Technical Memorandum NMFS-NWFSC-4

National Status and Trends Program

National Benthic Surveillance Project: Northeast Coast

Fish Histopathology and Relationships Between Lesions and Chemical Contaminants (1987-89)

December 1992

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

NOAA Technical Memorandum NMFS

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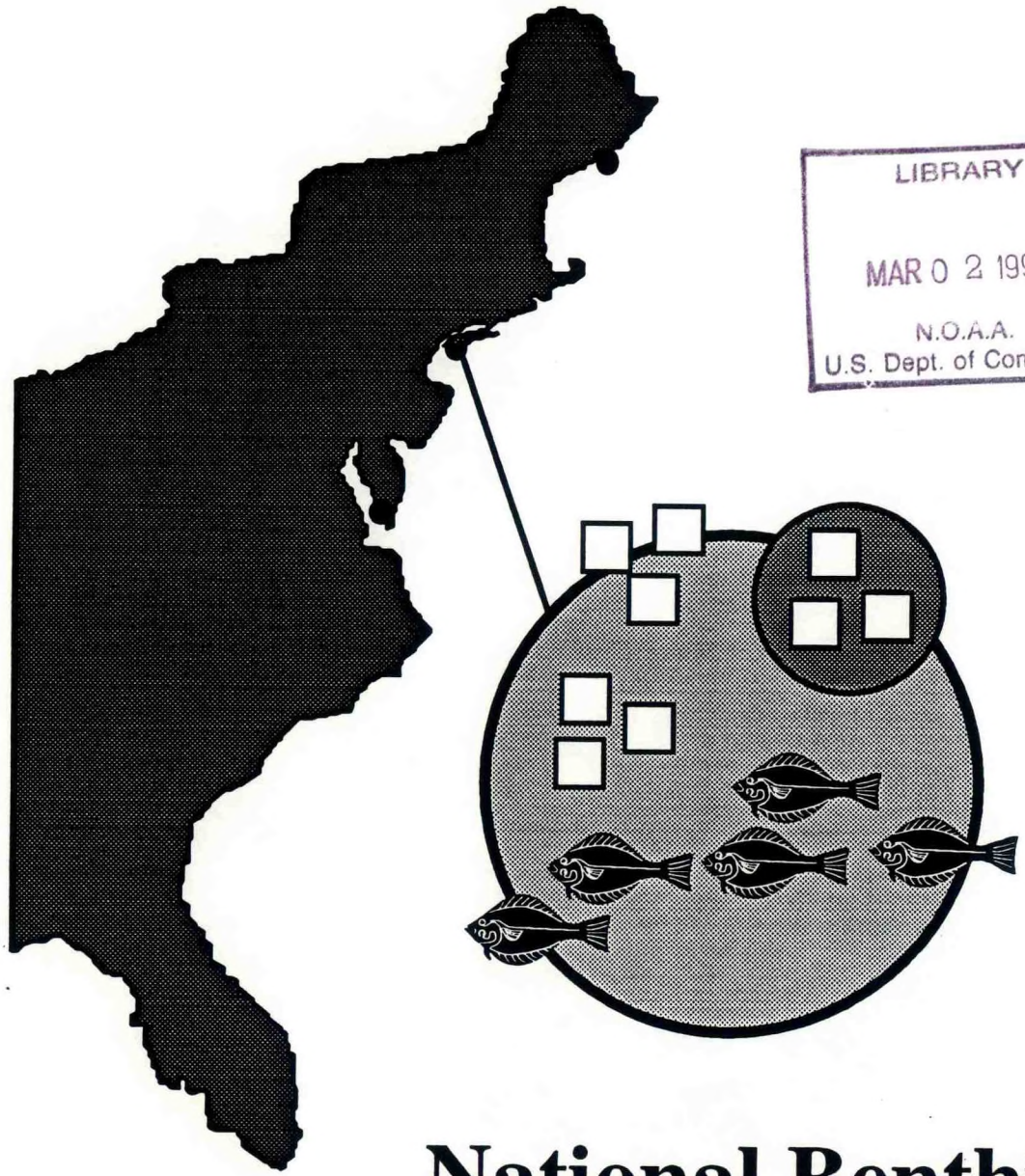
This document should be cited as follows:

Johnson, L. L., C. M. Stehr, O. P. Olson, M. S. Myers, S. M. Pierce, B. B. McCain, and U. Varanasi. 1992. National Status and Trends Program, National Benthic Surveillance Project: Northeast Coast, Fish histopathology and relationships between lesions and chemical contaminants (1987-89). U.S. Dep. Commer., NOAA Tech. Memo. NMFS-NWFSC-4, 96 p.

National Status and Trends Program



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Fish Histopathology and Relationships Between Lesions and Chemical Contaminants (1987-89)

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December 1992

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EXECUTIVE SUMMARY

This report presents and interprets the results of pathology studies conducted on winter flounder *(Pleuronectes americanus)* between 1987 and 1989 in conjunction with NOAA's National Status and Trends (NS&T) Program. In these studies, a variety of potentially contaminant-associated disease conditions were monitored in winter flounder collected from 22 Northeast Coast sites, and the relationship between disease occurrence and levels of organic chemical contaminants in sediment, stomach contents, and tissues was examined. Sampling was conducted primarily in spring of 1988 and 1989 (Cycles 5 and 6) as part of the National Benthic Surveillance Project (NBSP) of the NS&T Program, but to provide a more comprehensive view of pathological conditions in winter flounder from the Northeast Coast, data on winter flounder collected in Long Island Sound during 1987 and in Boston Harbor and adjacent embayments during the winter of 1988 were also included in this memorandum. In the three studies combined, a total of more than 1,500 fish were examined.

