig. 2). In embryos from this group, levels of BaP plus its metabolites increased throughout the exposure period ($R = 0.882$, 5 df, $P<0.01$). At day 15, BaP concentrations in treated embryos ranged from 0.459 (4 ppb initial) to 19.918 (869 ppb initial) ppm wet weight (Table 1). Tissue burdens from the initial BaP concentrations corresponded to bioaccumulation values of 127 to 23. Bioconcentration factors of 146-437 over steady-state BaP levels were measured.

Hatching

Hatching results are shown in Table 2. Low-level exposure to initial concentrations of BaP (4 and 7 ppb) had no significant effect ($P>0.05$) on the hatching abilities of exposed California grunion embryos, compared with the controls. However, with initial concentrations of 24 ppb and greater, significant differences ($P\leq0.05$) were observed between the control and experimental groups. At 24 ppb, 78% of the California grunion hatched, as compared with an average 95% hatching success in the controls. At 297 ppb only 6% of the California grunion hatched. After being exposed to 361 ppb BaP, only two of 95 larvae hatched, and both were abnormal. No eggs hatched after exposure to initial BaP concentrations of 869 ppb. Of those eggs which hatched on day 10, 99% had been exposed to 0 and 4 ppb initial BaP concentrations, 94% had been exposed to 7 ppb BaP, and 92% had been exposed to 24 ppb. All other hatchings occurred by day 13.

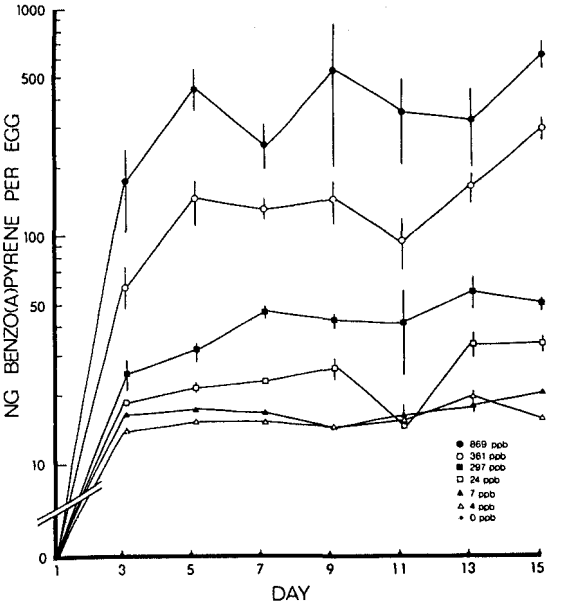


FIGURE 2.—Amount of benzo(a)pyrene (BaP) per California grunion egg corresponding to accumulated radioactivity (6.7 nCi ^{14}C-benzo(a)pyrene/ug BaP) during the 14-d incubation period. Initial dissolved BaP concentrations ranged from 0 to 869 ppb. Values shown are mean + standard deviation.

TABLE 1.—Tissue burdens of benzo(a)pyrene (BaP) and bioaccumulation factors in 15 d-old California grunion embryos.

Initial BaP concentration of seawater (ppb)	Embryo BaP concentration[1]		Bioconcentration factor	
	Wet weight (ppm)	Dry weight (ppm)	Initial BaP level in seawater	Steady-state BaP level in seawater
4	0.459±0.002	3.021±0.076	127±3	241±6
7	0.594±0.026	3.911±0.173	81±4	214±13
24	0.922±0.079	6.872±0.586	38±3	200±17
297	1.374±0.098	10.232±0.731	5±0	146±9
361	10.480±2.256	62.798±13.521	35±8	437±94
869	19.918±2.700	112.034±15.164	23±3	—[2]

[1] $\bar{x}$±SD, $n = 5$.
[2] Steady-state concentration not reached during study.

TABLE 2.—Percent hatching of California grunion eggs exposed to increasing BaP concentrations.

Initial BaP concentration (ppb)	Eggs		
	Total no.	No. hatched	% hatched[1]
0	88	84	95.4±5.5
4	87	81	93.3±2.8
7	92	83	90.3±11.2
24	84	65	78.1±8.2
297	81	5	6.2±6.0
361	95	2	2.1±1.8
869	90	0	0±0

[1] $\bar{x}$±SD, $n = 3$.

