EA Report HRF41A

A STUDY OF THE OCCURRENCE OF LIVER CANCER IN ATLANTIC TOMCOD (MICROGADUS TOMCOD) FROM THE HUDSON RIVER ESTUARY

FINAL REPORT

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1. INTRODUCTION

The relationship between environmental contamination and the occurrence of disease in fish has received considerable attention in recent years, both in the scientific literature (e.g., Sindermann 1979; Kraybill et al. 1977; Murchelano 1982) and popular press (e.g., Begley 1984; Anonymous 1984). Of particular concern have been the reports of high prevalence of neoplasia in feral fish populations from various contaminated sites throughout North America, including the Great Lakes and tributaries, Puget Sound, and the lower Hudson River estuary. Current research is focusing on the uses of potentially contamination-related diseases such as neoplasia as measures of environmental degradation and possible human health risk (Sloof 1983; Sonstegard 1976; Dawe and Harshberger 1969). However, despite ongoing interest in this area, the prevalence of neoplasia has been fully explored for only a few species in a limited number of localities, and the relationship between these diseases and associated environmental contamination has yet to be fully established.

The lower Hudson River estuary, and especially New York Harbor, is a highly impacted environment presently receiving numerous waste products from the upper Hudson River and tributary streams as well as direct discharge from the densely populated New York metropolitan region. These waste products include potentially toxic chemicals such as heavy metals, pesticides (e.g., DDT, dieldrin), polychlorinated biphenyls (PCBs), and polynuclear aromatic hydrocarbons (PAHs). Many of these contaminants are not particularly water soluble and tend to concentrate in sediments. Biological activity results in the passage of these compounds through the food chain where they tend to accummulate in fatty tissues including livers and gonads. Despite the confirmed existence of these contaminants, little is known on possible effects of these contaminants on those fish species which inhabit the New York Harbor complex, as well as those passing through the harbor area on their way to and from upper estuary spawning and nursery grounds.

The Atlantic tomcod (Microgadus tomcod) is a common benthic inhabitant of the lower Hudson River estuary. This small species is the only anadromous gadid ranging along the Atlantic Coast of North America northward to Labrador. The Hudson River appears to have the southern most spawning population. Spawning occurs in the middle reaches of the Hudson River estuary during the early winter months (McLaren et al. In Press). Young tomcod inhabit lower estuarine nursery areas, including New York Harbor, from late spring into fall. There the tomcod are exposed to a host of environmental contaminants input from tributary streams, as well as direct discharge from the densely populated New York metropolitan region. In these nursery areas, growth rates are rapid during spring and fall, and reduced during summer when water temperatures approach lethal levels. Juvenile tomcod average 50-80 mm at the beginning of summer and 175-200 mm at the end of the year. In the Hudson River, the first spawning population is comprised principally of individuals 11-13 months old. Older individuals (Ages 2+) comprise at most 5-10 percent of the adult stock.

This species has been the focus of a large scale environmental impact assessment program related to electric power generation in the Hudson River since 1973. Incidental observations during routine laboratory processing of the species revealed large numbers of individuals with enlarged or abnormal livers,

which under histological examination proved to be neoplastic or hepatocellular carcinoma (Smith et al. 1979). Subsequent observations continued to document the relatively high prevalence of these hepatomas in Hudson River tomcod, as well as to identify relatively high levels of polychlorinated biphenyls (PCBs) in selected tissues (Klauda et al. 1981).

In this report, EA summarizes the results of a year-long epidemiological survey of hepatomas in Atlantic tomcod from the Hudson River estuary. Specific objectives for this study were as follows:

1. To estimate the prevalence of hepatomas in adult Atlantic tomcod collected during the spawning period in the Hudson River estuary.

2. To investigate possible relationships between hepatoma occurrence at the size, age, sex, and overall condition of these tomcod adults.

3. To determine if neoplastic or pre-neoplastic lesions are evident in juvenile tomcod collected for lower Hudson River nursery areas.

4. To compare the prevalence of hepatomas in tomcod from the Hudson River to individuals collected from a relatively uncontaminated site in eastern Connecticut.

5. To document the occurrence of selected chemical contaminants in tomcod livers and to relate them to reported occurrences in the lower Hudson River.

6. To relate variations in liver concentrations of selected chemical contaminants to observed liver pathologies.

7. To explore ultrastructural changes in Atlantic tomcod livers preceding and coincident with hepatoma formation.

The results of this study as related to the first six objectives are included in the main body of the report. Studies related to the final objective were conducted independently and are included as a separate report in Appendix A.

2. MATERIAL AND METHODS

2.1 FIELD COLLECTIONS

Adult Atlantic tomcod were collected from the Hudson River estuary, principally from spawning areas near Garrison, New York (Figure 2-1), during the period 20 December 1983 through 7 February 1984. These individuals were caught in unbaited 1 m X 1 m X 2 m box traps set along a shoreline bulkhead. During this period, a small number of tomcod were also recovered from intake screens at the Roseton Generating Station north of Newburgh, New York. In addition, a small number of post-spawn individuals were collected by otter trawl in the lower estuary near Yonkers, New York, and the Tappan Zee Bridge on 16 and 19 March 1984.

To assess the possible occurrence of liver anomalies prior to maturity, young-of-the-year Atlantic tomcod were collected from the lower Hudson River estuary. Samples were collected using a 5-m head-rope otter trawl in Haverstraw Bay on 24 July 1984 and in New York Harbor on 11 October 1984.

In addition, adult Atlantic tomcod were collected from the Pawcatuck River estuary, principally from spawning areas near Westerly, Rhode Island (Figure 2-1), during the period 28-30 December 1984. This relatively unpolluted estuary was selected to provide control specimens for comparison to the Hudson River samples. All individuals were collected in unbaited 1 m X 1 m X 2 m box traps set along a shoreline bulkhead on the Connecticut side of the river.

2.2 LABORATORY PROCESSING

All tomcod collected were returned live to the laboratory for processing. Each individual selected was sacrificed, measured, sexed, and its liver and gonad were removed. The somatic weight of each fish was then determined and an otolith was removed for aging.

The livers of all fish were examined visually and placed into one of three categories and weighed. Class 1 livers included those which appeared normal. Class 2 livers included small (1-5 mm) clear or light grey lesions on the surface. Finally, Class 3 livers had larger, dark-colored lesions of varying size.

For the adults collected from the Hudson River, fish from the three gross pathological categories were then further divided into the following discrete age/length strata: Age 1 and <150 mm TL, Age 1 and >150 mm TL, and Age 2+. These strata were defined a priori to permit evaluation of age and length effects on liver condition and chemical contamination. Liver tissue samples from approximately 50 apparently normal and 10 of each type of lesion were randomly selected from each age/length strata for subsequent histological examination. The balance of the liver tissue within each of the nine groups was frozen for subsequent chemical analyses. In addition, any observed anomalies in other tissues were also saved for histology. Liver tissue samples