Embayments sampled included Salem Harbor, Boston Harbor, Massachusetts Bay, New Bedford Harbor, and Buzzards Bay in Massachusetts; Narragansett Bay in Rhode Island; Niantic Bay in Connecticut; several sites within Long Island Sound including New Haven, Norwalk, Bridgeport, and Rocky Point in Connecticut and Lloyd Point in New York; and Raritan Bay and Great Bay in New Jersey. The levels and types of chemical contaminants present in sediments from sampling sites within these embayments differed substantially. Among the most heavily contaminated sites were Mystic River and Quincy Bay in Boston Harbor, and Gravesend, West Reach, and East Reach sites in Raritan Bay. Sediments from these sites had elevated levels of all or most of the contaminants measured in this study, including aromatic hydrocarbons (AHs), polychlorinated biphenyls (PCBs), DDTs (DDT and its derivatives), and chlordanes. The New Bedford Harbor sampling site had very high concentrations of PCBs in sediments, but relatively low concentrations of other classes of contaminants. Lowest concentrations of nearly all classes of contaminants in sediment were found at the Plymouth Entrance site in Massachusetts Bay and at

the Rocky Point site in Long Island Sound. The Rocky Point site served as a reference site for analyses of intersite differences in lesion prevalences. Detailed information on concentrations of contaminants in sediments from these sampling sites will be reported in a forthcoming Technical Memorandum.

Winter flounder were examined for necrotic, sclerotic, and proliferative lesions in the kidney, fin erosion, and a number of pathological conditions in the liver, including:

1) neoplastic lesions (e.g., hepatocellular and cholangiocellular carcinomas, liver cell adenomas and cholangiomas);

2) putatively preneoplastic lesions (i.e., foci of cellular alteration (FCA));

3) several unique, presumably degenerative conditions (hydropic vacuolation, nuclear pleomorphism and megalocytic hepatosis, and spongiosis hepatis) which have been shown to be associated with toxicant exposure in previous studies with winter flounder or other fish species ;

4) nonneoplastic proliferative lesions such as hepatocellular or biliary regeneration or hyperplasia; and

5) a variety of nonspecific hepatocellular or biliary necrotic or degenerative conditions. Some of these lesions, such as liver neoplasms, FCA, and biliary or hepatocellular degenerative and regenerative lesions, are well established as histopathologic biomarkers of contaminant stress in fish and show strong evidence of a contaminant-associated etiology on the basis of previous field or laboratory studies with winter flounder or other fish species. Other lesions, such as necrotic and proliferative conditions in the kidney and fin erosion, have shown some promise as bioindicators of chemical pollution but require additional testing.

Prevalences of the lesion types described above were determined in winter flounder from each of the Northeast Coast sampling sites. In addition, logistic regression was used to examine the effects of selected biological and contaminant-associated risk factors (e.g., age, gender, site and season of capture, and levels of chemical contaminants in sediments and fish) on lesion occurrence. Logistic regression is a multivariate statistical method which is similar to stepwise multiple regression, but is uniquely suited to binomial or proportional data such as lesion presence

presence or absence or lesion prevalence. This technique is frequently applied in epidemiological and epizootiological studies and has a distinct advantage over simple intersite comparison of lesion prevalences because it allows biological factors such as age and gender to be taken into account when evaluating relationships between disease and site of capture or other parameters associated with contaminant exposure.

Relationships between lesion prevalences and the following major classes of chemical compounds were examined: high molecular weight AHs containing 4-6 benzene rings (HAHs), low molecular weight AHs containing 1-3 benzene rings (LAHs), total AHs (LAHs + HAHs), PCBs, DDTs, and chlordanes. These classes of chemical compounds were chosen as potential risk factors because 1) they are found at high concentrations in sediments from a number of Northeast Coast sites; 2) they are generally present in estuaries located near metropolitan areas throughout the United States and thus represent an index of urban pollution; and 3) they are generally recognized as toxicants in fish or mammals. Moreover, concentrations of these contaminants in sediments were highly correlated with levels of the same compounds or their derivatives in stomach contents, liver, and bile of winter flounder, indicating that these compounds were being taken up and accumulated or metabolized by the fish. Concentrations of organic contaminants in all compartments were correlated with disease prevalences to evaluate the consistency of observed relationships; however, contaminant levels in liver and bile were considered to have the greatest relevance as toxicologic risk factors because they correspond most closely to actual uptake of toxicants by animals.

Significant Findings

- Overall, hepatic lesions in winter flounder proved to be extremely reliable histological markers of chemical contaminant exposure. Hydropic vacuolation and nonspecific necrotic lesions showed particular utility as indicators of anthropogenic stress. Both lesions occurred at high prevalences (25 to 50%) in winter flounder from heavily contaminated sites, and showed a clear gradient in prevalence from heavily to

contaminated sites. In addition, they were closely correlated with levels of several classes of organic contaminants in fish liver and bile, sediments, and stomach contents.

