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Liver Lesions in Winter Flounder (*Pseudopleuronectes americanus*) from Jamaica Bay, New York: Indications of Environmental Degradation

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ABSTRACT: Liver sections of winter flounder (*Pseudopleuronectes americanus*) collected from Jamaica Bay and Shinnecock Bay, New York, in 1989, were examined microscopically to determine the pervasiveness of liver lesions observed previously in Jamaica Bay winter flounder. Neoplastic lesions were not detected in fish from Jamaica Bay or the Shinnecock Bay reference site. Twenty-two percent of Jamaica Bay winter flounder examined (n = 103) had unusual vacuolization of hepatocytes and biliary pre-ductal and ductal cells (referred to hereafter as the vacuolated cell lesion). The lesion, identical to that found in 25% of Jamaica Bay winter flounder examined in 1988, has previously been identified in fishes taken from highly polluted regions of the Atlantic coast (e.g., Boston Harbor, Massachusetts, and Black Rock Harbor, Connecticut). Prevalence of the vacuolated cell lesion in winter flounder from Jamaica Bay was significantly greater ($p < 0.0001$) than in 102 specimens collected from Shinnecock Bay. Current scientific literature indicates vacuolated hepatocytes and cholangiocytes are chronically injured and that the extent of their deformity is consistent with the action of a hepatotoxicant. The high prevalence of vacuolated hepatocytes in Jamaica Bay winter flounder and absence of the lesion in flounder from reference sites strongly supports the hypothesis that this impairment is a manifestation of a toxic condition in at least some portions of Jamaica Bay.

Introduction

The Jamaica Bay/Breezy Point Unit of Gateway National Recreation Area, Brooklyn, New York, confronts the resource manager with a striking juxtaposition of biotic wealth and environmental degradation. The 52-km² coastal estuary's diverse fishery includes 81 species of finfish and is heavily utilized for recreational angling (Heatwole and West 1983; Riepe et al. 1989). Jamaica Bay's shallow open water and salt marshes, part of which are designated Jamaica Bay National Wildlife Refuge, provide full-time or seasonal habitat for about 300 species of birds and are frequented by Atlantic flyway ducks and geese during fall migration. The environmental quality of this urban park continues to be degraded by a diversity of point and nonpoint pollutant sources, including four sewage

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treatment plants with a cumulative discharge of 320 million gallons per day, combined sewer and storm water overflows, atmospheric deposition, runoff from urban and commercial development, an international airport, and three defunct solid waste landfills (Tanacredi 1987, 1990). Two of the solid waste landfills are recognized as having received illegally deposited hazardous waste.

A number of environmental assessments indicate elemental, polycyclic aromatic hydrocarbon (PAH), and polychlorinated biphenyl (PCB) contamination of Jamaica Bay sediments, water, and biota (Tanacredi 1987, 1990; Seidemann 1991). The relative degree of sediment contamination in coastal and estuarine waters of the United States is monitored via the National Oceanic and Atmospheric Administration's National Status and Trends Program. National Status and Trends Program samples from Jamaica Bay indicate that average concentrations, in parts per million (ppm) dry weight, of silver (3.6 ppm), arsenic (18.0 ppm), cadmium (1.4 ppm), copper (110 ppm), chromium (160 ppm), lead (130 ppm), mercury (1.5 ppm), tin (18 ppm), zinc (200 ppm), total PCBs (0.48 ppm), and total PAHs (4.5 ppm) are at the high end of the overall national distribution (National Oceanic and Atmospheric Administration 1991). The PAHs and PCBs are of particular interest because of their ability to produce neoplasia and other cellular disorders in populations of feral fish, particularly in species residing or feeding in close association with contaminated sediments (Meyers and Hendricks 1982; Baumann 1989; Harshbarger and Clark 1990; Myers et al. 1990).

To assess potential impacts to resident biota from contaminant inputs to Jamaica Bay, the National Park Service and United States Fish and Wildlife Service have conducted histopathological evaluations of Jamaica Bay winter flounder (*Pseudopleuronectes americanus*). Winter flounder are estuarine-dependent, bottom-dwelling fish that feed primarily on benthic macroinvertebrates. Winter flounder predominately reside in their spawning area for the first years of their lives; as they approach sexual maturity, they migrate out of the estuary to colder, offshore waters during summer months (Murchelano 1988; Buckley 1989). These factors make them an ideal sentinel in environmental pollution monitoring (Stich et al. 1976; Murchelano 1988; Harshbarger and Clark 1990). The winter flounder's recreational importance in Jamaica Bay and use in earlier studies of the bay also supported its use for this study.