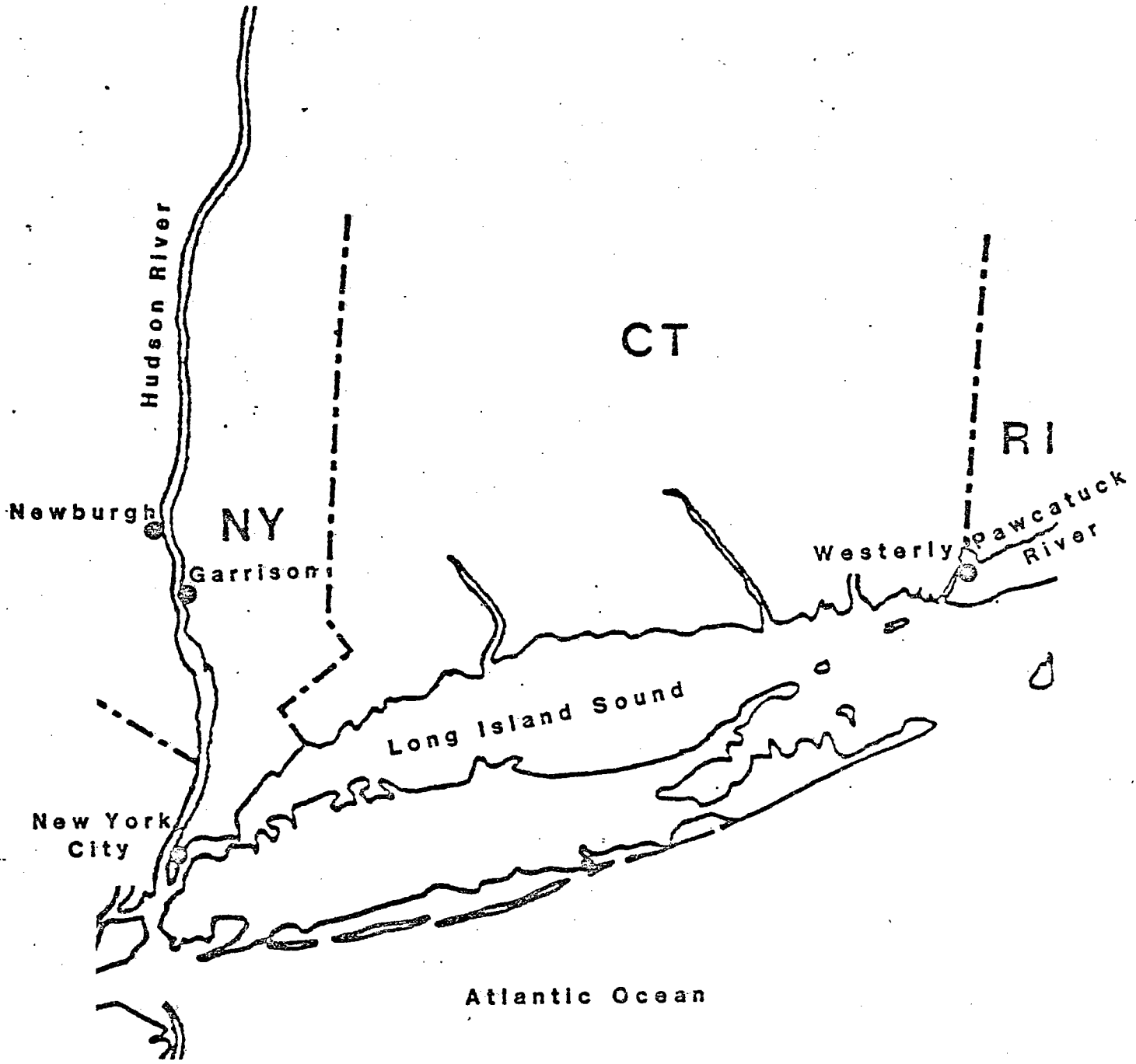


Figure 2-1. Sampling locations used for surveying hepatomas in Atlantic tomcod in 1983-1984.

from young-of-the-year tomcod from the Hudson River, as well as all controls from the Pawcatuck River, were preserved for subsequent histological and chemical analyses.

2.3 HISTOLOGICAL EVALUATION

All histological specimens were fixed in Bouin's solution for 24 hours and then transferred to 65 percent ethanol. Tissues were then dehydrated, embedded in paraffin, sectioned at 5 um, and stained with hematoxylin and eosin. Generally, four sections were examined for each tissue specimen. These sections were then shipped to the U.S. Fish and Wildlife Service, Fish Culture Development Center, Bozeman, Montana for evaluation.

All sections were evaluated blind; that is, the histologist had no knowledge of the age, length, sex, or gross pathology of the tomcod from which the sections were obtained. This step was implemented to insure unbiased estimates of the prevalance of liver alterations.

The purpose of this histological evaluation was two-fold. First, this evaluation was to document histological changes associated with each class of observed lesion. Secondly, this evaluation was to provide an estimate of the prevalance of histological changes in liver tissues visually judged to be normal. These prevalance estimates were combined with prevalance estimates for observed lesions to estimate the overall prevalance of hepatomas in tomcod. However, since not every possible section from all livers were evaluated, any prevalance estimates based on our histological evaluations must be considered minimal.

2.4 CHEMICAL ANALYSES

Chemical analysis was conducted on specific liver tissue composites (Table 2-1) to assess occurrence of selected organic and inorganic contaminants within the tomcod liver tissue from the Hudson River. Liver samples were prepared for analyses following the methods of U.S. EPA (1980). Heavy metals, including arsenic, cadmium, total chromium, lead, mercury, and nickel were assessed using atomic absorption spectrophotometry (U.S. EPA 1979). Halogenated hydrocarbon pesticides and polychlorinated biphenyls (PCBs) were assessed using gas chromatography with electron capture (U.S. EPA 1982). Polynuclear aromatic hydrocarbons (PAHs) were assessed using gas chromatography/mass spectrometry. In addition to these analyses, a single sample with the greatest amount of liver tissue was assessed for U.S. EPA priority pollutant base/neutral and acid phenolic compounds using gas chromatography/mass spectrometry (U.S. EPA 1982). All results are reported as ppm wet weight. The results of these chemical analyses were compared to the age and length of the fish as well as the pathological condition of the liver tissue.

TABLE 2-1 SPECIFIC CHEMICAL ANALYSES CONDUCTED ON COMPOSITES OF LIVER TISSUE FROM ATLANTIC TOMCOD FROM THE HUDSON AND PAWCATUCK RIVER SYSTEMS

COMPOSITE:										
SOURCE	Hudson River									Pawcatuck
TIME PERIOD	DEC 1983 - MAR 1984								JUL 1984	DEC 1984
AGE	1					2-3			1	1-3
LENGTH (MM TL)	109-149		150-235			183-302			58-85	120-340
GROSS PATHOLOGY*	1	2	1	2	3	1	2	3	1	1
CHEMICAL ANALYSES:										
Metals	X	X	X	X	X	X	X	X		
Halogenated hydrocarbon pesticides	X	X	X	X	X	X	X	X	X	X
Polychlorinated biphenols	X	X	X	X	X	X	X	X	X	X
Polynucleated aromatic hydrocarbons	X	X	X	X	X	X	X	X	X	X
U.S. EPA priority pollutants	X									

* 1 = apparently normal
2 = small, clear lesions
3 = large, dark lesions.

3. RESULTS

A total of 564 adult Atlantic tomcod (460 Age 1, 102 Age 2, 1 Age 3, and 1 not aged) were collected from the Hudson River estuary during the winter spawning season of 1983-1984. Age 1 individuals ranged from 109 to 235 mm TL while Age 2+ individuals ranged from 183 to 302 mm (Figure 3-1). For both age groups, females tended to be longer than males. These length ranges are similar to those reported during previous spawning seasons (McLaren et al. In Press). Approximately 73 percent of Age 1 and 55 percent of Age 2+ were males reflecting the tendency of male tomcod to spend a greater time on the spawning grounds than females, and are, therefore, more likely to be collected.

In addition to the primary samples collected during the spawning season, 44 juvenile (Age 0) tomcod, ranging in length from 58-148 mm TL, were collected in lower estuary nursery areas during July and October 1984. Finally, 44 adult tomcod, 34 Age 1, 8 Age 2, and 2 Age 3, ranging in length from 120-340 mm TL, were collected from the Pawcatuck River control site.

