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August 1988

Understanding the Estuary: Advances in Chesapeake Bay Research

Proceedings of a Conference March 29-31, 1988

> Chesapeake Bay Program



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Understanding the Estuary: Advances in Chesapeake Bay Research

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Some Histologic Gill Lessions of Several Estuarine Finfishes Related to Exposure to Contaminated Sediments: A Preliminary Report

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ABSTRACT

Collections were made during 1983, '84 and '85 in the Elizabeth River, whose sediments are heavily contaminated with PAHs, heavy metals and other anthropogenic materials. Comparison samples were from the "cleaner" Nansemond River, another subestuary feeding into Hampton Roads (the lower James River) nearby. Most samples from all stations included three transient quasi-catadromous nektonic sciaenids, Atlantic croaker (<u>Micropogonias undulatus</u>), spot (<u>Leiostomus xanthurus</u>) and weakfish (<u>Cynoscion regalis</u>), and two endemic estuarine benthic fishes, hogchoker (<u>Trinectes maculatus</u>) and oyster toadfish (<u>Opsanus tau</u>).

Processed gills of all species exhibited microscopic lesions of four general types or categories; Ballooning Dilatations (BD) or lamellar hyperemia; Hypertrophy (HPT) of filament and lamellar cells; Hyperplasia (HPL) of filament and lamellar cells; and, Growth Deformities (GD) of gill arches, filaments and lamellae. Various subtypes occurred within each category of lesion.

Occurring in individuals from both subestuaries, highest prevalences and/or severities, most often both, were in samples from the Elizabeth. Within the Elizabeth samples, occurrence and/or severity generally were higher (usually highest) in those individuals from ER Station 7, where sediments are most heavily contaminated by PAHs, and ER Upriver than at the ER Downriver station. Differences in occurrence and/or severity of lesions in the several species were also seen. Variations in exposure to the sedimentary toxicants and susceptibility to toxic insult are most probable causes.

INTRODUCTION

The Elizabeth River, a subestuary of the James River (Fig. 1), is heavily contaminated by industrial, domestic, urban, and agricultural wastes. Sediments of certain sites carry especially heavy burdens of

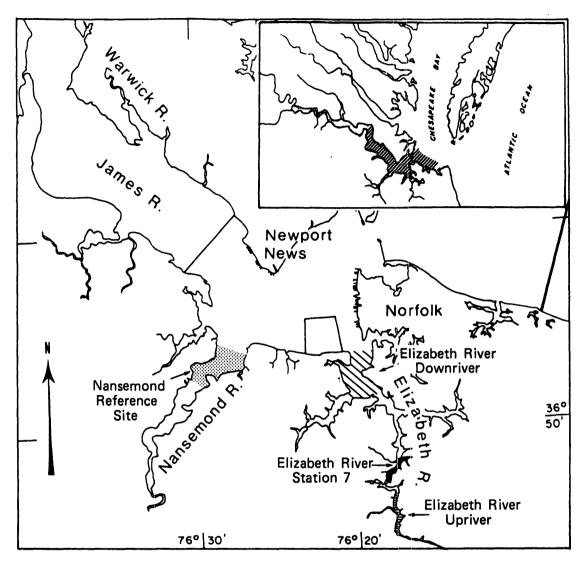


FIGURE 1 SAMPLING SITES ELIZABETH RIVER, CONTAMINATED NANSEMOND RIVER (REFERENCE) 'CLEANER' (Insert shows relative position of enlarged area.)

PAHs (up to 3900 ppm for example, Hargis et al. 1984). At others, especially near shipbuilding and ship repair yards, heavy metals abound. Numerous sewage and surface drainage outfalls make point source contributions, and non-point source effluvia enter all along.

Because of the extensive lockage and the freshwater drainage (or water supply) canal system of the Intracoastal Waterway at the upper, or southern, end of the Elizabeth River, fishes migrating in from higher salinity waters of the Chesapeake Bay and the adjacent Atlantic must enter at its mouth downriver at the northern end, (see Fig. 1). Therefore, oceanic or Bay migrants collected at ER Upriver had to have entered from Hampton Roads at the River's mouth and passed through ER Downriver and ER Sta. 7 and been exposed to the Elizabeth's waters, suspended or emulsified materials, and surficial sediments along the way.