Abnormalities

There was significant difference ($P\leq0.05$) between percent of abnormalities in yolk-sac larvae in the control groups and those in groups exposed to initial BaP concentrations of 24 ppb or greater (Table 3). Of hatched larvae exposed to 24 ppb BaP, almost 20% were abnormal, over twice the number of abnormalities found in the control group. From solutions

TABLE 3.—Percent of California grunion yolk-sac larvae with developmental abnormalities when exposed to increasing concentrations of BaP.

Initial BaP concentrations (ppb)	Eggs hatched		
	No.	No. with developmental abnormalities	% with developmental abnormalities[1]
0	84	8	9.4±3.4 (3)
4	81	7	8.8±2.8 (3)
7	83	13	15.5±7.5 (3)
24	65	13	20.0±2.4 (3)
297	5	2	41.6±11.8 (2)[2]
361	2	2	100.0±0 (2)[2]
869	0	0	0

[1] $\bar{x}$±SD (n)
[2] No eggs hatched in one replicate of these series, therefore n = 2.

containing 297 ppb BaP, 42% of the yolk-sac larvae were abnormal.

Gross abnormalities observed in yolk-sac larvae exposed to 24 ppb BaP included lateral foldings of the posterior one-fourth of the tail, absence of caudal fin folds, and hemorrhagic lesions or congested vasculature in the caudal region (Table 4).

In contrast, development of embryos exposed to 24-361 ppb BaP for 14 d was retarded and resembled that of the normal embryo at 2.5 and 5.5 d of age (Fig. 3a, b) (David 1939). Abnormalities included 1) malformed tails with congested vessels or hemorrhage, 2) sporadic heartbeat resulting in intermittent blood flow, 3) head displacement in relation to the yolk sac, and 4) lack of melanophores near the lateral line above the intestinal tract.

Embryos exposed to 869 ppb BaP for 14 d resembled normal embryos at 1.2-2.5 d of age (David 1939). However, affected embryos had a lateral curvature midbody with occasional melanophores found on the trunk. In general, those embryos with shorter notochord lengths were observed to have yolk sacs much larger than those of the controls.

TABLE 4.—Abnormalities observed in California grunion yolk-sac larvae and embryos exposed to increasing concentrations of BaP.

	BaP exposure (ppb)	Abnormality
Yolk-sac larvae	24	Lateral folding of posterior fourth of tail. Absence of caudal finfold. Congested vasculature on caudal region.
Embryos	24-361	14-d-old embryo retarded in growth (resembled normal embryo growth at 1.5-5.5 d of age). Sporadic heart beat. Displacement of head in relation to yolk sac. Absence of melanophores near lateral lines. Absence of lens formation. Lesions as in larvae (above).
	869	14-d-old embryo retarded in growth (resembled normal embryo growth at 1.2-2.5 d of age). Lateral curvature midbody. Absence of melanophores (except in trunk region). Unused yolk sac. Lesions as in larvae (above).

Notochord Length

Notochord lengths (NL) of embryos and yolk-sac larvae are shown in Figure 4. Larvae which hatched after exposure to initial levels of BaP up to 361 ppb were not significantly different from controls in length ($P > 0.05$). Shorter notochord lengths were observed in embryos exposed to BaP concentrations >7 ppb ($P < 0.05$). Upon hatching, the mean notochord length of the control larval group was 5.8 mm, while embryos of the same age exposed to 24 ppb BaP averaged 4.0 mm NL.

DISCUSSION

Toxic and teratogenic effects of the carcinogen BaP on developing fish were studied by incubating embryonic stages of California grunion to increasing concentrations of BaP. Eggs were hatched in seawater alone, although California grunion eggs are normally incubated in sand. David (1939) concluded that there was probably no special adaptation of embryo metabolism to sand incubation, and speculated that spawning in the sand was a mechanism to protect eggs from predation. Incubation in seawater without sand permitted optimal observation of embryonic development with minimum disturbance. Also, exclusion of sand reduced the possibility of fungal and bacterial overgrowth and eliminated any possible contamination and influence by sand and/or sand-absorbed materials, as well as eliminating a large, potentially adsorptive surface for BaP. Preliminary trials resulted in a hatching rate of 90-100% in our laboratory, comparable to values previously reported (David 1939; Hubbs 1965).