Histopathology provides a sensitive indicator of sublethal stress induced by environmental contaminants (Hunn 1988; Hinton and Lauren 1990). A previous histopathological investigation of Jamaica Bay winter flounder revealed a 25% prevalence of the vacuolated cell lesion in livers of specimens collected in the vicinity of the Pennsylvania Avenue landfill (United States Fish and Wildlife Service 1989). Similar, if not identical, lesions have been identified in winter flounder from highly polluted northeastern coastal waters including Boston Harbor and New Bedford Harbor, Massachusetts, and Black Rock Harbor, Connecticut (Murchelano and Wolke 1985; Gardner et al. 1987; Sass and Murchelano 1988).

This study was designed to reassess prevalence of the vacuolated cell lesion in Jamaica Bay winter flounder, and to determine if the lesion was present in winter flounder from a geographically similar but less environmentally impacted site. Shinnecock Bay is a shallow estuary on the southern shore of eastern Long Island. It is surrounded by a much less developed watershed than Jamaica Bay and serves as the reference location for this assessment. Shinnecock Bay sediments collected by the United States Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) were analyzed for a suite of organic and elemental contaminants in 1990. Shinnecock Bay sediment concentrations of silver (<1.0 ppm), cadmium (0.92 ppm), chromium (74.0 ppm), copper (35.5 ppm), lead (61.1 ppm), mercury (0.27 ppm), tin (4.81 ppm), total PCBs (0.006 ppm), total PAHs (0.50 ppm), and total chlordane (0.002 ppm) are one to two orders of magnitude lower than concentrations in Jamaica Bay sediments (R. Lattimer personal communication).

Methods

Collection of Samples

Prior to collections, we determined that a sample of 100 fish would yield sufficient power to detect a disease prevalence of 3% or greater in winter flounder from either bay. In Jamaica Bay, 103 winter flounder were collected with an otter trawl from October 16 to November 6, 1989; their average length was 23.5 cm (range 19.0–35.1 cm) and average weight was 180 g (range 90–580 g). Jamaica Bay trawl locations are shown in Fig. 1. Winter flounder from Shinnecock Bay (n = 102) were collected by otter trawl (n = 55) and trap net (n = 47) from November 9 to December 6, 1989; their average length was 29.1 cm (range 20.1–44.1 cm) and average weight was 360 g (range 80–>1,000 g).

Age of Jamaica Bay winter flounder was determined visually by counting annuli on plastic impressions of scales removed from the caudal peduncle (Fields 1988). Average age of specimens collected from Jamaica Bay was 3 yr (range 2–5 yr).

