

# EFFECTS OF BENZENE (A WATER-SOLUBLE COMPONENT OF CRUDE OIL) ON EGGS AND LARVAE OF PACIFIC HERRING AND NORTHERN ANCHOVY

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The effects of oil spills on the marine environment have received increasing attention over the past decade. A major portion of the research on these effects has followed a holistic approach of subjecting organisms to crude oil. Although these studies have contributed considerably to an understanding of the problem, oil is a complex amalgam of several components, varying in toxicity. It is difficult to ascertain specific effects on the survival and physiology of marine organisms using whole crude oil. While some studies have focused on the effect of tar components in oil (Bargmann, 1971; Moulder and Varley, 1971), little attention has been given to those compounds in crude oil thought most toxic to aquatic organisms, i.e., the low boiling point, water-soluble, aromatic hydrocarbons.

Most research to the present time at Tiburon Laboratory has concentrated on the effects of the aromatic component, benzene, on juvenile fishes (Brocksen and Bailey, 1973; Bailey and Brocksen, 1974; Benville and Korn, 1974; Korn and Macedo, 1974). Benzene is among the most abundant of the aromatic components, comprising at least 20% of the total aromatic hydrocarbons in crude oil. Benzene is relatively soluble in water (up to 2000 ppm in fresh water; Benville and Korn, 1974), and is among the most toxic of all oil components. Experiments are also in progress to test the effects of other toxic aromatics (toluene, xylene, 1, 2, 4-trimethylbenzene) on fishes, including synergistic and/or antagonistic effects of their combinations.

Since eggs and larvae are generally the life history stages most sensitive to environmental stress, and because few studies of the effects of oil components have been performed on these stages, experiments have been extended to test the effects of benzene on eggs and larvae of Pacific herring and northern anchovy.

The Pacific herring (Clupea pallasi) is an important commercial species in the San Francisco Bay area and along the coasts of Canada and Alaska (Eldridge and Kaill, 1973). In addition to a fishery for adults, there is a commercial fishery for the eggs which are principally exported to Japan. The eggs are demersal, being deposited on algae and substrate in bays from about October to March. Herring eggs and larvae occur inshore in bays where oil is likely to accumulate or be spilled.

The northern anchovy (Engraulis mordax) is the most abundant species with immediate harvest potential in the California Current System (Frey, 1971). It is also numerous in west coast bays where it has been found to be the most abundant fish, both numerically and in terms of biomass (Aplin, 1967). The anchovy spawns not only in San Francisco Bay but extensively throughout the California Current System of California (Ahlstrom, 1966). In offshore waters spawning occurs in every month of the year, usually peaking in late

winter and early spring with another minor peak in early fall. In San Francisco Bay, however, spawning has been delimited from about May through October. Pelagic anchovy eggs are abundant in surface layers of San Francisco Bay and, like herring, they may possibly be exposed to oil.

This paper presents the results of preliminary experiments testing lethal and sublethal concentrations of benzene on eggs and larvae of the Pacific herring and northern anchovy. Developing embryos were exposed at two stages to contrast their sensitivity to benzene: (1) eggs a few hours after spawning and fertilization and (2) larvae a few hours before or after completion of yolk absorption. Parameters measured include percent mortality, percent abnormal larvae, types of abnormalities, length of larvae and growth, yolk utilization, feeding and respiration.

#### METHODS

Herring eggs were collected inshore on the Tiburon Peninsula a few hours after spawning occurred. Eggs, attached to intertidal algae (several species of *Fucus*, *Laminaria*, and *Gracilaria*) were in early cleavage stages when collected. Anchovy eggs were collected from Raccoon Straits between Tiburon Peninsula and Angel Island with a 0.5 meter plankton net ( $333\mu$  mesh) towed on the surface. Although varying somewhat in developmental stage, most anchovy eggs were in gastrula stage when collected. Experiments performed on herring and anchovy eggs and larvae are summarized in Table 1.

After collection, eggs were sorted and placed into green polyethylene containers containing 8 liters of filtered seawater. Seawater was filtered through a series of two 5 $\mu$  cellulose filters and one charcoal filter, removing most organisms and debris. To increase hatching, erythromycin gluceptate was added to a final concentration of 5 to 10 ppm. Rearing methods

Summary of Conditions in Experiments Testing Effects of Benzene on Eggs and Larvae of Pacific Herring and Northern Anchovy. NM = Not Measured TABLE 1