To determine possible effects of contaminated sediments and sedimentexposed estuarine waters on health of feral fishes we have studied the gross (ulcerations, externally-visible cataracts and fin rot; Hargis and Colvocoresses 1986 and Huggett et al. 1987) and microscopic eye pathology (Hargis and Zwerner, 1988) of several marine/estuarine and estuarine-endemic finfishes collected from the Elizabeth and Nansemond Rivers. This preliminary report deals with the histopathology and features of the occurrence and severity of microscopic lesions in gill materials from some of those collections.

MATERIALS AND METHODS

From 1983 to 1985 standardized trawl samples from a series of upriverdownriver stations in the Elizabeth yielded over 70,000 individuals of 8 species. Comparison collections came from the nearby "cleaner" Nansemond. All were examined grossly. Five species, the hogchoker (Soleidae), the oyster toadfish (Batrachoididae) and the spot, Atlantic croaker and weakfish (all of the family Sciaenidae), were selected for histopathological examination because of their availability, presumed different susceptibilities to toxic damage and ecological similarities and differences.

Subsamples of individuals bearing externally-visible lesions (most from the Elizabeth River as it turned out) were selected where available. Animals were killed by severing the spinal column just behind the head, necropsied and organs fixed in Dietrich's AFA and 10% NBF. Lesionbearing individuals were chosen to determine if externally-visible lesions would be accompanied by internal ones and to increase the chances of finding internal lesions. Tissues collected routinely included areas of skin containing visible lesions, eyes, gills, livers, kidneys and intestines. Paraffin-embedded materials sectioned at 5-6 µm and stained in H&E were examined microscopically. Lesions were identified to subtype, enumerated, graded on a scale of increasing severity ranging from 1 to 5, recorded and representative microphotographs taken. Subtypes of lesions were grouped into categories based upon their basic affinities.

Occurrence data were based upon presence or absence of a particular lesion category in individuals. Mean severities presented were based

upon simple addition of the severity determinations for each subtype of lesion within each particular lesion category with the resultant sum divided by the number of individuals recorded as exhibiting that lesion category. In devising the rankings of percentage occurrence presented below differences of several tenths of a percentage point (<u>i.e.</u> = to or < 0.3%) were deemed insignificant and the data points involved to be essentially equal (<u>i.e.</u> \sim): For the mean severity rankings several hundredths (i.e. = to or < 0.03) was the criterion employed.

Microscopically-observed gill lesions were grouped into four primary categories; 1) <u>Ballooning Dilatation</u> (BD) (of lamellae), 2) hypertrophic responses (<u>Hypertrophy</u> - HPT), 3) hyperplastic growths (<u>Hyperplasia</u> - HPL), and 4) growth aberrations involving significant portions of filaments and/or arches, and lamellae collectively termed <u>Growth Deformities</u> (GD). All categories had several subtypes of lesions. Each subtype included some lesions which presented somewhat different appearances. Results on the occurrence and severity of the four types of general lesion categories are presented in Table I, Parts A and B, by species and by station respectively. Appearance of a normal gill is pictured in Figure 2.

RESULTS AND DISCUSSION

This report treats histologic lesions in the gill materials from five species taken in the 1983, '84 and '85 collections. Details are as follows:

Ballooning Dilatation (BD) (Figs. 3 and 8), or abnormal collection of blood in the lamellae (usually outside of the capillaries), called telangiectasia by some fish pathologists, occurred in 214/241 (88.8%) of all individuals and in all species. Ranked percentage occurrence of BD by species was; Hogchoker, 52.0% < Toadfish, 80.6% < Spot, 92.6% < Weakfish, 95.5% < Croaker, 98.3%: Ranked mean severity was; Toadfish, 1.40 < Hogchoker, 1.62 < Spot, 1.73 < Croaker, 1.98 < Weakfish, 2.17. Even with this ubiquitous lesion a larger proportion of each of the sciaenids had ballooning dilatations than the hogchoker and toadfish and were more severely affected.

