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**Liver pathology of winter
flounder:
Boston Harbor,
Massachusetts Bay,
and Cape Cod Bay-1991**

Massachusetts Water
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**LIVER PATHOLOGY OF WINTER FLOUNDER:
BOSTON HARBOR, MASSACHUSETTS BAY,
AND CAPE COD BAY - 1991**

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SUMMARY

Winter flounder have been shown to undergo a progressive liver disease whose extent is closely correlated with levels of exposure to chemical contaminants in the environment. This species is therefore an important sentinel for the biological effects of coastal contaminants on bottom-feeding vertebrates. This study was the fifth consecutive annual survey in which we have examined the pathology of these fish from Deer Island Flats, Boston Harbor. We have shown a continued prevalence of liver disease in winter flounder from this site. Questions have arisen as to the effect of time of year on sampling, of the prevalence of liver disease in winter flounder from the Future Outfall site, and of the possibility of using molecular methods to assess the biochemical impact of contaminants on these fish. To address these questions we collected winter flounder from five areas of the Massachusetts and Cape Cod Bays system. The prevalences of liver neoplasms, hydropic vacuolation and other liver lesions were determined by examination of multiple sections of liver tissue from 40 - 160 fish from each station.

- *Liver tumors and hydropic vacuolation were found in fish from Deer Island Flats and Broad Sound.*
- *Hydropic vacuolation was also present in fish from Nantasket Beach and from the Future Outfall site.*
- *In contrast, fish from Eastern Cape Cod Bay showed a very low prevalence of hydropic vacuolation.*
- *Lesion prevalences were found to increase with age of fish at the contaminated sites. Moreover, the above interstation differences were also statistically significant within single age classes.*

Eight to ten fish from each station were evaluated immunohistochemically for the incorporation of bromodeoxyuridine, a nucleotide analog, to assay for replicative DNA synthesis, a measure of exposure to chemicals that include epigenetic carcinogens (others have shown the concentrations of such carcinogens to be closely correlated with the prevalence of hydropic vacuolation).

- *DNA synthesis was elevated in fish showing cellular pathology.*

The same fish were also examined for expression of cytochrome P450IA, an integrative biochemical indicator of exposure to a range of compounds including some chlorinated and aromatic hydrocarbons.

- *Cytochrome P450IA expression was significantly elevated in fish from all stations examined, when compared to winter flounder from Georges Bank.*
- *We conclude that anthropogenic chemical waste is already having a significant biological effect on the benthic fauna of Nantasket Beach and the Future Outfall Site in addition to urban sites.*

To enable adequate monitoring of the Future Outfall it is important to establish whether our observations at the Future Outfall Site are a result of offshore dumping activity, or of transport from the existing shore-side outfalls. To allow adequate interpretation of future monitoring, this question should be answered before the offshore Boston Outfall is activated.

CONCLUSIONS

1. Winter flounder can be collected in adequate numbers from all the sites visited in this survey, including Boston Harbor, the Future Outfall site, and Eastern Cape Cod Bay.
2. Hydropic vacuolation in the liver of winter flounder was detectable at all the stations sampled. In contrast, liver neoplasia is a rare lesion, absent from all but the most contaminated sites. We have previously shown a close association between hydropic vacuolation and liver neoplasms in this species. Others have shown (Table 1) that hydropic vacuolation is closely correlated with a suite of chemical contaminants. At these latitudes this bottom-feeding species does not migrate substantially. Therefore, surveys for hydropic vacuolation prevalence are an appropriate long term monitor for the effects of benthic chemical contaminants on winter flounder at these latitudes. Hydropic vacuolation can be regarded as a harbinger of neoplastic risk, given adequate duration and level of exposure to carcinogens.
3. For a given site, the likelihood of encountering a significant prevalence of neoplasms, and related more common non-neoplastic contaminant-associated lesions is dependent on levels of chemical contamination, and age structure of the sample.
4. Comparison of age, length and lesion prevalences for three successive surveys of winter flounder livers from Deer Island showed a significant difference for fish caught in May compared to those caught in March or April.
5. Lesion prevalences are more completely investigated if three blocks of liver are examined per fish, as opposed to just one. However that improvement is in general statistically insignificant and probably does not warrant the increased effort. Furthermore, the improvement of information quality seen with two slides per liver block as opposed to one is not significant.

RECOMMENDATIONS

1. Gross and histological examination of winter flounder for liver disease prevalence should remain established as an essential tool in monitoring the level of chronic sublethal biological effect thought to result from exposure to sewage contaminants.
2. In future studies one slide from one liver slice from each of 50 fish per station should be examined histologically.
3. Future studies should concentrate on at least three stations: Deer Island Flats, the Future Outfall Site and a reference site in Eastern Cape Cod Bay.
4. Future studies should be tightly integrated with efforts to monitor levels of chemical contamination in benthic sediments and biota.
5. There is a seasonal trend of flounder size, age and lesion prevalence in the fish caught from Deer Island Flats. To maximize the sample size of comparable age classes, and avoid the effect of any seasonal variables, it is important to sample at the same time of year, for every year. Additionally, data analyses should always include age as a variable.
6. It may be advisable to define the peak period of winter flounder abundance at the Future Outfall Site to maximize survey efficiency. To do this an inshore dragger could be chartered to do a standard biweekly tow from March to June.
7. On the basis of currently available information, an annual survey cruise in the first week of April would seem appropriate.

INTRODUCTION

To monitor the biological effect of the chronic disposal of industrial and domestic wastes, it is important to examine a cumulative response that can potentially mirror the human health risks that the disposal area might present. For the Boston Harbor sewage outfall, the pathology of winter flounder liver is an appropriate indicator of such biological effects.

The literature on the biology of winter flounder is extensive, and has been reviewed by Klein-MacPhee (1978). A shallow-water coastal flatfish, this teleost feeds on small invertebrates, and prefers sand and mud substrates. Found from the Straits of Belle Isle to the Chesapeake Bay, a major factor affecting its movements is water temperature; 15°C being the upper optimum limit. South of Cape Cod this results in a summer migration to cooler deeper water. North of the Cape, with cooler water from the Labrador Current, the summer offshore migration is limited to 1-2 miles (Howe and Coates 1975). *Winter flounder is thus a better sentinel organism for investigating the effects of contaminants in a particular habitat at the latitude of Boston Harbor, than it is south of Cape Cod.*

Winter flounder in Boston Harbor grow to a total length of about 100 mm in the first year, to about 200 mm in the second year, and thereafter grow at variable rates. In the first two years males grow slower than females (Jay Barnett, unpubl. data). In the spring of the third year the fish become reproductively mature. Spawning occurs progressively later with increasing latitude, with the season being February to April in the Mystic River, CT. (Pearcy 1962), March in Boston Harbor (Adam Smith pers. comm., and pers. obs.) and April to June in Newfoundland (Kennedy and Steel 1971). Females ripen and spawn completely, whereas males remain in milt for an extended period through the spring (pers. obs.). Gravid females lay sticky demersal eggs on sand and mud. The eggs hatch after about three weeks, and remain as plankto-benthonic larvae until settling out as metamorphosed flatfish by two months of age (Sullivan 1915, Scott 1929, Williams 1975, Rogers 1976).

The presence of vacuolation and hepatic neoplasia in winter flounder from Boston Harbor was first reported by Murchelano and Wolke (1985); that report focused on some of the salient features of the end stage of the disease syndrome. More recently Gardner and Pruell (1989) presented findings suggestive of an association between neoplasm prevalence and contamination by polynuclear aromatic hydrocarbons. Additionally, Moore et al. (1989) described the earliest stage of vacuolation to be centrotubular, and Bodammer and Murchelano (1990) described the ultrastructure of vacuolated and aberrant hepatocytes from Boston Harbor winter flounder. The detailed morphology and postulated morphogenesis of this lesion in winter flounder has also been recently described (Moore 1991). In addition, the prevalence of hydropically vacuolated cells in winter flounder liver, and the

concentrations of chemical contaminants in bottom sediments, and winter flounder stomach contents, bile and liver tissue have been recently compared using data from the NOAA Status and Trends Program (Table 1). *Statistically significant associations were found between the prevalence of hydropically vacuolated cells and the following classes of contaminants measured in the above compartments: low and high molecular weight polynuclear aromatic hydrocarbons, chlordanes, dieldrin and total non-DDT pesticides.*

The influence of contaminant burdens in the progeny of winter flounder from Deer Island Flats were recently examined (NOAA 1990). Gravid fish from Deer Island Flats and a reference site in Long Island Sound were spawned in the laboratory. Resultant progeny from Deer Island showed reduced egg and larval size, reduced yolk sac volume, a mild increase in embryo mortality, and an increase in skeletal abnormalities. It was also shown that the concentrations of lipophilic contaminants, such as polychlorinated biphenyls, were high in larvae from Deer Island Fish, and presumably passed from adults to their progeny. In an experimental exposure of winter flounder eggs to DDT and dieldrin, Smith and Cole (1973) induced a high level of abnormal gastrulation and vertebral deformities. These and other chemical toxicities could have an incremental impact on growing flounder feeding in contaminated habitats.

This study represents a continuation and expansion of our annual surveys of the liver pathology of winter flounder from Deer Island Flats, Boston Harbor. In previous years, as described in our 1990 report to the Massachusetts Water Resources Authority (Moore and Stegeman 1990) and by Moore (1991), we found a prevalence of liver neoplasms in winter flounder of 5 to 10%. The prevalence of hydropic vacuolation was 50 to 60%. Hydropically vacuolated cells appeared to be a good indicator of the biological effect of contaminants that, at greater concentrations, and/or longer exposures, may also induce neoplasms in a minority of fish. Data analysis suggested two potentially important variables affecting the prevalence of liver disease, namely the year and the time of year that the fish were collected. To investigate this further, the *1991 field sampling was designed to collect a time series of samples from Deer Island Flats, and a single set of samples from a series of reference sites.*

The question of seasonal variation in disease prevalence was examined by sampling fish from Deer Island Flats on 3 occasions in 1991, one each in March, April and May. Additional sites were also sampled from Cape Cod Bay and Massachusetts Bay (Table 2 and Figure 1). In addition to routine histopathological analysis, a subset of fish from each station were processed to allow estimation of the induction in the liver of cytochrome P450IA protein, and of the rate of cell proliferation. Both of these assays are indices of exposure to different subsets of a range of chemical contaminants.

TABLE 1

Sediment, stomach, liver and bile contaminants whose concentrations correlate significantly (logistic regression, $p < 0.05$) with the prevalence of hydropic vacuolation in winter flounder liver. PAH = Polynuclear aromatic hydrocarbons, PCB = Polychlorinated biphenyls. ND = No data. ns = Not significant.

Percentages shown indicate the proportion of total variance in lesion prevalence accounted for by each risk factor, as assessed individually. Low and high molecular weight PAHs in bile are estimated by the levels of fluorescent aromatic compounds detected at the wavelengths of benzo(a)pyrene (high MW) and naphthalene (low MW).

Chemical class	Sediment	Stomach Contents	Liver	Bile
Low MW PAH	70%	53%	ND	24%
High MW PAH	63%	24%	ND	25%
PCBs	ns	ns	ns	ND
DDTs	ns	ns	23%	ND
Chlordanes	46%	78%	70%	ND
Dieldrin	6%	no data	33%	ND
Total non-DDT Pesticides	26%	69%	70%	ND

Data source: Pers. comm., L.L.Johnson and M.S.Myers, N.O.A.A./N.M.F.S.

METHODS

Stations and Sampling

Nine stations were sampled; 1 - Deer Island Flats, Boston Harbor; 2 - Nantasket Beach; 3 - Broad Sound; 4 - Future Outfall site; and 5, 6, 7, 8 and 9 - Eastern Cape Cod Bay (see Figure 1). In this report data from stations 5 to 9 were pooled to achieve comparable sample sizes. Heretofore, fish from those stations are referred to collectively as from station 5.

Bottom Trawling

All fish, except for those at the May 22 Station 1 sample were collected by commercial otter trawl: stations 1 to 4 were sampled using a commercial dragger, the F/V *Odessa*, from Gloucester, skipper William B. Crossen. The fishing gear comprised a western Atlantic trawl, with a 4 seam chain sweep, 53 foot head rope, 83 foot footrope, 10 fathom legs, 10 fathom ground cables, 400 pound bison steel doors, and 25 fathom of main wire for shallow stations with more for the deep stations. Trawling speed was approximately 2 knots. Stations 5 to 9 were sampled as part of the Massachusetts Division of Marine Fisheries Spring Bottom Fish Survey, using the R/V *Gloria Michelle* (NMFS/NOAA, Sandy Hook N.J.). Details of gear used are available elsewhere (Howe et al., in prep). The May 22nd sampling of station 1 was conducted aboard the R/V *Neritic*, UMass-Boston, using a skiff trawl with a 16 foot mouth. Trawl location and duration are given in Table 1. Fish were placed in through-flow sea water tanks prior to dissection.

Sample Numbers

Each fish was assigned a sample identification number, that was unique within our multi-year winter flounder archive and database. Datasheets and histological cassettes bearing this number were prepared in advance to ensure complete collection of both numerical information and samples.