Algae (No./ml)	None	None	(102)	(10 <sup>5</sup> )	None	None	None	None
Food Spec. (No./ml)	None	5-10 Artemia nauplii	9-20 (13) Brachionus	4-9 (6) Brachionus	None	None	None	None
Mean NH <sub>3</sub> (ppm)	WN	WN	.011910 (.128)	WN	.007030 (.014)	.005040	.010030	.010050
kange and pH	WN	WN	7.4-8.1 (7.9)	7.4-7.8 (7.6)	7.7-7.9 (7.8)	7.6-7.8 (7.7)	7.4-7.9 (7.6)	7.5-8.1 (7.9)
н 02 (ррм)	WN	WN	4.7-7.9 (7.0)	6.1-8.0 (7.3)	7.1-8.0 (7.5)	7.0-8.0 (7.5)	7.2-7.5 (7.4)	(6.9-7.6) (7.3)
sal. (ppt)	(24.0)	(24.0)	(22.0)	(10.0)	(29.5)	(28.0)	(28.0)	(30.0)
Temp. (°C)	14.0-17.5 (15.2)	10.0-17.0 (13.2)	11.5-14.0 (12.9)	12.2-13.5 (12.9)	17.5-19.2 (18.5)	16.5-18.0 (17.5)	17.0-18.2 (17.5)	16.8-18.4 (17.6)
Total Exposure (Hrs)	24, 48, 96	24,48,96	48	24, 48	24	24, 48	24,48	24,48
Mean Benzene Concentrations (ppm)	0,3.8,16.5,39.1	0,4.8,17.7,45.0	0,6.7,12.1	0,13.0,31.9	0,0.4,2.7,7.5	0,4.7,10.5,24.0	0,40.0	0,13.5,19.5,53.5
Day and Stage First Exposed	Egg Day O Early Cleavage	Egg Day O Early Cleavage	Larval Day 2	Larval Day 2	Day O	Day 0	Day 0	Day 0
Date Begun	2-05-73	3-01-73	11-28-73	1-22-74	7-06-73	7-31-73	8-14-73	8-22-73
Exp. No.	Herring 1	N	e	4	Anchovy 1	2	m	4

generally approximate those of Lasker et al. (1970) and Struhsaker et al. (1973).

All experiments were designed to permit multiway analysis of variance with two or more variables and three to five replicates per treatment combination. Replicate containers were placed randomly in water tables. In most experiments, 50 eggs were placed in each container. After sorting eggs into containers, various concentrations of benzene were added except for controls. On following days during development, benzene was added to give varying total exposure times of 0, 24, 48 and 96 hrs. Mean concentrations of benzene and exposure times are summarized in Table 1. After initial exposures, benzene concentrations declined over the 24-hr period due to volatization. For testing effects of 48- and 96-hr exposure times, additional benzene was added daily to achieve the initial concentration specified. The decline of three different initial benzene concentrations was measured every 2 hrs for 24 hrs under experimental conditions comparable to those in larval experiments. The decline in concentration is shown in Figure 1. The equation for the three calculated regression lines is  $\hat{Y} = ae^{-bX}$ . The regression coefficients for each concentration (Fig. 1) were not significantly different (P < 0.01) (Sokol and Rohlf, 1969). After 24 hrs, 25% to 30% of the initial concentration remained for all three concentrations. All benzene concentrations were measured with a Model MT-220 Tracor gas chromatograph to the nearest 0.05 ppm (Benville and Korn, 1974).

Ranges and means of physical variables, algae and food concentrations are also given in Table 1. During initial experiments with herring (1 and 2), the controlled seawater system was not completed and a greater range of conditions prevailed. In all other experiments these variables were more closely controlled.

Herring eggs or larvae were subsampled daily from each treatment combination and examined for indications of abnormal rates and modes of development.



Fig. 1. Exponential decline in three initial benzene concentrations over a 24-hr period. Each point is the mean of three replicate containers. Range of temperature:  $11.2^{\circ}C-13.5^{\circ}C$ . Equation for the calculated curve is:  $\hat{Y} = ae^{-bX}$ A-a = 47.8, b = .05697; B-a = 28.7, b = .05608;C-a = 13.9, b = .05648. All three regression coefficients were significant (P < 0.01). There was no significant difference between the three regression coefficients, (b) (Sokal and Rohlf, 1969).

In some experiments, measurements of the standard length of larvae (most anterior point of snout to end of notochord) were made to the nearest 0.1 mm.

Herring larvae hatched in the laboratory after 9 to 11 days of incubation, while anchovy larvae hatched after 1 to 2 days of incubation. For herring, developmental days were designated as egg day or larval day depending upon experiment being discussed. Egg day 0 corresponds to the first 24 hrs after fertilization and larval day 0 to the day that larvae hatch. For anchovy, day 0 corresponds to the day of collection (gastrula) and egg and larval days are not separated since larvae hatch only 1 to 2 days after collection. At hatching, the following counts were made: number of actively swimming normal larvae, number of dead eggs remaining and number of abnormal larvae. Percent hatching and percent abnormal larvae among those hatching were calculated for each treatment combination.