Because of the low solubility of BaP in seawater, it was necessary to select a solubilizing or dispersing agent to create a uniform distribution (Davis et al. 1942; Wilk and Schwab 1968; Neff 1979). Such an agent could affect early development by acting in an additive or synergistic manner with BaP. Solvents examined in preliminary studies were benzene, Triton X-100, trioctanoin, and acetone, all of which caused observable alterations except for acetone in low concentrations. Triton X-100 and trioctanoin proved lethal, whereas the carcinogen benzene induced tail malformations. The validity of utilizing a solvent to distribute high levels of BaP in seawater can be compared with the not-uncommon situation in nature whereby lipophilic compounds are solubilized by contaminating substances such as detergents or oils. Also, certain solvents in which BaP is soluble, such as benzene, toluene, and xylene, are present in varying quantities in crude and refined oil. However,

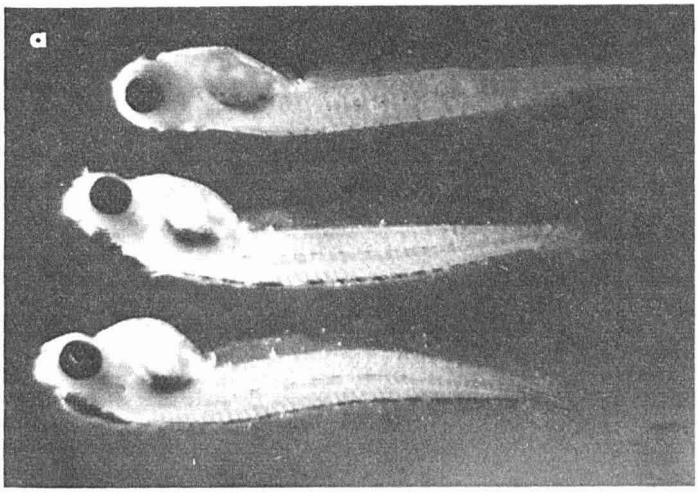
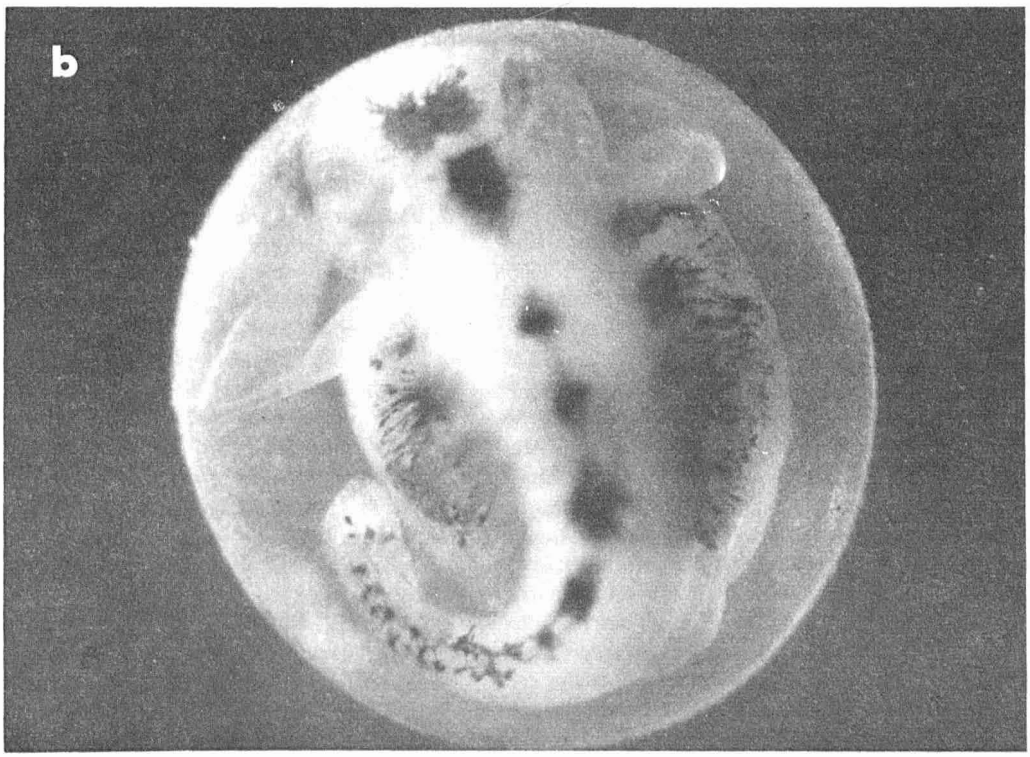