3.1 GROSS PATHOLOGY

None of the livers from young-of-the-year tomcod collected from lower Hudson nursery areas nor any from adults collected in the Pawcatuck River exhibited any gross liver lesions. These visually normal livers varied widely in size and ranged in color from cream to dark red-brown in freshly-killed specimens. The livers formed a discreet organ with four distinct areas: two elongate-triangular dorsal extensions and two smaller median projections, one directed rosturally and the other caudally (Figure 3-2a). In contrast, the spawning population from the Hudson River exhibited a wide range of gross pathologies. More than 32 percent of Age 1 individuals had observable lesions on their livers. The majority of these (27 percent of total population) had small, clear, or light-grey lesions on the surface (Class 2). These lesions, which often occurred in large numbers, gave the appearance of clear, fluid-filled cysts embedded within the hepatic tissue (Figure 3-2b). A smaller number (5.5 percent of the total population) had large, dark-colored lesions of varying size which occasionally involved the entire liver (Class 3). These lesions often contained large necrotic areas and cystic structures filled with clear, dark, grey-green fluid (Figue 3-2c).

Age 2+ individuals exhibited hepatic lesions in 77.7 percent of the total catch, with 43.7 percent being categorized as Class 3, while 34 percent were categorized as Class 2. In addition to being more prevalent, the large lesions (Class 3) were typically much more advanced in Age 2+ individuals than in Age 1. All tumors appeared to be restricted to liver tissues for both ages of metastasis, or non-neoplastic lesions such as papillomas were not evident in any of the Hudson adults.

Within age groups, the prevalence of hepatic lesions appeared to be greatly influenced by the length of the fish (Figure 3-3). The prevalence of lesions in Age 1 individuals increased in an almost linear fashion from 11.5 percent of the small (<125 mm TL) Atlantic tomcod to more than 73 percent of the largest (>200 mm TL) tomcod. These increases were a result of increases in the frequency of both Class 2 and Class 3 lesions. A general increase in the frequency of hepatic lesions was also evident in Age 2+ individuals increasing

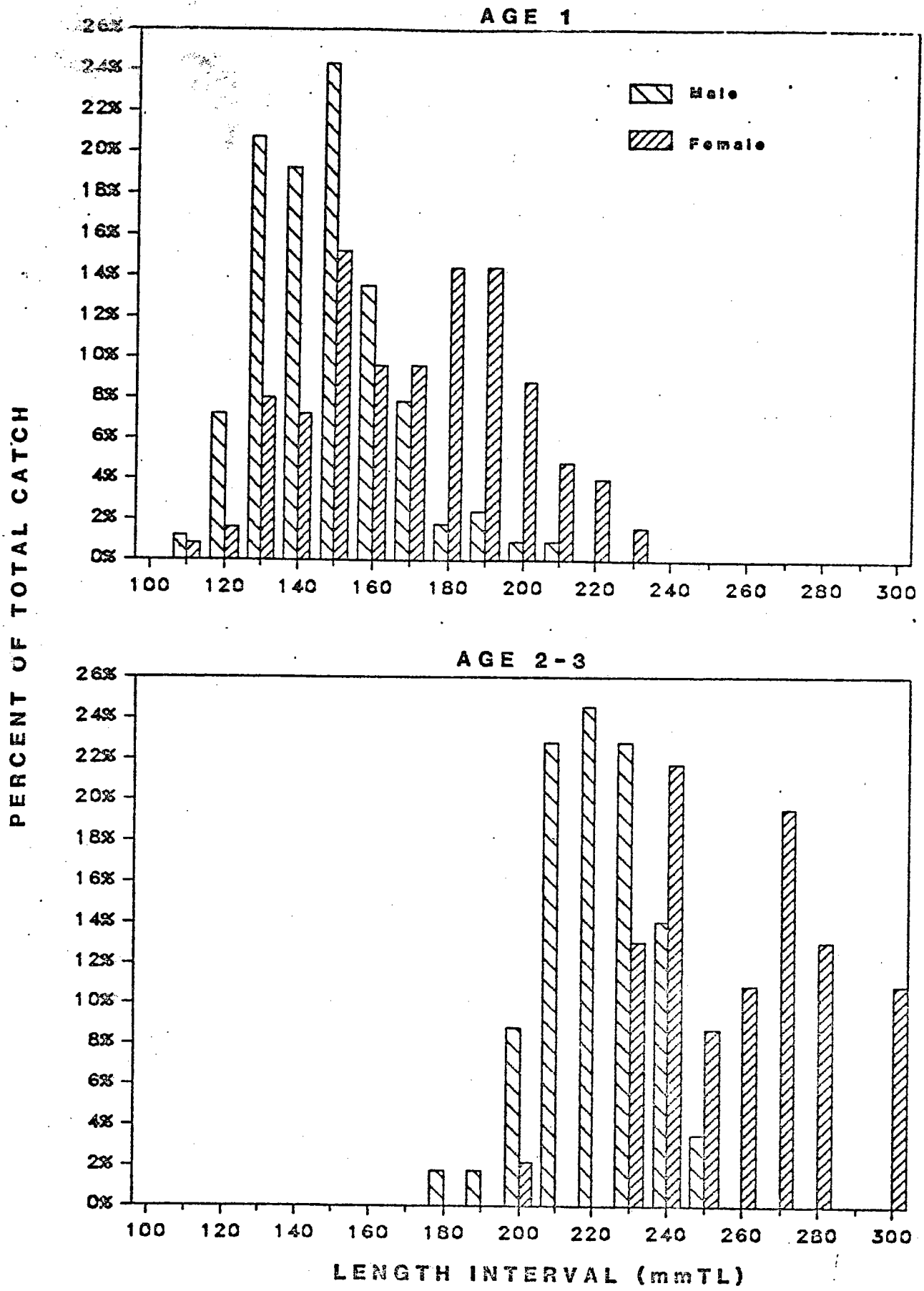


Figure 3-1. Length-frequency distribution of adult Atlantic tomcod collected from the Hudson River estuary, 1983-1984.