Labelling of a Subset of Fish with Bromodeoxyuridine

To allow estimation of proliferation indices, 8 to 10 fish from each station were injected with a mixture of two thymidine analogues: 30 mg bromodeoxyuridine (BrdU) and 3 mg flourodeoxyuridine (FdU) per kg body weight. The fish were maintained alive in flowing ambient sea water aboard the collecting vessel for 3-5 hours, and then killed and processed as described below.

Dissection of Fish

Fish were killed by cervical section. Fish were placed blind side up and measured for total and standard length. Their external condition was noted, and the condition of their fins with respect to erosion was scored on a scale of 0 - 4. An oval incision was made in the ventral body wall overlying the liver and anterior ventral gonad. Gonads were either white and triangular in males, pink and elongated caudally in females, or small and blue-gray in immature fish. Livers were removed by severance of the peritoneal attachments, and then cut into 4 mm thick slices, in a series of transverse cuts. Each slice was examined for grossly visible abnormalities and graded for gross abnormality 0 - 2. Routine samples were taken from three equidistant sections through the liver. Liver sections were immediately placed into pre-labelled histological cassettes. Other area(s) of visibly abnormal liver were also sampled. Tissue samples were fixed in 10% neutral buffered formalin. Other viscera, gonads, heart and gills were inspected for gross lesions. This resulted in three blocks of liver tissue from each animal. An exception to this was the fish from Station 3, where only one block per animal was routinely examined.

Age Determination

A sample of scales was removed from the dorsum of the caudal peduncle of each animal. Scales were placed in scale envelopes, labelled, and subsequently submitted to Jay Burnett, NMFS, Woods Hole, MA for age estimation by counting growth rings.

Histological Processing

Fixed specimens were returned to the laboratory and embedded in paraffin, sectioned at 5 μ m and stained with hematoxylin and eosin: these standard methods are described by Luna (1968). Each embedded block of liver tissue was sectioned at two levels, with skip sections between. Liver samples destined for immunohistochemical study were embedded in paraffin within 24 hours of dissection.

Histological Analysis

For each fish there were, with the exception of Station 3 fish, 3 tissue blocks, each with 2 slides, i.e. 6 slides in all. After an initial survey of the material, the intensity of the following lesions, which have been described in detail elsewhere (Moore 1991) were recorded:

- 1) Hydropic Vacuolation, seen in three forms:
 - a) Centrotubular vacuolation - isolated groups of 1-2 vacuolated cells in the center of the hepatic tubule.

- b) Tubular vacuolation - linear arrays of vacuolated cells, filling the hepatic tubule, often extending into biliary duct structures.
 - c) Focal vacuolation - foci of thirty to several hundred contiguous vacuolated cells.
- 2) Macrophage aggregation - circular golden brown cellular masses, often associated with fibrotic tracts, bile ducts and blood vessels..
 - 3) Biliary duct proliferation - branching ducts, often ensheathed by fibrosis.
 - 4) Neoplasms- focal, often grossly visible areas of cells fulfilling established criteria for neoplasia in this species (Moore 1991).

Data were recorded in Excel (Microsoft Corp., Redmond WA).

Severity of each lesion was scored as follows: Histological slides were examined under bright field illumination, at 25 x, 100 x, and 200 x. using a Zeiss Axioskop. For each slide, each lesion was scored by examining the whole selection at 25 x, and at least 5 views at 100 x and 200 x. Each lesion was scored at 0 - 4, 0 = absent, 1 = minor, 2 = moderate, 3 = severe and 4 = extreme. For each fish, and each lesion type, a histopathological index was then calculated as a mean of scores from six slides.

To allow comparison of 1991 data with previous data, lesion prevalence was also calculated for each lesion from each site. These data were derived from the histopathological indices by taking each fish with a mean lesion score of 1 or more to be scored as having the lesion present. This was a valid approach, given the fact that the criteria used to define "minor" were the same as those used to define the low point of "present" in previous years. In making between-year and interstation comparisons only data from the first block examined from each fish were considered. This was due to the fact that in previous years, and in the 1991 Broad Sound station, only one block per fish was examined (the Broad Sound material was sampled and processed before the current contract appeared secure). In this way comparisons were not biased by the increased sampling effort present in the majority of the 1991 study. Lesion data were generated using data from both one and three blocks per fish to allow an assessment of the cost-effectiveness of examining multiple blocks as compared to single blocks per fish, and to allow a comparison of the usefulness of scoring lesion severity as opposed to simply scoring presence or absence.

Immunohistochemical processing

Unstained slides were stained to highlight nuclei that were undergoing active replicative DNA synthesis, using an anti-bromodeoxyuridine monoclonal antibody (RPN 20), followed by routine chromogenesis, and counterstained as described previously

(Moore 1991). Adjacent sections were also stained with a monoclonal antibody reactive only with the cytochrome P450IA isozyme that is induced by exposure to foreign compounds such as polynuclear aromatic hydrocarbons, and some chlorinated hydrocarbons. These slides were stained with the monoclonal antibody (1-12-3) followed by routine chromogenesis and counterstaining, as described previously (Stegeman et al. 1991). Additional fish collected from Georges Bank in 1990 were also stained with the 1-12-3 monoclonal antibody.

Analysis of immunohistochemical slides

Anti-BrdU stained slides were evaluated for the number of hepatocytes and vacuolated cells per unit area, and the number of hepatocyte, vacuolated cell, and non-hepatocyte (endothelial and biliary preductular) nuclei that stained per unit area. Hepatocyte and non-hepatocyte labelling indices were expressed as a fraction of positive nuclei per 1000 hepatocytes, and vacuolated cell labelling indices were expressed as number of positive vacuolated cell nuclei per 1000 vacuolated cells.

Anti-P450IA stained slides were evaluated for the stain density of epithelia in liver, intestine and kidney on a range of 0 - 4.

Analysis of data

The histopathological indices and prevalences of lesions were compared between classes of fish defined by differences in station, age, sex, and length. Many lesions were found together: thus were they not statistically independent. Centrotubular vacuolation was regarded as the most sensitive histological indicator of exposure to chemical contaminants, and was thus tested for significant differences between groups of fish by analysis of variance using Statview (Abacus Concepts, Berkeley CA).

Quality assurance and control

At each stage of the project, protocols were defined prior to the start of work. Data sheets, tissue cassettes, scale envelopes, databases, and other processing systems, were all prepared in advance to receive each sample or data unit. Omissions were obvious, in routine cross- and back-checking, and rectified. At each stage data quality was assured by back-checking with the source material or data file at the completion of the task. Sample numbers were not station specific. Thus data were generated from the entire study set before specific stations were associated with specific groups of fish, avoiding bias of interpretation on the basis of expected outcome.

RESULTS AND DISCUSSION

Fish collected

Stations sampled are listed in Table 2 and their positions illustrated in Figure 1. Surface temperature and bottom depth are also listed in Table 2. The first attempt to collect fish at Deer Island Flats on February 5th was unsuccessful, as the flounder were seemingly absent. However, a sample was obtained from Broad Sound, at the mouth of Lynn Harbor (Station 3).

On the 12th of March, 40 winter flounder were obtained from Deer Island Flats. This sample consisted of 4 tows, with a total bottom time of 234 minutes (Table 3). This is an extremely poor catch rate for this station at that time of March on the basis of previous years samples, and suggests the *1991 abundance of winter flounder on Deer Island Flats was low*.

Between April 9th and 13th, winter flounder were collected from Deer Island Flats, Nantasket Beach, and the Future Outfall site, 9.5 miles east of Boston Harbor. This latter site proved hard to trawl, and lacking in winter flounder. However, a series of extended tows provided the necessary sample of adult fish. *The area of bottom adjacent to the Future Outfall site that is suitable for bottom trawling is very confined and not without obstructions*. The extent of this area was defined in discussion with local draggersmen, and in consultation with M. Bothner, USGS, using sidescan sonar data. The two sources essentially recommended identical sampling areas. This area is known to inshore draggersmen as "Rosie's Hole", and is bounded by a quadrilateral area defined approximately by the following 4 loran lines: 44287, 13960, 44282, and 13955. This area has patches of hard bottom, boulders of all sizes, and at least 5 significant "hang-ups".

During the April sampling period, winter flounder in cleaner sites to the east of "Rosie's Hole" were apparently not available. For this reason, an appropriate reference collection was made on May 12th in Eastern Cape Cod Bay by pooling 5 tows made in the Massachusetts Division of Marine Fisheries Spring Bottom Survey (Stations 5 to 9), referred to in this report collectively as Station 5

The data in Table 2 were analyzed for the catch per unit effort, for stations sampled by the *Odessa*, using the same trawl technique. The catch rate of flounder per minute of bottom time is given in Table 4.

The poor rate of catch for the Outfall Site suggests that it may be necessary in future years to establish the optimum time of year to maximize sampling efficiency. Other fish species that were available included windowpane flounder, little skate, and sand dab, but

FIGURE 1

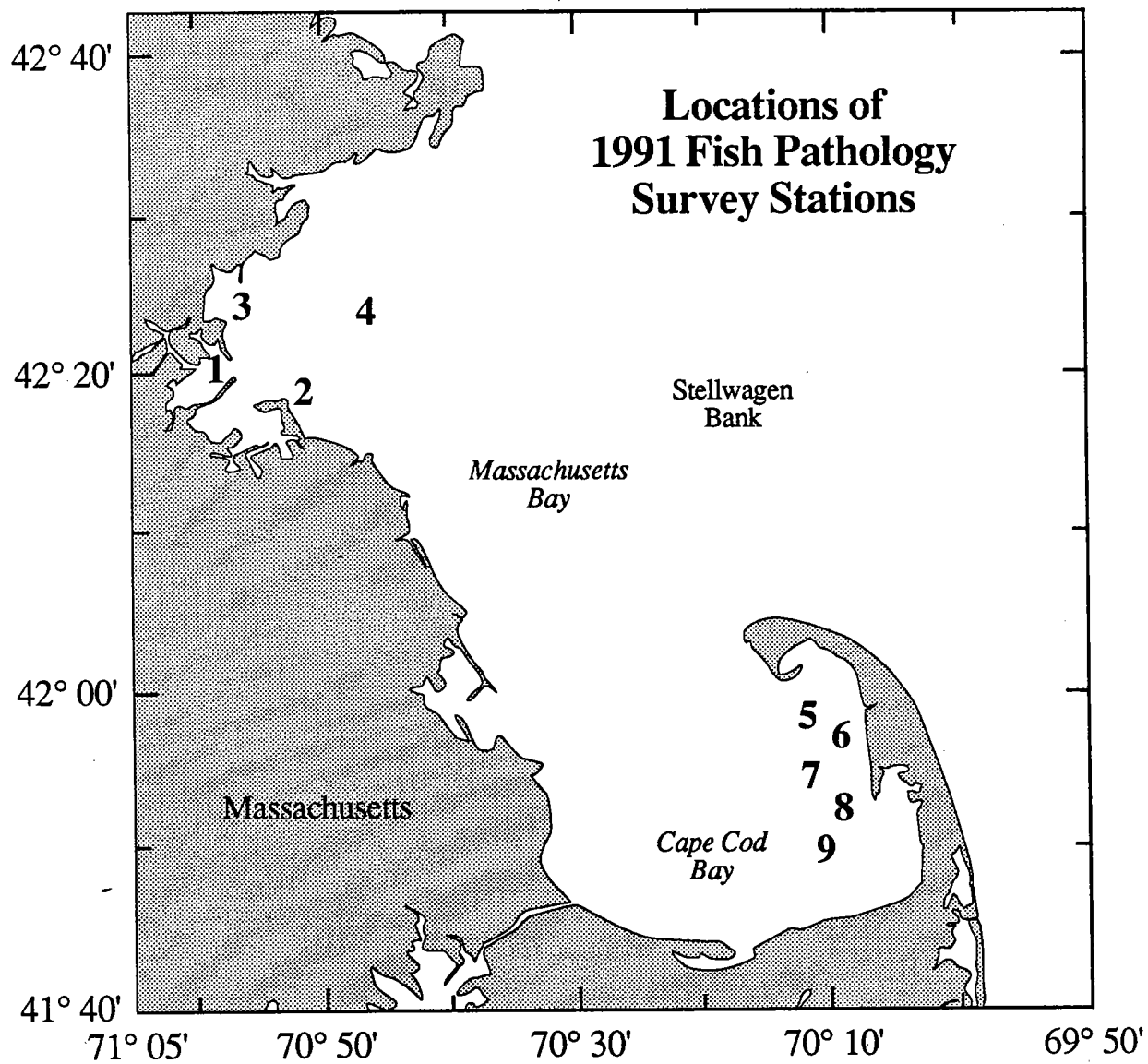


TABLE 2 - SUMMARY OF TRAWLS MADE IN 1991 WINTER FLOUNDER LIVER PATHOLOGY SURVEY

Date	Station ¹	Trawl #	Start Position ²	Stop Position	Bottom time	Surface Temp. ³	Depth ⁴
02 05 91	3	1	13995.9/44304.9	14000.1/44306.4	25		25
		2	13995.4/44303.9	13998.2/44304.0	20		25
03 12 91	1	1	14025.5/25848.7	14026.1/25850.1	25		23
		2	14025.2/25848.7	14026.2/25845.0	42		21
		3	14026.1/25849.1	14025.7/25849.0	37		21
		4	14025.9/25848.5	14025.2/25847.2	54		22
04 08 91	1	1	14025.7/25849.4	14026.1/25850.0	56	8.5	22
		2	14025.3/25848.3	14024.3/25845.6	30	9.0	24
		3	14026.2/25848.9	14024.9/25846.8	47	9.5	22
		4	14026.8/25849.0	14025.4/25845.8	32	10.4	25
	2	1	14001.6/44256.4	13996.4/44254.4	45	9.7	18
		2	13996.4/44254.0	12997.2/44255.3	60	9.0	32
	4	1	13957.0/44282.6	13955.8/44286.6	20	7.9	108
		2	13956.2/44285.0	13954.4/44283.2	110	7.8	108
04 09 91	4	1	13958.9/44284.7	13956.0/44279.2	80	8.1	108
04 13 91	4	1	13958.3/44285.2	13960.5/44281.3	120	8.1	106
		2	13958.9/44282.2	13960.2/44286.4	120	6.9	112
		3	13960.7/44286.4	13960.1/44283.9	65	7.0	114
		4	13959.9/44283.2	13958.4/44285.4	77	7.0	115
05 12 91	5 (37) ⁵	1	13818.7/44074.3	13822.5/44070.2	20	11.2	88
	6 (38)	1	13807.5/44071.4	13811.1/44067.9	17	12.0	46
	7 (39)	1	13851.3/44048.2	13843.7/44051.5	20	10.9	79
	8 (40)	1	13850.6/44039.2	13842.6/44041.5	13	12.2	43
	9 (41)	1	13863.3/44026.3	13856.0/44029.9	20	11.8	26
05 22 91	1	1	42 20.79/70 58.62	42 20.75/70 58.29	25	12.8	23
		2	42 20.75/70 58.2	42 20.80/70 58.56	20	13.0	21
		3	42 20.80/70 58.76	42 20.76/70 59.03	25	13.6	20
		4	42 20.76/70 59.10	42 20.67/70 58.92	26	14.0	23
		5	42 20.72/70 59.01	42 20.69/70 58.96	20	13.5	18