FIGURE 3.—California grunion incubated in decontaminated seawater, 14-d postfertilization: a) newly hatched larvae, with no benzo(a)pyrene (BaP) present (control) (90×); b) embryos, initially containing 24 ppb BaP (540×).



it should be emphasized that this study was not undertaken to duplicate field conditions of environmental exposure of California grunion eggs incubating in contaminated sands, although our results suggest that such studies are warranted.

Another difficulty was the decline of dissolved BaP over the 2-wk span in which the embryos were exposed (Fig. 1). This decline occurred despite precautions such as wrapping the jars to prevent photooxidation and opening the jars for daily inspection only under subdued light filtered free of ultraviolet wavelengths which degrade BaP. Loss of BaP could be caused, perhaps, by oxidation and adherence to glass, in addition to the uptake and metabolism of BaP by the embryos themselves. Felton et al. (1982) demonstrated that 16-20 ppb of crystalline BaP could be dissolved with agitation. This concentration could not be maintained,

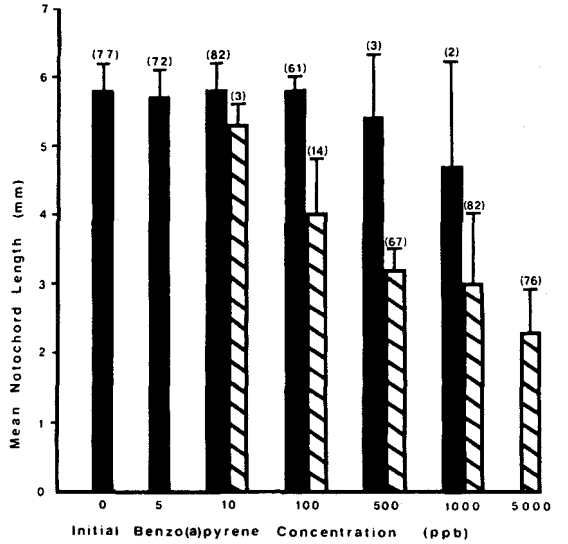


FIGURE 4.—Mean notochord length of California grunion embryos (slashed bars) and yolk-sac larvae (black bars) after 14-d incubation in seawater containing 0-5,000 ppb benzo(a)pyrene. Vertical lines = SD; numbers in parentheses = total no. embryos or larvae.

however, without continued addition of BaP; in fact, it decreased to about 1 ppb after several hours. Additional BaP dissolved in ethanol resulted in levels that decreased to near zero in 24 h. Struhsaker (1977) reported a similar difficulty in maintaining a stable concentration of benzene in seawater and attributed this to the volatility of benzene.