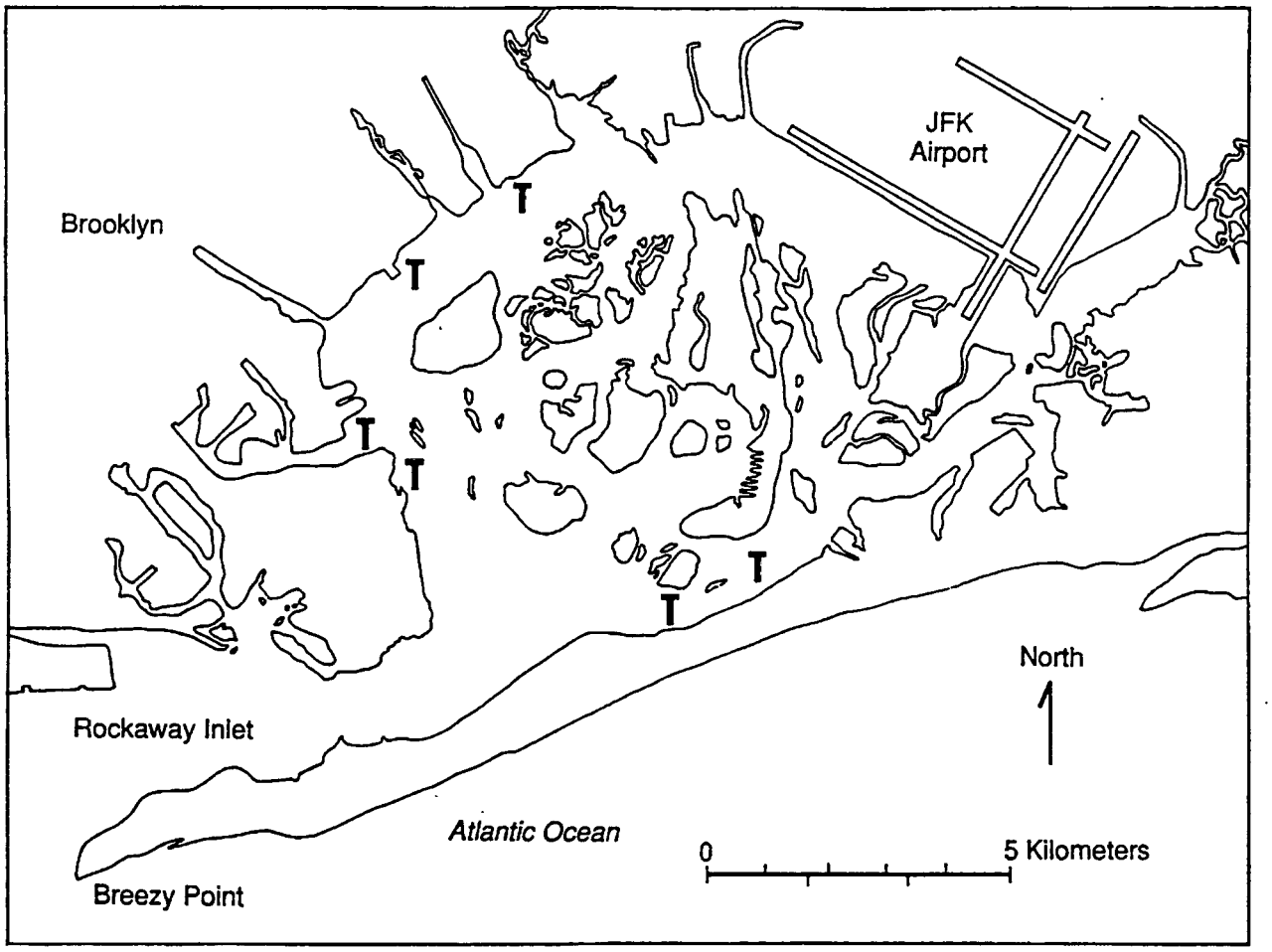

Fig. 1. Jamaica Bay study area and winter flounder trawling locations (T).

Age was not determined for fish collected from Shinnecock Bay. However, from the length and weight data reported above, it is assumed that Shinnecock Bay specimens are of comparable age or older than those collected from Jamaica Bay. Sex of all specimens was determined by visual examination of gonads. Sixty-two percent of the Jamaica Bay fish were female, 38% male. Fifty percent of Shinnecock Bay winter flounder were female, 50% male.

Histopathology

Fish were placed on ice in plastic coolers, and necropsies were performed within 24 h. Length, weight, and results of an internal and external examination were recorded on individual data sheets. The liver and several scales were removed and placed in 10% buffered formalin. Incisions were made in most large livers to ensure adequate fixation of tissues.

Tissues were delivered to the National Fish Health Research Laboratory in Kearneysville, West Virginia. Livers were large enough to permit portions to be removed from three different areas for embedding; this increased the probability of finding unevenly distributed lesions. Liver portions were embedded in paraffin, cut to four μm, and stained with hematoxylin and eosin. Special stains were prepared as necessary to aid in interpretation. For each specimen, the severity of vacuolated cell lesions was scored on a subjective scale of 1 to 4 (rare to extensive); other pathologies were noted only as present or absent. Necrotic foci and lymphocytic infiltrates were not reported for individual specimens because they occurred as very small lesions, were low in prevalence, and occurred in specimens from both bays.

Statistical Analysis

Fisher's Exact Test (Lehmann 1975) was used to compare the prevalence of liver lesions in winter flounder from Jamaica Bay and Shinnecock Bay.