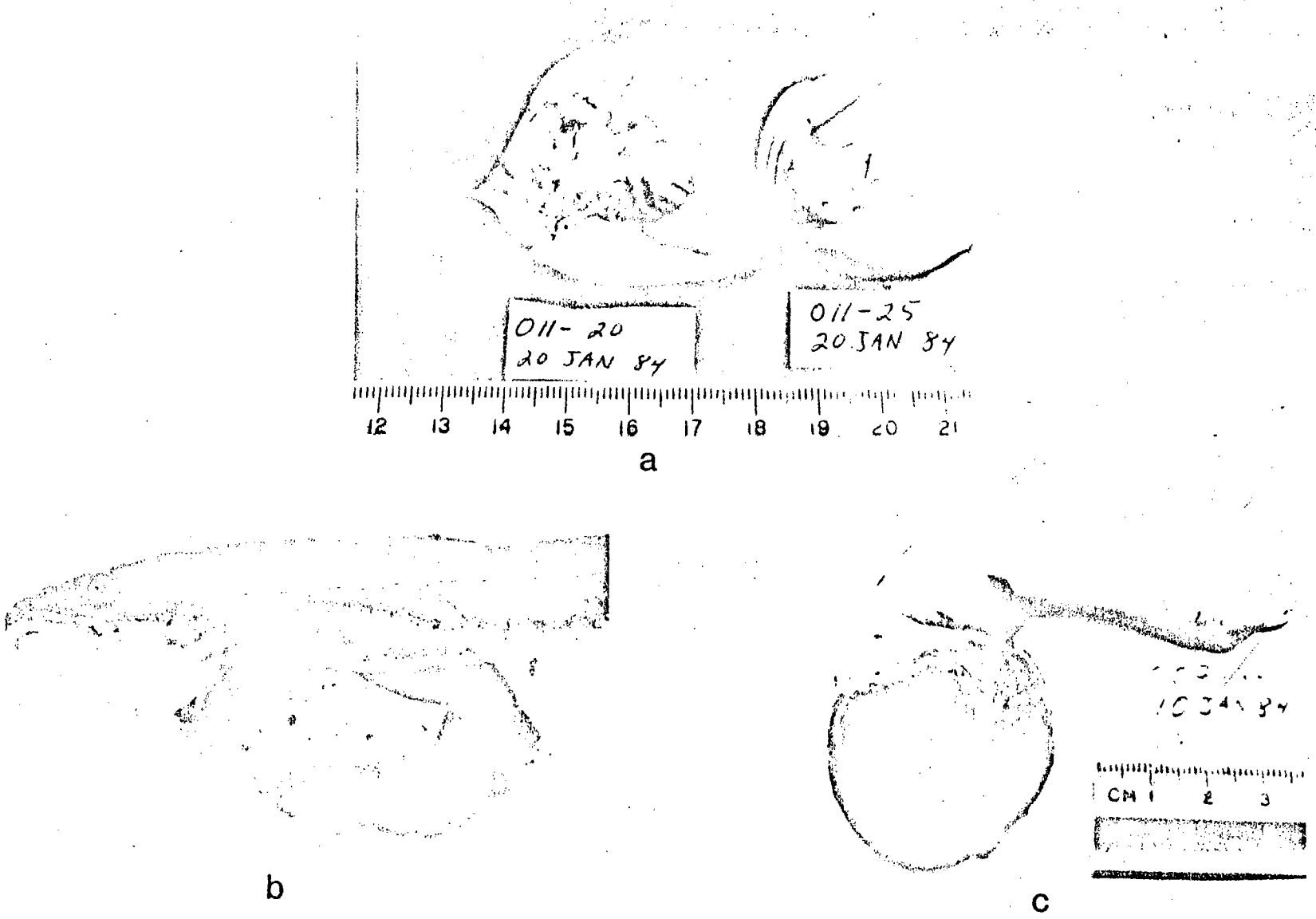


Figure 3-2 Liver pathologies observed in Atlantic tomcod from the Hudson River Estuary, 1983-1984. a. normal (Class 1) b. small clear lesions (Class-2) c. large dark lesions (Class 3)

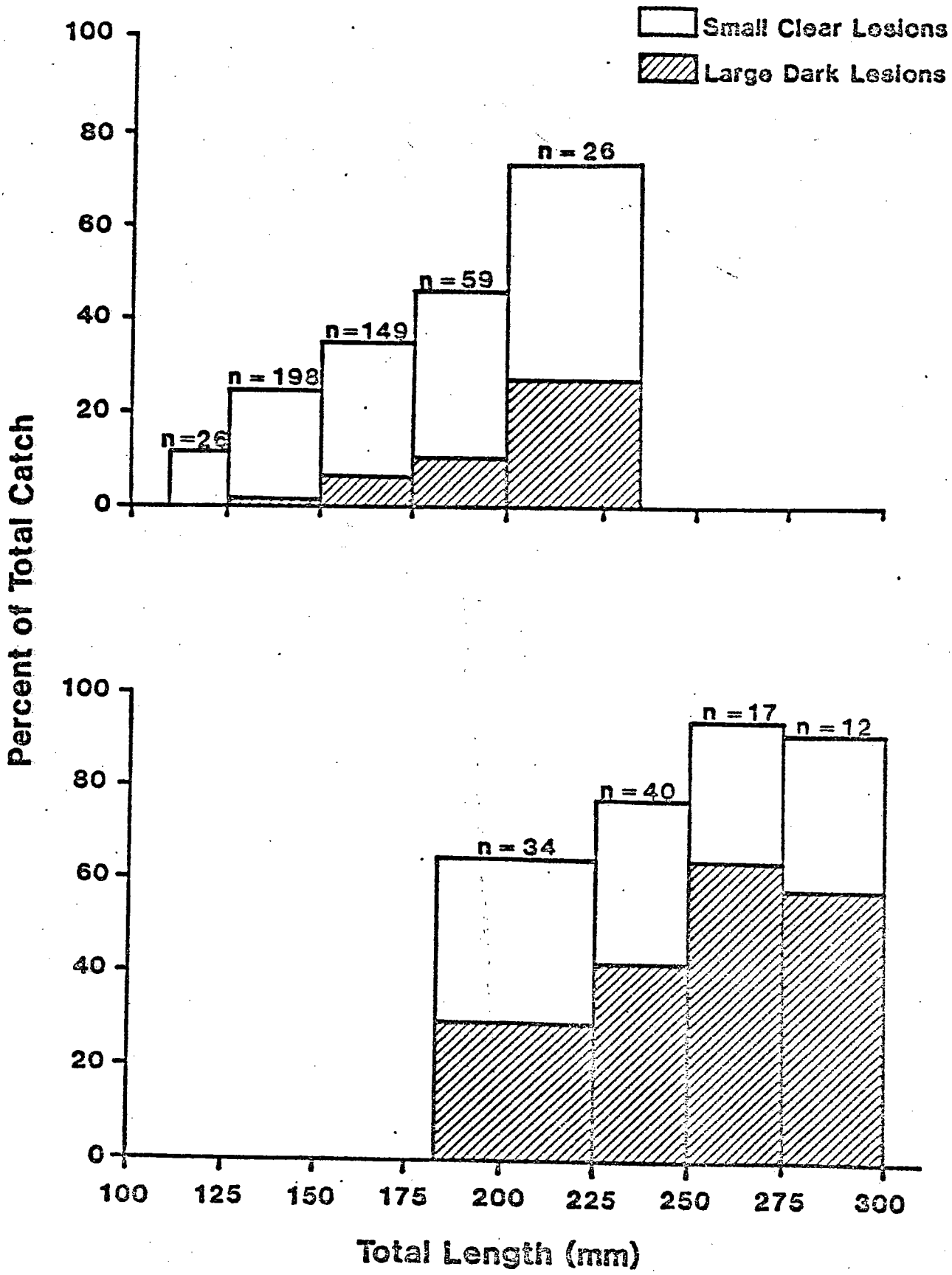


Figure 3-3. Relationship between the prevalence of liver lesions and age and length of Atlantic tomcod adults collected from the Hudson River estuary, 1983-1984.

from 64.7 percent in fish <225 mm TL to 94.1 percent in individuals from 250 to 274 mm TL. This increase was primarily produced by an increasing frequency of large lesions (Class 3). However, the relationship between hepatic lesions frequency and length did not appear as strong for Age 2+ individuals as it was for those Age 1.

The frequency of hepatic lesions was higher for females than for males for both age groups. However, when the two sexes were subset to have the same length distribution, the frequency of lesions was not significantly different (Age 1, X^2=0.15, P=0.7; Age 2+, X^2=1.18, P=0.3). This result indicates that the differences in overall lesion frequency between the two sexes was due to the generally larger size of females than males

In addition to the effects of age and length, the prevalence of lesions also depended upon the time of collection. For all age groups and length intervals, lesion prevalence increased from the pre-spawning period to the post-spawning period (Figure 3-4). Hepatic lesions were 10-30 percent more prevalent near the end of the spawning season than in the beginning. For Age 1 individuals, this apparent increase with time was primarily due to increases in the small, clear lesions (Class 2). While for Age 2+ individuals, this increase was more a result of increases in large lesions (Class 3).

Despite the occurrence of these lesions, which could on occassion involve almost the entire liver, the tomcod collected from the Hudson River appeared normal with no obvious signs of stress. Individuals with hepatic lesions appeared to mature normally with no apparent effects on size, gonad size, or fecundity. Only a single individual out of the entire collection had an obvious abnormal external appearance. This Age 2 female appeared severely emaciated with a distinct yellow cast to its skin. Upon dissection, the liver was found to be totally involved with no normal tissue apparent.

The liver somatic index (liver weight divided by somatic weight) exhibited considerable variation both within and between stocks (Figure 3-5). The index for Pawcatuck River fish averaged 2.7 percent of body weight with a total range from 1.4 to 5.7 percent. The livers from Hudson River adults were relatively larger as well as more variable, averaging 5.1 percent, and ranging from 1.9 to 15.1 percent of body weight. The liver somatic indexes from apparently normal (Class 1) adults from the Hudson River were significantly higher than the indexes from the Pawcatuck River (t=11.57, $P \leq 0.001$). Differences in the index among the three gross pathological classes of Hudson River fish were not significant although Class 3 individuals were slightly larger and clearly more variable than the other two classes.