In herring experiments 1 and 2 an attempt was made to feed larvae Artemia salina nauplii. Few larvae were able to feed (about 5%) because the nauplii were too large. In herring experiments 3 and 4, larvae were given rotifers (Brachionus plicatilus), on which they fed readily. Culture methods for rotifers are given by Theilacker and McMaster (1971). Nephroselmis sp., isolated from San Francisco Bay, was added to cultures in the latter two experiments. Concentrations of food organisms and Nephroselmis are in Table 1. Counts of rotifers and Nephroselmis were made with a Model ZBI Coulter counter. In experiments exposing herring larvae, benzene was added on larval day 2, just after disappearance of the yolk sac. Daily counts from hatching to larval day 7 were made to determine percent survival.

Daily counts were not made in experiments with anchovy, because eggs and larvae were too small to be seen readily. Counts were made at the termination of the experiment and percent survival and abnormal larvae calculated. The standard length and size of yolk sac of surviving larvae were also measured at

the end of the experiment. At the time of these experiments, rotifers had not been cultured and anchovy larvae were not fed. The experiments were ended prior to occurrence of starvation mortality (counted on days 3 or 6).

Larvae were observed for indications of abnormal swimming and feeding behavior. Also the percent of subsamples with food in guts was determined. All samples were preserved for more detailed studies of the effects of benzene on various morphometric characters.

Respiration of larvae was measured using a Gilson Respirometer. Ten larvae and 10 ml of seawater were placed in each 25 ml flask. Correction was applied from blanks containing only seawater.

Data were analyzed using the UCLA BMD computer programs OlV and O2V (Dixon, 1970).

# RESULTS

#### HERRING

# Exposed Eggs; Effect on Early Development and Hatching

After exposure of eggs to benzene, the developmental sequence appeared normal except at the higher concentrations (35-45 ppm range). In embryos treated with an initial mean concentration of 45.0 ppm, developmental rate was obviously delayed over that in controls. The heartbeat rate of normal embryos ranged from about 70 to 90 beats per minute. In embryos at 45.0 ppm the heartbeat rate was often irregular, sometimes increasing to 110 beats a minute, while at other times a few beats were missed altogether.

Hatching occurred from 9 to 11 days in the laboratory. At hatching, approximately 20% to 25% of the larvae appeared abnormal in all treatment combinations, including controls. Eggs from the same

spawn collected in the field just prior to hatching also exhibited 20% to 25% abnormal larvae at hatching. Of these abnormal embryos, many simply did not develop and died before hatching. Others appeared to be retarded at an earlier stage of development, characterized by incomplete development of the body, fins and jaw. Yolk was not absorbed to the degree observed in normal larvae and the embryos remained dorsoventrally coiled over the yolk sac instead of straightening as in normal larvae at time of hatching. The high percent of abnormal, inviable larvae was observed among those hatching in all experiments with herring eggs collected from San Francisco Bay. In one experiment, using eggs from Tomales Bay, fewer abnormal larvae were observed. The possible reasons for this high percent of abnormal larvae at hatching are discussed below.

For brevity, and because data from the two experiments exposing eggs are comparable, only results from the second experiment (Table 1) are presented to illustrate the effects of benzene on eggs. The percent larvae hatching and percent of abnormal larvae at hatching are shown in Figure 2. Analysis of variance of the data showed a significant difference (P < 0.01) between concentrations, but not between exposure times. Significantly fewer larvae survived and significantly more larvae were abnormal at 45.0 ppm. Although the effects of different times of exposure were not significantly different, there is an indication that more larvae were abnormal at 48 hrs than at 24 and 96 hrs. At higher concentrations the time of exposure may have been more influential. The reason for the unusually high percent abnormal larvae at 17.7 ppm, 48 hrs is unclear. Most of these abnormal larvae occurred in only one replicate of the treatment combination and may be due to errors in application of benzene (too high concentration) added to the containers at the beginning of egg day 2. Other replicates at 17.7 ppm and 48 hrs were comparable to those at 24 and 96 hrs. Generally, it appears that herring eggs are relatively resistant to





benzene exposure. It should be remembered, however, that the benzene concentration declined over each 24-hr exposure period (Fig. 1). An initial concentration of 45 ppm is only 12 ppm or less after 24 hrs.

From the results of exposing herring eggs in experiment 1, it was noted that the 50% mortality level among larvae at time of hatching occurred at an initial mean concentration of approximately 40 to 45 ppm benzene when exposed for 96 hrs.