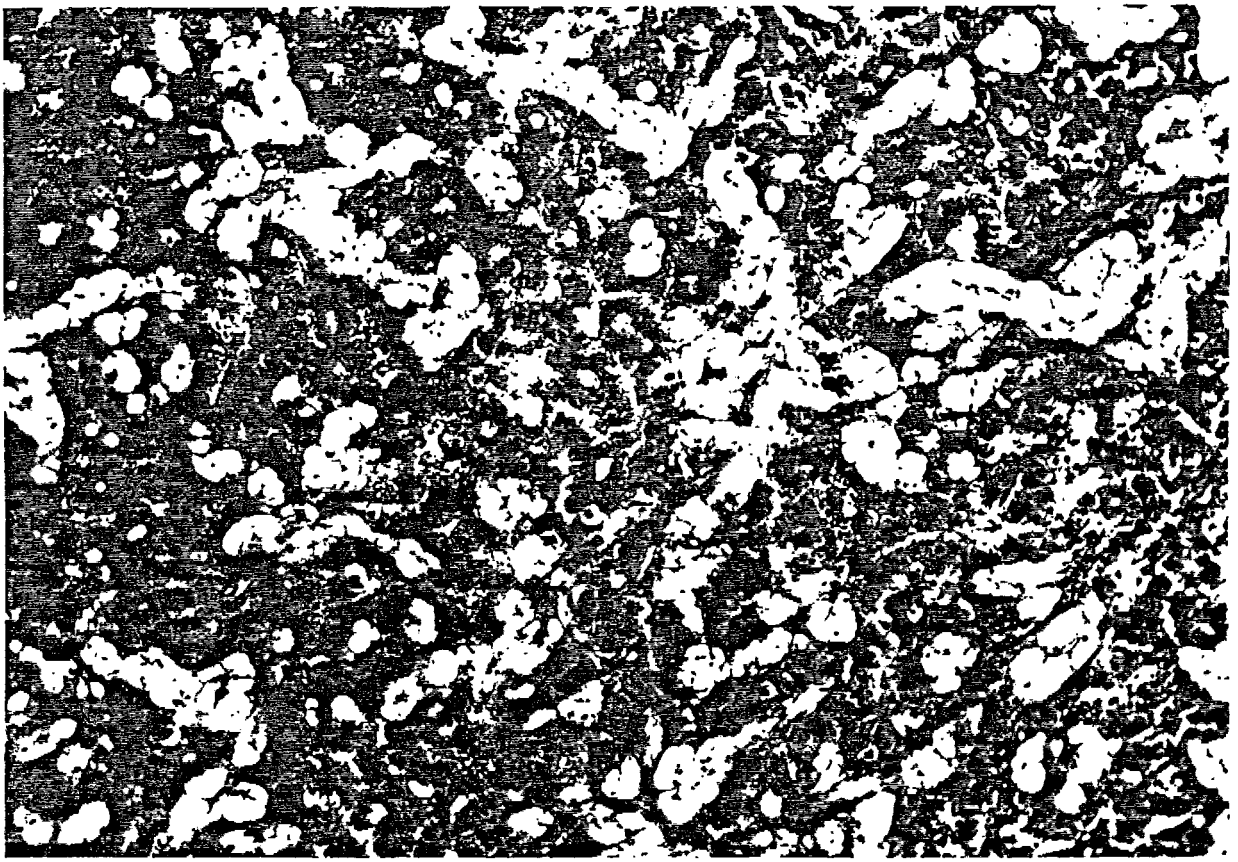

Fig. 2. Section of Jamaica Bay winter flounder liver with extensive vacuolated cell lesions (X 40).

Two-tailed tests were performed using SAS software (Statistical Analysis System 1985).

Logistic regression (Hosmer and Lemeshow 1989), also performed with SAS software, was used to test for the association between lesions and sex, weight, length, age, and liver weight in winter flounder from Jamaica Bay. This approach is appropriate because the dependent variables are categorical (i.e., lesion presence or absence, or the estimated severity of the lesion on a scale of 1 to 4) and the independent variables are either continuous (i.e., length, weight, and liver weight) or ordinal (i.e., age). A likelihood ratio test (Hogg and Craig 1978) was used to test the statistical significance of each of the explanatory variables from the regressions by testing if the logistic regression slope was equal to zero. Sample sizes was 103 for all regressions except age, where n = 102 (one sample was lost). Because eight of the fish were not sexed (not recorded), the sample sizes when sex was included in the analyses were n = 95 and n = 94, respectively.