3.2 HISTOPATHOLOGY

Microscopic examination of the liver from spawning adults from the Hudson River revealed a range of pathological changes similar to those previously described by Smith et al. (1979). The observed tissue changes appeared as a continuum, ranging from a pronounced basophilia of the cytoplasm indicating minor subcellular alteration to extensive hemorrhage and necrosis. There is general agreement that the areas of altered cytoplasmic staining, specifically the basophilic foci, are the earliest microscopic indicators of neoplastic change, and that the basophilic cell type is the common pathway to neoplasia in fish (Hendricks et al. 1984).

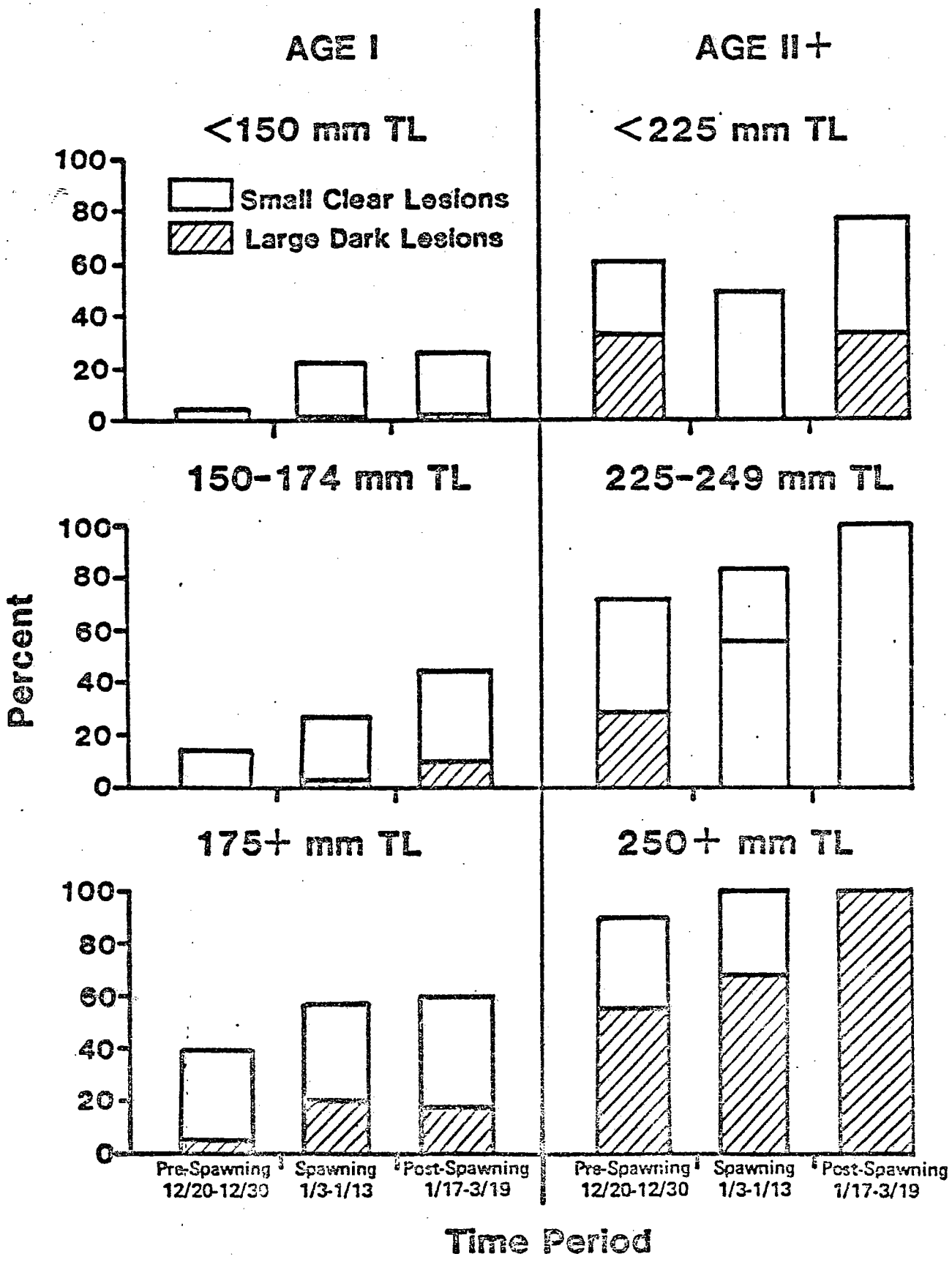


Figure 3-4. Relationship between prevalence of liver lesions and time of capture for adult Atlantic tomcod from the Hudson River estuary, 1983-1984.

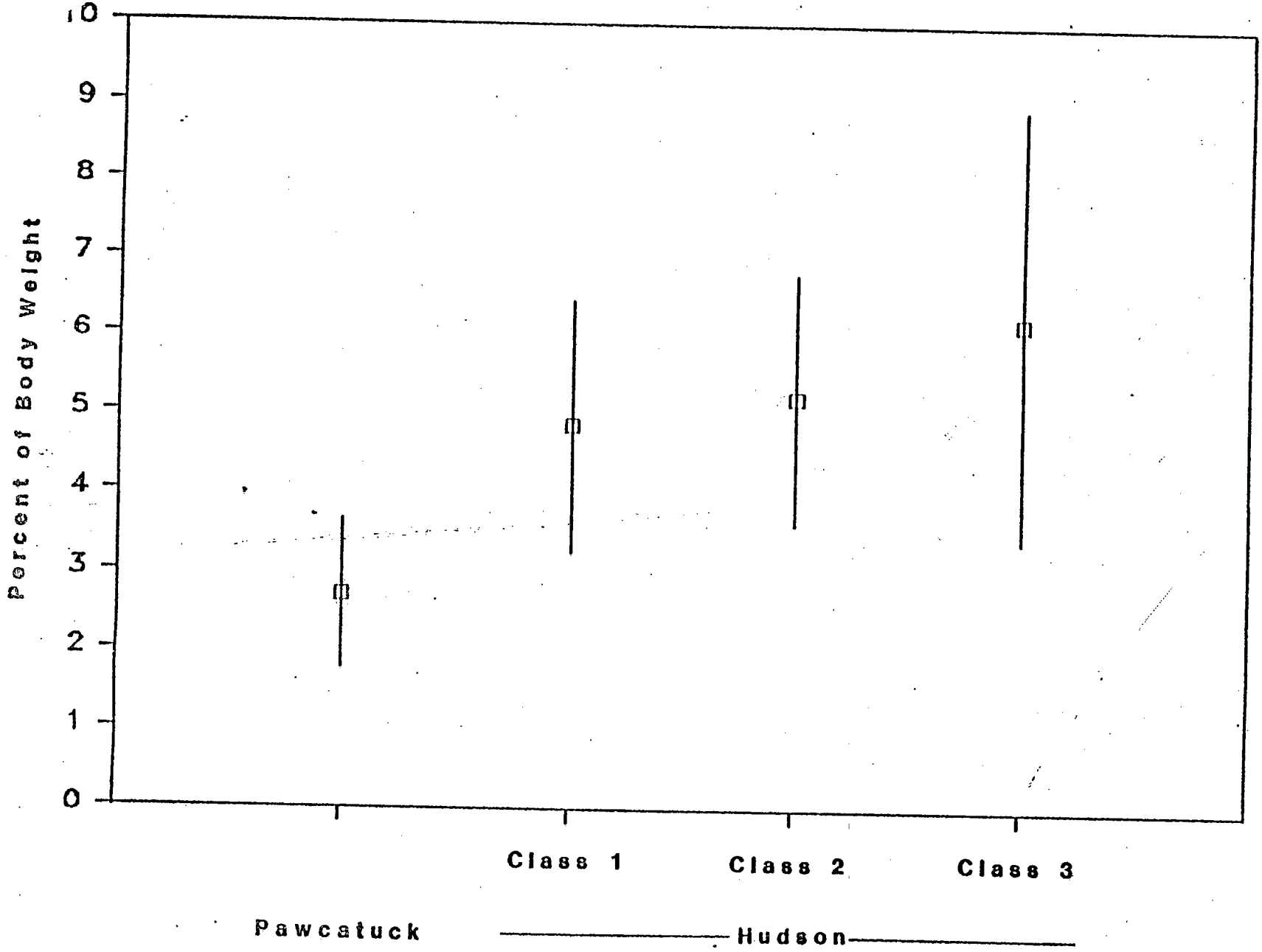


Figure 3-5. Mean liver-somatic index (±1 S.D.) for adult Atlantic tomcod collected from the Hudson River and Pawcatuck River estuaries, 1983-1984.