¹See Fig. 1. ²Loran coordinates, except for 05 22 91 trawls, which are in latitude and longitude. Many of the trawls were circuitous, especially at stations 1,2, and 4. ³Degrees celsius. ⁴Feet. ⁵Numbers in parentheses refer to station numbers in Mass. Div. Mar. Fish. Spring '91 bottom trawl survey, the source of the fish caught on 05 12 91.

TABLE 3 - SUMMARY OF ADULT WINTER FLOUNDER COLLECTED, AND OTHER SPECIES AVAILABLE IN 1991 WINTER FLOUNDER LIVER PATHOLOGY SURVEY.

DATE	STATION	TOTAL BOTTOM TIME (minutes)	# WINTER FLOUNDER	% GROSS LESIONS	OTHER SPECIES PRESENT ¹
02 05 91	3	45	57	23	wp, ls, lf
03 12 91	1	158	39	21	wp, ls
04 08 91	1	165	62	8	ls, sd,
04 08 91	2	105	50	4	ls, sd,
04 08 91	4	210	11	2	ls, sd
04 13 91	4	382	51	4	yt, sd, ls, wf, sr, op
05 12 91	5,6,7,8,9 ²	90	60	0	he, aw, sh, op, ls, tt, wp, sm, po, sr,
05 22 91	1	116	63	3	ls, wp

¹wp = windowpane flounder, ls = little skate, sd = sand dab, yt = yellowtail flounder, sr = sea robin, op = ocean pout, ss = short-horned sculpin, wf = wolf fish, cf = cod fish, lf = lumpfish, he = sea herring, aw = alewife, sh = silver hake, tt = tautog, sm = smelt, po = pollock.

²For data analysis these stations were pooled, and will henceforth be collectively referred to as Station 5.

TABLE 4

CATCH PER UNIT EFFORT FOR WINTER FLOUNDER CAUGHT BY F/V
ODESSA USING A STANDARD TRAWL METHOD.

Station	Flounder per minute of bottom time
Broad Sound	1.26
Deer Island	0.24, 0.37
Nantasket	0.47
Future Outfall	0.10

none were represented in all stations, and none have a comparable legacy of data of information for studies such as this to compare with the winter flounder.

Age/ length parameters

Mean age and length of fish from each station are listed in Table 5. Of particular note is the Broad Sound station, where the fish were, on average, significantly older and longer than from any of the other sites. These relationships are further examined in Figures 2 and 3. The mean lengths- at -age for fish captured at each station are shown in Table 6. These numbers show the *similarity of mean length for a given age in the samples from all stations*, suggesting that the fish come from a genetic stock that has a unified growth pattern. This is in distinct contrast to the winter flounder from Georges Bank, which grow at a significantly higher rate, especially as juveniles (Jay Burnett, pers. comm.). For a given age, adult males are on average shorter than females. The small differences present in Table 6 would be even less obvious if the data were segregated for males and females, as the sex ratio did vary between stations (Table 9).

In contrast to the similarity of mean length for a given age at all the sites, the *number of fish in each length or age class compared between stations was markedly different for the Broad Sound fish compared to all the other sites*(Figures 2 and 3). This is not a sex ratio effect. Data presented in Table 9 shows that there was not a preponderance of females in the Broad Sound sample. Rather, the preponderance of old, large fish from Broad Sound presumably reflects a lower mortality rate compared to the other stations. This lowered rate probably results from reduced exploitation. Recreational and commercial fishing pressure is low compared to the Boston Harbor area.

It is important to note that a preponderance of large, old fish has never been seen in any of our Boston Harbor collections, nor has it been seen in any inshore samples examined by Jay Burnett, at Woods Hole NMFS. *It is thus reasonable to assume that the amount of mixing between Broad Sound fish and those in Boston Harbor is limited.* This conclusion, when considered with data from earlier tagging studies (Howe and Coates 1975) that suggested limited migration for winter flounder north of Cape Cod, strongly supports the study of winter flounder pathology to monitor for the chronic non-lethal biological effects of coastal contaminants.

Interstation Comparison of lesion prevalence

As described above, lesion prevalences were calculated for each lesion at each given station. Table 7 lists that data. For all stations except Broad Sound the prevalence is given both for observations on one tissue block, and three. Statistically significant increases

TABLE 5 - SUMMARY OF COLLECTIONS OF WINTER FLOUNDER MADE IN THE SPRING OF 1991 IN MASSACHUSETTS AND CAPE COD BAYS

Station Number	Station Name	Date of collection	Sample size	Total length (mean \pm S.D.)	Age (mean \pm S.D.)	Grossly visible lesions (%)	Neoplasia (%)
1	Deer Island	3/12/91, 4/8/91, 5/22/91	167	360 \pm 39cde	4.8 \pm 1.3cde	8	2
2	Nantasket Beach	4/8/91	57	353 \pm 33c	4.6 \pm 1.0c	3	0
3	Broad Sound	2/5/91	56	396 \pm 40abde	6.4 \pm 1.1abde	21	7
4	Future Outfall Site	4/8-13/91	66	338 \pm 45ac	4.2 \pm 1.0ac	6	0
5	Eastern Cape Cod Bay	5/12/91	59	334 \pm 33ac	4.1 \pm 1.1a	0	0

Prevalence of neoplasia limited to observations on one tissue block to allow direct comparison to include Broad Sound, where only one block was examined. Significant interstation differences (ANOVA $p < 0.05$, using Fisher's Protected Least Significant Difference Test) shown as: Station 1:a, 2:b, 3:c, 4:d, 5:e.

TABLE 6 - RELATIONSHIP BETWEEN AGE AND TOTAL LENGTH FOR WINTER FLOUNDER FROM 5 AREAS OF MASSACHUSETTS AND CAPE COD BAYS

Age	Deer Island	Nantasket Beach	Broad Sound	Future Outfall	Eastern Cape Cod Bay
3	310 ± 14 (10)	321 ± 27 (5)		308 ± 47 (15)	312 ± 13 (18)
4	333 ± 20 (69)	331 ± 20 (21)	346 ± 19 (2)	330 ± 39 (28)	330 ± 17 (25)
5	368 ± 20 (40)	355 ± 17 (24)	365 ± 16 (8)	361 ± 23 (17)	361 ± 10 (8)
6	400 ± 19 (24)	406 ± 16 (5)	378 ± 23 (19)	372 ± 57 (6)	
7	400 ± 18 (10)	429 (1)	411 ± 34 (15)	400 (1)	400 (1)
8	420 ± 30 (4)	445 (1)	447 ± 33 (8)		
9	442 ± 25 (3)				
10			435 (1)		

Length given as mean ± S.D. (sample size).