Of the 214 cases of BD, 22, or 10.3%, were from Nansemond fishes; 192, or 89.7%, came from the Elizabeth River. Of the Nansemond fishes alone BD occurred in 22 of 27 individuals, or 81.5%; Elizabeth's were 192 of 214, or 89.7%. Ballooning Dilatation occurred at all stations and its percentage occurrence by station was closely grouped from 81.5% in the "reference" Nansemond to 90.6% at the ER Upriver site. The percentage occurrence ranking was Nansemond, 81.5% < ER Downriver, 89.0% < ER Sta. 7, 89.7% < ER Upriver, 90.6%. Ranked mean severity by station was; Nansemond, 1.41 < ER Downriver, 1.75 < ER Upriver 2.04 \sim ER Sta. 7, 2.05. Clearly BD, though found in fishes from all stations, occurred least in the "cleaner" Nansemond, and most in the more polluted Elizabeth: Likewise, severity was lowest in the Nansemond and highest in the Elizabeth. Within the Elizabeth, percentage occurrence and mean severity were lowest at ER Downriver and highest at the upstream stations, ER Sta. 7 and ER Upriver. Occurrences and severities at ER Sta. 7 and ER Upriver were quite close or equal, respectively.

TABLE 1

Occurrence and Severity of Gill Lesions by Species and by Station

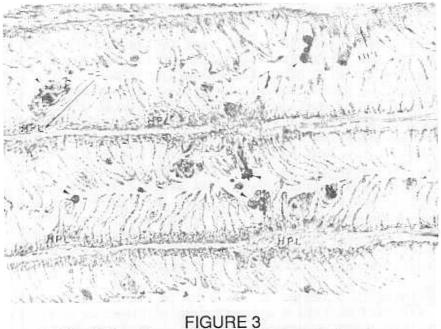
[Symbols: No. = Total Individuals; Occ. = No. with Lesions (Occurrence); % 0. = % Occurrence; X Sev. = Mean Severity; BD = Ballooning Dilatation, HPT = Hypertrophy, HPL = Hyperplasia, GD = Growth Deformities. Calculations of mean severity involve only affected animals, no negatives included.]

				BD		HPT			HPL			GD		
A.	SPECIES	No.	0	% 0.	X Sev.	Occ.	% 0.	X Sev.	Occ.	% 0.	X Sev.	Occ.	% 0.	X Sev.
	Weak.	67	64	95.5	2.17	36	53.7	1.79	50	74.6	3.33	14	20.9	1.07
	Croak.	59	58	98.3	1.98	28	47.5	1.95	45	76.3	2.70	10	17.0	1.05
	Spot	54	50	92.6	1.73	15	27.8	1.97	44	81.5	2.20	9	16.7	0.89
	Toadf.	36	29	80.6	1.40	20	55.6	1.65	16	44.4	1.54	26	72.2	1.98
	Hogch.	25	13	52.0	1.62	17	68.0	2.35	10	40.0	2.80	1	4.0	1.00*
	Totals	241	214	88.8		116	48.1		165	68.5		60	24.9	
			BD			HPT			HPL			Q)		
в.	STATION	No.	0.	20.	X Sev.	0cc.	%0.	Χ̄ Sev.	Occ.	% 0.	X Sev.	000.	% 0.	X Sev.
	Nanse.	27	22	81.5	1.41	9	33.3	1.12	19	70.4	2.38	1	3.7	1.00**
	ER Down.	82	73	89.0	1.75	36	43.9	1.79	60	73.2	2.26	17	20.7	1.87
	ER Sta. 7	68	61	89.7	2.05	39	57.4	1.91	41	60.3	3.51	24	35.3	1.42
	ER Up.	64	58	90.6	2.04	32	50.0	2.26	45	70.3	2.50	18	28.1	1.06
		241	214	88.8		116	48.1		165	68.5		60	24.9	

* 1 case only. ** Also 1 case only; further, GD noted involved lamellae only and not filaments.