The cytoplasm of normal liver tissue appear vacuolated with the muralium duplex, or two-celled arrangement of the hepatic cords, evident (Figure 3-6a). For the purposes of the current study, the distinction between the basophilic focus and neoplastic nodule was based less on the size of the lesion than on individual cell size, the degree of continuity of the affected cells with the surrounding parenchyma, and the extent of nuclear change: enlargement, vesicularization, hyperchromaticity, and prominence of the nucleoli. Thus, the cells comprising a basophilic focus were minimally altered in terms of these criteria and the cytoplasm was usually vacuolated (Figure 3-6b). Neoplastic nodules typically contained hypertrophied cells with an enlarged, vesicular nucleus and a single swollen nucleolus. The cytoplasm was intensely basophilic and relatively non-vacuolated. Mitotic figures were uncommon. The muralium duplex, was more pronounced within the nodules than in the adjoining tissue, thereby serving to demarcate the area (Figure 3-6c).

Lesions designated as hepatocellular carcinomas were larger, and the contents less uniform. Focal to extensive areas of hemorrhage and necrosis were a common feature as were cystic areas containing eosinophilic debris (Figure 3-6d). The carcinoma conformed to the description of the trabecular type with varying degrees of anaplasia or deviation from normal appearance of the tumor cells and their arrangement. Muralia and sinusoids in the surrounding liver tissue were often compressed and arranged tangentially to the cords of tumor cells.

Liver sections with small, clear, or grey lesions (Class 2) typically contained neoplastic nodules and those with larger, darker-colored lesions (Class 3), hepatocellular carcinomas. Approximately 28 percent of the livers exhibiting no obvious macroscopic lesions were found to contain basophilic foci, and/or neoplastic nodules on microscopic examination.

In order to continue this histological information with the prevalent gross pathologies, each liver section was classified into one of the four categories: apparently normal, basophilic foci, neoplastic nodules, and hepatocellular carcinoma, according to the most advanced lesion observed.

Using this combined histology/gross pathology database, estimates of the prevalence of hepatomas (neoplastic nodules or hepatocellular carcinoma) ranged from 26 percent in small Age 1 tomcod to more than 86 percent in large Age 1 individuals (Figure 3-7). Among Age 2+ individuals, the prevalence of hepatomas ranged from 87 to 100 percent depending on length. For the Age 1 individuals, most of the hepatomas were classified as neoplastic nodules, whereas, the hepatomas were more evenly divided between neoplastic nodules and hepatocellular carcinoma for Age 2+ individuals. Weight average prevalence rates of hepatomas was 44.3 and 92.9 percent for Age 1 and Age 2+ individuals, respectively.

Microscopic examination of liver tissues from young-of-the-year individuals collected in the lower estuary revealed no apparent histological changes, while a similar examination of individuals collected from the Pawcatuck River revealed neoplastic nodules in 2 out of the 43 livers examined. One of these was Age 1 and one was Age 2, producing hepatoma prevalence of 3 percent for Age 1 and 10 percent for Age 2+ individuals.

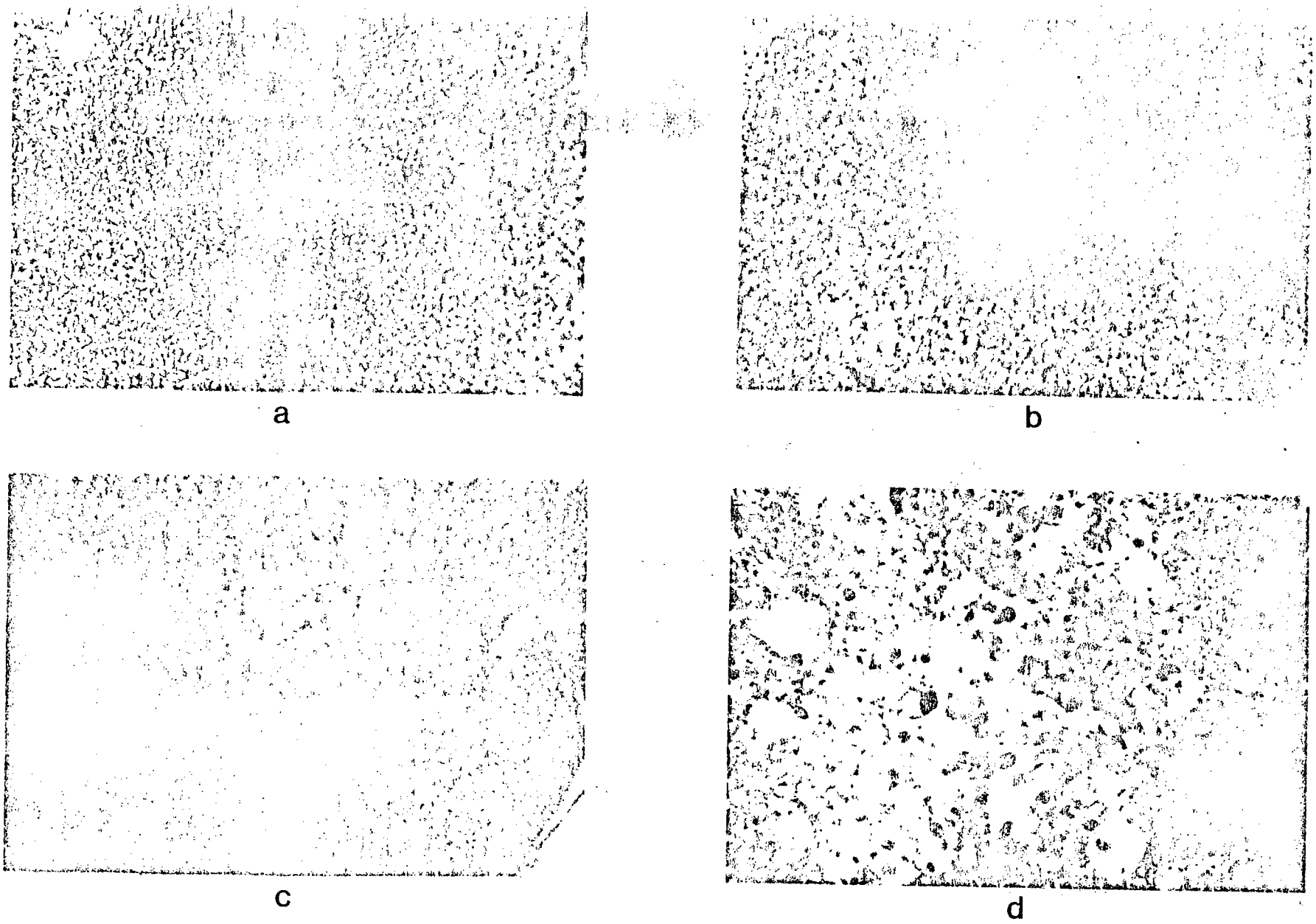


Figure 3-6 Histopathological categories used for evaluation of Atlantic tomcod livers.

