Marine Environmental Research 14 (1984) 337–354

Effects of Contaminated Sediments and Sediment-Exposed Effluent Water on an Estuarine Fish: Acute Toxicity*

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ABSTRACT

The Elizabeth River is Virginia's most heavily populated, industrialized and contaminated subestuary. Its sediments contain the residues of wastes discharged for several hundred years. To examine some of the effects of sediment from a particularly noxious location, we chose the spot (Leiostomus xanthurus), *a bottom-feeding sciaenid and naturally occurring spring–summer migrant of the river whose juveniles adapt readily to laboratory conditions. Spot from the Ware River, a nearby uncontaminated reference subestuary, were placed in two 380 liter (100 US gal), flow-through tanks (70 each). One tank contained Elizabeth River sediments contaminated with PAHs. The second tank contained uncontaminated sediment from the York River as a control. 13 spot each were placed in two aquaria that received the overflow from the sediment-containing tanks. Animals from the reference estuary were examined and processed to provide baseline information.*

Results obtained to date show the following: (1) spot experimentally exposed to contaminated sediment developed penetrating integumental lesions within 8 days after exposure began and later severe fin and gill erosion; (2) their hematocrits were significantly reduced and no weight gain occurred; (3) pancreatic and liver alterations were observed in some of the chemically stressed fish; (4) control fish exhibited no fin erosion or integumental lesions; (5) control fish showed no hematocrit or growth reduction; and (6) dead fish were first observed in the contaminated-sediment tank after 8 days while no control fish died. Clearly, one or more

* Contribution No. 1164 from the Virginia Institute of Marine Science and School of Marine Science, College of William and Mary.

Marine Environ. Res. 0141-1136/84/$03·00

factors associated with Elizabeth River sediments and/or water closely associated with those sediments (probably the heavy concentrations of PAHs) are capable of causing serious injury, disease and death in experimental spot populations.

INTRODUCTION

Interest in the effects of environmental stresses on health and disease in fish and other marine organisms has increased in recent years (Snieszko, 1970; Sindermann, 1979; Sindermann *et al.*, 1980). Sindermann (1979) reviewed much of the research prior to 1978 and presented an extensive bibliography. Several researchers have devoted attention specifically to fishes (Couch, 1975; Couch *et al.*, 1977; Haensly *et al.*, 1982; McCain *et al.*, 1978, 1982; Middaugh *et al.*, 1977, 1980; Overstreet & Howse, 1977; Overstreet, 1978; Pierce *et al.*, 1980; Rowe *et al.*, 1983*a*,*b*; Schultz & Schultz, 1982; Solangi & Overstreet, 1982). Neff & Anderson (1981) considered the responses of marine animals to petroleum and some specific hydrocarbons. They too provided an extensive reference list.

The Elizabeth River is a three-branched subestuary becoming increasingly heavily populated, industrialized and polluted. The site of civilian and military shipbuilding, shipping and shoreside commerce, and associated manufacturing and processing industries for over 300 years, it is located on the southern shore of the lower James River estuary, Virginia (Fig. 1).

The Elizabeth River is recognized as the most heavily contaminated subestuary in the lower Chesapeake region. During geochemical studies, Bieri *et al.* (1982) found heavy concentrations of PAHs in sediments in the upper Elizabeth (Southern Branch) which had received wastes from a wood treatment plant using creosote. Another such plant and several oil transfer piers and storage tank farms are nearby. Downstream sites and those in other branches contained far less. Lu (1982) reported benzo[*a*]pyrene concentrations ranging from 10 μg/g in the top 2 cm to greater than 200 μg/g at 30 cm in a bottom core taken from the most heavily PAH-contaminated location in the Elizabeth River.

Consequently, sediments from this site (Station 217) were chosen to examine the responses of fishes exposed to contaminated sediment in controlled-exposure conditions. For preliminary studies the fish, spot (*Leiostomus xanthurus* Lacépède: Sciaenidae, Perciformes), was selected