Among larvae exposed to a mean initial concentration of 45 ppm, all three exposure times, and larvae at 17.7 ppm, 96-hr exposure, abnormalities were observed that did not occur in controls and

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lower concentrations. Most common was some form of flexure along the body. Many larvae exhibited severe lateral or ventral bends just posterior to the head. Several were laterally bent to varying degrees of severity in the body and tail region; some both in the nape and tail region forming an s-shaped larva. A few had only one eye. Development of the lower jaw appeared incomplete. Of the abnormal larvae hatching, those most severely retarded or bent died shortly after hatching. A few of those less severely bent survived for several days, but were unable to swim or feed normally and eventually died.

# Exposed Eggs; Survival of Larvae after Hatching

In herring experiment 2 (Table 1), an attempt was made to rear larvae past the period of yolk absorption by feeding larvae Artemia salina nauplii as reported by Talbot and Johnson (1972). The percent of larvae feeding and surviving daily for 33 days after hatching was recorded. It was observed that only a few larvae were feeding on the nauplii (5-10%), although these began feeding on larval day 1. The inability of most larvae to feed was probably due to the nauplii being too large. Yolk was absorbed by larval days 2 to 3 in all treatment combinations except at 45 ppm (approximately 1 day later). From larval days 1 to 7, survival of larvae at 4.8 ppm at all three exposure times was comparable to controls. In larvae at 17.7 ppm, 96 hrs, and 45 ppm, 48 and 96 hrs, survival was less than controls for these This was due to the death of larvae with periods. less obvious abnormalities. Starvation mortality began on about larval day 6 in all treatment combinations. Survival of feeding larvae was not significantly different between treatment combinations for the remainder of the experiment (33 days).

The standard length of surviving larvae (33 days from spawning) was measured and larvae of similar size from the field examined. Most major developmental

characteristics were comparable. The standard length of laboratory larvae ranged from 12.4-17.0 mm; mean, 14.7 mm; there were two modal groups of 12.4-13.0 mm and 14.8-16.1 mm; most larvae were from 14.8-16.1 mm. There was no significant difference in standard length between larvae from different treatment combinations.

Larvae surviving for 33 days after higher benzene exposures of eggs appeared normal in all respects. At least some larvae are able to recover from all effects of the early exposure. However, at least 30% to 50% more larvae at 35 to 45 ppm benzene suffered irreversible effects leading ultimately to their death than did control larvae. The effects of benzene on exposed eggs are summarized in Table 2. Subsamples and surviving larvae were preserved and a more complete study of their morphometrics and abnormalities is underway and will be published separately.

# Exposed Larvae; Effects on Feeding, Behavior, Development and Survival

Eggs were incubated for 9 to 11 days to obtain larvae for benzene exposure. Only normal larvae were used in experiments. Because brine shrimp nauplii were obviously inadequate food, larvae were given rotifers (Brachionus plicatilus) immediately after hatching. Larvae were observed feeding on rotifers on larval day 1. Nephroselmis were also added to provide food for rotifers. After completion of yolk absorption on larval day 2, benzene was added to containers (concentrations in Table 1). We chose to expose larvae at this stage in the assumption that they would be most sensitive to pollutant stress. Larvae reacted immediately to the benzene, swimming erratically and contracting violently. During the 7-day experimental period, the most obvious effect on larvae was on their swimming and feeding behavior. Many exposed larvae were lethargic, often lying on the bottom with reduced swimming and feeding movements, while controls were actively swimming or

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TABLE	

Summary of Effects of Benzene on Eggs and Larvae of Pacific Herring. Percents Are Approximate Estimates of the Significant Differences from Control Levels  $(P < \overline{0.05})$ . NS = Not Significant, LD = Larval Day

Stage Exposed	Approximate Concentration Range (ppm)	Exposure Time (Hrs)	Survival Hatching LD 0	Survival Larvae LD 3	Abnormal Larvae Hatching	Mean % Feeding LD 1-7	Respiration Rate	Stand. Length LD 1-7	Developmental Rate
Egg Day 0	3- 5 15-20 35-45	24	NS NS -15%	NS NS -10%	NS NS +20\$				NS NS Delayed
Egg Day 0, 1	3- 5 15-20 35-45	48	NS NS -15%	NS NS -10%	NS NS +20%				NS NS Delayed
Egg Day 0, 1, 2, 3	3- 5 15-20 35-45	96	NS NS -15&	NS -10% -10%	NS NS + 25%				NS Delayed Delayed
Larval Day 2	5-10 10-15 30-35	24	Not exp. until LD 2	LD 7 NS -10% -25%		30%	 +25% +45%	SN SN SN	NS Delayed Delayed
Larval Day 2,3	5-10 10-15 30-35	48		NS NS -70%		-25% -25% -50%		Smaller Smaller Smaller	Accelerated Delayed Delayed

suspended in the water column and feeding normally. This lethargic behavior was most notable during the first 1 to 2 days after exposure. Subsequently, surviving larvae recovered, exhibiting normal swimming and feeding behavior. Their ability to recover and feed is shown by the percent of larvae with food observed in the gut (Table 3) over the 7-day period.