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FIGURE 2 Weakfish, *C. regalis,* from Nansemond River showing appearance of "normal" filaments and lamellae. Dietrich's AFA fixative often causes lamellar delamination (Arrows)! Dietrich's, H&E, Ca. 100X



Weakfish, *C. regalis,* from ER Sta. 7 showing ballooning dilatation (**BD**) (Arrows). Hyperplasia (**HPL**) of the basal interlamellar type also present. Dietrich's, H&E, Ca. 100X

Hypertrophy (HPT) (Fig. 4), abnormally enlarged cells of soft tissues of the lamellae and filaments, occurred in 116/241 (48.1%) of all individuals and in all species. Ranked percentage <u>occurrence</u> of HPT by species was; Spot, 27.8% < Croaker, 47.5% < Weakfish, 53.7% < Toadfish, 55.6% < Hogchoker, 68.0%. Mean <u>severity</u> was; Toadfish, 1.65 < Weakfish, 1.79 < Croaker, 1.95 \sim Spot, 1.97 < Hogchoker, 2.35. Hypertrophy occurred more frequently in the hogchoker than in the toadfish and the sciaenids. Its severity was greatest in hogchoker as well. Percentage occurrence in toadfish was greater than in the spot and other sciaenids, while mean severity in toadfish was lower than in all other species.

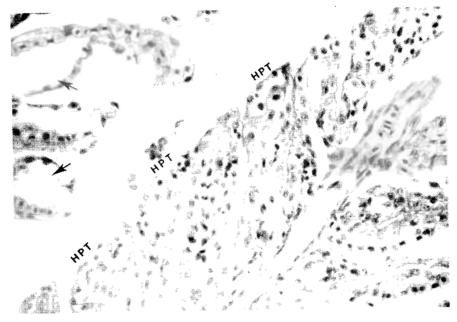
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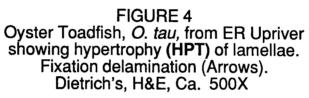
Of the 116 cases of HPT, 9, or 7.8%, were in Nansemond fishes while 107, or 92.2%, occurred in Elizabeth River fishes. Of the Nansemond River fishes alone, 9 of 27, or 33.3%, were affected, while 107 of 214, or 50.0%, of those from the Elizabeth had HPT. Hypertrophy occurred at all stations and its percentage occurrence by station was; Nansemond, 33.3% < ER Downriver, 43.9% < ER Upriver, 50.0%, < ER Sta. 7, 57.4%: Mean severity was; Nansemond, 1.12 < ER Downriver, 1.79 < ER Sta. 7, 1.91 < ER Upriver, 2.26. Though HPT occurred at all stations both occurrence and severity were less in the Nansemond and at ER Downriver than at the upstream stations, ER Sta. 7 and ER Upriver.

Hyperplasia (HPL) (Figs. 3, 5 and 6), abnormally large numbers of cells of soft tissues of the lamellae and filaments, occurred in 165/241 (68.5%) of individuals collected and in all species. Ranked percentage <u>occurrence</u> of HPL by species was; Hogchoker, 40.0% < Toadfish, 44.4% < Weakfish, 74.6% < Croaker, 76.3% < Spot, 81.5%: Mean <u>severity</u> was; Toadfish, 1.54 < Spot. 2.20 < Croaker, 2.70 < Hogchoker, 2.80 < Weakfish, 3.33.

Of all 165 HPL cases, 19, or 11.5%, were in Nansemond fishes, while 146, or 88.5%, came from the Elizabeth. Of the Nansemond River fishes alone HPL occurred in 19 of 27 individuals, or 70.4%: In the Elizabeth it was 146 of 214, or 68.2%. Hyperplastic lesions occurred at all stations and their percentage <u>occurrence</u> by station was; ER Sta. 7, 60.3% \leq ER Upriver, 70.3% \sim Nansemond, 70.4% \leq ER Downriver 73.2%. ER Upriver and Nansemond were essentially equal. That its occurrence was lowest at heavily contaminated ER Sta. 7 instead of highest seems anomalous. Mean <u>severity</u> by station was; ER Downriver, 2.26 \leq Nansemond, 2.38 \leq ER Upriver, 2.50 \leq ER Sta. 7, 3.51. Severity indices of ER Downriver and Nansemond were close: ER Upriver was somewhat higher than Nansemond and ER Downriver, while ER Sta. 7 was considerably higher. Thus, the anomaly of a lower percentage occurrence at heavily-contaminated ER Sta. 7 was balanced somewhat by the far greater severity of HPL at that station.