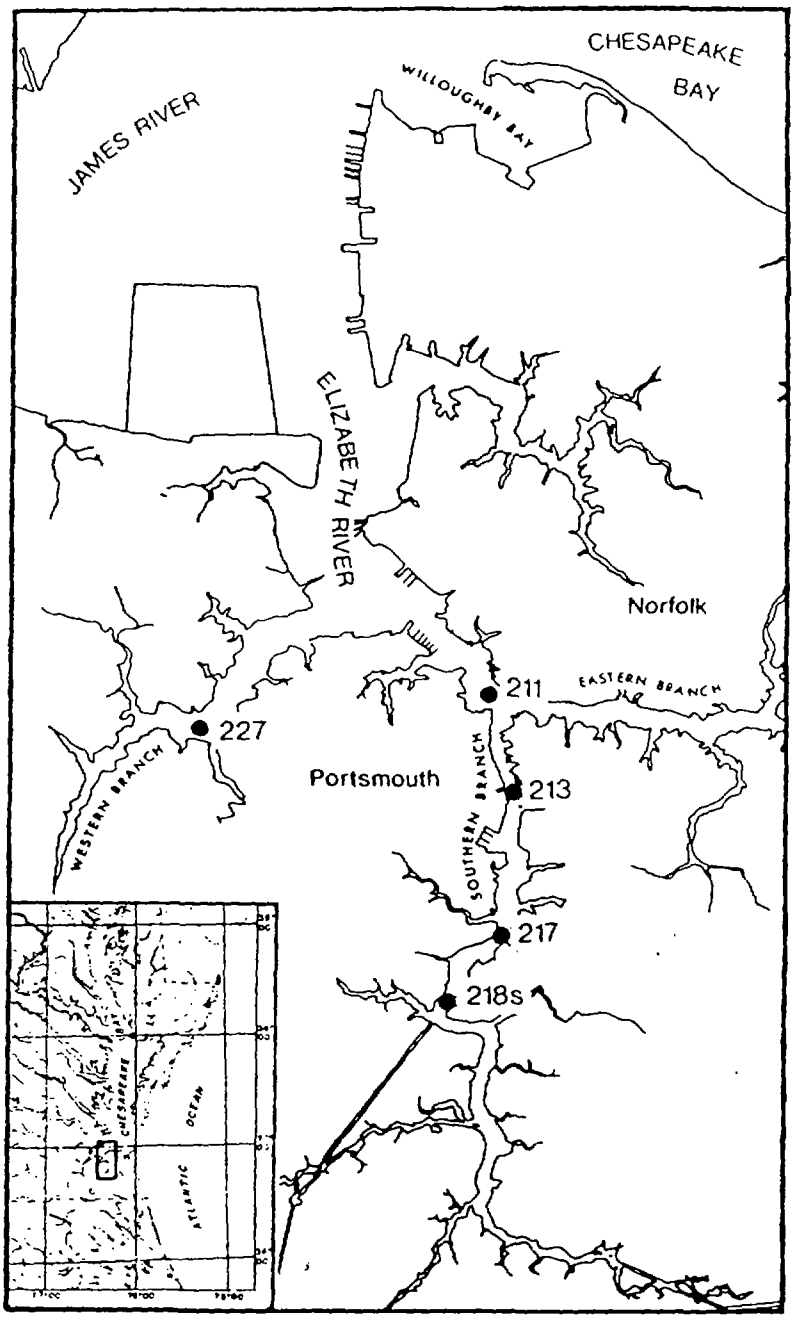

Fig. 1. The Elizabeth River, showing stations. (Inset depicting Chesapeake Bay shows location of Elizabeth River.)

because it is a naturally occurring spring and summer migrant of the region, is a bottom-feeder in close contact with sediments and is also adaptable to laboratory conditions. Of principal interest were the effects of contaminated sediments and sediment-associated water on the gross morphology (external and internal) and other manifestations of health or disease. Weeks & Warinner (this volume, pp. 327–35) are studying immune responses of these fishes from the Elizabeth River and reference waters.

MATERIALS AND METHODS

Experimental studies were begun on 23 August 1982. Two 380 liter (100 gal) fiberglass tanks containing 70 spot each and receiving a flow of about 10 liters/min York River water were established (Fig. 2). One tank contained a layer of about 10 cm in depth of contaminated sediment from Station 217 in the Elizabeth River. The other contained a like amount of similar, but relatively clean, sediment from the York River, a less contaminated estuary. Aeration was provided to maintain adequate oxygen concentrations. The test fish, ranging in size from 8·2 cm TL to 12·5 cm TL, were collected from the Ware River, Virginia, a rural,

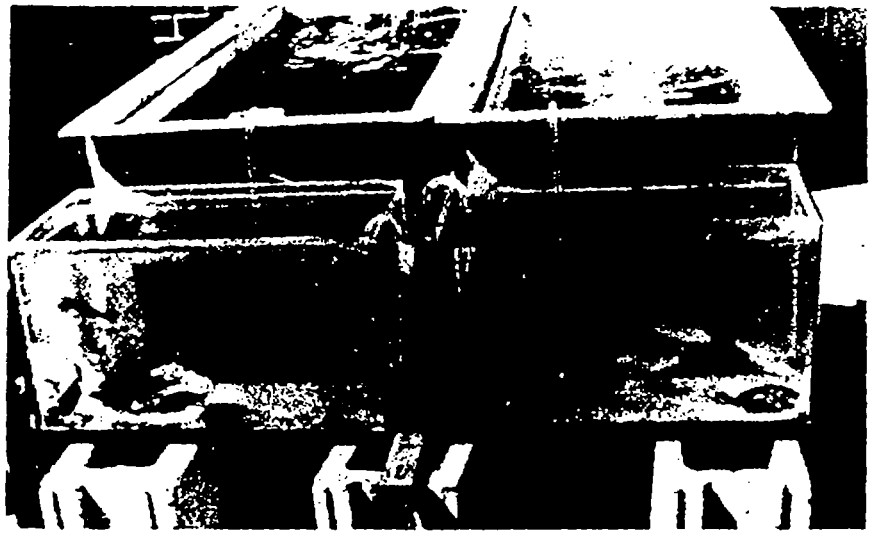

Fig. 2. Experimental arrangements showing 100 gal main, sediment-containing tanks and 10 gal effluent-receiving aquaria. PAH-contaminated sediments in 100 gal tank to left.

undeveloped system similar in salinity and other essential features to the Elizabeth and yet sufficiently distant from it to serve the purpose. A baseline group of 10 randomly chosen individuals was weighed to provide data for feeding calculations and later weight comparisons. 34 other baseline fish from the Ware were measured, weighed, necropsied and processed at the beginning of the experiment. Fish were fed measured amounts of Zeigler's trout chow (No. 3, starter pellets) each day. This simple experiment was designed to determine whether spot exposed to contaminated Elizabeth River sediments would develop symptoms of acute or subacute toxicity, or disease.