To test for the effect of sex, a logistic "analysis of covariance" was performed. First, a full model was fit to the data that estimated separate slopes and intercepts for each sex and for each morphometric variable analyzed separately. Then a reduced model was fit, pooling over sexes, for each morphometric variable. A likelihood ratio chi-square test was constructed from the difference in the log likelihoods between the models for each morphometric variable. It tests for a difference between sexes in how the presence or severity of lesions relates to each of weight, length, age, and liver weight. If the test for sex effect is not significant, then it is appropriate to pool over sexes in subsequent analyses.

Results

Neoplastic lesions were not detected in winter flounder collected from either location. Twenty-two percent of Jamaica Bay winter flounder examined had abnormally vacuolated liver cells similar if not identical to those reported by Murchelano and Wolke (1985) in flounder sampled from Boston Harbor. These cells appeared in groups arranged in acinar or tubular patterns (Fig. 2). The cells were enlarged by clear vacuoles,

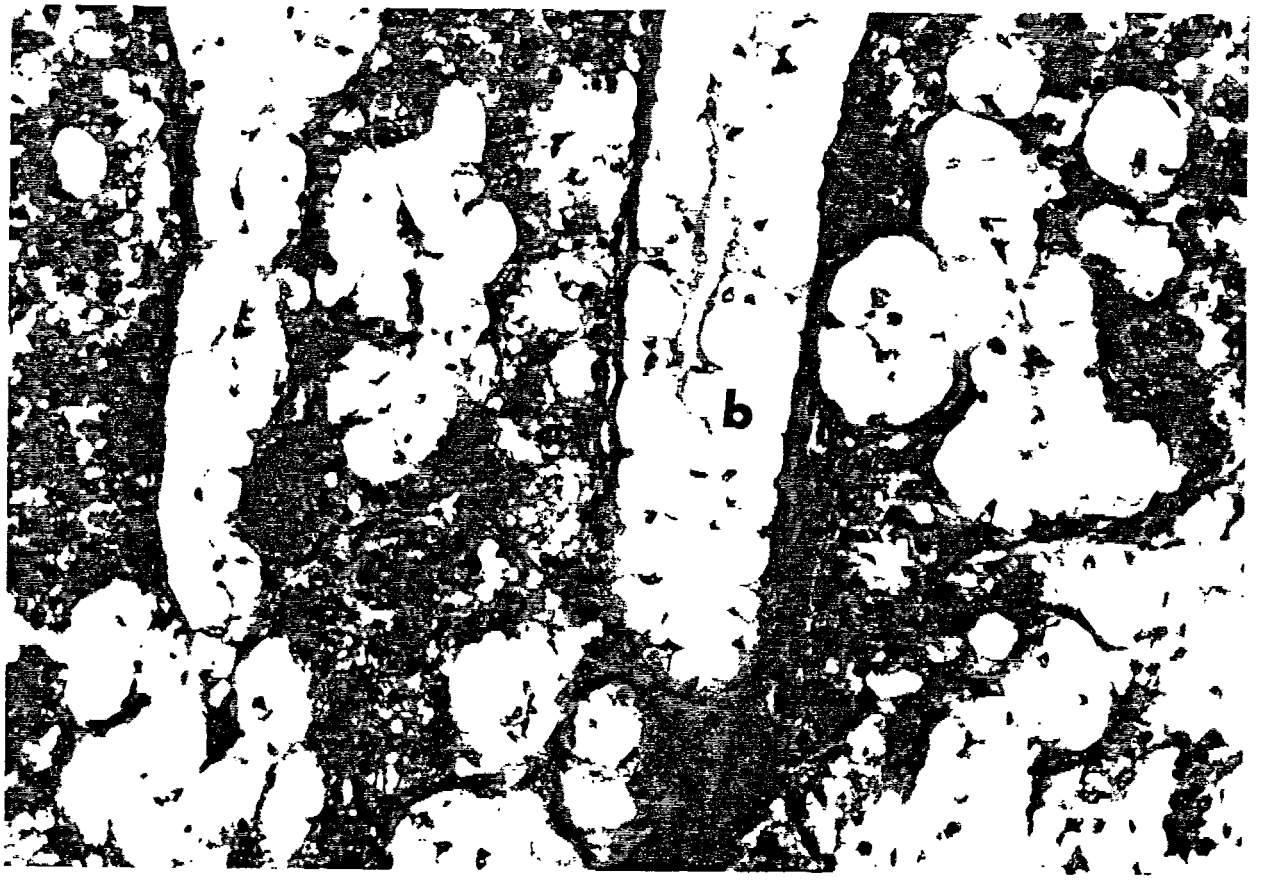

Fig. 3. Section of Jamaica Bay winter flounder liver showing vacuolated cell lesions involving bile ducts (b) (X 100).

which compressed the cytoplasm and nucleus to the cell margins (Fig. 3). Fibrous sheaths surrounded many of the vacuolated cell structures.