a. normal b. basophilic focus c. neoplastic nodule d. hepatocellular carcinoma

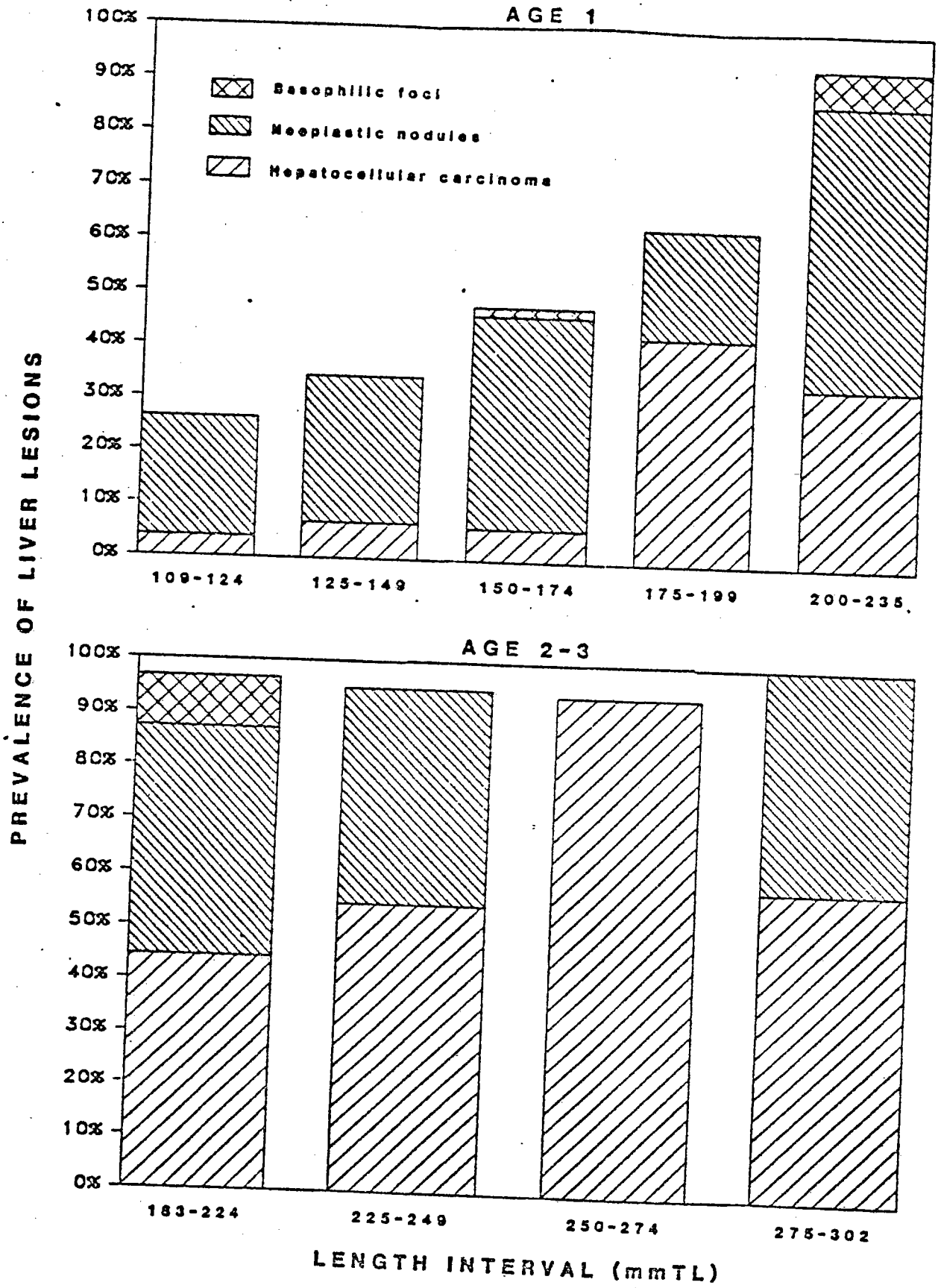
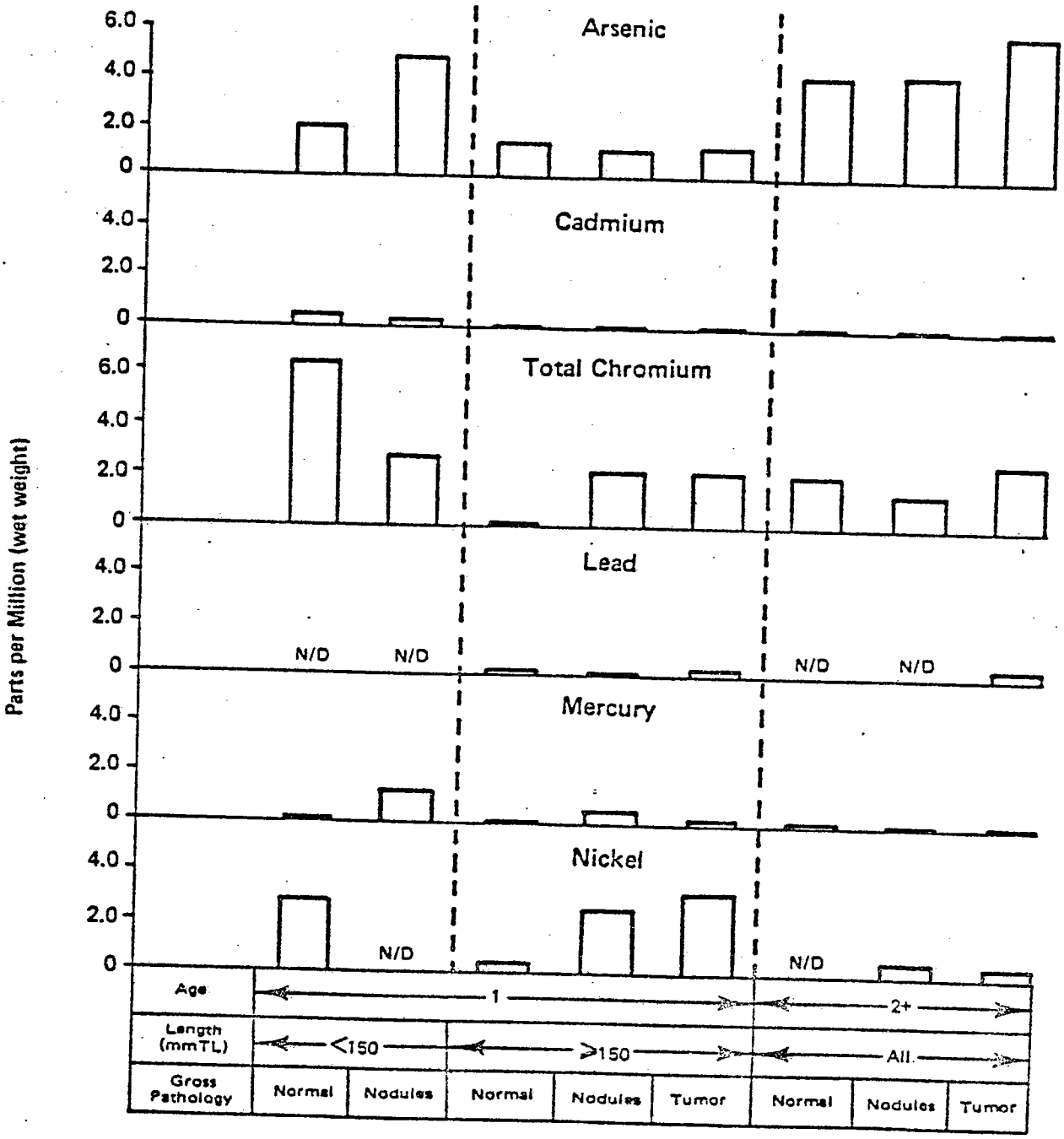


Figure 3-7. Relationship between liver pathology and length and age for adult Atlantic tomcod collected from the Hudson River estuary, 1983-1984.