We intended the experiment to continue for about 100 days with half of the fish to be sampled and processed at day 50 and the other half upon its termination. The sediment proved extremely toxic, producing lesions and death in some fish within 8 days. Consequently the experiment was allowed to continue for only 28 days.

When lesions and deaths began appearing in the experimental animals, a corollary experiment was begun exposing 13 fish to effluent from each sediment-exposure tank in 38 liter (10 gal) aquaria (Fig. 2). This test, designed to examine toxicity of the effluent water from the sediment tanks, continued for 24 days.

Survival for both sediment-exposure main tanks was determined only twice during the experiment since this required their total drainage. Survival for the overflow tanks was monitored daily as were environmental conditions in all tanks.

On 10 September 1982 (after 18 days of exposure) the experiment was interrupted and the main, sediment-containing tanks were drained. All fish were counted and examined superficially, remaining dead animals were removed and 10 live individuals were sacrificed from each of the sediment-exposure tanks for detailed examination and processing. The experiment was resumed with 33 fish in each tank on the same day. The effluent exposure experiment was also continued after a brief interruption. Regular feeding and monitoring continued as before. Among the 10 fish removed from the contaminated-sediment tank were those with obvious lesions (ulcerations) and the most severe fin erosion. This selection was made to allow examination of severely affected individuals while still alive. The sediment-exposure phase of the experiment was terminated on 20 September 1982, 10 days later, when all fish were removed from the sediment-containing tanks and a sample of 15 each was taken from each tank for processing. All remaining surviving

contaminated-sediment tank fish had been exposed to experimental conditions for 28 days.

At both sampling periods the fish were measured, weighed and examined externally; blood samples were taken for hematocrits; internal examinations conducted; smears of blood, bile and gill tissues examined microscopically; and samples of tissues from live animals (including skin lesion, olfactory rosettes, gills and livers and occasionally other interesting tissues) were quickly killed and immediately fixed in 10% neutral-buffered formalin for histological processing.

Histological specimens were infiltrated with and embedded in paraffin, sectioned at 6 μm and stained with Harris's hematoxylin and eosin. When necessary, tissues were decalcified in 0·1 N HCl prior to embedding. Permanently stained blood slides were prepared using Giemsa stain. Slides were examined using bright field microscopy. 35 mm macro- or microphotographs were routinely made of all interesting phenomena using Kodak Ektachrome® 160 Tungsten film. Black and white photographs have been made from the resulting slides where required.

Sediment cores were collected from each exposure tank at the beginning and end of the experiment and frozen. Samples were freeze-dried and extracted with methylene chloride. The extracts were fractionated with gel permeation chromatography and high-performance liquid chromatography and then analyzed by glass capillary gas chromatography using a FID detector (Bieri *et al.*, 1982).

The effluent experiment, begun with surplus control animals on 14 September 1982, was continued later (but not longer) than the main tank experiment (with several fish placed in each sediment-containing tank to serve as agitators or 'mud-stirrers') and was terminated on 8 October after 24 days total exposure of the fish to the effluent from the sediment-containing tanks.

The experimental containers were outside and subject to ambient weather conditions. Control and experimental (contaminated) tanks were side by side and covered except during tending (Fig. 2). Water in the sediment-containing tanks was monitored regularly for salinity, sediment and water temperature, water pH and dissolved oxygen.

All tanks were examined twice daily and dead fish recovered and frozen. Later these fish were examined externally, measured and weighed, unless too badly decomposed. A few fish were never recovered from the main, sediment-containing tanks since they did not always float, decomposed rapidly, and were difficult to see from above in the deep sediment-

exposure tanks in which sediments were being continuously resuspended. Skeletal remains found in the drainage system later confirmed this occurrence.

RESULTS

The results reported here are for a preliminary experiment. The severity of response in the exposed fish, with rapid appearance of external lesions and mortality and other signs of stress and injury, are especially noteworthy.

Analysis of the tank-contained sediments (main tanks) disclosed that the Elizabeth River Station 217 sediments were heavily contaminated with PAHs as compared with the York River sediments used in the control tank. A sample from the contaminated sediment (Station 217) tank on day 0 contained 2500 ppm PAH per dry weight of sediment vs. 1·2 ppm in the control tank sediment. At the end of the experiment, day 45, the concentrations were 3900 ppm vs. 0·7 ppm respectively. The increase in the contaminated sediment tank measured reflects uneven distribution of PAHs in sediments.