Twenty-one percent of Jamaica Bay winter flounder examined had what we designated as "foamy cell" lesions. These were focal lesions involving only a few cells. Cell outlines within the lesions were often undetectable, granular to stringy cytoplasmic material was variable, and nuclei, if present, tended to be pycnotic. Compression of surrounding cells was associated with the larger lesions (Fig. 4).

Only three fish, two female and one male, from Jamaica Bay had both vacuolated cells and "foamy cell" lesions. Neither type of lesion was seen in livers of winter flounder from the Shinnecock Bay reference site (Table 1).

Table 2 lists results of the logistic regressions used to test the statistical significance of morphometric variables versus the presence and severity of anomalies. Prevalence and severity of the vacuolated cell lesion in Jamaica Bay winter flounder were significantly ($p < 0.05$) correlated with fish age, length, weight, and liver weight. Because sex was never a significant factor ($p > 0.05$) in any of the logistic regressions, sexes were pooled for the logistic regressions in Table 2.

Discussion

Prevalence of the vacuolated cell lesion in winter flounder from Jamaica Bay was significantly greater than in specimens collected from Shinnecock Bay ($p < 0.0001$). Although neoplasms were not found in liver tissues from either location, the 22.3% prevalence of livers with vacuolated cell lesions in Jamaica Bay winter flounder is of concern because these lesions have been associated with neoplasia in other coastal ecosystems.

Vacuolated cells were first identified in winter flounder from Boston Harbor (Murchelano and Wolke 1985) and have been well described by Moore et al. (1989), Bodammer and Murchelano (1990), and Murchelano and Wolke (1991). Table 3 is a compilation from various sources documenting the prevalence of the vacuolated cell lesion in winter flounder from the northeast United States Atlantic coast. Of particular interest is the absence or low prevalence of the lesion in relatively uncon-