<u>Growth</u> <u>Deformity</u> (GD) (Figs. 7, 8 and 9), which can appear as branched lamellae, deformed or forked filaments or deformed arches, with one or more in the same gill, occurred in 60/241 (24.9%) of all individuals collected and in all species. In some cases of deformed arches filament forking occurred very close to the arches, themselves. A majority of gill filaments affected by GD were well-formed otherwise and appeared functional. Percentage <u>occurrence</u> by species was; Hogchoker, 4.0% < Spot, 16.7% \sim Croaker, 17.0% < Weakfish, 20.9% <





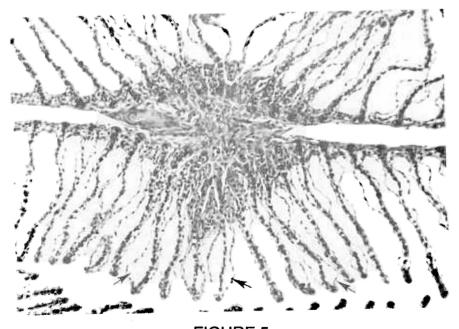


FIGURE 5 Weakfish, *C. regalis*, from ER Upriver showing the peculiar "medusa-shaped" interlamellar hyperplastic (HPL) lesion frequently seen in this species. Fixation delamination (Arrows). Dietrich's, H&E, Ca. 300X

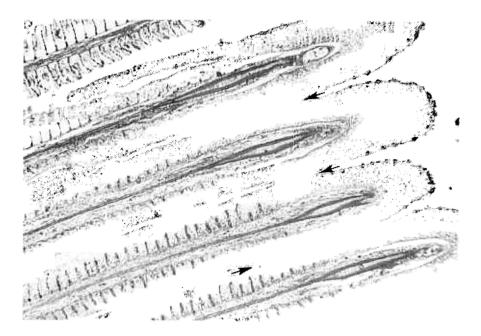


FIGURE 6 Croaker, *M. undulatus*, showing hyperplasia (HPL) with spongiosis. Note marked overgrowth of lamellae and filaments and interlamellar hyperplastic growth (fusion). See also "bridges" between filaments in lesion (Arrows). Dietrich's, H&E, Ca. 225X

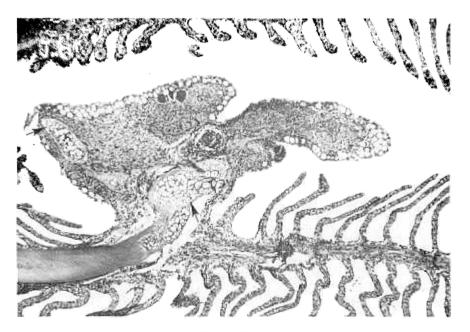


FIGURE 7 Toadfish, *O. tau,* from ER Sta. 7 showing gnarled growths on a filament exhibiting growth deformity (GD). Note cartilaginous deformities (Arrows). Dietrich's, H&E, Ca. 100X

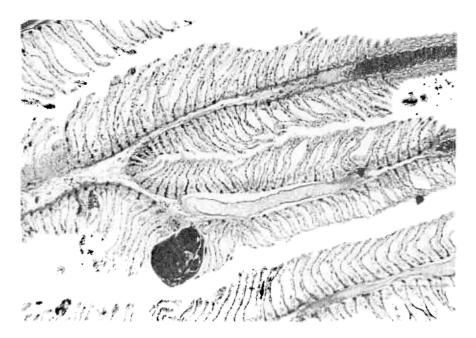


FIGURE 8 Weakfish, *C. regalis,* showing the growth deformity **(GD)** forked or branched filament. A ballooning dilatation (BD) also appears. Dietrich's, H&E, Ca. 125X