The concentrations of the 20 selected species of PAH which were most plentiful in these sediment samples are shown in Table 1. 19 of the 20 compounds were present in both control and experimental sediments. Phenanthrene and fluoranthracene were the two most abundant species in both sediments, each accounting for 5–12% of the total PAH. A number of higher molecular weight species, e.g. benzo[*a*]fluorene and benzo[*b*]fluorene, were relatively more abundant in contaminated than in control sediment. Concentrations of low molecular weight PAHs (i.e. those lighter than anthracene) were not measured accurately because of losses due to volatilization during sample processing.

Water quality was similar in the two main test tanks (Table 2). Water temperatures declined from 27 °C to 21 °C during the 46-day experiment. Dissolved oxygen averaged 5·9 mg/liter, but was as low as 2·5 mg/liter at one point. This concentration, stressful to spot, resulted primarily from resuspension of the reduced sediments by the fish in both tanks. Aeration, introduced when reduced oxygen was observed, maintained the oxygen concentration at acceptable levels thereafter. There was no evidence of bacterial fouling of the water in either of the sediment tanks or effluent tanks.

TABLE 1
Concentrations of 20 Selected PAHs
(ppm dry weight of sediment)

PAH species	*23 August*		*7 October*	
	Control	*Experimental*	*Control*	*Experimental*
Benzothiophene	0·014	0	0·000	0
2-Methylnaphthalene	0·025	15	0·009	20
1-Methylnaphthalene	0·037	25	0·015	47
Biphenyl	0·016	8	0·008	16
Fluorene	0·079	75	0·032	137
Dibenzothiophene	0·016	23	0·007	51
Phenanthrene	0·140	268	0·082	468
Anthracene	0·020	85	0·008	125
Fluoranthracene	0·054	230	0·048	324
Pyrene	0·040	155	0·035	226
Benzo[*a*]fluorene	0·007	65	0·010	86
Benzo[*b*]fluorene	0·007	63	0·009	82
Benz[*a*]anthracene	0·008	60	0·009	82
Chrysene	0·024	78	0·028	105
Benzofluoranthrene	0·025	73	0·034	94
Benzo[*e*]pyrene	0·011	33	0·017	35
Benzo[*a*]pyrene	0·008	35	0·009	43
Perylene	0·006	10	0·004	12
Indeno[1,2,3-*cd*]pyrene	0·004	13	0·005	20
Benzo[*ghi*]perylene	0·006	13	0·007	16

TABLE 2
Calculations of Regular Observations of Conditions in Sediment-Containing Tanks During Experimental Period

	Control			*Experimental*		
	$\bar{x}$	*SD*	*Range*	$\bar{x}$	*SD*	*Range*
Salinity (‰)	19·5	0·5	18·4–20·3	19·5	0·5	18·3–20·4
Sediment temperature (°C)	23·7	2·1	19·8–27·1	23·4	1·9	20·0–26·9
Water temperature (°C)	24·0	2·1	20·0–27·8	24·0	2·0	20·7–27·8
Water pH	7·5	0·2	7·3–7·9	7·5	0·1	7·2–7·8
Oxygen (mg/liter)	5·9	1·5	2·5–8·9	5·9	1·4	2·7–8·5

TABLE 3
Survival of Spot (*Leiostomus xanthurus*) Exposed to Control and Contaminated Sediments in Flowing Seawater Tanks or Effluent (SAW) from These Tanks

Date	*Sediment tank*			*Effluent tank*		
	Day	*No. of fish*	*%S*	*Day*	*No. of fish*	*%S*
Control sediments:						
23 Aug.	0	70	—			
10 Sept.	18	70	100			
10 Sept.	18	33				
14 Sept.	22	—		0	13	
17 Sept.	25	—		3	0[a]	0
20 Sept.	28	33	100			
20 Sept.	28			6	12[b]	
8 Oct.	46			24	11[c]	100[c]
Contaminated sediments:						
23 Aug.	0	70	—			
10 Sept.	18	49	70			
10 Sept.	18	33				
14 Sept.	22	—	—	0	13	
17 Sept.				3	13	100
20 Sept.	28	15	45			
20 Sept.	28			6	13	
21 Sept.				7	12	92
8 Oct.	46			24	10[d]	92[d]

[a] Resulted from loss of water flow to this tank and consequent oxygen depletion. Repopulated with fish from control tank population, main experiment.
[b] Only 12 were added since one fish had died in the contaminated-sediment effluent tank.
[c] 11 spot remained in the control tank at the end of the experiment, one is presumed to have jumped out during tending. No dead fish were found near by and none appeared in the aquarium. The presumed escaped fish was not included in survival calculations.
[d] Fish disappeared from contaminated-sediment effluent tank. A dead fish found nearby and presumed to have jumped out during tending is not included in survival calculations.

Obvious skin lesions and deaths were observed in the contaminated-sediment tank within 8 days after exposure. In response to these acute toxic effects, we shortened the experiment and took the first sample on 10 September 1982 after 18 days exposure. Survival in the contaminated-sediment main tank was 70% (49 individuals of the original 70) after 18 days from first exposure (Table 3). During the second phase (after resumption of the contaminated-sediment exposure experiment) 18 fish died in the contaminated-sediment main tank. Survival on 20 September 1982, then, was 15 individuals out of the 33 reintroduced at restart on 10 September 1982, or 45%. No fish died in the control, clean-sediment main tank during the entire 28-day exposure period.