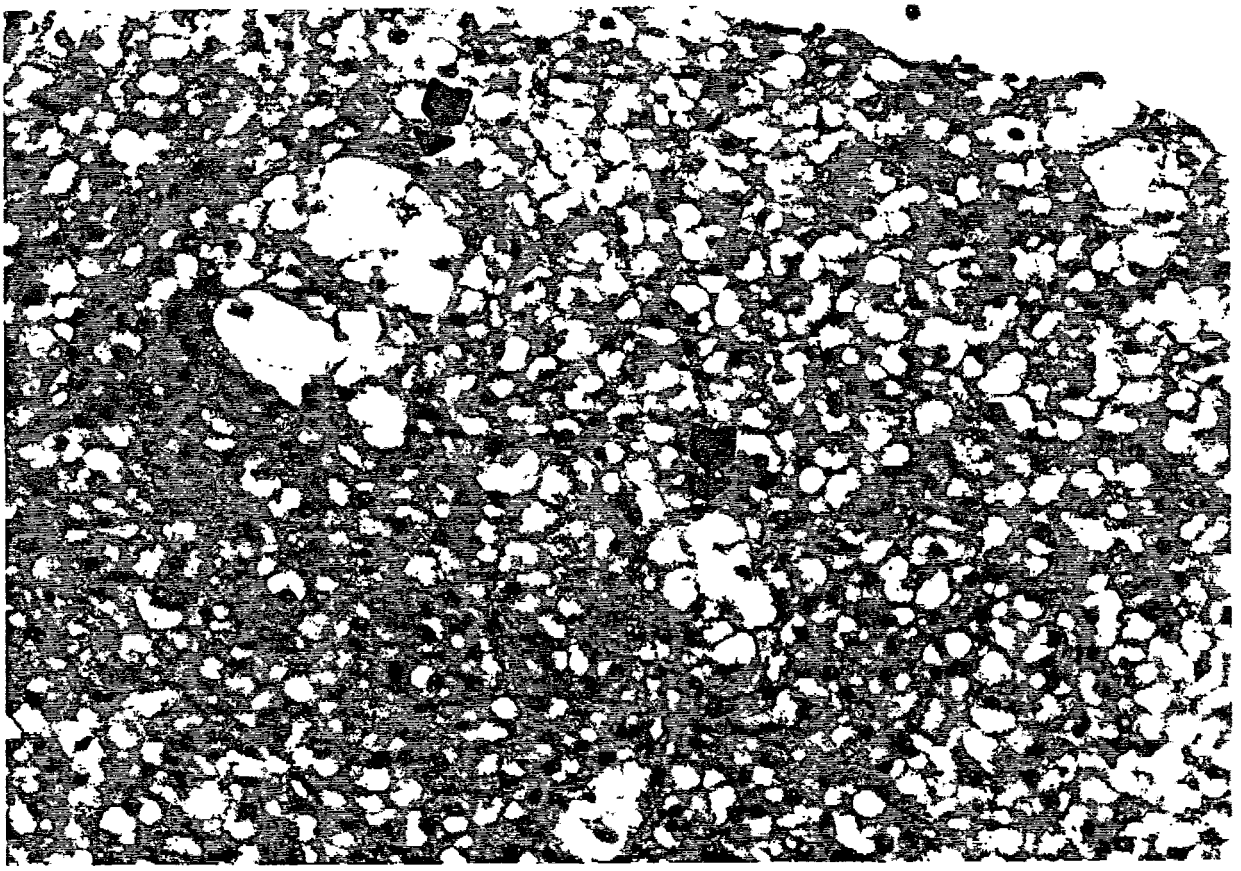

Fig. 4. Section of Jamaica Bay winter flounder liver showing foamy foci, some with compression of adjacent cells (X 100).

taminated areas and the relation between the lesion and hepatic neoplasia in highly contaminated areas. While there is uncertainty as to whether the lesion is part of a proliferative process (Moore et al. 1989; McMahon et al. 1990) or a form of modified apoptosis (Murchelano and Wolke 1991), there appears to be consensus on the following points: vacuolated hepatocytes and cholangiocytes are chronically injured; neoplastic lesions are frequently found in close proximity to vacuolated cells, and tumor prevalence is often associated with increasing prevalence of vacuolated cells; and the extent of deformity and cell injury in vacuolated cells is consistent with the action of a hepatotoxicant. Some evidence exists that supports the inference of a contaminant-induced etiology. Vacuolated cell lesions were induced in young-of-the-year and 1-yr-old winter flounder exposed in the laboratory to contaminated sediments from Black Rock

TABLE 1. Prevalence and statistical significance of anomalies detected in livers of winter flounder from Jamaica Bay (n = 103) and Shinnecock Bay (n = 102), New York.

Lesion or Abnormality	Location	Prevalence	Fisher's Exact Test
Vacuolated cells in the liver	Jamaica Bay	22.3%	F = 30.94 p < 0.0001
	Shinnecock Bay	0.0%	n = 205
Foamy cell focus	Jamaica Bay	21.3%	F = 29.35 p < 0.0001
	Shinnecock Bay	0.0%	n = 205
Nematodes present	Jamaica Bay	9.7%	F = 5.568 p = 0.0332
	Shinnecock Bay	1.8%	n = 205

TABLE 2. Results of the logistic regression analysis testing for the association of histopathological anomalies and various morphometric variables in Jamaica Bay winter flounder. Sexes were pooled because sex was not a significant factor in any of the regressions (p > 0.05).

	Vacuolate Cell Lesion (Presence/Absence)		Vacuolate Cell Lesion (Severity)[a]		Foamy Cell (Presence/Absence)	
Variable	Chi-sq	p-Value	Chi-sq	p-Value	Chi-sq	p-Value
Weight	5.32	0.021	4.70	0.030	0.04	0.836
Length	4.61	0.032	4.51	0.034	0.09	0.762
Age	5.09	0.024	5.83	0.016	1.32	0.251
Liver weight	8.99	0.003	6.34	0.012	0.12	0.726

[a] Severity ranked on a subjective scale of 0 to 4 (scarce to extensive).

TABLE 3. Prevalence of the vacuolated cell lesion (VCL) and neoplasms in liver of winter flounder from northeast United States Atlantic coastal waters.