Note: N/D indicates not detected.

Figure 3-8. Relationship between concentrations of selected metals and age, length, and liver pathology for adult Atlantic tomcod from the Hudson River estuary, 1983-1984.

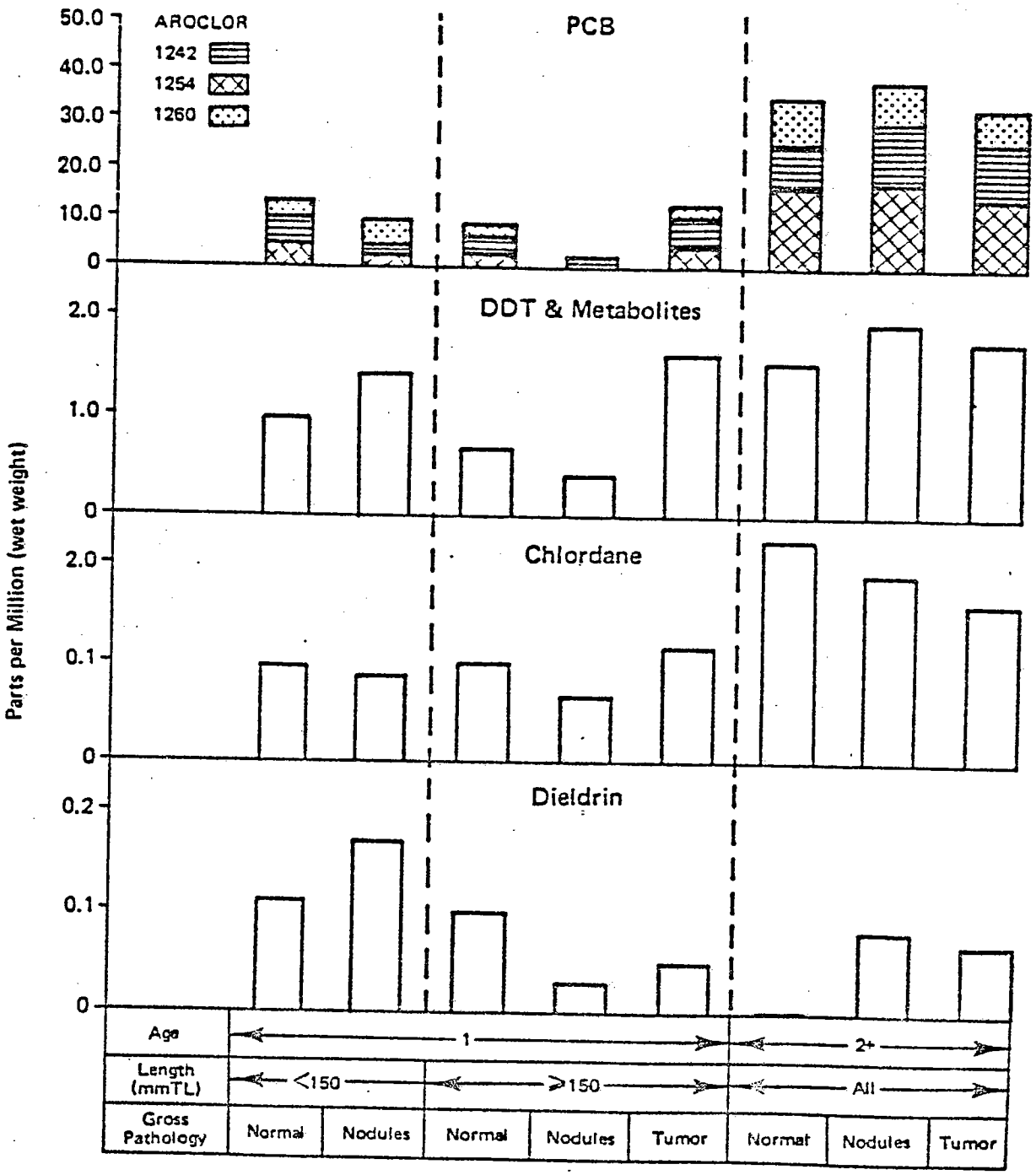


Figure 3-9. Relationship between concentrations of selected organics and age, length, and liver pathology for adult Atlantic tomcod from the Hudson River estuary, 1983-1984.

3.3 CHEMICAL ANALYSES

In addition to the pathological and histological examinations, composite liver tissue samples were analyzed for selected metal and oraganic contaminants. The purpose of this analysis was to identify chemicals which may be related to the observed tumor formation, and to assess any possible relationships between the concentration of observed chemical contaminants and gross liver pathology.

Each of the six selected metals as encountered in virtually all of the eight liver samples tested from the Hudson River. There was, however, no relationship apparent between the concentration of any of the metals and the age of the fish or gross pathology of the liver (Figure 3-8). Highest average concentrations were arsenic (3.1 mg/kg) and total chromium (2.5 mg/kg), and lowest were cadmium (0.1 mg/kg) and lead (0.2 mg/kg). Total chromium exhibited the highest, single liver concentration (6.5 mg/kg), and nickel concentrations were extremely variable ranging from <0.3 to 3.2 mg/kg.

Despite extensive, organic chemical analysis, including a complete U.S. EPA priority pollutant scan, only four groups of compounds were above detection limits in any of the composite liver samples. These four groups were chlordane, dieldrin, DDT and metabolites, and polychlorinated biphenols (PCBS). All groups with the exception of dieldrin, exhibited higher concentrations in older individuals, undoubtedly, a reflection of bioaccumulation (Figure 3-9). However, there was no apparent relationship between the concentration and any length group, gross liver pathology, and size within an age class among adult tomcod from the Hudson River.

PCBs consisting of Aroclors 1242, 1254, and 1260 exhibited the highest concentrations among each of the four groups encountered. Concentrations ranged from 2.5 to 38.2 mg/liter wet weight. Among Age 2+ liver composites, Aroclor 1254 predominated, although there was no consistent pattern among Aroclors for the younger adults. PCBs were also detected in relatively high concentrations (14 mg/kg) in livers from the juvenile tomcod collected from the lower Hudson River nursery areas. These PCBs were largely in the form of Aroclor 1254. Finally, a relatively low concentration (<0.1 mg/kg) of a single Aroclor (1254) was detected in the control specimens from the Pawcatuck River estuary.

DDT and metabolites, consisting of p,p′-DDE; p,p′-DDD; p,p′-DDT, and o,p′-DDD, ranged in concentrations from 0.5 mg/kg in Age 1 composites to 2.0 mg/kg in Age 2+ composites mg/kg. Most of this group consisted of the first two isomers. Also found was p,p′-DDE in relatively high concentrations in the juvenile fish collected from the lower Hudson River nursery areas and in relatively minute concentrations (<0.01 mg/kg) in the control fish from the Pawcatuck River estuary.

Chlordane, including alpha-chlordane, ranged in concentrations from 0.8 mg/kg in Age 1 composites to 2.8 mg/kg in Age 2+ composites from Hudson River adults. Chlordane was typically 3-5 times more abundant than alpha-chlordane in the liver samples. Chlordane was not detected in either the juvenile livers collected in the lower Hudson River nursery area, or the control liver samples from the Pawcatuck River estuary.

Dieldrin ranged in concentration from <0.01-0.17 mg/kg in adult livers from the Hudson River. This compound was also detected in intermediate concentrations in the juvenile liver samples collected in the lower Hudson River nursery areas. Dieldrin was not detected in livers from adult tomcod collected at the Pawcatuck site.

Polynuclear aromatic hydrocarbons (PAHs) were not detected in any of the composite samples at detection limits ranging from 0.1-0.6 mg/liter. In addition, no other U.S. EPA priority pollutants were detected in the single, liver composite analyzed. For this analysis, detection limits for individuals were generally less than 1 mg/kg.