As Table 3 also shows, mortality was much less in the effluent aquarium. A single individual died on day 7 in the aquarium receiving effluent from the contaminated tank during the 24-day exposure period of this group of fish. It also had an integumental lesion. Survival in the contaminated-effluent aquarium remained at 92% after this death. On day 3 (17 September 1982) of the effluent experiment, water delivery to the control tank stopped and all control fish died of anoxia. The dead fish were physically in good shape except for clear signs of death by asphyxiation. The control aquarium was re-established. None of the 12 replacement control fish (originally in the control main tank) died during the 21-day remainder of the effluent experiment (Table 3). One fish disappeared and was presumed to have jumped out during tending (a dead fish was found near by).

Subacute effects were pronounced in the contaminated-sediment tank also. Of the 10 live spot extracted from this experimental container during

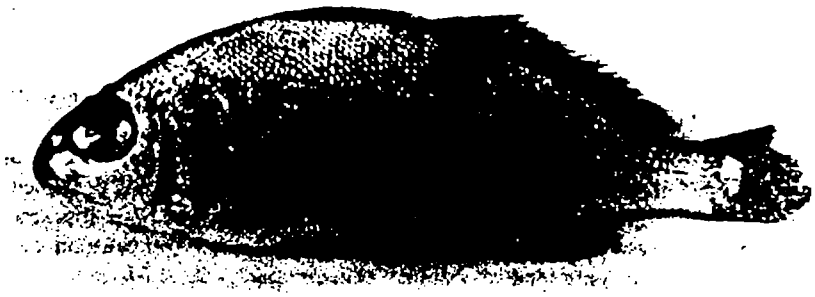

Fig. 3. Spot (*L. xanthurus*) from contaminated-sediment tank with severe fin erosion. Pectoral fin has eroded to the falciform shape frequently observed during experiment. (All fish approximately same size.)

Fig. 4. Spot (*L. xanthurus*) from contaminated-sediment tank with severe fin erosion. Only stumps remain of pectorals and caudal is almost gone.

the first sampling period on day 18, *all* exhibited significant fin erosion. 7 fish had severely eroded fins, with entire fins gone in most (Figs. 3 and 4). The pectoral and caudal fins were almost completely eroded away in 3 instances. Examination under the stereomicroscope revealed gill erosion in 7 of the 10 animals. Gill erosion in 2 fish was slight, but heavy in 5. External lesions were apparent in 6 (or 12%) of the 49 individuals surviving. Most lesions extended into the dermis, some into the musculature (Fig. 5). None of these signs appeared in individuals from the

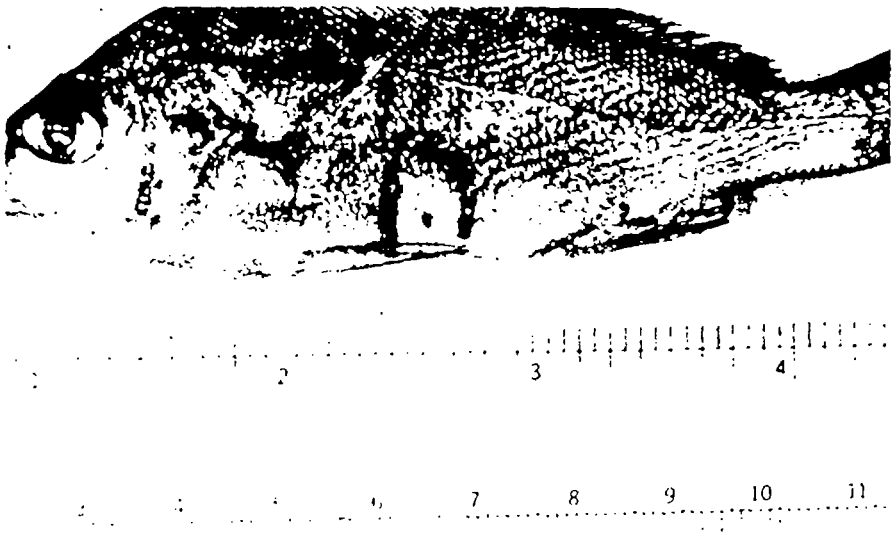

Fig. 5. Spot (*L. xanthurus*) from contaminated sediment tank with penetrating lesion on lower left side.

control, clean-sediment, container. One control fish lacked its left eye but the wound was well-healed and there was no trace of eye or nerve tissue remaining in the empty eye-socket.

Evidence of injury was observed also in *all* 15 of the second and final sample of fish from the contaminated-sediment tank at termination on 10 September 1982, 28 days after initiation of the experiment and 10 days after the first sampling period. Fin erosion ranging from moderate to severe was clearly displayed in *all* individuals. The pectoral fins (13 of 15 individuals) and caudal fins (9 of 15) were most heavily damaged, with 6 fish (of 15) showing severe erosion of the pelvic fins. Thus, the fins in closest contact with the sediments during swimming and feeding (stirring) were most seriously affected. 4 of the fish with eroded fins seemed to show signs of repair and regeneration. 10 of the 15 animals displayed significant hyperemia. 3 fish, or 20% of the survivors, had moderate to heavy integumental lesions.