#### TABLE 3

Percent of Herring Larvae Subsample with Food (Rotifers) in Gut. Total Benzene Exposure of 48 Hrs, LD 0 and 1. Experiment 3

Concentration (ppm)	0	1	2	Larv 3	val I 4	ay 5	6	7	Mean (%)
0 - control		100	100	100	100	100	100	100	100
6.7		45	40	100	85	85	85	85	75
12.1		35	50	65	65	100	100	100	74

Unlike larvae hatching from exposed eggs, exposed larvae showed few obvious morphological abnormalities. Most notable was the delay in development. The developmental sequence appeared unaltered, but the treated larvae were approximately 1 day behind control larvae in rate of development. While most exposed larvae regained ability to feed, some appeared to feed rarely and subsequently the foregut and hindgut collapsed. After this occurred, no further feeding took place and larvae died. This was particularly noticeable in larvae exposed to higher concentrations of benzene (above 10 ppm). All larvae were preserved for further study of their morphometrics and abnormalities.

Figure 3 shows percent survival of larvae on day 7, 48-hr exposure of 6.7 and 12.1 ppm benzene

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(herring experiment 3). Analysis of variance of the data shows a significant difference between concentrations (P < 0.05). Significantly fewer larvae survived in 12.1 ppm than in controls. Apparently, larvae exposed to 6.7 ppm for 48 hrs were able to recover sufficiently to survive through the critical period of yolk absorption. Most remaining larvae from all treatments survived beyond larval day 7 without further significant mortality for 21 days.



Fig. 3. Percent survival of herring larvae on larval day 7 after 48-hr exposure on larval day 2 and 3 to different concentrations of benzene. Herring experiment 3.

Figure 4 shows percent survival of larvae on the larval day preceding starvation mortality (herring experiment 4). The density of rotifers was inadequate to sustain normal growth and survival in this experiment (see Table 1). Significant differences in survival between treatments were found (P < 0.05). There was a significant difference between both

concentrations and times of exposure. Fewer larvae survived at either concentration of benzene than in controls. Significantly fewer larvae survived at 48 hrs than at 24 hrs.



Fig. 4. Percent survival of herring larvae on larval day preceding starvation mortality after exposure to benzene on larval day 2 and/or 3. Herring experiment 4.

The 50% mortality level on larval day 7 is approximately a mean initial concentration of benzene of 20 to 25 ppm for 48 hrs exposure. Larvae seem to be more sensitive to lower concentrations of benzene than are eggs. Larvae, however, have the capacity to recover to some degree from the exposure while the affected embryos in eggs undergo irreversible effects.

### Exposed Larvae; Effect on Growth and Respiration

Although many larvae treated with higher concentrations showed reduced feeding and delayed development, many were able to recover. Table 4 shows the mean standard length of larvae sampled on larval day 8

at the end of the experiment. Exposed larvae were significantly smaller than control larvae (P < 0.05). A difference of approximately 1.0 mm suggests that larval growth was retarded by benzene. In the future, measurements will be taken for a longer period to determine if exposed larvae are able to recover and attain size of control larvae.

Larvae exposed to benzene were placed in respiration flasks in the Gilson respirometer on larval day 3, 24 hrs after initial exposure. The total amount of oxygen consumed over a 24-hr period was recorded (Table 5). The results are preliminary, but show a significant increase (P < 0.05) in respiration exposed larvae over that of controls. Additional experiments are being performed on eggs and larvae of different developmental stages. The oxygen consumption is being measured at shorter 3-hr intervals for a total period of 24 hrs.

A summary of results of exposure of herring larvae is in Table 2.

## TABLE 4

Mean Standard Length of Surviving Larvae (Day 8). Total Exposure of 48 Hrs. Experiment 3

Concentration (ppm)	Exposure Time (hrs)	Mean Standard Length (mm)	N
0 - control		10.3	80
6.7	48	9.4	80
12.1	48	9.2	80

TABLE 5 Summary of Preliminary Experiment Measuring Respiration in Herring Larvae (Larval Day 3) Exposed to Benzene for 24 Hrs. Larvae Acclimated for 30 Min Before Test. Test Run for 24 Hrs

Mean Concentration Benzene	Flask Number	Number Larvae	Oxygen C in 24 Microlite	onsumed Hrs rs/Larva
0 - control	1	10	7.5	
	2	10	6.9 7 7	
	3 4	Malfunction	· • /	
Mean				7.4
13.0 ppm	1 2	10 10	8.0 9.7	
	3	10	12.3	
Mean	4	9	10.4	10.1
39.1 ppm	1	10	13.2	
	2	10	13.6	
	3	10	15.0	
	4	10	10.5	
Mean				13.1

A significant difference exists between all three treatments (P < 0.05).