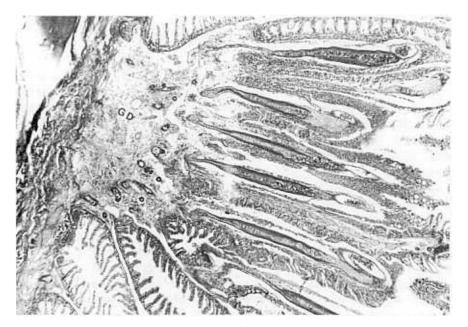


FIGURE 9 Croaker, *M. undulatus*, from ER Upriver showing growth deformity **(GD)** at bases of several adjacent filaments. Dietrich's, H&E, Ca. 100X Toadfish, 72.2%: Mean <u>severity</u> was; Spot, 0.89 < Croaker, 1.05 \sim Weakfish, 1.07 < Toadfish, 1.98. (Hogchoker is not included in the mean severity ranking because only a single individual was involved and no average was possible). Toadfish had larger numbers and more severe GD than all other species. Within the sciaenids, weakfish had more and more severe GD lesions than the other two species. Croaker GD, though essentially equal in occurrence to that in spot, was somewhat greater in severity. All sciaenids were fairly close in occurrence and severity of this lesion.

Of the 60 cases of GD the Nansemond had 1, or 1.7%, while the Elizabeth had 59, or 28.3%. Of the Nansemond River fishes alone only 1 of 27, or 3.7%, had GD, while 59 of 214 of the Elizabeth River fishes, or 27.6%, were affected. Percentage occurrence of GD by station was; Nansemond, 3.7% < ER Downriver, 20.7% < ER Upriver, 28.1% < ER Sta. 7, 35.3%. Mean severity by station was; ER Upriver, 1.06 < ER Sta. 7, 1.42 < ERDownriver, 1.87. [As indicated, only 1 fish in the Nansemond was affected by a subtype of this lesion category. It was rated 1.0 in severity. Because 1) it was the only Nansemond individual with GD and 2) only the "soft" lamellae, as opposed to the cartilaginous or bony elements of the filaments and arches of most other cases, were involved it is accorded little weight in these comparisons.] Essentially, Nansemond exhibited no GD lesions which were comparable to those in most Elizabeth fishes. Within the Elizabeth, occurrence was lowest at ER Downriver and highest at ER Sta. 7. That mean severity was higher at ER Downriver (1.87) than at ER Sta. 7 (1.42) and ER Upriver (1.06) was due to the fact that a single affected individual at ER Downriver showed a uniquely high mean severity datum of 8.0 making the calculated mean very high in comparison with the mean severities of GD observed at other stations.

DISCUSSION AND CONCLUSIONS

1. Microscopic studies of gills from 241 individuals of 5 species of fishes revealed lesions of 4 categories; Ballooning Dilatation, Hypertrophy, Hyperplasia and Growth Deformities. All species were affected. The first three histologic gill lesions (BD, HPT and HPL) occurred in both subestuaries, the highly-contaminated Elizabeth River and the "cleaner" Nansemond. The single GD seen in the Nansemond River involved the soft lamellae alone while most others of this lesion category involved the bony or cartilagenous support elements of filaments and/or arches. Consequently, the Nansemond fishes could be considered essentially GD-free. There were differences in occurrence and severity of all four lesions (BD, HPT, HPL and GD) between rivers, stations of the Elizabeth and species also, apparently reflecting the geographical variations in levels of chronic toxicity of sediment-borne contaminants. These differences also reflect variations in time and nature of exposure to the toxicants and susceptibility of the fishes to toxic insult by biologically active contaminants. Ballooning dilatation (BD), the most ubiquitous lesion probably was brought about by handling in the most severe cases, as well as by toxic stress. HPT, HPL and GD are all clearly related to chronic toxicity and, perhaps, other long-term environmental stressors and not to handling.

- 2. Though exceptions (explainable) occurred, several general patterns emerged:
 - Ballooning dilatations (BD) were the most numerous of the four lesions: Growth Deformities (GD) were the least. The ranked sequence of mean occurrence of lesion categories was GD, 24.9%
 < HPT 48.1% < HPL, 68.5% < BD, 88.8% (Table I, Part A).
 - b. A lower percentage of lesions of all types occurred in the Nansemond than the Elizabeth (Table I, Part B). Severity was lower in the Nansemond River fishes as well. Undoubtedly, this reflects the more polluted condition of the sediments and waters of the Elizabeth and, probably, the greater toxicity of the contaminants involved as well. At ER Sta. 7 PAHs related to creosote and other petroleum hydrocarbons predominate and are most likely candidates as primary contributors to occurrence and severities of the microscopic gill lesions seen in our collections, especially those taken at the two upstream stations, ER Sta. 7 and ER Upriver.