Fish exposed to contaminated sediment differed from control fish at both observation times with respect to body weight and general appearance. Those from the PAH-contaminated tank showed no growth and were often hollow-bellied, whereas those from the control tank had gained an average of 12 g per fish by day 18.

Hematocrits taken from the two groups of fish were clearly different. The mean hematocrit for 22 fish (including those from both samplings) exposed to contaminated sediment was $22{\cdot}6 \pm 3{\cdot}17$ (range = 15·5–30·0, Student's $t_{0{\cdot}01}$ (40 d.f.) = 2·704, $P < 0{\cdot}01$), significantly lower than that for 22 control fish ($\bar{x} = 30{\cdot}7 \pm 6{\cdot}52$, range 24·0–49·0).

Preliminary evaluation of the histology of the liver sections shows clear tinctorial and structural differences in the liver parenchyma (Fig. 6) of some and signs of atrophy of interhepatic pancreatic nodules in many fish exposed to contaminated sediments. The livers of the few control individuals examined thus far do not display these signs. Some contaminated-sediment exposed fish showed filament hyperplasia and lamellar clubbing of the gills.

Taken together, the results from both sampling periods (involving 70 fish each from the sediment-containing tanks) indicate that in the contaminated-sediment tank, 21 of 70 (or 30%) fish died during the first 18 days of exposure and an additional 18 died during the next 10 days (Table 3). Thus, 39 of the 70 original fish (or 56%) were killed during the 28-day experiment. 6 others smothered themselves in the mud after becoming greatly agitated during the first sampling period. These

Fig. 6. Stained (Harris's hematoxylin and eosin) section of liver of spot (*L. xanthurus*) from contaminated-sediment tank showing clear-cell lesion of parenchyma. Note textural difference between damaged area containing heavily vacuolated hepatocytes and adjacent, more normal tissue. Tinctorial differences appeared also. (800 ×)

combined-cause deaths occurring throughout the experiment left only 25 live animals for processing.

As indicated separately above, *all* 25 living spot obtained from the main tank, containing PAH-contaminated sediments from Elizabeth River Station 217, displayed external and internal symptoms of injury or disease. All had suffered fin erosion, ranging from severe in most to moderate in some. 9 of the total of 25 surviving animals (36%) had developed fulminating, penetrating lesions. 6 of the first 10 individuals and 3 of the last 15 had them. 7 of 10 individuals from the first sampling exhibited gill erosion. This feature was not noted in the last 15. 22 of the 25 survivors (or those from which blood and hematocrit samples could be obtained) had low hematocrits and most showed no growth. No deaths and none of these symptoms of injury or disease occurred in the 70 control fish from the uncontaminated-sediment tank.

The effects on spot in the tanks receiving effluent from the sediment exposure tanks were somewhat less marked. The single fish which had

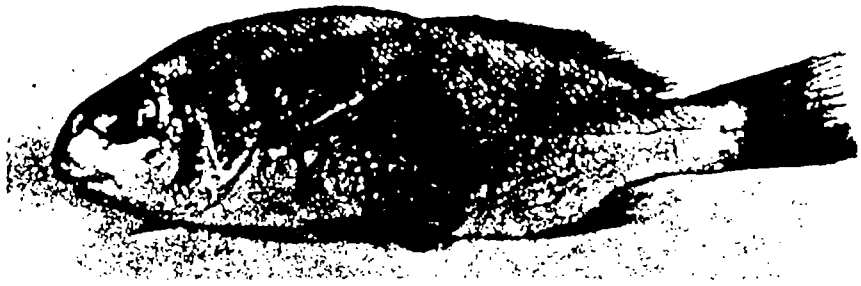

Fig. 7. Spot (*L. xanthurus*) exposed to contaminated effluent showing especially severe penetrating lesion in lower left side. Fish alive at time of necropsy. Lesion about 23 mm in diameter.

died in the contaminated-sediment effluent tank on day 7 (Table 3) had developed a severe skin lesion within 4–6 days after exposure. The remaining 10 fish—those which survived the full 24-day exposure period—were examined and processed. 5 of the 11 exposed animals (including the dead one), or 45%, displayed lesions. One of the lesions borne by a living fish was massive, extending over a large portion of the side, completely penetrating the muscles and exposing eroded rib ends and a large portion of the body cavity. The internal organs were clearly visible (Fig. 7). Two lesions were noted on another individual. When introduced into the tanks, neither control nor experimental fish exhibited lesions or other damage from collecting or transfer operations and control fish never developed lesions or other signs of distress.

Of the 4 live fish collected from the effluent aquaria at the end of the experiment, and suffering from severe, penetrating lesions, 3 showed signs of healing in at least one section of the perimeter of the lesion when the experiment was terminated.

All 10 contaminated effluent survivors had opaque or cloudy eyes (appearing like cataracts in humans). Only one fish showed severe fin erosion. Fins of the rest were only slightly eroded or not at all.