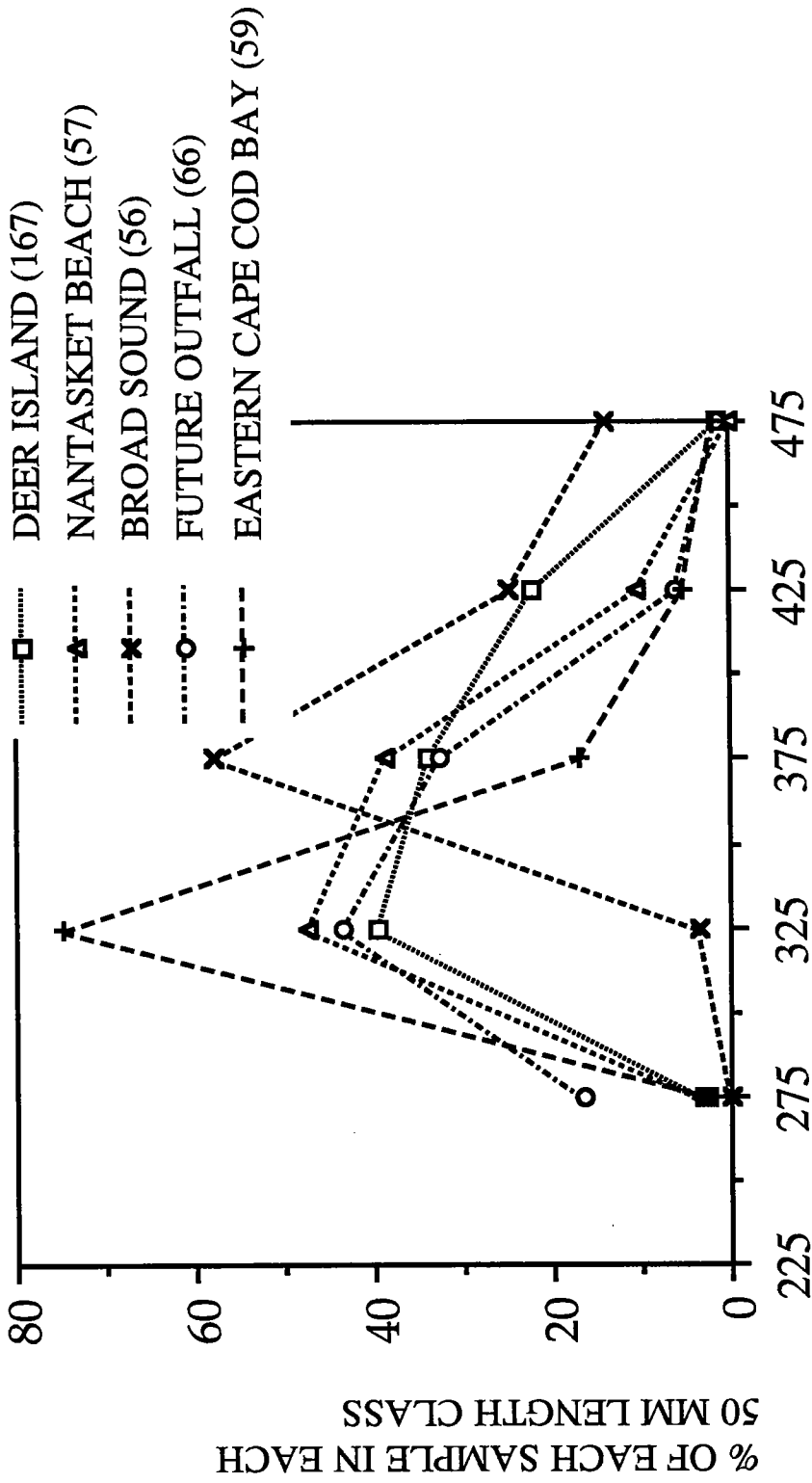


FIGURE 2 - DISTRIBUTION OF SAMPLES FROM EACH STATION BY LENGTH CLASS

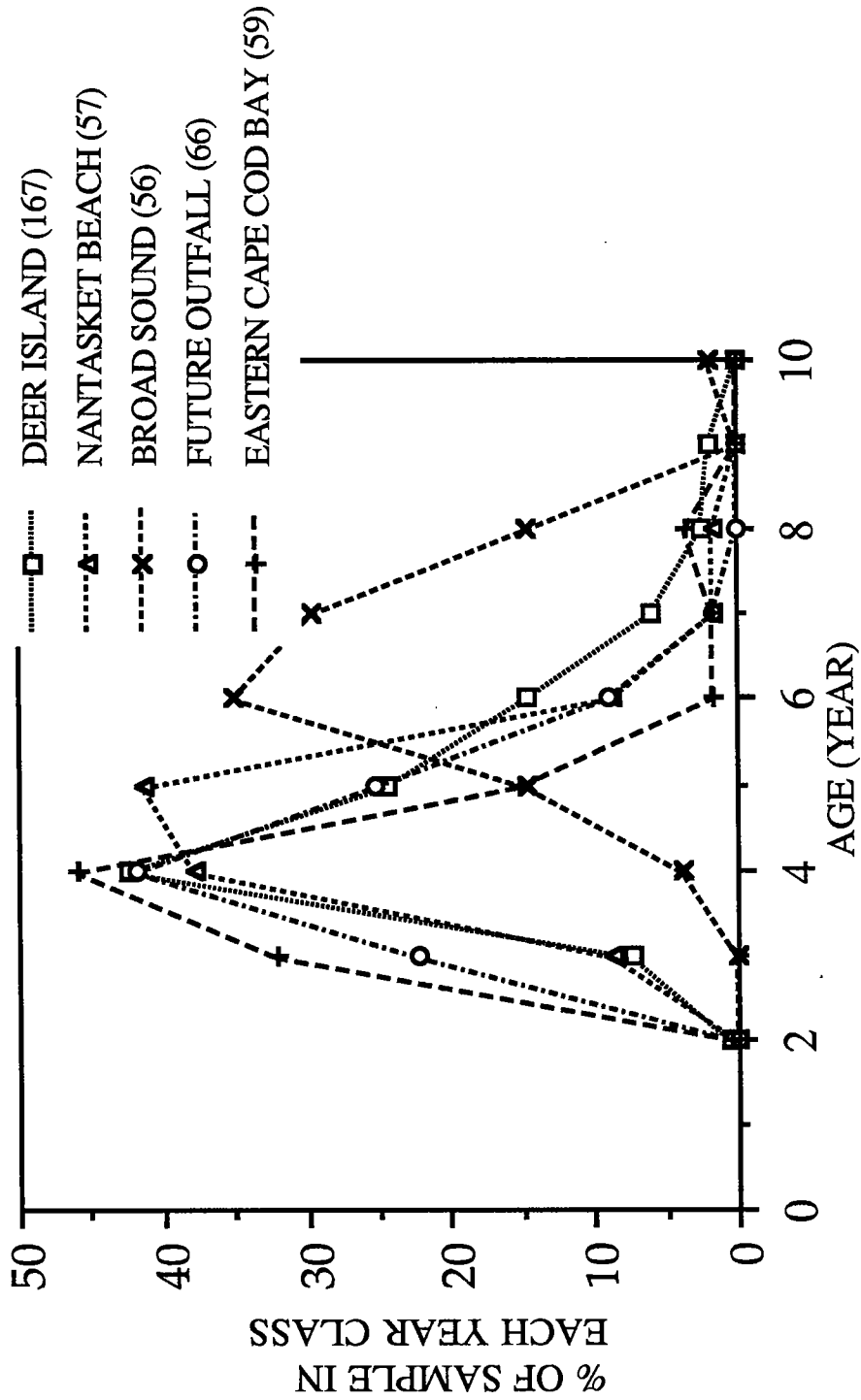


FIGURE 3 - DISTRIBUTION OF SAMPLES FROM EACH STATION BY AGE CLASS

TABLE 7 - 1991 HISTOPATHOLOGICAL LESION PREVALENCES FOR WINTER FLOUNDER LIVERS FROM MASSACHUSETTS AND CAPE COD BAYS

Sampling Intensity ¹	Neoplasms		Focal hydropic vacuolation		Tubular hydropic vacuolation		Centro-tubular hydropic vacuolation		Macrophage aggregation		Biliary proliferation	
	1	3	1	3	1	3	1	3	1	3	1	3
Deer Island 3/12/91	3	3	5	5	37	37	45	50	50	79 ³	29	39
Deer Island 4/8/91	3	3	6	6	43	43	62	72	77	80	39	48
Deer Island 5/22/91	2	3	2	3	18	20	32	35	35	42	5	10
Nantasket Beach	0	0	2	2	14	16	36	40	66	74	12	24
Broad Sound ²	7		11		44		61		79		26	
Future Outfall site	0	0	0	1	25	27	28	28	37	45	10	22 ³
Eastern Cape Cod Bay	0	0	0	0	0	0	3	3	22	22	10	15

¹Prevalences calculated as the percentage of fish exhibiting each lesion. Presence of a lesion defined by the observation of a pathological index of greater than zero. Prevalence calculated in two ways: 1 - data only taken from the first tissue block sampled, for grossly normal fish, and for multiple blocks for visibly abnormal fish, and 3 - Lesion regarded as present if seen in any or all of three tissue blocks examined.

²Prevalence 3 unavailable as only one tissue block examined per visibly normal fish for this station.

³Prevalence significantly increased by examining three blocks per fish as compared to one, when compared by Fisher's Exact Test. Between station differences are compared in Table 8.

in lesion prevalence were only seen at one of five stations for each of macrophage aggregation and biliary proliferation. Thus the increased prevalence of critical lesions, i.e. hydropic vacuolation and neoplasms were statistically insignificant at all stations. Likewise, examination of the raw data in Table 20 shows a very strong similarity for data generated from different sections from the same block. Therefore, neither repeat sections from blocks, nor repeat blocks seem justified. The salient important trends in Table 7 are illustrated in Figure 4. The data are all for observations from one block per animal, to allow inclusion of the Broad Sound site. *Both vacuolation and neoplasms were prevalent in winter flounder livers from Broad Sound and Deer Island Flats. However, the prevalence of vacuolation was also significantly elevated at the Nantasket Beach and Future Outfall sites. In contrast, the prevalence of vacuolation in Eastern Cape Cod Bay flounder livers was very low, although not zero.* In contrast, flounder from Georges Bank have been shown to have a zero prevalence (0/36) of vacuolation (Moore 1991). The statistical significance of the differences in lesion prevalence between each site and the reference site are compared in Table 8. Centrotubular and tubular vacuolation are thus shown as powerful discriminators between all of the sites and the reference site.

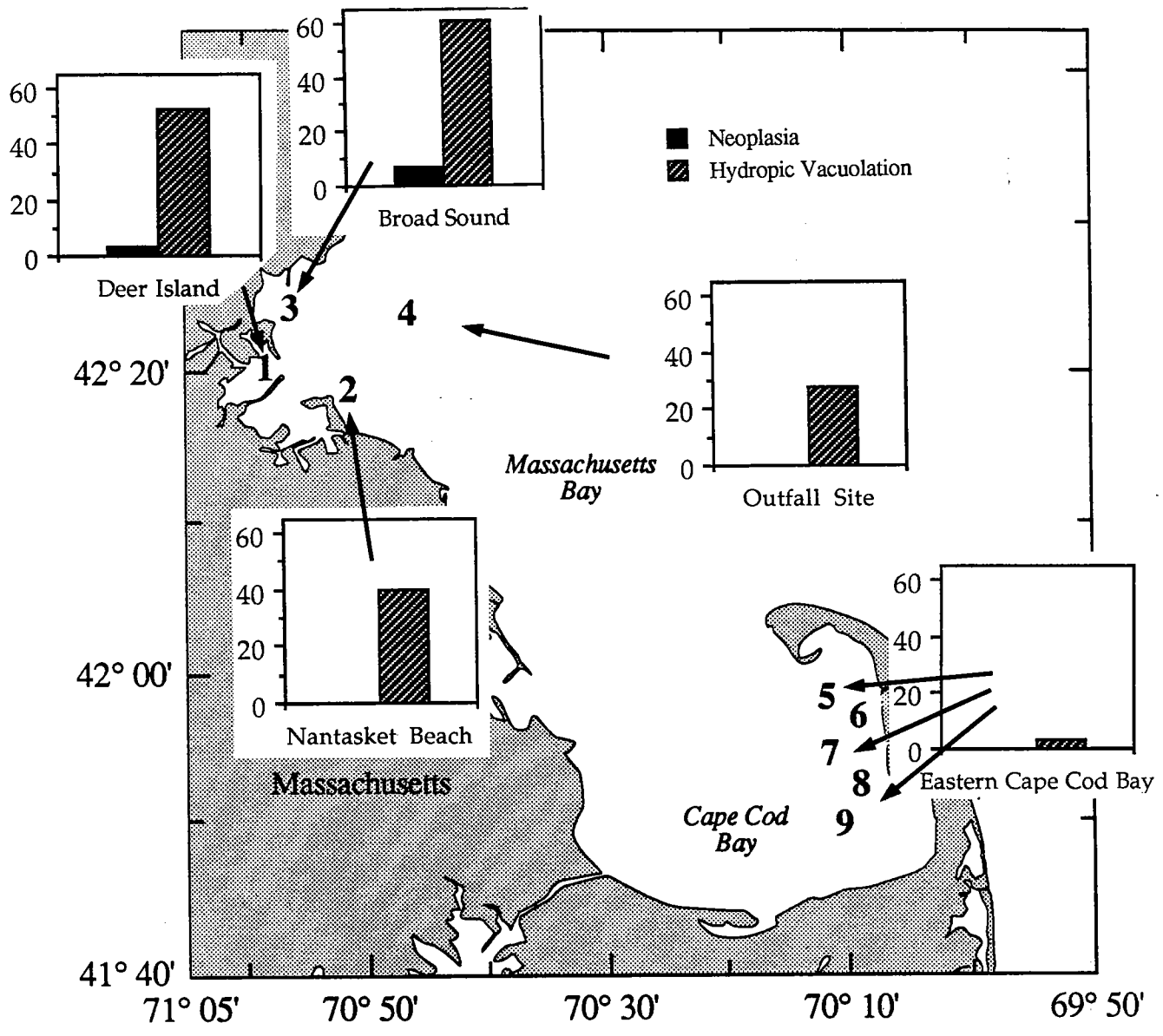
The observation of a marked difference in vacuolated cell prevalence among Eastern Cape Cod Bay, the Future Outfall site, and Boston Harbor, coupled with the close association of vacuolated cell prevalence with the concentrations of specific groups of chemical contaminants (Table 1), strongly supports the assessment of hydropic vacuolation in winter flounder liver as a reliable biomarker of the effects of these chemicals. In contrast, the prevalence of hepatic neoplasms was too low, even from highly contaminated sites, to make it a particularly useful parameter for comparing between less contaminated sites.

The relative prevalence of lesions in males vs. females has been an issue in the past, however this study reiterated our previous finding (Moore 1991) of no significant difference in hydropic vacuolation prevalence between genders at any station (Table 9).

Interstation Comparison of histopathological indices.

Table 10 lists histopathological indices for each lesion type at each station. These data show that the severity, as well as the prevalence of liver disease, increases in urban and nearshore coastal stations. Furthermore, those differences are statistically significant. Lesion prevalence was only examined statistically for centrotubular vacuolation, as the different lesion types co-vary, and are thus statistically dependent. Centrotubular

FIGURE 4 - Prevalence of liver neoplasms and centrotubular hydropic vacuolation in winter flounder from 5 areas of Massachusetts and Cape Cod Bays in 1991



Percentages of fish affected with neoplasia and hydropic vacuolation of the liver of adult winter flounder, sampled in the Spring of 1991, from 5 areas of Massachusetts and Cape Cod Bays. Sample sizes were as follows: station 1: 167, station 2: 57, station 3: 56, station 4: 66, and stations 5 to 9 (pooled): 59.

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TABLE 8 - SIGNIFICANCE OF LESION PREVALENCE DIFFERENCES BETWEEN REFERENCE SITE (EASTERN CAPE COD BAY), AND EACH OTHER SITE - DATA FROM TABLE 7, 1 BLOCK PER FISH.

Data for the 3 Deer Island samples pooled. Differences compared, for each lesion type except biliary proliferation using Fisher's Exact Test (one tailed). Biliary proliferation was tested by chi-square, with continuity correction, given high frequencies seen at Deer Island.

Site	Neoplasia	Focal Hydropic vacuolation	Tubular Hydropic Vacuolation	Centrotubular Vacuolation	Macrophage Aggregation	Biliary Proliferation
Broad Sound	ns (0.053)	0.012	0.0000001	0.0000001	0.0000001	0.019
Deer Island Flats	ns	ns	0.0000001	0.0000001	0.0000001	0.0345
Nantasket Beach	ns	ns	0.001	0.0000008	0.0000001	ns
Future Outfall Site	ns	ns	0.0000003	0.000009	0.005	ns

TABLE 9 - PREVALENCE OF CENTROTUBULAR VACUOLATION BETWEEN STATIONS, COMPARED BETWEEN GENDERS

Station	Sex ratio (% Female)	Prevalence (%) of centrotubular vacuolation	
		Males	Females
1	75	44	48
2	50	38	34
3	65	65	60
4	73	17	33
5	65	5	3

No significant difference between prevalence of centrotubular vacuolation in males vs females for any station (Chi-square p > 0.05)

TABLE 10 - COMPARISON OF HISTOPATHOLOGICAL INDICES FOR WINTER FLOUNDER FROM MASSACHUSETTS AND CAPE COD BAYS CAUGHT IN THE SPRING OF 1991

Site number	Site Name	Neoplasia	Focal hv	Tubular hv	Centrotubular hv	Macro-phage	Biliary prolif.
1	Deer Island 5/22/91 4/8/91 5/22/91	0.02 ± 0.10	0.07 ± 0.42	0.55 ± 0.92	0.86 ± 0.10 ^{bde}	0.84 ± 0.85	0.32 ± 0.57
2	Nantasket Beach 4/8/91	0.0 ± 0.0	0.01 ± 0.04	0.22 ± 0.63	0.52 ± 0.75 ^{ace}	0.73 ± 0.65	0.16 ± 0.35
3	Broad Sound 2/5/91	0.05 ± 0.24	0.06 ± 0.21	0.67 ± 0.98	0.95 ± 1.0 ^{bde}	1.25 ± 1.01	0.32 ± 0.65
4	Outfall site 4/8 to 13/91	0.0 ± 0.0	0.0 ± 0.04	0.32 ± 0.63	0.49 ± 0.82 ^{ace}	0.46 ± 0.64	0.11 ± 0.24
5	Eastern Cape Cod Bay 5/12/91	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.24 ^{abcd}	0.22 ± 0.44	0.06 ± 0.15

Each histological condition was scored on a scale of 0 to 4 for severity, with 0 = absent to 4 = severe. Two skip sections from each of three 3 blocks per fish were examined, except that for visibly normal fish from Broad Sound, only one block per fish was examined. Mean indices for each fish were averaged for each station. Data given as mean ± S.D. for each station. Significant (ANOVA $p < 0.05$, using Fisher's Protected Least Significant Difference Test) interstation differences shown as: Station 1:a; 2:b; 3:c; 4:d; 5:e. Lesion co-dependence precluded testing of more than one lesion type statistically. Centrotubular vacuolation was regarded as the most sensitive indicator of exposure to chemical contaminants.