- Hepatic neoplasms, foci of cellular alteration, and nonneoplastic proliferative lesions such as biliary hyperplasia were also found in winter flounder from several heavily contaminated sites, but these lesions were not highly correlated with chemical contaminant levels in liver, bile, stomach contents, or sediments. The lack of correlation can be partly attributed to the relatively low prevalences (2 to 10%) of neoplastic, preneoplastic, and proliferative lesions in winter flounder sampled in this study. Also, there is a long latency period for the development of neoplasms and related lesions. Consequently, even if contaminant exposure is a critical factor in their etiology, they may not be highly correlated with contaminant levels in sediment at the sampling site, or with levels of contaminants in the animals at the time of collection. This is especially likely to be the case in younger animals, or in a relatively mobile species such as winter flounder. The problem is compounded by the fact that for certain key environmental carcinogens such as AHs, current methodologies estimate only short-term, not chronic exposure. There is substantial evidence from both field and laboratory studies that neoplastic and preneoplastic lesions in fish are associated with exposure to environmental toxicants. However, because of their relatively low prevalence in many marine species and their long latency period, they may be less suitable as biomarkers of anthropogenic stress in field monitoring programs than earlier-occurring and more prevalent toxicopathic lesions.
- The compounds most frequently identified as risk factors for pathological conditions in winter flounder were AHs, DDTs, and chlordanes. In addition to these chemical contaminants, several biological factors, particularly age and sampling season, had a significant influence on disease prevalence. However, because AHs, DDTs, and chlordanes generally occurred together in sediments, and animals were exposed to them simultaneously, it was difficult to evaluate the relative importance of these toxicants in the development of pollution-associated disease in winter flounder.

- PCBs did not emerge as significant risk factors for any pathological condition observed in winter flounder. Not only did PCB levels in sediment, stomach contents, and liver fail to correlate with lesion prevalences, but perhaps more importantly, fish sampled from one site, New Bedford Harbor, that had high levels of PCBs in sediments but relatively low levels of other contaminants including AHs, had low prevalences of all pathological conditions monitored in this study. These data, however, do not exclude PCBs from a role as secondary etiologic agents for neoplastic lesions or regenerative and degenerative conditions in winter flounder.
- Prevalences of renal lesions were not highly correlated with levels of organic contaminants in sediments, stomach contents, bile or liver of winter flounder. Of the three classes of lesions monitored in this study (proliferative, necrotic, and sclerotic), only necrotic lesions showed any evidence of a relationship to contaminant exposure.
- Fin erosion prevalences were relatively high (e.g., 25 to 30%) at certain heavily contaminated sites especially within Boston Harbor, and were significantly correlated with contaminant concentrations in sediments and stomach contents, and with biliary FAC concentrations. However, because biotic factors such as fungal or bacterial pathogens as well as xenobiotic contaminants may be associated with the development of fin erosion, it is not as specific a biomarker as other toxicopathic lesions.
- The toxicopathic response (e.g., hydropic vacuolation of hepatocellular and biliary cells and biliary hyperplasia, and a predominance of cholangiocellular neoplasms over hepatocellular neoplasms) typically exhibited by winter flounder sampled from urban sites is quite different from patterns of lesion occurrence associated with the histogenesis of neoplasia in mammals and fish species such as medaka, rainbow trout, and English sole. The types of lesions found in winter flounder in contaminated areas have also been noted in other bottomfish (e.g., starry flounder (*Platichthys stellatus*) and white croaker (*Genyonemus lineatus*)) at contaminated sites on the West Coast sampled in the NBSP. These differences between starry flounder, white croaker, and winter flounder and

species such as English sole suggest that toxicopathic response, and possibly histogenesis of neoplasia, may follow more than one pathway in teleost fish.

In these studies we were not able to evaluate the effects of a number of potential toxicological risk factors such as certain metals, especially biologically-active forms of metals including organometals, dioxins, and coplanar PCBs, because appropriate cost-effective methodologies amenable for large-scale biomonitoring are not currently available. Development of such methodologies and their incorporation into the NBSP could bring about significant advances in our understanding of the toxicopathic effects of such contaminants and their importance in the development of pollution-associated disease. Our ability to identify relationships between environmental toxicants and disease conditions in benthic fish would be further enhanced by the utilization of indicators (e.g., xenobiotic-DNA adducts) that measure chronic exposure to labile toxicants, such as AHs, and by determination of concentrations of selected contaminants, including CHs, in individual fish. Such data may be used to develop predictive dose-response models linking contaminant levels in individuals with the risk of disease.

Although biomonitoring studies such as those reported here could benefit greatly by inclusion of a broader suite of contaminants and biological endpoints, the data gathered thus far provide significant indications that certain environmental contaminants are potential risk factors for pathological conditions observed in winter flounder, a benthic fish used as an indicator species for the assessment of environmental quality on the Northeast Coast. This correlational evidence, while being circumstantial, provides valuable insights into the potential involvement of certain groups of contaminants in disease initiation and progression, and will serve as a basis for the formulation of definitive cause-and-effect studies with potential etiologic agents present in urban environments.