Within the Elizabeth there were fewer and less severe gill lesions at the ER Downriver station, with the single exception of GD. Usually, lesions were highest at the upstream locations, ER Sta. 7 where sediments are most heavily contaminated by PAH's, among other pollutants, or ER Upriver. Between ER Sta. 7 and ER Upriver occurrence and severity of BD were essentially equal: Occurrence and severity of GD was greater at ER Sta. 7 than at E.R. Upriver. With HPT and HPL the relative position of one or the other of these lesions at the same two upstream stations was reversed. Significance of these last findings is not clear at this point.

Basically the patterns of general occurrence of microscopic lesions of gills agreed with those of earlier studies which employed the occurrence of gross lesions as a basis for analysis of the possible effects of contaminated sediments on the health of finfishes in the Elizabeth River, between stations in the Elizabeth and between the Elizabeth and Nansemond Rivers (Hargis and Colvocoresses 1986 and Huggett et al. 1987). Gross lesions were virtually non-existent in the Nansemond samples. Within the Elizabeth, where the largest number of both gross and microscopic gill lesions occurred, fewer were downstream than upstream generally. Both were usually more prevalent (occurrence) or severe at ER. Sta. 7 and/or ER Upriver, the upstream stations.

c. Of the 5 species of finfishes studied, the most "resistant" to toxic effects from contaminants in the Elizabeth (and Nansemond) in terms of occurrence and/or severity of microscopic lesions of the gills were the more-or-less endemic bottom-dwelling hogchoker and toadfish, usually in that order (Table I, Part A). Generally the least resistant were the more pelagic, ocean-spawning, migratory sciaenids. One exception was Hypertrophy (HPT), with the hogchoker highest in occurrence at 68.0% and the toadfish second at 55.6%. Comparative mean

severity of HPT was also highest in the hogchoker (2.35) but lowest in the toadfish (1.65). HPT may require close and frequent contact with the source of toxicants, the sediments, for its initiation and/or continuance. This condition would be met by hogchokers and toadfish since they live nearest the bottom and are in closest and most frequent contact with the sediments and with those waters affected most by sedimentassociated toxicants. The soleid flatfish, hogchoker, usually is found on or in the bottom sediments. That HPL was more severe though not more prevalent in hogchoker than in all other species except the weakfish is probably related to the longterm and regular close contact with bottom sediments and bottom water of this benthic fish.

The other exception was Growth Deformity (GD) in which toadfish was highest by far in both percentage occurrence (72.2%) and mean severity (1.98). Since initiation of most gill GD must occur early in the development of the organ systems involved and toadfish are the single species of those studied here whose eggs, larvae and, probably, early juveniles develop closest to the bottom, it is not surprising they were most severely affected. Indeed, it would have been surprising had they not been.

d. Of the three sciaenids, weakfish generally were highest in occurrence and/or severity of lesions, while spot were least. Where reversals in either were observed (Table 1, Part A) as in HPT, spot and croaker (and in that order) were more affected than weakfish. This probably reflects their more intimate contact with the source of the toxicant, the contaminated sediments, during feeding. The greater occurrence of HPT in these two species agrees with the likelihood that a closer association with contaminated sediments is involved with this lesion as discussed above when considering its occurrence and severity in the bottom-dwelling hogchoker and toadfish.

Generally, our histopathological findings confirm shipboard and laboratory experiences that hogchoker and toadfish are most resistant to trauma or ecological insult, with spot, croaker and weakfish increasingly susceptible and in that order. Weakfish, the predator operating at the highest trophic levels of the three sciaenids, seems generally the most susceptible of all five species studied to toxic stress in relation to likelihood of direct exposure to the contaminated sediments and closely-associated benthic waters.

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