11 fish survived in the control effluent aquarium (Table 3). None of these fish exhibited noticeable fin erosion or any of the other signs of disease noted in the contaminated effluent tank inhabitants. The caudal fin of one was missing but the completely healed, truncated peduncle suggests that it had suffered this injury much earlier, probably prior to the experiment.

This brief experiment exposing spot to effluent water from the sediment

exposure tanks indicates that the water passing over contaminated sediments from Elizabeth River Station 217 is capable of producing recognizable disease signs in spot, even at the dilutions which occurred in this continuously running, flow-through system. Direct contact with large volumes of contaminated sediments is not necessary to produce chronic effects.

Examination of the histological materials from these sediment- and effluent-exposed fish is not complete.

As the results of these simple exploratory experiments show, under the experimental conditions pertaining, *all* of the spot exposed to Elizabeth River Station 217 sediment or to the effluent from the tank and obtained alive were injured (i.e. had induced disease signs) or killed (i.e. 56% of the original population of 70 in the main sediment-containing tanks). Those exposed to sediments from the reference station in the York River, or to the effluent from the tank, were not injured under comparable experimental conditions in the control tank.

DISCUSSION

Our experiments involving flow-through tank exposure of spot (*L. xanthurus*) to sediments from Station 217 in the Elizabeth River, contaminated with 3900 ppm of low and high molecular weight PAHs, demonstrate clearly that disease and even death can be induced by contact with the sediments and/or the water associated with those sediments. One and usually more disease signs appeared in *all* 25 of the surviving fish exposed to contaminated sediment.

Also, *all* 11 of the fish exposed to contaminated effluent water, which were recovered, developed signs of stressor-induced disease. (Opacity of the eye of exposed spot was noted in all 10 living fish remaining in the contaminated effluent tank. This finding seems significant, but the process is not understood.) Direct contact with these PAH-contaminated sediments apparently was not necessary to produce signs of disease, or even death.

The acute lethality of the contaminating materials in these sediments manifested itself as early as day 8, when the first death in the contaminated-sediment tank was observed. Ultimately many fish in this experimental tank died during the 28-day course of the experiment (i.e. 39

of 70, or 56%; Table 3). One spot exposed to the contaminated effluent died as well (on day 7). While the precise cause or causes of these deaths is not known, the sediments from Station 217 in the Elizabeth River clearly contain extremely high concentrations of very toxic chemicals, PAHs (Table 1). The water exposed to those sediments undoubtedly does likewise.

Spot are largely bottom-feeders, actively agitating the surface of the sediments with their fins and body movements while foraging. This would account for the high incidence of severe fin erosion of the pectoral, caudal and pelvic fins and of obvious hyperemia around these fins. In some, the pectorals, caudals and pelvics were so severely eroded that mere stumps remained (Fig. 4).

The development of penetrating and rapidly progressing (or fulminating) lesions (Figs. 5 and 7) is of especial interest, but the process of their development is not understood. Smears were inconclusive as to possible bacteriological involvement. Sections have not been fully read. It is not clear whether dissolved chemicals alone are sufficient to cause the observed skin and fin erosion and lesions or whether chemical suspensoids or particles of contaminated sediment are involved, but spot exposed to contaminated sediments directly, or only to flowing water which has passed over the sediment, can be induced to form integumental lesions.

Spot exposed to contaminated Elizabeth River sediments in these experiments evidently fed only poorly. There was no weight gain among fish exposed to contaminated sediment. However, many remained alive long after starvation would have killed non-feeding fish. Also, the hematocrits of these fish were significantly lower than those of the controls. These phenomena may be connected. Our colleagues (Weeks & Warinner, personal communication) have found the cell-mediated (macrophage) immune response to be much lower in spot from Station 217 in the Elizabeth River than in comparable fish from a reference estuary.

The extent and significance of the liver and pancreatic injuries discovered in spot exposed to contaminated sediment is not clear. We seem to have induced significant tinctorial and structural disturbances in the liver parenchyma (clear-cell foci and other lesions) and atrophy of pancreatic nodules in some fish. Completion of the microscopical examinations will be necessary before the full nature and extent of these histopathological effects are known.

oil from experimentally oiled sediments to English sole (*Parophrys vetulus*) and pathological consequences. *J. Fish. Res. Bd Can.* **35**, 657–64.

McCain, B. B., Myers, M. S., Varanasi, U., Brown, D. W., Rhodes, L. D., Gronlund, W. D., Elliott, D. G., Palsson, W. A., Hudgins, H. O. & Malins, D. C. (1982). Pathology of two species of flatfish from urban estuaries in Puget Sound. Final Report of the Federal Interagency Energy/Environment Research and Development Program, NOAA and EPA, 100 pp.

Middaugh, D. P., Crane, A. M. & Couch, J. A. (1977). Toxicity of chlorine to juvenile spot, *Leiostomus xanthurus*. *Water Res.* **11**, 1089–96.

Middaugh, D. P., Burnett, L. E. & Couch, J. A. (1980). Toxicological and physiological responses of the fish, *Leiostomus xanthurus*, exposed to chlorine-produced oxidants. *Estuaries* **3**, 132–41.