CONTENTS

PREFACE

The National Benthic Surveillance Project (NBSP) was initiated in 1984 as a component of the National Oceanic and Atmospheric Administration's (NOAA's) National Status and Trends (NS&T) Program, which was designed to assess the status of, as well as long-term changes in, the environmental quality of the Nation's coastal and estuarine waters. The NBSP is a cooperative effort between the National Marine Fisheries Service and the Coastal Monitoring and Bioeffects Division of the Office of Ocean Resources Conservation and Assessment of NOAA's National Ocean Service. The project's specific objectives are to measure concentrations of chemical contaminants in sediment and in bottom-dwelling fish species at selected sites in urban and nonurban embayments, to determine the prevalences of pathological conditions and other bioindicators of contaminant exposure in these same fish species, and to evaluate temporal trends in the above-mentioned parameters. One hundred sites in embayments along the Atlantic, Gulf and Pacific coasts, including Alaska, have been sampled since 1984, with each annual sampling referred to as a "cycle" (e.g., 1984 = Cycle 1). Prior to Cycle 5, the Northeast Fisheries Center was responsible for Northeast Coast NBSP sampling, and results of these studies are presented in Zdanowicz et al. (1986) and NOAA (1987, 1988).

This Technical Memorandum presents and interprets the results of histopathological studies conducted on winter flounder *(Pleuronectes americanus* formerly *Pseudopleuronectes americanus)* sampled from Northeast Coast sites between 1987 and 1989 as part of the NS&T Program. Pathological conditions described include a variety of idiopathic liver, kidney, and fin lesions. Some of these lesions, such as liver neoplasms and related lesions, are well established as histopathologic biomarkers of contaminant stress, while others have shown some promise as indicators but require additional testing. Prevalences of these lesions in winter flounder sampled from sites with different degrees of chemical contamination are compared. In addition, lesion prevalences are correlated with chemical exposure data to better understand the relationship between environmental contaminants and fish disease. This memorandum is not meant to

comprehensively review the marine pollution literature; however, pertinent references are included in discussions of the most significant findings.

In addition to this publication, a separate Technical Memorandum is in preparation to provide detailed information on contaminant levels in winter flounder tissues and in sediments at the Northeast Coast sampling sites (Brown et al. in prep.). While data on sediment and tissue contaminant concentrations will be utilized in this pathology memorandum, the chemistry report should be consulted for more comprehensive information on tissue and sediment chemistry for Northeast Coast NBSP studies conducted during 1988 and 1989.

INTRODUCTION

The National Benthic Surveillance Project (NBSP), a component of the National Oceanic and Atmospheric Administration's (NOAA's) Status and Trends (NS&T) Program, is designed to assess the status of the Nation's coastal and estuarine waters and monitor long-term changes in marine environmental quality. A key component of the NBSP is the utilization of histopathologic alterations in fish tissues as indicators of the effects of exposure to environmental contaminants on marine organisms. These histopathologic biomarkers are pathological conditions that either morphologically resemble lesions induced by the experimental exposure of mammals or fish to toxicants, or show evidence of a contaminant-associated etiology in field studies because they occur either exclusively or at significantly increased prevalences in fish from contaminated sites.

Probably the most commonly used histopathologic biomarkers are neoplastic and preneoplastic liver lesions, although recent studies indicate that certain associated degenerative and regenerative conditions may be equally or even more useful as biomarkers of anthropogenic stress (Myers et al. in press, Varanasi et al. in press). Liver cancer and related lesions have been reported in several species of bottomfish (e.g., English sole *(Pleuronectes vetulus*, formerly *Parophrys vetulus)* (McCain et al. 1977; Malins et al. 1984, 1985; Myers et al. 1987), white croaker *(Genyonemus lineatus)* (Malins et al. 1987; Myers et al. 1991, 1992); Atlantic tomcod *(Microgadus tomcod)* (Smith et al. 1979; Cormier 1986)) from chemically contaminated coastal areas. Associations between concentrations of chemical contaminants in sediments and fish tissues and certain of these lesions have been observed in field surveys (Malins et al. 1984, 1985; McCain et. al. 1988; Vogelbein et al. 1990), and have been further substantiated by statistical analyses and epizootiological models (Rhodes et al. 1987, Myers et al, 1990, 1991, 1992; Landahl et al. 1990). Moreover, in some species, including English sole, cause-and-effect relationships between selected contaminants and hepatic lesions similar to those observed in field-sampled animals have been demonstrated in long-term laboratory studies (Varanasi et al. 1987, Schiewe et al. 1991). Other pathological conditions, including fin erosion and renal lesions, have also shown some promise as

bioindicators of contaminant exposure in fish (Dethlefsen 1980, Cross 1985, Wellings et al. 1976, Sherwood and Mearns 1977, Cross et al. 1985, Sindermann 1990, Reimschuessel et al. 1990, Hawkins et al. 1989, McCain et al. 1992), but require additional testing in both the field and the laboratory to confirm their chemical etiology.