# ANCHOVY

# Exposed Eggs and Larvae; Effect on Development and Survival

The effect of benzene on anchovy eggs and larvae was tested by exposing both stages in each experiment. Four experiments were done (Table 1). The first experiment exposed eggs to a single 24-hr exposure; the latter three studies included both 24- and 48-hr exposures. At the time of the experiments, rotifers had not been cultured and larvae were not fed. Survival of larvae on days 3 and 6 was determined. Because eggs at time of collection varied in developmental stage, the results of anchovy experiments are more variable than those for herring.



Figure 5 shows percent survival of anchovy larvae on days 3 and 6 for different mean initial

EXPOSURE TIME (HOURS)

Fig. 5. Percent survival of anchovy larvae on days 3 and 6 after exposure of eggs and larvae to benzene. Anchovy experiment 2.

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concentrations and exposure times. Refer to Figure 1 for actual concentrations over the 24-hr exposure period. On day 3, 24-hr exposure, survival of larvae was significantly lower (P < 0.05) at 4.7 ppm than in other concentrations and controls. Development at this concentration was accelerated over controls, whereas development at the higher concentrations was delayed (Table 6). On day 3, 48-hr exposure, an indication of lower survival is shown for larvae at 4.7 and 10.5 ppm. The difference, however, is not significant. On day 6, both 24- and 48-hr exposure, the pattern of larval survival was similar to that for day 3, 24 hrs, but differences were not significant. Except for larvae at 10.5 ppm, 48 hrs, on day 3, the same survival pattern persists throughout. There appears to be an acceleration of development and decreased survival at 4.7 ppm and delayed development and increased survival at 10.5 and 24.0 ppm. Larvae died earlier at 4.7 ppm, while at higher concentrations death of larvae was probably delayed due to narcotization. The slightly lower survival of larvae at 24.0 ppm was due to increased mortality of grossly abnormal larvae not occurring in other treatments. For both days, larval survival was significantly higher at 48 hrs because of delayed development. Additional experiments are planned to determine effects later in larval development when larvae are fed.

Figure 6 shows percent survival of anchovy larvae on day 6 for a concentration range overlapping that shown in Figure 5. When the two figures are compared on day 6, similarities are seen. Mean initial concentrations of 10.5, 13.5, 19.5 and 24 ppm show survival comparable to, or higher than, controls because of delayed development and mortality. At 53.5 ppm, however, survival is significantly less (P < 0.05) than controls and other treatments. Many abnormal larvae died at this concentration and, among those surviving (34%), approximately 30% were still abnormal in some way. Development of the surviving larvae (in 53.5 ppm) was greatly delayed over that of controls and they were also inactive. Even when fed

TABLE 6

Summary of Effects of Benzene on Eggs and Larvae of Northern Anchovy. Approximate Estimates of the Differences from Control Levels (P < 0.05) Derived from the NS = Not Significant Combination of All Experiments.

lopmental Rate	3 Day 6	era Accelera	era. Accelera.	era. Accelera	ed Delaved	sd Delayed	ita. Delaved		d Delayed		d Delayed	
Deve	Day	Accel	Accel	Accele	Delave	Delaye	Accele	avelad	Delave		Delaye	
d Length	Day 6	NS	SN	SN	SN	Smaller	Larger	Larger	Larger	repre.]	Smaller	
Standar	Day 3	NS	SN	SN	SN	Smaller	SN	SN	SN	NS	Smaller	
Yolk	UTILIZATION Day 3	Accelerated	Accelerated	Accelerated	Delayed	Delayed	Accelerated	Delayed	Delayed	Delayed	Delayed	
ıl Larvae	Day 6	+20%	+20%	+25%	+20%	+20%	NS*	*SN	*SN	*SN	+308*	9
Abnorma	Day 3	+25%	+20%	SN	SN	+20%	+20%	+20\$	+25%	+50%	+50%	
Survival	Day 6	NS	SN	SN	SN	SN	SN	SN	SN	SN	-15%	
Larval	Day 3	<b>8</b> 0 T	SN	NS	NS	SN	-10%	-108	-108	NS	SN	6. thus
Exposure Time	(hrs)			24					48			ed bv đav
<b>Approximate</b> Concentration	Range (ppm)	4- 5	5-10	10-15	20-25	40-55	4- 5	5-10	10-15	20-25	40-55	rmal larvae di
Stage Exposed				Day O Egg					Day O Day 1		Egg and Larva	*Most abno

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EXPOSURE TIME (HOURS)



they would die a few days later. Assuming these abnormal larvae will die, the lowest mean initial concentration range at which 50% mortality occurs is approximately 20 to 25 ppm.