Because the uptake of polycyclic aromatic hydrocarbons occurs primarily via the aqueous BaP fraction, rather than by direct accumulation from surrounding sediment (Roesijadi et al. 1978), the exposure of grunion eggs to BaP in these experiments may simulate what occurs in the natural environment. For example, an oil spill may result in initially high concentrations of hydrocarbons, but photooxidation, adsorption into sediments and water-column particulates, and tidal action will decrease the concentrations of various oil constituents (such as BaP) over time. We have periodically monitored unfiltered waters of the Los Angeles Harbor for BaP concentration during the period 1977-81. Levels of BaP fluctuated from below the limit of detectability (<0.1 ppb) up to 5.4 ppb (Puffer et al. 1979). Niaussat and Auger (1970) have reported levels of 1.6 ppb BaP in seawater collected from a remote, isolated atoll in the eastern tropical Pacific Ocean. BaP levels in sand collected offshore of Cabrillo Beach in the Los Angeles Harbor ranged from 223 to 471 ppb (Duncan and Puffer 1982) and as high as 18,000 ppb in sediments from the inner Los Angeles Harbor (Gossett et al. 1983). Concentrations of BaP in sediments worldwide have ranged from nondetectable levels up to 15,000 ppb (Neff 1979). This suggests that embryos may be exposed to high levels of BaP in interstitial water during incubation in sand. As there were no prior studies regarding the effect of BaP on early grunion development, we utilized a wide range of BaP concentrations to achieve various tissue burdens. This not only reflects the broad range of exposure in nature, but also affords an opportunity to assess the sensitivity of eggs incubated under controlled conditions in seawater containing various concentrations of BaP and to correlate observed effects with known tissue BaP levels in embryos.

The extent of BaP uptake by California grunion embryos was directly proportional to initial and steady-state BaP concentration in seawater. By day 15, embryos accumulated BaP at levels 146-437 times the steady-state BaP concentration in seawater.

Comparative BaP bioaccumulation factors range from 5,142 to 21,000 for rainbow trout alevins and flatfish larvae, respectively (Hose et al. 1981; Hannah et al. 1982) and 861 for the clam *Macoma inquinata* (Roesijadi et al. 1978). While Hannah et al. (1982) noted an increase over time in BaP concentrations in embryonic rainbow trout, tissue BaP levels in California grunion remained essentially constant from day 3 to day 15. Eldridge et al. (1978) demonstrated that tissue levels of benzene in Pacific herring, *Clupea harengus pallasii*, reached equilibrium within 6-12 h at 11 times the initial water concentration. Steady-state tissue levels of BaP probably represent an equilibrium between pollutant absorption, embryonic metabolism, and excretion of the more hydrophilic metabolites (Binder and Stegeman 1980).

The observed alterations in development of California grunion exposed to BaP include 1) hatching, 2) abnormalities, and 3) reduction of notochord length. The earliest consequence of egg exposure to BaP was a reduction in hatching rate. Initial concentrations of BaP >24 ppb caused a significant mortality of yolk-sac larvae. These results are consistent with those reported by Ernst et al. (1977) who showed a 25/25 (100%) hatching rate of *Fundulus grandis* eggs exposed to 1.1 ppm water-soluble fraction of No. 2 fuel oil, a 4/25 (16%) hatching rate when exposed to 2.2 ppm, and 0/25 (0%) when exposed to 4.4 ppm. BaP has long been known to be embryo toxic in rodents (Rigdon and Rennels 1964) and more recently in sand sole, *Psettichthys melanostictus*, (Hose et al. 1982) and following maternal exposure in flathead sole, *Hippoglossoides elassodon*, (Hose et al. 1981). Furthermore, petroleum hydrocarbons, including

BaP, can alter the duration and time of teleost hatching (Ernst et al. 1977; Leung and Bulkley 1979; Hannah et al. 1982). Normally, hatching of California grunion eggs occurs in 10-14 d. Since most hatched eggs in this study did so on day 10 and no later than day 13, no effect was noted on duration and time of hatching of California grunion eggs. The dramatic and significant effect was on hatching rate.

The second effect noted was increased abnormalities of the developing yolk-sac larvae embryos. Of those yolk-sac larvae observed, 20% had a midbody lateral curvature when exposed to 24 ppb BaP or greater, as compared with 9% of the controls. Vascular abnormalities observed in embryos included stasis in yolk-sac vessels, apparent hemorrhages in the caudal regions, intermittant heart beat, and distinctly underdeveloped bodies with nonutilized yolk hydrocarbons (Ernst et al. 1977; Lonning 1977), particularly BaP (Hose et al. 1981, 1982; Hannah et al. 1982). Depressed heart rates of fish embryos treated with high levels of petroleum hydrocarbons have been attributed to inhibition of metabolism and/or neurotransmission (Whipple et al. 1981) and can result in partial or complete mortality (Anderson et al. 1977).