Neff, J. M. & Anderson, J. W. (1981). *Response of marine animals to petroleum and specific petroleum hydrocarbons*. Applied Science Publishers, London, 177 pp.

Overstreet, R. M. (1978). *Marine maladies? Worms, germs and other symbionts from the northern Gulf of Mexico*. Mississippi–Alabama Sea Grant Consortium, Ocean Springs, Miss., 140 pp.

Overstreet, R. M. & Howse, H. D. (1977). Some parasites and diseases of estuarine fishes in polluted habitats of Mississippi. *Ann. NY Acad. Sci.* **298**, 427–62.

Pierce, K. V., McCain, B. B. & Wellings, S. R. (1980). Histopathology of abnormal livers and other organs of starry flounder *Platichthys stellatus* (Pallas) from the estuary of the Duwamish River, Seattle, Washington, USA. *Fish Dis.* **3**, 81–91.

Rowe, D. W., Sprague, J. B. & Heming, J. A. (1983*a*). Sublethal effects of treated liquid effluent from a petroleum refinery. I. Chronic toxicity to flatfish. *Aquat. Toxicol.* **3**, 149–59.

Rowe, D. W., Sprague, J. B., Heming, T. A. & Brown, I. T. (1983*b*). Sublethal effects of treated liquid effluent from a petroleum refinery. II. Growth of rainbow trout. *Aquat. Toxicol.* **3**, 161–9.

Schultz, M. E. & Schultz, R. J. (1982). Induction of hepatic tumors with 7,12-dimethylbenz[*a*]anthracene in two species of viviparous fishes (genus *Poeciliopsis*). *Environ. Res.* **27**, 337–51.

Sindermann, C. J. (1979). Pollution-associated disease and abnormalities of fish and shellfish: a review. *Fish. Bull.* **76**, 717–49.

Sindermann, C. J., Bang, F. B., Christensen, N. O., Dethlefsen, V., Harshbarger, J. C., Mitchill, J. R. & Mulcahy, M. F. (1980). The role and value of pathobiology in pollution effects monitoring programs. *Rapp. P.-v. Reun. Cons. Int. Explor. Mer* **179**, 135–51.

Snieszko, S. F. (Ed.) (1970). *A symposium on diseases of fishes and shellfishes*. Special Publication No. 5, American Fisheries Society, Washington, DC, 526 pp.

Solangi, M. A. & Overstreet, R. M. (1982). Histopathological changes in two estuarine fishes, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. *J. Fish Dis.* **5**, 13–35.

ACKNOWLEDGEMENTS

The assistance of our colleagues must be acknowledged: Dr Eugene M. Burreson, Dennis A. Thoney and Maura E. Jansen in making collections and preparations; Jessica K. Proctor in preparing the sections; Dr Robert J. Huggett in arranging for chemical analyses; Kay B. Stubblefield and William W. Jenkins for artwork and photography; Daniel J. Fisher, John Hudak, Lynn Arnold and Charles J. Strobel for monitoring the laboratory experiments. To Alice Lee Tillage and Shirley O. Sterling for typing the numerous drafts that three authors combined with four other reviewers can generate, our sincere thanks. Their interest and patience were extremely valuable. Dr R. J. Huggett, Dr Herbert M. Austin and James E. Price, colleagues at the Institute, reviewed the manuscript and their comments assisted greatly; however, responsibility for the work and its quality remains with us. To all of these helpful persons we extend our grateful appreciation.

REFERENCES

Bieri, R. H., Hein, C., Huggett, R. J., Shou, P., Slone, H., Smith, C. & Su, C.-W. (1982). Toxic organic compounds in surface sediments from the Elizabeth and Patapsco Rivers and Estuaries. Final Report to EPA, Chesapeake Program.

Bieri, R. H., Hargis, W. J., Huggett, R. J., Roberts, M. H., Weeks, B. A. & Zwerner, D. E. (1983). Chemical and biological studies of an estuary highly contaminated by polynuclear aromatic hydrocarbons. Abstract of a paper presented at the Second International Symposium on Responses of Marine Organisms to Pollutants, Woods Hole, MA, *Aquat. Toxicol.* (in press).

Couch, J. A. (1975). Histopathological effects of pesticides and related chemicals on the livers of fishes. In: *The pathology of fishes* (Ribelin, W. E. & Migaki, A. (Eds)). University of Wisconsin Press, 559–84.

Couch, J. A., Winstead, J. T. & Goodman, L. R. (1977). Kepone-induced scoliosis and its histological consequences in fish. *Science* **197**, 585–7.

Haensly, W. E., Neff, J. M., Sharp, J. R., Marris, A. C., Bedgood, M. F. & Boem, P. D. (1982). Histopathology of *Pleuronectes platessa* L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the *Amoco Cadiz* crude oil spill. *J. Fish Dis.* 5, 365–91.

Lu, M. Z. (1982). Organic compound levels in a sediment core from the Elizabeth River of Virginia. Master's thesis, School of Marine Science, College of William and Mary, Gloucester Point, Virginia, 157 pp.

McCain, B. B., Hudgins, H. O., Gronlund, W. D., Hawkes, J. W., Brown, D. W., Myers, M. S. & Vandermuelen, J. H. (1978). Bioavailability of crude