Previous field studies suggest that liver lesions and other disease conditions in winter flounder (*Pleuronectes americanus* formerly *Pseudopleuronectes americanus*) may be reliable bioindicators of environmental degradation on the Northeast Coast. The occurrence of neoplastic and non-neoplastic liver disease in fish from contaminated areas, especially from sites within Boston Harbor, is well-documented, both in earlier NBSP sampling conducted by the Northeast Fisheries Center (Zdanowicz et al. 1986, NOAA 1987, 1988) and in studies by other investigators (Murchelano and Wolke 1985, 1991; Moore 1991, Gronlund et al. 1991). Elevated prevalences of fin erosion have also been reported in flounder from contaminated sites along the Northeast Coast (Williams 1981, Ziskowski and Murchelano 1975). At present, however, there is relatively little information available on how levels of specific contaminants in sediments and winter flounder tissues are related to disease occurrence, or on the effects of biological factors such as age and gender on disease prevalence in this species.

This report presents the results of pathological studies conducted on winter flounder collected from Northeast Coast sites between 1987 and 1989 as part of the NS&T Program. The major objectives of the study were 1) to determine the prevalences of fin erosion and selected hepatic and renal lesions in winter flounder from the Northeast Coast sampling sites; 2) to examine the effects of biological variables such as age, gender, and sampling season on lesion occurrence; and 3) to assess the relationship between levels of chemical contaminants in sediments and fish and lesion prevalences.

Sampling was primarily conducted during Cycles 5 and 6 (1988 and 1989) of the Northeast Coast NBSP, but to provide a more comprehensive view of pathological conditions in winter flounder from the Northeast Coast, data from winter flounder collected in Long Island Sound in 1987 (Gronlund et al. 1991) and in Boston Harbor and adjacent embayments during the winters of

1988 and 1989 (Johnson et al. 1992, in press) are also included in this report. Histopathological examination of tissues and chemical analyses were carried out in our laboratory in a uniform fashion for all three studies, so all data included here are comparable.

Lesion types monitored in winter flounder included fin erosion; necrotic, sclerotic, and proliferative lesions in the kidney; and a number of suspected toxicopathic liver lesions such as neoplasms, putatively preneoplastic foci of cellular alteration, and nonneoplastic proliferative and degenerative conditions. A variety of other pathological conditions, such as parasitic infections, were noted in flounder examined in this study, but they will not be described in detail in this report as there is little evidence that they have a chemical etiology (Hinton et al. 1992).

The following classes of chemical contaminants were included in the analysis as potential risk factors : high molecular weight aromatic hydrocarbons (AHs) containing 4-5 benzene rings (HAHs), low molecular weight AHs containing 2-3 benzene rings (LAHs), total AHs (LAHs + HAHs), polychlorinated biphenyls (PCBs), DDT and its derivatives, DDD and DDE (DDTs), and chlordanes. These classes of chemical compounds were chosen because 1) they were present at high concentrations in sediments from a number of Northeast Coast urban sites; 2) they are among the most prevalent contaminants in estuaries adjacent to metropolitan areas throughout the United States, and thus represent an index of urban pollution (Varanasi et al. 1988, 1989a); and 3) they are generally recognized as toxicants in fish or mammals (Klaassen et al. 1986). Concentrations of these organic contaminants or their metabolites were measured in several compartments (i.e., sediments, stomach contents, liver, and bile) to give a comprehensive picture of exposure and relationships between contaminant levels in all three compartments and idiopathic disease. However, contaminant levels in liver and bile were considered to have the greatest relevance as toxicologic risk factors, because they are most representative of the actual uptake of toxic compounds by fish. It should be noted that other types of contaminants, such as dioxins or organometals, may also play a role in the development of disease in winter flounder and other fish species. However, because methodologies for measuring concentrations of these compounds in

fish and sediments that are cost-effective and appropriate for a large-scale monitoring program are not currently available, it was not possible to include them in the NBSP at this time.

Trace metal analyses of sediments, liver tissue, and stomach contents were conducted by the Southeast Fisheries Science Center, and data on metal concentrations will not be presented here. Analyses of metals from previous NBSP samplings on both the West Coast (Varanasi et al. 1988, 1989a) and the Northeast Coast (NOAA 1988) indicated that there was little relationship between tissue concentrations of metals and concentrations of metals in sediment in any of the fish species examined. These findings suggest that metals may not be bioavailable to fish, or that their uptake and accumulation are governed by complex processes that make tissue concentrations of metals a poor indicator of environmental exposure. Consequently, no attempt was made to correlate metal concentrations with fish disease.

Logistic regression (Breslow and Day 1980, Schlesselman 1982) was used to assess the influence of biological and contaminant-associated risk factors on lesion occurrence. The logistic function is considered a basic model for dose-response relationships and logistic regression analysis is frequently applied in epidemiological and epizootiological studies. This technique is similar to commonly-used stepwise multiple regression procedures, but is uniquely suited to binomial or proportional data (e.g., lesion prevalence or lesion presence or absence). Results of these analyses and their relationship to other studies of pollution-associated disease in winter flounder will be discussed below.

MATERIALS AND METHODS

Field Sampling

Samples were collected from 22 Northeast Coast sites. Site names and abbreviations, the locations of these sites, the types of samples collected, and the frequency of sampling are presented in Table 1. Figure 1 illustrates the locations of the sampling sites. Fifteen of these sites were located in or near urban embayments, and the seven remaining sites were in nonurban embayments, one of which (Long Island Sound, Rocky Point) served as a comparison site.

Comparison sites were selected in order to collect from minimally contaminated environments the same fish species obtained at urban sites. Observations of these "reference" fish were used to help interpret the significance of pathological conditions observed in the respective species from urban sites.