Vacuolation was chosen as this was the lesion showing the most significant intersite differences, and appears to be the most sensitive biomarker.

Age effects on lesion prevalence.

We have previously shown that lesion prevalence in winter flounder from Deer Island Flats increases with both length and age (Moore 1991). It was thus important to establish whether the above trends were genuine station-related effects, or confounded by differences in the age of fish from different stations. Indices of centrotubular vacuolation were compared for three age classes for each station; see Table 11. The differences are less extreme than for the samples taken as a whole, but nonetheless, *statistically significant between-station differences were found for age-normalized prevalences of centrotubular vacuolation.*

Comparison of lesion indices from three successive collections on Deer Island Flats.

Table 12 describes 3 samples of winter flounder from Deer Island Flats. There are significant differences in the age and length characteristics of the 3 samples. The May 22nd sample is particularly striking in that both the age and length means are distinctly lower than the earlier samples.

Table 13 compares the histopathological indices for the three Deer Island samples. Again, there are significant differences in centrotubular vacuolation prevalence between the March and April samples and the May sample. However, when these differences were normalized for age (Table 14), the number of significant differences in the severity index for centrotubular hydropic vacuolation among the sampling periods was less, showing a difference only between the April and May sampling periods for four year old fish. This age class represented the largest overall sample size among the sampling periods. Part of this diminution in significant differences among the sampling periods is undoubtedly due to a reduction in the sample sizes for groups tested, caused by splitting the data into year classes. Future incorporation of an age-related factor in as multivariate analysis of the entire data set may yield a more appropriate analysis. Nonetheless, *these observations reinforce the need to normalize histopathological data by age*, and to establish a routine sampling time that is adhered to on an annual basis to maximize the chance of collecting matched age distributions on an annually repeatable basis.

TABLE 11 - COMPARISON BETWEEN AGE CLASSES, OF HISTOPATHOLOGICAL INDICES [Mean \pm S.D. (Sample size)] OF CENTROTUBULAR VACUOLATION IN WINTER FLOUNDER FROM MASSACHUSETTS AND CAPE COD BAYS CAUGHT IN THE SPRING OF 1991

Station Number	Station Name	3 & 4 Year	5 & 6 Years	7 & 8 Years
1	Deer Island	0.54 \pm 0.86 (81) ^e	1.13 \pm 1.08 (64) ^{be}	1.29 \pm 1.05 (14) ^e
2	Nantasket Beach	0.53 \pm 0.75 (27) ^e	0.39 \pm 0.62 (29) ^{ac}	2.25 \pm 0.82 (2) ^e
3	Broad Sound		1.0 \pm 0.94 (27) ^{be}	0.91 \pm 0.93 (24)
4	Future Outfall site	0.35 \pm 0.71 (43) ^e	0.78 \pm 0.94 (23)	
5	Eastern Cape Cod Bay	0.0037 \pm 0.025 (46) ^{abd}	0.18 \pm 0.58 (10) ^{ac}	0.0 \pm 0.0 (3) ^a

Each histological condition was scored on a scale of 0 to 4 for severity, with 0 = absent to 4 = severe. Two skip sections from each of three 3 blocks per fish were examined, except that for visibly normal fish from Broad Sound, only one block per fish was examined. Mean indices for each fish were averaged for each station. Significant (ANOVA $p < 0.05$, using Fisher's Protected Least Significant Difference Test) interstation differences shown as: Station 1:^a, 2:^b, 3:^c, 4:^d, 5:^e.

TABLE 12 - COMPARISON OF WINTER FLOUNDER DATA FOR 3 SUCCESSIVE SURVEYS FROM DEER ISLAND FLATS IN 1991

Station Number	Station Name	Date of collection	Sample size	Total length (mean ± S.D.)	Age (mean ± S.D.)	Grossly visible lesions (%)	Neoplasia (%)
1a	Deer Island	3/12/91	40	357 ± 42 ^{b,c}	5.2 ± 1.5	16	3
1b	Deer Island	4/8/91	68	379 ± 36 ^{a,c}	5.2 ± 1.3	9	3
1c	Deer Island	5/22/91	59	339 ± 26 ^{a,b}	4.2 ± 0.8	2	3

Neoplasm prevalences calculated for observations from 3 tissue blocks per animal. Significant interstation length differences shown as: Station 1a:^a, 1b:^b, 1c:^c (ANOVA $p < 0.05$, using Fisher's Protected Least Significant Difference Test)

TABLE 13 - COMPARISON OF HISTOPATHOLOGICAL INDICES FOR WINTER FLOUNDER FROM DEER ISLAND FLATS FOR 3 SURVEYS IN 1991

Station Number	Station Name	Neoplasms	Focal hydropic vacuolation	Tubular hydropic vacuolation	Centrotubular hydropic vacuolation	Macrophage aggregation	Biliary proliferation
1a	Deer Island 3/12/91	0.01 ± 0.05	0.12 ± 0.65	0.76 ± 1.23	0.98 ± 1.21 ^c	0.86 ± 0.85	0.42 ± 0.74
1b	Deer Island 4/8/91	0.03 ± 0.18	0.09 ± 0.42	0.68 ± 0.91	1.16 ± 0.98 ^c	1.19 ± 0.86	0.48 ± 0.62
1c	Deer Island 5/22/91	0.02 ± 0.01	0.02 ± 0.14	0.28 ± 0.64	0.45 ± 0.77 ^{ab}	0.44 ± 0.67	0.07 ± 0.22

Each histological condition was scored on a scale of 0 to 4 for severity, with 0 = absent to 4 = severe. Two skip sections from each of three 3 blocks per fish were examined, except that for visibly normal fish from Broad Sound, only one block per fish was examined. Mean indices for each fish were averaged for each station and expressed as mean ± S.D. Significant interstation differences for centrotubular vacuolation tested by ANOVA $p < 0.05$, using Fisher's Protected Least Significant Difference Test. Interstation length differences shown as: Station 1a:a, 1b:b, 1c:c Lesion co-dependence precluded testing of more than one lesion type statistically. Centrotubular vacuolation was regarded as the most sensitive indicator of exposure to chemical contaminants.

TABLE 14 - COMPARISON OF INDICES [Mean \pm S.D. (Sample size)] OF CENTROTUBULAR VACUOLATION FROM DEER ISLAND FLATS WITHIN AGE CLASSES, BETWEEN SURVEYS IN 1991

Station Number	Station Name	4 Years	5 Years	6 years
1a	Deer Island 3/12/91	0.55 \pm 1.2 (13)	0.86 \pm 0.8 (12)	1.93 \pm 1.9 (5)
1b	Deer Island 4/8/91	0.86 \pm 0.8 (17) ^c	1.2 \pm 1.0 (21)	1.34 \pm 1.2 (16)
1c	Deer Island 5/22/91	0.36 \pm 0.7 (39) ^b	0.66 \pm 1.0 (7)	0.39 \pm 0.5 (3)

Each histological condition was scored on a scale of 0 to 4 for severity, with 0 = absent to 4 = severe. Two skip sections from each of three 3 blocks per fish were examined, except that for visibly normal fish from Broad Sound, only one block per fish was examined. Mean indices for each fish were averaged for each station. Significant interstation differences tested by ANOVA, $p < 0.05$, using Fisher's Protected Least Significant Difference Test.

Annual lesion prevalence trends: 1984 to 1991

Table 15 lists data compiled from 3 sources: Murchelano and Wolke (1985), Moore (1991), and this study. Data are not normalized for age. *There is an apparent trend for both neoplasms and centrotubular vacuolation prevalence to decrease in recent years* (Figures 5 and 6). Age data are only available for collections made in or after 1989. Age-specific analysis of data for these years (Table 16) fails to support the existence of a temporal trend showing a reduction in prevalence of centrotubular hydroptic vacuolation. However, that does not necessarily mean that the 1989 - 1991 period does not in fact represent a lower prevalence than earlier years. To explore this possibility, the prevalence of centrotubular hydroptic vacuolation was compared for 3 length classes for each of years 1987 to 1991; see Table 17. A trend is suggested for the two shorter length classes, especially in the shortest length class since 1988, with decreasing prevalence in recent years. These trends are shown in Figure 7. The data for the longest length class have been omitted for this figure. It is not unreasonable to expect the longest, and hence probably oldest fish to be the last group to demonstrate a reduced prevalence, if, as seems possible, they were exposed as juveniles to heavier levels of contamination than were the juveniles of more recent years. *However, because analysis of this apparent temporal trend by the Spearman rank correlation coefficient failed to detect any significant decreasing trend in the prevalence of this lesion over the five years represented, the establishment or refutation of the validity of these suggested trends will require at least three additional years of age specific data.*

Cell Proliferation studies

To further investigate interstation differences in this study, a subset of fish from each station was processed to allow an estimation of cell proliferation in the liver (Table 18). The most obvious trends in these data is the increasing number of hepatocytes per unit area in inshore stations, the high labelling index for hydroptically vacuolated cells, and the increasing labelling index for hepatocytes in fish from the cleaner sites. These trends will have to await larger sample size before meaningful statistical analysis can be conducted.

The increase in hepatocyte number should not be over-interpreted, because paraffin-embedded material is notoriously poor for the generation of absolute numbers concerning cell number and volume. Artifactual shrinkage can be extremely variable among individuals, especially where different quantities of lipid and glycogen are stored, which is the case in comparing among these stations. To evaluate the possibility of a trend in cell number per unit area, a new sample of fish from these stations should be collected, the weight and volume of each liver determined, their livers embedded in epoxy, sectioned at 1

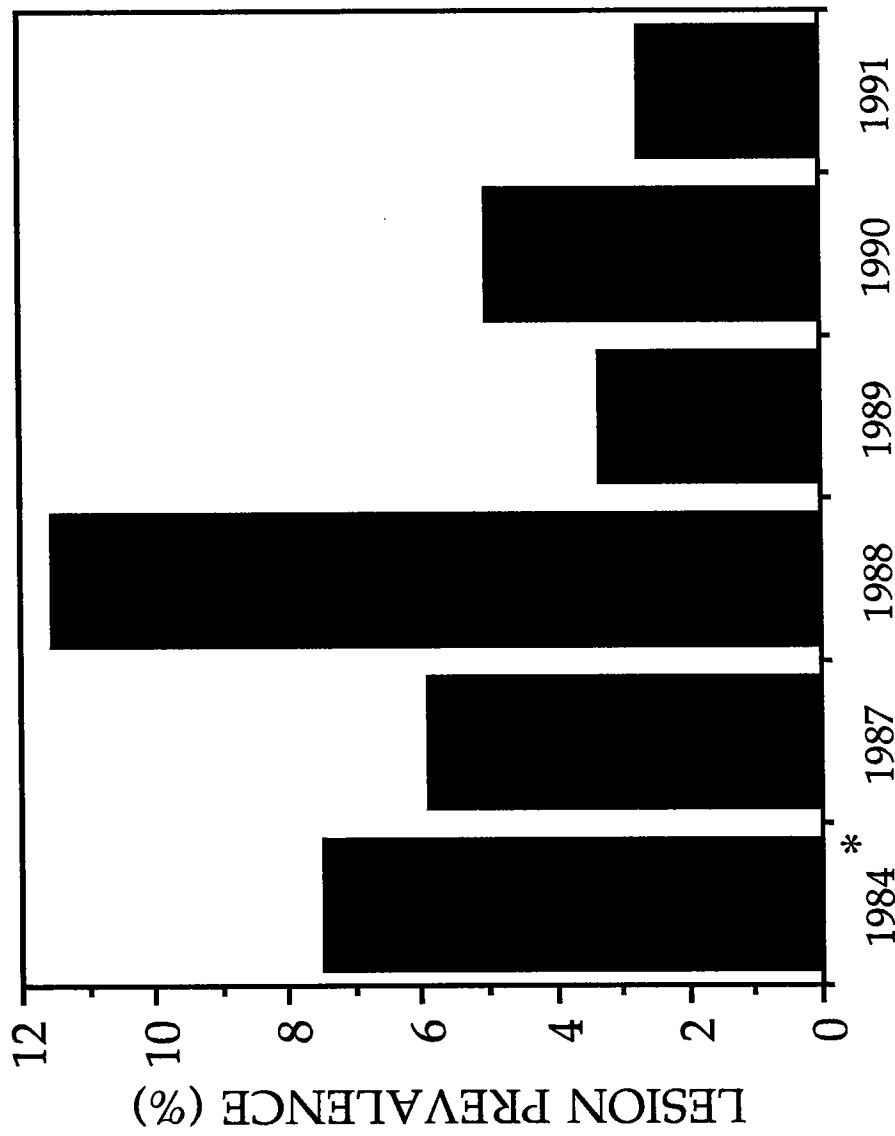
TABLE 15 - SUMMARY OF DATA ON DEER ISLAND FLATS WINTER FLOUNDER 1984 TO 1991

Year sampled	1984 ¹	1987 ²	1988 ²	1989 ²	1990 ²	1991 ³
Date sampled	2 April & 6 June	21 April	18 March	13 May	30 April	12 Mar, 8 Apr, 22 May
Sample size	200	51	52	29	100	167
%Female	-	57	92	66	71	73
Body length ± S.D.	353	364 ± 32	378 ± 38	367 ± 31	370 ± 31	358 ± 35
Gross lesion (%)		17.7	28	13.3	10	9
Neoplasm (%)	7.5	5.9	11.5	3.3	5	2.7
Centrotubular HV ⁴ (%)	68	77	65	40	39	46
Tubular HV (%)		71	48	30	29	32
Focal HV (%)		14	22	10	2	3
Macrophage (%)	68	75	63	30	43	54