The third response observed in California grunion embryos exposed to BaP was reduction in notochord length. The notochord length of embryos exposed to 24 ppb BaP averaged 70% of that of controls. At 297 ppb BaP or greater, the notochord length of affected embryos was generally <50% of the notochord length of the control group. Retarded growth was also evident in rainbow trout alevins reared in 0.08-2.99 ppb BaP (Hannah et al. 1982) and may result from the inhibitory effects of polycyclic aromatic hydrocarbons on DNA synthesis (Santodonato et al. 1981) and, hence, mitosis (Bourne and Jones 1973; Kocan et al. 1981).

At the end of 14 d, embryos exposed to initial concentrations of 24-297 ppb BaP resembled normal embryos at 2.5-5.5 d of development. This retarded growth was characterized by a lack of lens formation, absence of caudal fin folds, and a reduced number of melanophores. However, there was one exception to this trend of slow development: The pectoral fins of embryos treated with 24-297 ppb BaP appeared to be of normal size and maturity, whereas all other aspects of embryo development seemed severely delayed. Irregular cleavage and retarded development in oil-treated fish embryos have been previously described (Lonning 1977), and assessment of the developmental effects of petroleum hydrocarbons on marine fish eggs has been reviewed by Kuhnhold (1977). These include sublethal effects such as chromosomal aberrations and morphological anomalies as well as direct toxicity (Rosenthal and Alderdice 1976). Toxic hydrocarbon levels reportedly correlated with mitotic errors in eggs of Atlantic mackerel, *Scomber scombrus*, (Longwell and Hughes 1980). Anderson et al. (1977) also noted lack of pigmentation in estuarine killifish, and a histological examination of *Fundulus grandis* embryos exposed to the water-soluble fraction of No. 2 fuel oil revealed pathological lens, liver, kidney, and epthelial tissues (Ernst et al. 1977).

In summary, the effects observed in California grunion embryos exposed to the carcinogen BaP were threefold: Decreased hatching rates, increased number of morphological abnormalities, and shortened notochord lengths. These grossly visible alterations would be detrimental to the potential growth and survival of fish in the wild (Rosenthal and Alderdice 1976). The number of fish reaching adulthood would decrease directly as a result of the lethal effects of BaP on embryos and indirectly as a result of decreased ability of affected fish to elude predators. Also, short-term observations such as these do not address the problem of carcinogenesis, although recent experiments have demonstrated that the polycyclic aromatic hydrocarbon, 7,12-dimethylbenz(a)anthracene, is tumorigenic in freshwater fish (Schultz and Schultz 1982).

It is predicted that BaP will increase in the environment unless restrictions can be imposed upon its production. However, reduced production of BaP is unlikely, as this compound is an unavoidable by-product of incomplete combustion and petroleum usage (National Academy of Sciences 1972). Therefore, the results of our experiments indicate that the short- and long-term effects of BaP on the developmental stages of fish and other marine life warrant further investigation.

ACKNOWLEDGMENTS

We thank Gilbert Jones, Basil Nafpaktitus, James Kremer, Gary Brewer, and Steve Kottmeier for their suggestions, and Bob Arthur, Melanie Posner, and Havens Newman for technical assistance.

LITERATURE CITED

ANDELMAN, J. B., AND M. J. SUESS.
1970. Polynuclear aromatic hydrocarbons in the water environment. Bull. W.H.O. 43:479-508.

ANDERSON, J. W., D. B. DIXIT, G. S. WARD, AND R. S. FOSTER.
1977. Effects of petroleum hydrocarbons on the rate of heart beat and hatching success of estuarine fish embryos. *In* F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B.

Vernberg (editors), Physiological responses of marine biota to pollutants, p. 241-258. Acad. Press, N.Y.

BINDER, R. L., AND J. J. STEGEMAN.
1980. Induction of aryl hydrocarbon hydroxylase activity in embryos of an estuarine fish. Biochem. Pharmacol. 29:949-951.

BOURNE, E. W., AND R. W. JONES.
1973. Effects of 7, 12-dymethylbenz (a) anthracene (DMBA) in fish cells in vitro. Trans. Am. Microsc. Soc. 92:140-142.