Site selection was based on 1) the availability of bottom-feeding fish, 2) location in a subtidal, sedimentary-depositional zone, 3) location outside of a point source or authorized dumpsite, and 4) a location not subject to dredging, scouring or slumping. Each site consisted of three stations generally located less than 0.4 km apart and positioned in a manner designed to characterize the entire site, but not the entire embayment in which the site was located. Sites for sediment collection were situated along trawl lines for fish collection. In addition to standard NBSP sites, areas sampled during intensive studies of Long Island Sound in 1987 and Boston Harbor in 1988 were also included, to increase the sample size for statistical analyses and thus improve our ability to detect any relationships between contaminant levels and fish lesions. The target species was winter flounder, a bottom-feeding flatfish species. Winter flounder was chosen as a target species because it is widely distributed in both urban and nonurban estuaries along the northern Atlantic coast. Additionally, suspected toxicopathic lesions had been previously reported in flounder from heavily urbanized embayments such as Boston Harbor (e.g., Murchelano and Wolke 1985), indicating that the species was sensitive to environmental toxicants.

The sites along the Northeast Coast were sampled using the NOAA ship *Ferrel*, and research vessels *Gloria Michele* and *Kyma*. To maximize comparability of data over space and time, standardized collection gear and sampling methods were used. All research vessels were equipped with navigational equipment to determine the precise location (latitude/longitude) of the sampling stations.

Fish were collected with otter trawls equipped with netting that was not chemically treated. Fish equal to or above 15 cm in length were randomly selected from each haul and assigned a unique identification number. These fish were kept alive in fresh seawater to prevent autolysis of tissues until necropsies were performed. The necropsy procedure involved 1) weighing (total

weight in grams) and measuring (total length in millimeters) each fish; 2) removing otoliths for age determination; and 3) excision of the gall bladder, liver, kidney, gonad and stomach contents. Approximately 30 winter flounder were necropsied at each site in 1987, and the number was increased to a maximum of 60 animals in 1988 and 1989. If the target number of animals could not be captured, as many animals were sampled as possible. Sites where less than 15 animals could be obtained are not included in this report. For flounder sampled as part of NBSP or the 1987 Long Island Sound study, tissue sections of the central portion of the liver and the posterior portion of the kidney, and a sagittal section of the gonad were taken and preserved in Dietrich's fixative (Gray 1954) for routine histological processing and histopathological examination. Only liver and gonad samples were collected during the Boston Harbor reproductive study conducted in winter 1988.

The bile from the gall bladder and a portion of the remaining liver tissue were placed in separate, solvent-rinsed glass vials and frozen at -80°C for later analyses for fluorescent aromatic compounds (FACs) and organic compounds, respectively. Another portion of the liver was placed in a plastic vial and stored frozen until analyzed for selected metals. Approximately 60 liver samples for organic chemistry, 30 liver samples for metals, and a minimum of 10 bile samples were collected per site. Individual liver samples were subsequently composited in the laboratory into 3 composites of 10-20 individuals each per site, and chemical analyses were conducted on the composite samples. The total contents of stomachs from at least 10 fish per target species at each site were removed and composited in a solvent-rinsed glass jar and frozen for chemical analyses. In a similar manner, the stomach contents from another five fish at each site were composited into a jar containing 10% buffered formalin. These latter composites were returned to the laboratory for taxonomic classification of the food organisms.

Surface sediments were collected as described in the previous NBSP reports (Varanasi et al. 1988, 1989a; McCain et al. 1989).

Laboratory Analyses

Fish Age Determination

In selected animals collected over NBSP Cycles 5 and 6, and in the Long Island Sound and Boston Harbor studies, fish age was estimated by counting the number of clearly defined opaque zones of whole otoliths under a binocular dissecting microscope (Kimura et al. 1979, Chilton and Beamish 1982). Ages were determined by examination of otoliths in all animals collected at each site for at least one sampling cycle. For the remaining animals age was estimated from length based on age-length curves constructed from data on fish whose ages had been determined from otoliths. Both gender-specific and gender-combined age-length curves were generated for each major geographic region sampled (e.g., winter flounder females, Boston Harbor) using simple or polynomial regression techniques. Resultant age-length curves and equations (Appendix Table A2) were then chosen on the basis of best fit to the data (i.e., highest adjusted R-squared value). Fish age was then estimated to the nearest year from these gender-specific age-length equations. If no gender data were available, or insufficient numbers of a particular gender existed to perform meaningful age-length regression, ages were estimated by applying age-length equations for both genders combined.

Histopathological Analyses

Fish tissues preserved in the field for histopathological examination were processed for paraffin embedding by routine histological procedures (Preece 1972). After embedding, the tissues were sectioned at 4-5 mm thicknesses. Paraffin sections were stained with Mayer's hematoxylin and eosin-phloxine (Luna 1968). For further characterization of specific lesions, additional sections were stained using special staining methods (Thompson 1966, Luna 1968, Preece 1972). Lesion classification followed previously described diagnostic criteria (Jones and Butler 1975, Stewart et al. 1980, Myers et al. 1987, Robbins et al. 1984, Squire and Levitt 1975).