¹Murchelano and Wolke 1985 (Fish from Deer Island and elsewhere in Boston Harbor);

²Moore 1991; ³This study; ⁴HV = Hydropic vacuolation

FIGURE 5 - PREVALENCE OF LIVER NEOPLASIA IN WINTER FLOUNDER FROM DEER ISLAND FLATS 1984 TO 1991



*1984 Data from Murchelano and Wolke 1985

FIGURE 6 - PREVALENCE OF CENTROTUBULAR VACUOLATION IN WINTER FLOUNDER FROM DEER ISLAND FLATS 1984 TO 1991

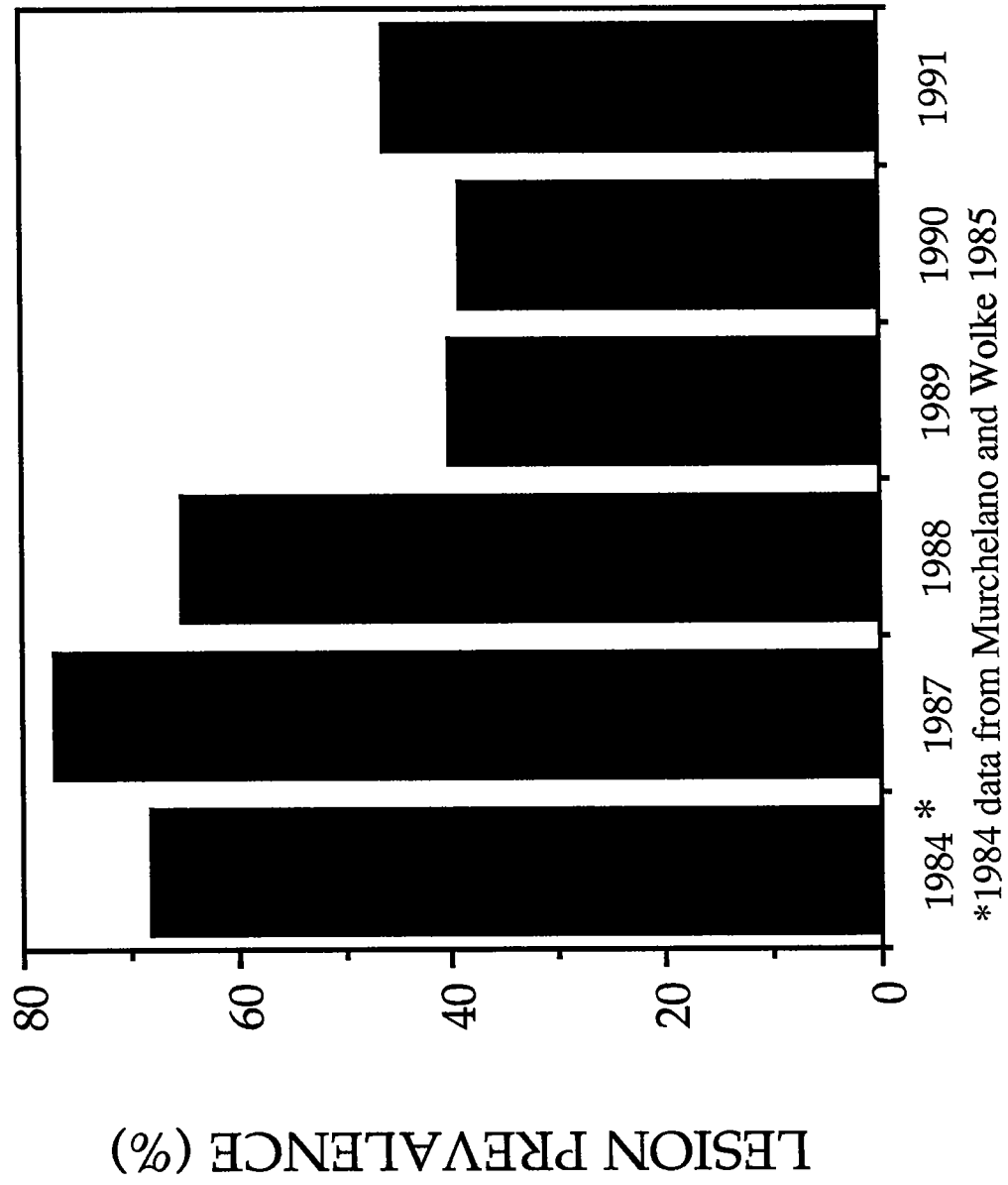


TABLE 16 - ANALYSIS BY AGE CLASS OF PREVALENCE OF CENTROTUBULAR HYDROPIC VACUOLATION IN WINTER FLOUNDER FROM DEER ISLAND FLATS, BOSTON HARBOR, CAUGHT IN 1989, 1990, AND 1991

	All fish (%)	3 year olds	4 year olds	5 year olds	6 year olds	7 year olds	8 year olds	9 year olds
1989	51 (25)	0 (2)	29 (7)	33 (9)	100 (4)	100 (2)	0 (1)	
1990	39 (99)	0 (4)	38 (29)	48 (31)	35 (23)	50 (4)	50 (4)	0 (1)
1991	47 (167)	33 (12)	32 (69)	55 (40)	67 (24)	60 (10)	75 (4)	67 (3)

Prevalence given as a % of sample affected, with sample size in parentheses. Nine fish were not analyzed for age.

TABLE 17 - PREVALENCE OF CENTROTUBULAR VACUOLATION IN FEMALE WINTER FLOUNDER FROM DEER ISLAND FLATS, BOSTON, BY LENGTH CLASS, FOR 1987 TO 1991.

YEAR	TOTAL LENGTH (mm)		
	300 - 350	350 - 400	400 - 450
1987	75 (8)	79 (14)	50 (6)
1988	89 (9)	50 (14)	60 (10)
1989	0 (1)	39 (13)	100 (2)
1990	33 (21)	46 (26)	36 (22)
1991	28 (39)	53 (47)	71 (35)

Prevalence given as a % of sample affected, with sample size in parentheses.

FIGURE 7 - PREVALENCE OF CENTROTUBULAR VACUOLATION FOR TWO LENGTH CLASSES OF FEMALE WINTER FLOUNDER FROM DEER ISLAND FLATS, 1987 TO 1991

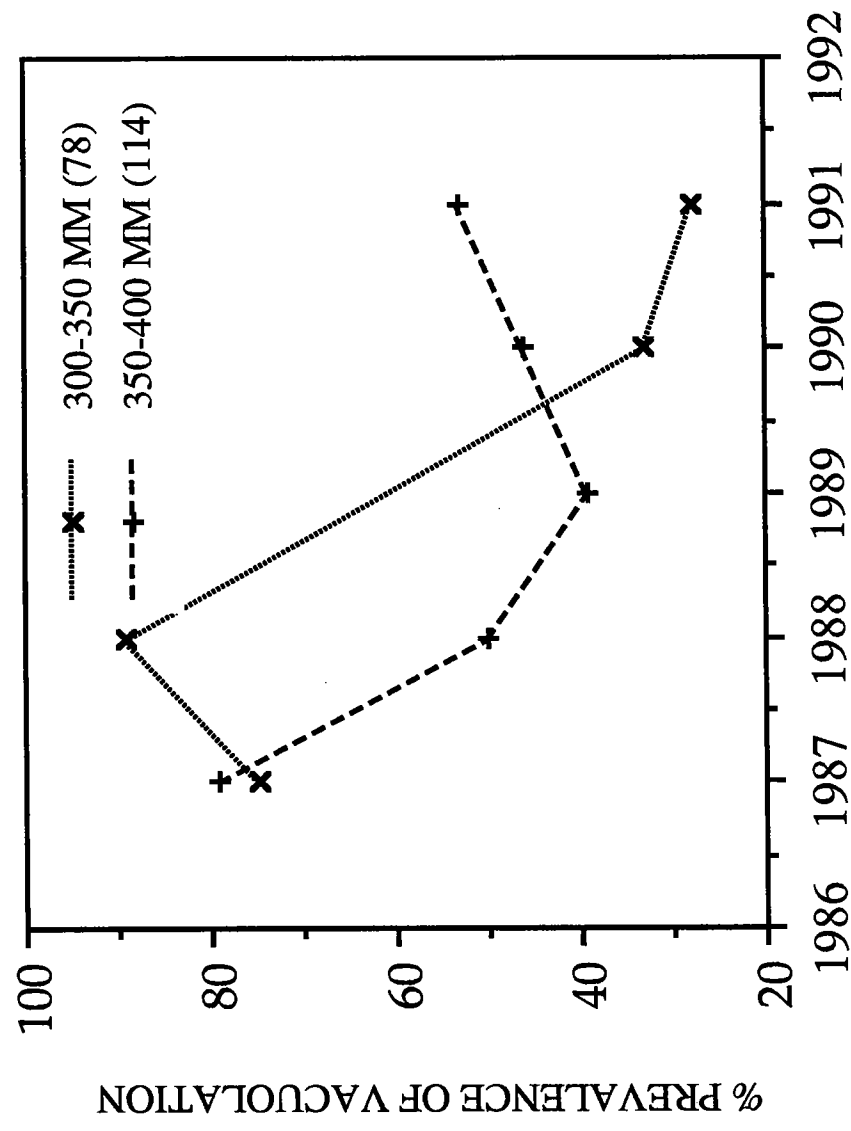


TABLE 18 - NUMBER OF HEPATOCYTES PER UNIT AREA AND LABELLING INDICES (L.I.; MEAN \pm S.D.) OF WINTER FLOUNDER TISSUES TO COMPARE CELL PROLIFERATION

STATION	NUMBER OF FISH	NUMBER OF HEPATOCYTES PER UNIT AREA	HEPATOCYTE L.I.	NON-HEPATOCYTE L.I.	VACUOLATED CELL L.I. (NUMBER OF FISH)
DEER ISLAND	8	1214.57 \pm 191.69	0.27 \pm 0.19	0.97 \pm 1.41	32.98 \pm 7.09 (5)
NANTASKET BEACH	10	1222.50 \pm 108.56	0.24 \pm 0.20	0.72 \pm 0.76	0.54 \pm 0.93 (3)
NEW OUTFALL	10	941.30 \pm 202.72	1.11 \pm 1.40	1.34 \pm 1.41	18.80 (1)
EASTERN CAPE COD BAY	9	770.22 \pm 368.97	3.02 \pm 4.53	1.38 \pm 1.07	

Hepatocyte and non-hepatocyte labelling indices (L.I.) were expressed as a fraction of positive nuclei per 1000 hepatocytes, and vacuolated cell labelling indices were expressed as number of positive vacuolated cell nuclei per 1000 vacuolated cells.

µm, and cellular morphometrics estimated using established stereological methods. Such an analysis cannot be attempted on the material in hand.

In contrast, the labelling indices are valid in paraffin embedded material as the index is a ratio, not an absolute number. Elevated indices were recorded for vacuolated cells. This confirms our earlier observations of *vacuolated cells undergoing increased levels of replicative DNA synthesis* (Moore and Stegeman, in press). Experimentally we have induced a proliferative epithelial lesion in winter flounder liver using technical grade chlordane (Moore 1991). The same lesion was seen in flounder caught from Deer Island Flats in this study. Concentrations of the same compounds (in technical grade chlordane) also correlate with hydropic vacuolation in field-caught winter flounder livers (Table 1).

The trend of increased hepatocyte labelling in the cleaner sites was somewhat of a surprise, however one could postulate that the fish from the more polluted sites are undergoing hepatocyte toxicity, with reactive hydropically vacuolated cells filling in the deficit, whereas in cleaner sites the hepatocytes can sustain their population internally. We are currently investigating chemical and biological parameters that affect labelling indices in winter flounder, and until further understanding is reached, interpretation of these data will be incomplete.