DAVID, L. R.
1939. Embryonic and early larval stages of the grunion, *Leuresthes tenuis*, and of the sculpin, *Scorpaena guttata*. Copeia 1939:75-81.

DAVIS, W. W., M. E. KRAHL, AND G. H. A. CLOWES.
1942. Solubility of carcinogenic and related hydrocarbons in water. J. Am. Chem. Soc. 64:108-110.

DUNCAN, K. L., AND H. W. PUFFER.
1982. Levels of benzo(a)pyrene in marine organisms and sediments in the Los Angeles Harbor. (Abstr.) Proceedings of the 13th International Cancer Congress, 8-15 Sept. 1982, Seattle, Wash. Thirteenth International Cancer Congress, Inc., Seattle, Wash., p. 545.

DUNN, B. P.
1976. Techniques for determination of benzo(a)pyrene in marine organisms and sediments. Environ. Sci. Technol. 10:1018-1021.

DUNN, B. P., AND J. FEE.
1979. Polycyclic aromatic hydrocarbon carcinogens in commercial seafoods. J. Fish. Res. Board Can. 36:1469-1476.

EHRLICH, K. F., AND D. A. FARRIS.
1971. Some influences of temperature on the development of the grunion *Leuresthes tenuis* (Ayres). Calif. Fish Game 57:58-68.

ELDRIDGE, M. B., T. ECHEVERRIA, AND S. KORN.
1978. Fate of ^{14}C-benzene in eggs and larvae of Pacific herring (*Clupea harengus pallasi*). J. Fish. Res. Board Can. 35:861-865.

ERNST, V. V., J. M. NEFF, AND J. W. ANDERSON.
1977. The effects of the water-soluble fractions of No. 2 fuel oil on the early development of the estuarine fish, *Fundulus grandis* Baird and Girard. Environ. Pollut. 14:25-35.

FELTON, S. P., M. L. LANDOLT, W. T. IWAOKA, B. S. MILLER, D. DI-JULIO, AND B. LLOYD.
1982. Techniques for the waterborne administration of benzo(a)pyrene to aquatic test organisms. U.S. Environ. Prot. Agency, EPA-600/9-82-013, p. 148-162. Gulf Breeze, Fla.

GOSSETT, R. W., H. W. PUFFER, R. H. ARTHUR, JR., AND D. R. YOUNG.
1983. DDT, PCB, and benzo(a)pyrene levels in white croaker (*Genyonemus lineatus*) from Southern California. Mar. Pollut. Bull. 14:60-65.

HANNAH, J. B., J. E. HOSE, M. L. LANDOLT, B. S. MILLER, S. P. FELTON, AND W. T. IWAOKA.
1982. Benzo(a)pyrene-induced morphologic and developmental abnormalities in rainbow trout. Arch. Environ. Contam. Toxicol. 11:727-734.

HEIDELBERGER, C.
1975. Chemical carcinogenesis. Ann. Rev. Biochem. 44:79-121.

HOSE, J. E., J. B. HANNAH, D. DIJULIO, M. L. LANDOLT, B. S. MILLER, W. T. IWAOKA, AND S. P. FELTON.
1982. Effects of benzo(a)pyrene on early development of flatfish. Arch. Environ. Contam. Toxicol. 11:167-171.

HOSE, J. E., J. B. HANNAH, M. L. LANDOLT, B. S. MILLER, S. P. FELTON, AND W. T. IWAOKA.
1981. Uptake of benzo(a) pyrene by gonadal tissue of flatfish (family Pleuronectidae) and its effects on subsequent egg development. J. Toxicol. Environ. Health 7:991-1000.

HUBBS, C.
1965. Developmental temperature tolerance and rates of four Southern California fishes, *Fundulus parvipinnis*, *Atherinops affinis*, *Leuresthes tenuis*, and *Hypsoblennius* sp. Calif. Fish Game 51:113-122.

KOCAN, R. M., M. L. LANDOLT, J. BOND, AND E. P. BENDITT.
1981. *In vitro* effect of some mutagens/carcinogens on cultured fish cells. Arch. Environ. Contam. Toxicol. 10:663-671.