Figure 7 shows percent abnormal larvae among those surviving on days 3 and 6 for the same experiment as in Figure 5. On day 3, 24- and 48-hr exposure, there was a significant difference (P < 0.05) between controls and exposed larvae. On day 3, 48 hrs, there were significantly (P < 0.05) more abnormal larvae at 24 ppm than controls and other treatments. On day 6, there were fewer abnormal larvae among survivors than on day 3, because most grossly abnormal larvae had already died. The only significant difference in percent abnormal larvae on day 6 was between controls and larvae exposed to 24 ppm, 48 hrs (P < 0.05).



EXPOSURE TIME (HOURS)



The development of embryos before hatching was not observed in these experiments. When the experiment was ended, control larvae were examined and compared to larvae at approximately the same stage collected from the bay. No obvious differences were observed. Anchovy larvae treated with benzene exhibited abnormalities similar to those described earlier for herring larvae, the most obvious being bending of the notochord and somatic musculature ("bent larvae"). They were preserved for study of their morphometrics and abnormalities.

Unlike herring, few anchovy larvae were abnormal in the controls. The maximum percent of abnormal larvae observed in any experiment was from 10% to 15%. These abnormalities were not severe and usually occurred either when eggs were not treated with antibiotics before hatching or in eggs spawned later in the spawning season.

Benzene affected the developmental rates of the exposed eggs and larvae. Figure 8 shows the percent of larvae with yolk remaining on day 3 after 24-hr exposure of the egg. This illustrates the accelerated utilization of yolk at lower concentrations. Up to 10 ppm the differences from controls are significant (P < 0.05). Utilization of yolk at 24 ppm and, in other experiments, at 40 to 55 ppm is delayed. A difference in rate of yolk utilization occurs in larvae at concentrations of 4.7 and 10.5 ppm depending upon the length of exposure. When exposed for only 24 hrs, yolk is utilized more rapidly in both these concentrations than in controls. When exposed for 48 hrs, yolk utilization is delayed at both concentrations (see Table 6).

This pattern of yolk absorption is also reflected in the mean standard length of larvae on day 3 (Fig. 9). Differences in mean standard length are



MEAN INITIAL CONCENTRATION (ppm)



not significant between concentrations or exposure times on day 3. On day 6, however, there are significant differences (P < 0.01) in the size of larvae correlated with developmental rate and yolk absorption. Control larvae decreased in size from day 3 to day 6. They were active, but not fed, and thus resorbed tissue. When eggs were exposed for 24 hrs to 4.7 ppm, larvae on day 6 were larger than in controls, but smaller than those in 10.5 ppm. They utilized yolk more rapidly, but size remained about the same as day 3. Even though more active than larvae at higher concentrations, they were not as active as control larvae and resorbed less tissue. When eggs were exposed for 24 hrs to 10.5 ppm, larvae



EXPOSURE TIME (HOURS)

Fig. 9. Mean standard length of anchovy larvae on days 3 and 6 after exposure of eggs and larvae to benzene. Anchovy experiment 2.

on day 6 were larger than in other treatments. They were probably narcotized and absorbed yolk more slowly. Because the larvae were less active, energy derived from yolk was channeled into growth. Resorption of tissue would probably occur later. When eggs were exposed for 24 hrs to 24 ppm, larvae on day 6 were larger than controls because of slower yolk absorption and development. Larvae at 24 ppm were less in mean standard length than those at 10.5 ppm because smaller, abnormal larvae were included in the measurements.

When eggs and larvae were exposed for 48 hrs, by day 6 all exposed larvae were larger than controls. Only normal larvae in all treatments were measured. At all concentrations, development was delayed and larvae were relatively inactive. Most energy derived from yolk absorption was expended in growth and tissue was not resorbed through activity as in control larvae. Whether the exposed larvae are able to recover later in development as did herring larvae is not known. Experiments are planned for next spawning season in which larvae will be fed and the delayed effects examined.

#### DISCUSSION

Benzene induces considerable physiological stress on eggs and larvae of herring and anchovy over much of the initial concentration range tested (approximately 5 to 50 ppm). Both lethal and sublethal effects were noted in measurements of survival and other parameters indicative of metabolic rate. The lethal effects of benzene are summarized in Table 7 as 50% mortality levels at certain stages. Because benzene volatilizes, calculated concentrations are given for various times. Maintenance of a constant concentration of benzene in an open system, such as that used in tests with juveniles and adults (Benville and Korn, 1974), was not possible. When a constant concentration is maintained, a fungus-like organism, apparently