Cytochrome P450IA Immunohistochemistry

The same fish that were examined for labelling indices were also stained for the expression of cytochrome P450IA (Table 19). In addition, a set of fish collected from Georges Bank in 1990 were also examined. There are no obvious differences among the coastal and urban stations, but *all four of these stations show an obvious difference compared to Georges Bank, which is considerably lower in stain density for cytochrome P450IA*. This presumably indicates that all the inshore stations have a higher exposure to compounds that induce the synthesis of this protein than do the fish from Georges Bank. This observation reinforces a previous study (Monosson and Stegeman 1991), where P450IA levels in Georges Bank winter flounder were significantly lower than in a coastal sample.

TABLE 19 - STAIN DENSITY (MEAN \pm S.D.) OF WINTER FLOUNDER TISSUES TO COMPARE INDUCTION OF CYTOCHROME P450IA (CYPIA) BETWEEN STATIONS

Stain density recorded on a scale of 0 - 4. Data expressed as a mean of individual observations for each cell type for each station (one observation per cell type per fish).

STATION	NUMBER OF FISH	HEPATOCYTE CYPIA	GUT CYPIA	KIDNEY CYPIA
DEER ISLAND	8	2.38 \pm 1.06	1.25 \pm 0.71	1.88 \pm 1.46
NANTASKET BEACH	10	2.10 \pm 0.99	1.10 \pm 0.74	1.90 \pm 0.60
NEW OUTFALL	10	3.00 \pm 0.47	1.60 \pm 0.70	1.80 \pm 1.03
EASTERN CAPE COD BAY	9	2.22 \pm 0.44	1.89 \pm 0.60	2.17 \pm 0.41
GEORGES BANK	8	1.29 \pm 0.76	0.13 \pm 0.35	0.0 \pm 0.0

TABLE 20 (See following 12 pages)

HISTOLOGICAL DATA RECORDED FOR EACH FISH COLLECTED IN 1991

Column headings are abbreviated as follows:

ID	Individual fish identification number. In the multi-year archive, and in all sample labels these numbers are all prefaced with 91-
ST	Station number see Table 2 and Figure 1
YR	Age of fish in years
SEX	Gender: 1 = female, 0 = male
TL	Total Length (mm)
FIN	Fin-rot score 0 - 4: 0 = absent, 1 = minor, 2 = moderate, 3 = severe and 4 = extreme
GR	Gross lesions: 0 = absent, 1 = present
GS	Gross score: 0 = absent, 1 = mild, 2 = severe
MEAN	Mean histological index for that lesion type for that fish. Each lesion was scored at 0 - 4, 0 = absent, 1 = minor, 2 = moderate, 3 = severe and 4 = extreme
P1	Prevalence score (from one tissue block); 1 = present, 0 = absent
P3	Prevalence score (from three tissue blocks); 1 = present, 0 = absent
HV	Hydropic vacuolation
CENTRO	Centrotubular hydropic vacuolation
BILIARY PRO.	Biliary proliferation

WINTER FLOUNDER DATA- MOORE AND STEGEMAN 1991

ID	CENTRO	HV	MEAN	P1	P3	MACROPHAGE	MEAN	P1	P3	BILIARY PRO.	MEAN	P1	P3
218	0	0	0	0	0	0	0	0	0	0	0	0	0
219	2	1	2	2	2	1.83	1	1	1	0	0	0	0
220	0	0	0	0	0	0.00	0	0	0	0	0	0	0
221	0	0	0	0	0	0.00	0	0	0	0	0	0	0
222	0	0	0	0	0	0.00	0	0	0	2	2	2	2
223	0	0	0	0	0	0.00	0	0	0	0	0	1	1
224	0	0	0	0	0	0.00	0	0	0	0	0	0	0
225	0	0	0	0	1	0.17	0	0	0	0	0	0	0
226	1	1	1	1	1	1.00	1	1	1	1	1	1	1
227	0	0	1	1	1	0.67	0	1	0	0	0	0	0
228	1	1	1	1	1	1.00	1	1	1	0	0	0	0
229	2	2	2	2	2	2.00	1	1	1	2	2	2	2
230	0	0	0	0	0	0.00	0	0	0	0	0	0	0
231	0	0	1	2	2	1.17	0	1	0	0	0	1	1
232	0	0	0	0	0	0.00	0	0	0	1	1	0	0
233	0	0	0	0	0	0.00	0	0	0	1	1	1	1
234	0	0	0	0	0	0.00	0	0	0	0	0	1	1
235	0	0	0	0	0	0.00	0	0	0	2	2	2	2
236	1	1	1	1	1	1.00	1	1	1	0	0	0	0
237	1	1	1	1	1	1.00	1	1	1	0	0	0	0
238	1	1	1	1	1	1.00	1	1	1	0	0	0	0
239	0	0	0	0	0	0.00	0	0	0	1	1	1	0
240	2	3	2	2	2	2.17	1	1	1	2	2	2	2
241	0	0	0	0	0	0.00	0	0	0	0	0	0	0
ID	CENTRO	HV	MEAN	PREV	MACROPHAGE	MEAN	PREV	BILIARY PRO.	MEAN	PREV			
246	2	2	2	2	2	2.00	1	1	1	1	1	1	1
247		1	2	1	1	0.83	1	1		0	0	0	0
248	2	2	1	2	1	1.50	1	1	2	2	2	2	1
249	2	2	2	2	3	2.17	1	1	2	2	2	2	2
251	1	1	1	1	2	1.33	1	1	2	1	0	0	0
252	1	1	1	1	1	1.00	1	1	2	2	1	1	1
253	2	2	2	2	1	1.83	1	1	1	2	1	1	1
254	1	1	0	0	0	0.33	1	1	1	1	0	0	0
255	3	3	3	3	3	3.00	1	1	1	2	2	2	1
256	0	1	1	1	0	0.50	1	1	3	3	3	3	3
257	2	2	2	2	2	2.00	1	1	0	0	0	1	1
258	2	2	2	2	2	2.00	1	1	2	2	1	2	2
259	1	1	1	1	2	1.17	1	1	1	1	1	1	1
260	0	0	0	0	1	0.50	0	1	1	1	2	2	1
261	0	0	0	0	0	0.00	0	0	2	2	2	2	3
262	1	1	1	1	2	1.17	1	1	0	1	1	0	1
263	2	2	2	2	2	2.00	1	1	0	0	0	0	0
264	2	2	2	3	2	2.33	1	1	3	2	2	2	3
266	2	3	3	2	2	2.50	1	1	1	1	1	1	2
267	0	0	0	1	1	0.50	0	1	1	1	1	1	1
268	3	3	3	3	3	3.00	1	1	2	2	2	2	3
269	0	0	1	1	1	0.50	0	1	0	0	0	0	0
270	0	0	1	1	1	0.67	0	1	1	1	1	1	1
271	1	0	0	0	1	0.50	1	1	1	1	1	1	1
272	2	2	2	3	2	2.00	1	1	1	1	1	1	1
273	0	0	0	0	0	0.00	0	0	1	1	0	0	0
274	0	0	0	0	0	0.00	0	0	0	0	0	0	0
275	2	2	2	2	2	2.00	1	1	1	1	1	1	1
276	0	0	0	1	1	0.50	0	1	1	1	1	1	1
277	0	0	1	1	1	0.67	0	1	1	1	1	1	1
278	2	2	2	2	2	2.00	1	1	1	1	1	1	1
279	3	2	2	2	2	2.17	1	1	1	1	2	2	2
280	0	0	0	0	0	0.00	0	0	0	0	0	0	0
281	2	2	3	3	2	2.33	1	1	2	2	2	2	3
282	2	2	2	2	2	2.00	1	1	2	2	1	2	2
283	3	3	2	3	2	2.67	1	1	2	3	2	2	2
284	1	1	2	2	1	1.33	1	1	1	1	1	1	1
285	0	0	0	0	0	0.00	0	0	0	0	0	0	0
286	2	2	2	2	2	2.00	1	1	2	2	2	2	2
287	3	3	3	3	3	3.00	1	1	2	2	2	2	2
288	0	0	0	0	0	0.00	0	0	0	0	0	0	0
289	3	3	3	3	3	3.00	1	1	3	3	3	3	3
290	0	0	0	0	0	0.00	0	0	0	0	0	0	0
291	0	0	0	0	0	0.00	0	0	0	0	0	0	0
292	0	0	0	0	0	0.00	0	0	0	0	0	0	0
293	0	0	0	0	0	0.00	0	0	0	0	0	0	0
294	0	0	0	0	0	0.00	0	0	0	0	0	0	0

ID	CENTRO HV	MEAN P1 P3	MACROPHAGE	MEAN P1 P3	BILIARY PRO.	MEAN P1 P3
295	2 2 2 2 2 2	2.00 1 1	1 1 1 1 2 2	1.33 1 1	0 0 0 0 0 0	0.00 0 0
296	0 0 0 0 0 0	0.00 0 0	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0
297	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
298	0 0 0 0 0 0	0.00 0 0	2 2 3 3 2 2	2.33 1 1	0 0 0 0 0 0	0.00 0 0
299	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
300	0 0 0 0 1 1	0.33 0 1	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
301	1 1 2 2 2 2	1.67 1 1	2 2 2 2 2 3	2.17 1 1	1 2 1 1 1 1	1.17 1 1
302	0 0 0 0 0 0	0.00 0 0	1 1 2 2 1 1	1.33 1 1	0 0 1 1 1 1	0.67 0 1
303	1 1 1 1 1 1	1.00 1 1	1 1 1 1 1 1	1.00 1 1	0 0 1 1 1 1	0.67 0 1
304	1 1 1 1 1 1	1.00 1 1	1 1 1 1 1 1	1.00 1 1	1 1 0 0 0 0	0.33 1 1
305	0 1 1 1 1 1	0.83 1 1	1 1 1 1 1 1	1.00 1 1	0 0 1 1 1 1	0.67 0 1
306	2 2 1 1 2 1	1.50 1 1	2 2 1 1 1 1	1.33 1 1	0 0 0 0 0 0	0.00 0 0
310	2 2 2 2 2 2	2.00 1 1	1 1 2 2 2 2	1.67 1 1	1 1 1 1 1 1	1.00 1 1
311	2 2 2 2 2 2	2.00 1 1	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0
460	0 0 0 0 0 0	0.00 0 0	3 3 3 3 3 3	3.00 1 1	0 0 0 0 0 0	0.00 0 0
461	2 2 2 2 2 2	2.00 1 1	3 3 3 3 2 2	2.67 1 1	1 1 1 1 1 1	1.00 1 1
462	0 0 0 0 0 0	0.00 0 0	1 2 1 1 1 1	1.17 1 1	2 1 1 1 1 1	1.17 1 1
463	1 1 1 1 1 1	1.00 1 1	1 1 1 1 1 1	1.00 1 1	1 1 1 1 1 1	1.00 1 1
464	2 2 2 2 2 2	2.00 1 1	3 3 3 3 3 2	2.83 1 1	2 3 2 3 2 2	2.33 1 1
466	0 0 0 0 0 0	0.00 0 0	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0
467	2 2 1 1 1 1	1.33 1 1	0 0 0 0 0 0	0.00 0 0	1 1 0 0 0 0	0.33 1 1
468	3 3 2 2 2 2	2.33 1 1	1 1 1 1 1 1	1.00 1 1	1 1 0 0 0 0	0.33 1 1
312	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
313	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
314	0 0 0 0 0 0	0.00 0 0	0 1 1 0 1 0	0.50 1 1	0 0 1 0 0 0	0.17 0 1
315	0 0 0 0 0 0	0.00 0 0	0 0 1 1 0 0	0.33 0 1	0 0 0 0 0 0	0.00 0 0
316	0 0 0 0 0 0	0.00 0 0	1 1 1 1 0 1	0.83 1 1	0 0 0 0 0 0	0.00 0 0
317	1 1 1 1 1 1	1.00 1 1	1 1 0 0 1 1	0.67 1 1	0 0 0 0 0 0	0.00 0 0
318	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
319	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 1	0.17 0 1	0 0 0 0 0 0	0.00 0 0
320	1 1 2 2 1 1	1.33 1 1	0 1 1 1 1 1	0.83 1 1	0 0 0 0 0 0	0.00 0 0
321	1 1 1 1 1 1	1.00 1 1	1 1 1 0 0 0	0.50 1 1	0 0 0 0 0 0	0.00 0 0
322	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
323	0 0 0 0 1 1	0.33 0 1	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
324	1 1 1 1 1 1	1.00 1 1	2 3 2 2 1 1	1.83 1 1	0 0 0 0 1 1	0.33 0 1
325	3 2 2 2 2 2	2.17 1 1	1 2 2 2 2 2	1.83 1 1	0 0 0 0 1 1	0.33 0 1
326	0 0 0 0 0 0	0.00 0 0	0 0 1 1 1 1	0.67 0 1	0 0 0 0 0 0	0.00 0 0
327	2 2 1 1 2 2	1.67 1 1	2 2 1 1 1 1	1.33 1 1	0 0 0 0 0 0	0.00 0 0
328	1 1 1 0 0 0	0.50 1 1	1 1 1 2 2 1	1.33 1 1	0 0 0 0 0 0	0.00 0 0
329	3 3 2 3 3 3	2.83 1 1	3 3 3 3 3 3	3.00 1 1	1 1 1 2 3 3	1.83 1 1
330	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
331	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
332	0 0 0 0 0 0	0.00 0 0	2 2 1 1 1 1	1.33 1 1	0 0 0 0 0 0	0.00 0 0
333	2 2 2 2 1 1	1.67 1 1	1 1 1 1 1 1	1.00 1 1	0 0 1 1 0 0	0.33 0 1
334	3 3 1 1 1 0	1.50 1 1	1 1 0 1 1 1	0.83 1 1	0 0 0 0 0 0	0.00 0 0
335	2 2 2 1 1 1	1.50 1 1	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0
336	2 2 2 2 2 2	2.00 1 1	2 2 2 2 2 2	2.00 1 1	0 0 0 0 0 0	0.00 0 0
337	0 0 0 0 0 0	0.00 0 0	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0
338	0 0 0 0 0 0	0.00 0 0	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0
339	1 1 1 1 1 1	1.00 1 1	1 1 1 1 1 1	1.00 1 1	1 1 1 1 0 0	0.67 1 1
340	0 0 0 0 0 0	0.00 0 0	1 1 0 0 0 0	0.33 1 1	0 0 0 0 0 0	0.00 0 0
341	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
342	0 1 1 1 1 1	0.83 1 1	1 1 1 1 0 0	0.67 1 1	0 0 0 0 0 0	0.00 0 0
343	0 0 0 0 1 1	0.33 0 1	1 1 1 2 1 1	1.17 1 1	0 0 0 0 0 0	0.00 0 0
344	2 1 2 2 2 2	1.83 1 1	1 1 1 1 1 1	1.00 1 1	1 1 1 0 0 0	0.50 1 1
345	2 2 2 2 2 2	2.00 1 1	2 2 2 2 2 2	2.00 1 1	0 0 1 1 1 1	0.67 0 1
346	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
347	0 0 0 0 0 0	0.00 0 0	0 0 0 0 1 0	0.17 0 1	0 0 0 0 0 0	0.00 0 0
348	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
349	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
350	0 0 0 0 0 0	0.00 0 0	1 1 1 1 1 2	1.17 1 1	0 0 0 0 0 0	0.00 0 0
351	1 1 1 1 1 1	1.00 1 1	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0
353	0 0 0 0 0 0	0.00 0 0	1 1 1 0 0 0	0.50 1 1	0 0 0 0 0 0	0.00 0 0
354	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
355	0 0 0 0 0 0	0.00 0 0	1 1 1 1 1 1	0.67 1 1	0 0 0 0 0 0	0.00 0 0
356	0 0 0 0 0 0	0.00 0 0	1 1 0 0 0 0	0.33 1 1	0 0 0 0 0 0	0.00 0 0
357	1 1 1 0 0 0	0.75 1 1	1 1 1 0 0 0	0.75 1 1	0 0 0 0 0 0	0.00 0 0
358	0 0 0 0 0 0	0.00 0 0	0 1 1 1 1 2	1.00 1 1	0 0 0 0 0 0	0.00 0 0
359	0 0 0 0 0 0	0.00 0 0	1 1 1 1 1 1	1.00 1 1	1 1 1 1 1 1	1.00 1 1
360	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0	0 0 0 0 0 0	0.00 0 0
469	1 1 1 1 1 1	1.00 1 1	2 2 2 2 1 1	1.67 1 1	0 0 0 0 0 0	0.00 0 0
470	2 2 2 2 2 1	1.83 1 1	1 1 1 1 1 1	1.00 1 1	0 0 0 0 0 0	0.00 0 0