For reporting and statistical analyses, hepatic lesions were grouped into the following categories: neoplasms, foci of cellular alteration (FCA), nuclear pleomorphism, spongiosis hepatis of liver parenchyma, hydropic vacuolation (synonymous with RAM cell areas or atypical

vacuolation (Moore et al. 1989, Bodammer and Murchelano 1990)), proliferative lesions, and nonspecific degenerative or necrotic lesions. Kidney lesions were grouped into the following categories: 1) proliferative lesions, 2) necrotic lesions, 3) and sclerotic lesions. A complete description of the types of individual lesions included in these categories is given in Table 2. Data concerning biological characteristics (e.g., length, weight, and sex) and lesions in individual fish were stored in the Reflex[1] database system. Lesions were identified using a numerical coding system adapted from File Type 013 of the National Ocean Data Center (NODC) coding system (NODC 1991).

Chemical Analyses of Sediments, Stomach Contents, Liver Tissue, and Bile

Liver tissue, stomach contents, and sediments were analyzed for a broad spectrum of aromatic hydrocarbons (AHs) and chlorinated hydrocarbons (CHs) (McCain et al. 1988, Varanasi et al. 1988, 1989a, Krahn et al. 1988). Fluorescent aromatic compounds in fish bile were measured using the procedures of Krahn et al. (1984, 1986, 1987). Bile analyses were performed on samples from individual fish, but chemical analyses of liver tissue were perfomed on composite samples. For each site, three 3 g composites of 10-20 livers were analyzed. For statistical analyses, the AHs and CHs whose concentrations were measured in sediments, stomach contents, and fish tissues were grouped into five broad categories: LAHs, HAHs, DDTs, PCBs, and chlordanes. The specific chemicals included in each of these categories are listed in Table 3. As noted previously, trace metal analyses of sediments, liver tissue, and stomach contents were conducted by the Southeast Fisheries Science Center, and data on metal concentrations will not be presented or correlated with disease conditions in this memorandum.

Stomach Contents Taxonomy

Samples of fish stomach contents to be taxonomically characterized were transferred from 10% neutral buffered formalin to 70% ethanol prior to analysis. Food organisms were examined

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[1]Mention of tradenames is for information only and does not constitute endorsement by the Department of Commerce.

with a dissecting microscope and grouped into four main categories: arthropods, annelid worms, molluscs, and nematodes. For each sample, the organisms in each of these categories were weighed to nearest milligram, and the percentage of organisms (on a wet weight basis) in each taxonomic category was determined.

Statistical Analyses

Heterogeneity in Lesion Prevalence

To determine if statistically significant differences in lesions prevalences existed between the reference site (Rocky Point, Long Island Sound) and the individual test sites, the G-statistic (Sokal and Rohlf 1981) was computed using the lesions prevalence at the reference site as the expected value. A lesion prevalence was considered to be different (either higher or lower) from the overall mean prevalence for that lesion type if the difference was significant at $p \leq 0.05$. Sites at which less than 15 fish were collected were omitted from this analysis.

Logistic Regression and Risk Factor Analysis

Stepwise logistic regression (Breslow and Day 1980, Schlesselman 1982) was used to identify statistically significant relationships between potential risk factors and lesion occurrence in winter flounder. In the logistic model (Fig. 2), the probability of disease *(p)* is given by:

$$p = 1/\{1 + \exp[-(\beta_0 + \beta_1 x_1 + \ldots + \beta_p x_p)]\}$$

where $x_1, x_2, \ldots, x_p$ represent potential risk factors, and the β's are coeffecients that represent the effects of the x's on the risk (probability) of disease.

Two types of logistic regression analyses were conducted: 1) an analysis to examine the relative risk of disease in individual fish in relation to site of capture, gender, season of capture, and age or estimated age; and 2) an analysis to detect significant relationships between lesion prevalences at the sampling sites and mean concentrations of contaminants in sediments, fish stomach contents, and fish tissues. A number of chemical contaminants were measured in this study, and for the second analysis, it would have been desirable to evaluate their relative importance as risk factors for hepatic disease using a multivariate logistic model. In practice,

however, it was not feasible to construct such a model because of the large number of highly intercorrelated risk factors and the relatively low number of samples available for analysis. Consequently, relationships between various classes of chemicals and lesion prevalences were examined separately while adjusting for potential effects of the mean fish age and gender ratio at the sampling sites.

Logistic regression models were fitted using the PECAN analysis module of the EGRET statistical package (Version III, Statistics and Epidemiology Research Corporation, Seattle, WA) using a procedure similar to stepwise regression. The estimated odds ratio or relative risk for the occurrence of a lesion in an individual fish, which is a measure of the degree of association between a risk factor and lesion occurrence (Fleiss 1981), was calculated from variable coefficients of the logistic regressions (Anderson et al. 1980, Schlesselman 1982). The relative odds or risk of disease in individuals with different values for the risk factors (e.g., in animals of differing ages, or from contaminated vs. reference sites) is given by:

$$\exp[\Sigma_i \beta_i (x_i^* - x_i)]$$

where the β's are the coeffecients for risk factors $x1$, $x2$., . . .x_i, and the x^*_i's and x_i's represent the values of the risk factors for the two individuals which are being compared (Schlesselman 1982). Odds ratios or relative risks greater than 1 indicate an increased probability of disease, and odds ratios less than 1 indicate a decreased probability of disease. For all logistic regression analyses, risk factors were considered significant at $p \leq 0.05$.