Location (Year)	N[a]	Length[b]	VCL[c]	Neoplasia[c]	Reference
Jamaica Bay, New York (1989)	103	23.5	22.3	0	1
Jamaica Bay, New York (1988)	80	26.1	25.0	0	2
Shinnecock Bay, New York (1989)	102	29.1	0	0	1
New Bedford Harbor, Massachusetts (1985)	36	NR[d]	16.7	0	3
New Bedford Harbor, Massachusetts (1985–89)	100	30.4	>50.0	26	6
Massachusetts coastal waters (1985)	589	NR	0.7	0.3	3
Boston Harbor, Massachusetts (1984)	200	35.3	68.0	8.0	4
Boston Harbor, Massachusetts (1984–85)	325	36.5	74.0	15.0	5
Martha's Vineyard, Massachusetts (1985–89)	100	24.2	0	0	6
Narragansett Bay, Rhode Island (1985–89) (Fox Island)	100	26.5	>20.0	2.0	6
Narragansett Bay, Rhode Island (1985–89) (Gaspee Point)	100	30.9	>50.0	8	6
Black Rock Harbor, Connecticut (1985–86)	105	22.8	15	18	7

References: (1) present study; (2) United States Fish and Wildlife Service 1989; (3) Sass and Murchelano 1988; (4) Murchelano and Wolke 1985; (5) Murchelano and Wolke 1991; (6) Gardner et al. 1989; (7) Gardner et al. 1987.

[a] N = number of specimens in the referenced study.

[b] Average length (in centimeters) of specimens in the referenced study.

[c] Prevalence expressed as a percentage of N.

[d] NR = not reported.

Harbor, Connecticut (Gardner et al. 1991). Another study, by the National Marine Fisheries Service, identified statistically significant correlations between the prevalence of the vacuolated cell lesion in winter flounder and the concentration of PAHs and organochlorine pesticides in sediment (Johnson et al. 1992). The vacuolated cell lesion was also correlated with PAH metabolites in flounder bile and total PAHs and organochlorine pesticides in flounder stomach contents. Limited sampling from previous studies in Jamaica Bay has detected elevated concentrations of these compounds in various environmental media (Tanacredi 1987); however, no detailed analyses have been performed to determine PAH burdens in Jamaica Bay fish.

The vacuolated cell lesion was present in 15.5% of the 2-yr-old winter flounder in our sample. Because young winter flounder do not migrate and their exposure to toxicants is entirely within their spawning grounds, presence of liver anomalies in second-year winter flounder from Jamaica Bay is of concern for water and sediment quality at that location. The high prevalence of vacuolated hepatocytes in Jamaica Bay winter flounder, absence of the lesion in flounder from Shinnecock, and detection of the lesion in 2-yr-old Jamaica Bay winter flounder all support the hypothesis that this condition is a manifestation of a toxic condition in at least some portions of Jamaica Bay.

The absence of neoplastic lesions in Jamaica Bay winter flounder suggests a lower degree of liver impairment than that diagnosed in flounder from highly polluted harbors of the Northeast. This lesser degree of impairment could be a function of a lower degree of contamination or different types of contaminants than in areas reporting neoplastic lesions, or of the age structure of winter flounder in Jamaica Bay, where 80% of the population is less than 3 yr old.

In this study, 58% of the winter flounder collected from Jamaica Bay were less than 3 yr old, and 97% were less than 4 yr old (despite an intentional sampling bias to omit fish less than 19.0 cm total length from analyses). Prevalence and severity of the vacuolated cell lesion are not related to sex but are significantly ($p < 0.05$) related to age, weight, length, and liver weight (Table 2). These explanatory variables are highly correlated and are not giving independent information in the logistic regressions, but they do indicate that a population (actual or sample) where older and larger individuals are better represented would be expected to have a higher overall prevalence of the vacuolated cell lesion. If the lesion is a biomarker for the conditions leading to neoplasia, then a Jamaica Bay sample population of older or larger organisms may show the presence of liver tumors.

The necrotic "foamy cell" foci detected at a prevalence of 21.3% in the Jamaica Bay winter flounder are of unknown etiology. They suggest a more acute type of toxicosis than the vacuolated cell lesions, but determination of their true significance will require more information.

In summary, the vacuolated cell lesion in Jamaica Bay winter flounder was the major anomaly detected of known concern for fish health. Although of uncertain etiology, the available evidence indicates that vacuolated cells are chronically injured, toxicant-induced, and serve as biomarkers of the environmental conditions and physiological processes that may lead to neoplasia. The elevated prevalence and severity of the vacuolated cell lesion suggests impaired fish health and is another

reason for efforts to reduce pollutant loading to Jamaica Bay.

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