ID	CENTRO HV			MEAN	P1	P3	MACROPHAGE			MEAN	P1	P3	BILIARY PRO.			MEAN	P1	P3
601	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
602	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
603	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
604	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
605	1	1	1	1.00	1	1	1	1	1	0.67	1	1	0	0	0.00	0	0	
606	3	3	3	3.00	1	1	2	2	2	2.00	1	1	1	1	1.00	1	1	
607	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
608	0	0	0	0.00	0	0	0	1	1	0.67	0	1	0	0	0.00	0	0	
609	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
610	0	0	0	0.00	0	0	1	1	2	1.67	1	1	1	1	0.83	1	1	
611	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
612	0	0	0	0.00	0	0	0	1	0	0.67	1	1	0	0	0.00	0	0	
613	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
614	1	1	1	1.00	1	1	1	1	1	1.00	1	1	0	0	0.17	0	1	
615	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
616	1	1	1	0.67	1	1	1	1	1	0.67	1	1	0	0	0.00	0	0	
617	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
618	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
619	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
620	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
621	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
622	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
623	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
624	0	0	0	0.00	0	0	1	1	1	0.83	1	1	0	0	0.00	0	0	
625	1	1	1	1.00	1	1	0	0	1	0.67	1	1	0	0	0.00	0	0	
626	3	3	3	3.00	1	1	3	2	2	2.17	1	1	0	0	0.00	0	0	
627	1	1	1	0.83	1	1	0	0	0	0.00	0	0	0	0	0.00	0	0	
628	1	1	1	1.00	1	1	1	1	1	1.00	1	1	0	0	0.00	0	0	
629	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
630	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
631	1	1	0	0.83	1	1	1	1	0	0.33	1	1	0	0	0.00	0	0	
632	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
633	1	1	1	1.00	1	1	1	1	1	1.00	1	1	0	0	0.00	0	0	
634	0	0	0	0.00	0	0	1	0	0	0.17	1	1	0	0	0.00	0	0	
635	3	3	3	2.67	1	1	2	2	3	2.67	1	1	0	0	0.33	0	1	
636	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
637	1	1	1	1.00	1	1	1	1	1	1.00	1	1	0	0	0.00	0	0	
638	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
639	0	0	0	0.00	0	0	0	0	1	0.33	0	1	0	0	0.00	0	0	
640	1	1	1	1.00	1	1	1	1	1	1.00	1	1	0	0	0.00	0	0	
641	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
642	0	0	0	0.17	0	1	1	1	0	0.50	1	1	0	0	0.00	0	0	
643	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
644	1	1	1	1.00	1	1	0	0	1	0.67	0	1	0	0	0.00	0	0	
645	0	0	1	0.67	0	1	0	0	0	0.00	0	0	0	0	0.00	0	0	
646	1	1	1	1.00	1	1	1	1	0	0.67	1	1	0	0	0.00	0	0	
647	1	1	1	0.67	1	1	0	0	0	0.00	0	0	0	0	0.00	0	0	
648	0	0	0	0.00	0	0	0	0	0	0.00	0	0	1	1	1.00	1	1	
649	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
650	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	
651	2	2	2	2.00	1	1	1	1	2	1.67	1	1	0	0	0.33	0	0	
652	0	0	0	0.00	0	0	0	0	1	0.50	0	1	0	0	0.00	0	0	
653	0	0	0	0.00	0	0	0	0	0	0.00	0	0	0	0	0.00	0	0	

TABLE 21

IMMUNOHISTOCHEMISTRY DATA - 1991 (see following 2 pages)

I.D.	Individual fish identification number. In the multi-year archive, and in all sample labels these numbers are all prefaced with 91-
STN	Station number see Table 2 and Figure 1
HN1 to HN5	Number of hepatocytes per 1/5 of unit grid (5 replicates)
HN M	Mean of HN1 to HN5 multiplied by 5
BH1 to BH5	Number of BrdU positive hepatocyte nuclei per unit grid (5 replicates).
MBH	Mean of BH1 to BH5
LIHEP	Hepatocyte labelling index (MBH divided by HN M)
BN1 to BN5	Number of BrdU positive non-hepatocyte nuclei per unit grid
LI N	Non-hepatocyte labelling index (Mean of BN1 to 5 divided by HN M)
VC+	Number of BrdU positive vacuolated cells counted
VA TO	Total number of vacuolated cells counted
VLI	Vacuolated cell labelling index (VC+ divided by VA TO)
CYPIA	Cytochrome P450IA labelling indices: 0 = absent, to 4 = strong.
L	CYPIA labeling index for hepatocytes
G	CYPIA labeling index for gut epithelia
K	CYPIA labeling index for kidney epithelia

IMMUNOHISTOCHEMISTRY DATA - MWRA 1991

STN I.D.	HN1	HN2	HN3	HN4	HN5	HN M	BH1	BH2	BH3	BH4	BH5	MBH	L IHERB N1	BN2	BN3	BN4	BN5	LN	VC+	VA TO	VLI	CYPLA			
																						L	G	K	
1	460	DIED BEFORE ADEQUATE BDU UPTAKE																							
1	461 B	205	253	228	262	274	1222	0	0	0	2	0.4	0.33	0	0	2	0	0	0.33	0	1000	0.00	1	1	1
1	462 B	291	299	271	307	258	1426	1	1	0	0	0.6	0.42	0	0	2	2	2	0.84				2	1	1
1	463 B	215	180	260	181	271	1107	0	1	0	0	0.4	0.36	1	2	0	0	1	0.72	15	834	17.99	1	1	1
1	464 B	171	254	84	223	143	875	0	2	0	0	0.4	0.46	3	0	1	7	7	4.11	4	502	7.97	4	2	4
1	466 B	234	242	207	243	282	1208	1	0	0	1	0.4	0.33	0	1	0	0	1	0.33				3	2	2
1	467 B	214	267	250	265	233	1229	0	0	0	0	0	0.00	0	0	1	0	0	0.16	3	561	5.35	3	2	2
1	468 B	311	266	267	342	249	1435	0	0	0	0	0	0.00	0	2	0	0	0	0.28	1	595	1.68	3	1	4
2	469 B	241	218	214	284	221	1178	0	0	2	0	0.5	0.42	0	0	2	0	3	0.85	0	400	0.00	1	1	1
2	470 B	178	153	226	324	213	1094	0	0	0	1	0.2	0.18	0	1	0	0	1	0.37	1	621	1.61	3	1	2
2	471 B	240	261	244	272	250	1267	1	1	0	0	0.4	0.32	2	3	5	4	2	2.53	0	450	0.00	3	1	2
2	472 B	200	249	272	294	302	1317	0	0	1	0	0.6	0.46	0	1	1	0	0	0.30				2	1	2
2	473 B	366	236	242	207	188	1239	0	0	0	1	0.2	0.16	0	1	0	0	0	0.16				1	1	2
2	474 B	218	230	249	178	122	997	0	1	0	0	0.4	0.40	0	0	0	0	0	0.00				1	1	3
2	475 B	262	313	278	174	305	1332	0	0	0	0	0	0.00	2	1	1	0	1	0.75				1	1	2
2	476 B	249	242	246	201	258	1196	0	0	2	0	0.6	0.50	1	2	0	0	1	0.67				3	3	1
2	477 B	245	314	188	240	320	1307	0	0	0	0	0	0.00	0	0	1	6	2	1.38				3	0	2
2	478 B	328	213	226	286	245	1298	0	0	0	0	0	0.00	0	0	1	0	0	0.15				3	1	2
4	450 B	94	85	101	115	96	491	5	2	2	0	1.8	3.67	2	1	2	1	0	2.44				3	3	1
4	451 B	182	202	191	227	187	989	0	0	0	0	0	0.00	0	0	1	1	0	0.40				3	2	2
4	452 B	154	150	147	135	142	728	0	0	0	0	0	0.00	2	0	1	0	1	1.10				3	1	2
4	453 B	211	208	302	235	210	1166	0	0	0	0	0	0.00	1	0	0	0	0	0.17				4	2	3
4	454 B	210	196	235	141	226	1008	1	1	1	0	0.8	0.79	0	0	1	0	0	0.20				3	1	1
4	455 B	210	181	159	167	152	869	1	0	0	1	0.4	0.46	0	1	1	1	1	0.92				3	2	2
4	456 B	244	192	231	268	175	1110	1	1	2	6	2.2	1.98	0	5	3	3	2	2.34	11	585	18.80	3	2	4
4	457 B	272	282	190	152	167	1063	2	7	3	4	3.6	3.39	5	6	5	5	3	4.52				3	1	1
4	458 B	200	184	215	189	278	1066	0	0	1	0	0.2	0.19	0	0	0	0	0	0.00				3	1	1
4	459 B	183	170	231	191	148	923	0	2	0	0	0.6	0.65	2	1	3	0	0	1.30				2	1	1

IMMUNOHISTOCHEMISTRY DATA - MWRA 1991

STN I.D.	HN1	HN2	HN3	HN4	HN5	HN M	BH1	BH2	BH3	BH4	BH5	MBH	LIHEFBN1	BN2	BN3	BN4	BN5	LIN	VC+	VA TO	VLI	CYPIA		
5	583 B	148.6	133.2	172.6	144	139	147					4.18	0.60					0.41				2	1	2
5	585 B	119.6	127	118.4	107	93	113					1.12	13.80					1.76				2	2	2
5	586 B	240	300	300								0.70	0.7					2.14				2	3	2
5	587 B	176.2	165.2	174.4								0.82	0.82					1.35				2	2	x
5	588 B	79	89	97								4.80	4.8					3.4				2	2	x
5	590 B	92.6	105.6	84								0.42	0.42					0.35				3	2	x
5	591 B	280	272.4	257.6								0.00	0					0.62				3	2	2
5	592 B	111.4	129.2	143								0.50	0.5					0.26				2	1	2
5	593 B	87	92.4	103.4								5.50	5.5					2.12				2	2	3

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