	ť		Approxi	mate	calc	ulate	ğ	Initial Conc.	Exposure
Setcede	Exposed	age 50% Mortality	Concentra Initial	ttion 6	ls Ové 12	er 24 18	Hrs <sup>*</sup> 24	Range (ppm)	Time (Hrs)
Herring	Eggs	At Hatching	40	29	20	15	10	40-45	96
			45	32	23	16	12		
Herring	Larval	Larval Day 7	20	14	10	7	ŝ	20-25	48
	Day 2		25	18	13	ი	2		
Anchovy	Egg and	Larval Day 3	20	14	10	7	ŝ	20-25	48
	Larval Day l		25	18	13	ი	7		

Summary of Concentrations and Exposure Times of Benzene Causing 50% Mortality in Herring and Anchovy Eggs and Larvae

TABLE 7

\*Calculated from the equation  $\underline{\hat{Y}} = ae^{-.0565X}$  (refer to Fig. 1).

using benzene as an energy source, grows over the chorionic surface and the eggs die. We are trying to identify this organism and attempting to define its growth requirements as well as means of eliminating it from laboratory cultures.

A single exposure of benzene may approximate what occurs in the field. The initial benzene put into solution in the field undoubtedly volatilizes and the concentration declines over time. We plan to conduct spatial and temporal sampling in San Francisco Bay in order to determine actual concentrations of benzene and other aromatic components in the water column, both in areas of chronic oil pollution and in the event of a spill. Benzene may interact synergistically with other water-soluble aromatic components to produce greater effects than that of benzene alone. Data on concentrations of aromatics in either estuaries or oceans, however, are incomplete.

Sublethal effects of benzene on yolk absorption, growth and respiration show that benzene influences metabolic rate and energy utilization of embryos and larvae. At lower concentrations, metabolic rate is accelerated with a resultant energy cost in the alleviation of stress. At higher concentrations, metabolic rate is delayed, probably because larvae are narcotized. We plan to do additional measurements of respiration and growth to determine effects of the aromatic components on the energy budget of the larvae.

Reference to Table 7 shows that a greater exposure of benzene is required to affect mortality in exposed eggs than in exposed larvae. The exposure of eggs, however, induces abnormalities in the embryos. The effect is permanent, larvae eventually dying. It is interesting that many exposed herring and anchovy larvae are able to recover even though they undergo temporary effects. Those that survive 3 days after the last exposure often recover all feeding and swimming activity. There may be, however, delayed effects in development and growth not obvious until later in larval development. Herring and anchovy larvae showed 50% mortality at approximately the same concentration range of 20 to 25 ppm (Table 7).

Brocksen and Bailey (1973) found similar effects of benzene on the metabolic rate of juvenile striped bass and chinook salmon, evaluated by measuring their respiration rate at different concentrations and exposure times. They found an increase in respiration at lower concentrations and shorter exposure times. In contrast, at higher concentrations and longer exposures, narcotization occurred and respiration was reduced. They also found that fish recovered from exposures. Brocksen and Bailey (1973) provide an explanation of the possible biochemical pathways involved in benzene metabolism which may account for these effects on respiration.

Most other studies of the effect of oil on larval fish have been with whole crude oil or crude oil fractions. Kühnhold (1969, 1970) has tested the effect of several crude oils and their water-soluble fractions on Atlantic herring (Clupea harengus) larvae. At the National Marine Fisheries Service laboratory at Auke Bay, Alaska, the effect of watersoluble fractions of Prudhoe Bay crude oil was tested on Pacific herring (C. pallasi) and pink salmon fry (Oncorhynchus gorbuscha) (Stanley Rice, personal communication). Since we are studying the isolated physiological effects of benzene, our results cannot be legitimately compared.

It is probable that oil, acting synergistically with other pollutants such as pesticides deleteriously affects the young stages of fish and invertebrates in polluted estuaries. Many of these fish and invertebrates are important sport and commercial fisheries resources. Although the decline in their populations cannot be attributed solely to the effects of oil, oil and other pollutants may act together in significantly reducing larval recruitment in estuarinedependent species.

The higher percent of abnormal larvae observed in larval herring hatching from eggs spawned in

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San Francisco Bay than in the relatively unpolluted waters of Tomales Bay farther north may indicate that San Francisco Bay water is already polluted sufficiently to stress eggs after spawning. Although some genetic variation in viability could be expected in species producing such large numbers of eggs, it is unlikely that 20% to 25% would be genetically inviable. Abnormal eggs may also result from deleterious effects of pollutants on the development of eggs in gonads of adults. Since aromatics are highly soluble in lipid tissues, such as eggs, we plan to study this problem further.

The applied benefit of this research is obvious in view of the increasing possibility of oil spills, blowouts, effluents, and ballast dumping in the future. Recommendations on allowable levels of oil must involve knowledge of specific effects at sublethal concentrations as well as immediate lethal effects.

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