KÜHNHOLD, W. W.
1977. The effect of mineral oils on the development of eggs and larvae of marine species. A review and comparison of experimental data in regard to possible damage at sea. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 171:175-183.

LEE, R. F., R. SAUERHEBER, AND G. H. DOBBS.
1972. Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. (Berl.) 17:201-208.

LEUNG, T. S.-Y., AND R. V. BULKLEY.
1979. Effects of petroleum hydrocarbons on length of incubation and hatching success in the Japanese medaka. Bull. Environ. Contam. Toxicol. 23:236-243.

LONGWELL, A. C., AND J. B. HUGHES.
1980. Cytologic, cytogenetic, and developmental state of Atlantic mackerel eggs from sea surface waters of the New York Bight, and prospects for biological effects monitoring with ichthyoplankton. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 179:275-291.

LONNING, S.
1977. The effects of crude Ekofisk oil and oil products on marine fish larvae. Astarte: J. Arctic Biol. 10(1):37-47.

MILLER, E. C.
1978. Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. Can. Res. 38:1479-1496.

NATIONAL ACADEMY OF SCIENCES.
1972. Particulate polycyclic organic matter. Biological effects of atmospheric pollutants. Nat. Acad. Sci., Wash., D.C., 361 p.

NEFF, J. M.
1979. Polycyclic aromatic hydrocarbons in the aquatic environment: Sources, fates and biological effects. Appl. Sci. Publ. Ltd., Lond., 262 p.

NIAUSSAT, P., AND C. AUGER.
1970. Mise en evidence et repartition du benzo-3-4-pyrene et du perylene chez differents organismes de la biocoenose lagunaire de Clipperton. [Distribution of 3-4-benzopyrene and perylene in different organisms of lagoon biocenosis of Clipperton.] C. R. Acad. Sci. (Paris), Ser. D. 270:2702-2705.

PUFFER, H. W., K. L. DUNCAN, E. H. VON HOFE, D. L. WINKLER, G. D. BREWER, AND S. K. MONDAL.
1979. Benzo(a)pyrene: Studies of the effects of this ubiquitous pollutant on fishes. *In* Oceans 79, Proceedings of the 5th Annual Conference, Institute of Electrical and Electronics Engineering, Inc., p. 398-400.

RIGDON, R. H., AND E. G. RENNELS.
1964. Effect of feeding benzpyrene on reproduction in the rat. Experientia 20:224-226.

ROESIJADI, G., J. W. ANDERSON, AND J. W. BLAYLOCK.
1978. Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: Influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. J. Fish. Res. Board Can. 35:608-614.

ROSENTHAL, H., AND D. F. ALDERDICE.
1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Board Can. 33:2047-2065.

SANTODONATO, J., P. HOWARD, AND D. BASU.
1981. Health and ecological assessment of polynuclear aromatic hydrocarbons. J. Environ. Path. Toxicol. 5:1-364.

SCHULTZ, M. E., AND R. J. SCHULTZ.
1982. Induction of hepatic tumors with 7, 12-dimethylbenz(a)anthracene in two species of viviparous fishes (Genus *Poeciliopsis*). Environ. Res. 27:337-351.

SOKAL, R. R., AND F. J. ROHLF.
1969. Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Co., San Franc., 776 p.

STRUHSAKER, J. W.
1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring, *Clupea harengus pallasi.* Fish. Bull., U.S. 75:43-49.

WALKER, B. W.
1952. A guide to the grunion. Calif. Fish Game 38:409-420.

WHIPPLE, J. A., M. B. ELDRIDGE, AND P. BENVILLE, JR.
1981. An ecological perspective of the effects of monocyclic aromatic hydrocarbons on fishes. *In* J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg (editors), Biological monitoring of marine pollutants, p. 483-551. Acad. Press.

WILK, M., AND H. SCHWAB.
1968. Zum transportphänomen und wirkungsmechanismus des 3,4-benzpyrens in der zelle. Z. Naturforsch Z3B:431-438.

ZOBELL, C. E.
1971. Sources and biodegradation of carcinogenic hydrocarbons. *In* Proceedings of the Joint Conference on Prevention and Control of Oil Spills, p. 441-451. Am. Petrol. Inst., Wash., D.C.