The statistical analyses described above were applied to the following categories of hepatic lesions: 1) neoplasms, 2) FCA, 3) nuclear pleomorphism and megalocytic hepatosis, 4) spongiosis hepatis, 5) hydropic vacuolation, 6) proliferative lesions, and 7) non-specific degenerative or necrotic lesions. Additionally, the same analyses were applied to the following categories of renal lesions: 1) proliferative lesions, 2) necrotic lesions, 3) and sclerotic lesions; and to fin erosion. Temporal trends in lesion occurrence were not evaluated because only 2 years of data were available for most sampling sites. The Rocky Point site in Long Island Sound was designated as the reference area, and calculated odds ratios or relative risks associated with site of

capture were interpreted relative to this site. Calculated odds ratios for age (in years) were interpreted for each additional year of age.

In addition to the risk factors outlined above, the effect of sampling season on lesion prevalence was also examined for stations sampled in winter of 1988-89 during the intensive reproductive success study, and in spring during the routine NBSP sampling. Because no male fish were sampled during the reproductive studies, the analysis of the impact of season of capture on lesion prevalence is limited to female fish only.

Relationships between tissue contaminant concentrations in individual fish and disease risk were not evaluated for this report because data on contaminant exposure levels in individual fish were available only for biliary FACs. In future NBSP samplings, levels of various chlorinated hydrocarbons, including DDTs, PCBs, and selected non-DDT pesticides will be determined in tissues of selected individual fish, and a more comprehensive analysis will be presented when these data are available.

RESULTS

Chemical Characterization of Sampling Sites

Sediment Contaminant Levels at Sampling Sites

In Table 4, the 19 of the 22 sampling sites for which chemistry data are available are ranked according to sediment concentrations of the major classes of chemical contaminants (i.e., LAHs, HAHs, total AHs, PCBs, DDTs, and chlordanes) measured in this study. Specific compounds included in the major classes are listed in Table 3. In addition to chlordanes, a number of other chlorinated non-DDT pesticides (i.e., lindane, mirex, heptachlor, heptachlor expoxide, aldrin, dieldrin and hexachlorobenzene) were present in sediments and tissues from some Northeast Coast sampling sites (Brown et al. in prep.), but because of their relatively low concentrations (e.g. < 100 ng/g dry wt in liver and 3 ng/g dry wt in sediments), they were not included as risk factors for pathological conditions in the logistic regression analyses.

The highest concentrations of contaminants were found at the Mystic River and Quincy Bay sites in Boston Harbor. Sediments from these two sites contained high concentrations of a wide variety of organic compounds, including both low and high molecular weight AHs, PCBs, DDTs, and chlordanes. Several sites within Raritan Bay (e.g., Gravesend Bay, East Reach, and West Reach) also had relatively high levels of AHs, DDTs, PCBs, and chlordanes. New Bedford Harbor had the highest level of PCBs in sediment of all the sampling sites, but concentrations of other classes of contaminants were low to moderate. Buzzards Bay in Massachusetts, Rocky Point in Long Island Sound, and the Plymouth Entrance site in Massachusetts Bay had the lowest concentrations of all classes of contaminants in sediment. Detailed information on actual concentrations of compounds in sediment at the Northeast Coast sampling sites is given in Brown et al. (in prep.).

Patterns of Occurrence of Contaminants Within Sediment

In many cases, different classes of chemical contaminants tended to occur together at polluted urban sites (Table 5). Concentrations of aromatic hydrocarbons (Total AHs, HAHs, and LAHs) measured in sediment were highly intercorrelated ($0.8 > r > 0.9$). Various classes of chlorinated hydrocarbons (i.e., chlordanes, PCBs, and DDTs) also tended to co-occur ($0.8 < r < 0.9$). In addition, sediment AHs levels highly correlated ($r = 0.9$) with concentrations of chlordanes in sediment. The correlation was particularly strong because of the influence of Mystic River and Quincy Bay in Boston Harbor. These two sites, which had the highest levels of AHs in sediment of all the Northeast sites, also had high levels of chlordanes in sediment. Weaker, but still significant correlations ($0.7 > r > 0.75$) were also observed between sediment AH concentrations and levels of PCBs and DDTs in sediment. Although in general, AHs, PCBs, DDTs, and chlordanes tended to co-occur in sediments at the Northeast sampling sites, the Clarks Point site in New Bedford Harbor was a notable exception; this site had the highest levels of PCBs of all the Northeast sites but low to moderate levels of DDTs and AHs.

Contaminant Levels in Sediments, Stomach Contents, and Tissues

For all major classes of contaminants measured in this study, chemical concentrations in sediment were positively correlated with concentrations in winter flounder stomach contents (Table 6), indicating that the organisms which serve as prey items for winter flounder take up and accumulate a variety of organic contaminants from sediment. Moreover, for concentrations of all organic contaminants measured in this study, significant correlations were observed between contaminant concentrations in stomach contents and liver or bile (Table 6), suggesting that, in winter flounder, an important route of exposure to environmental pollutants is through the diet. Significant correlations between concentrations of several contaminants in sediment (i.e., AHs, PCBs, and chlordanes) and levels of the same compounds or their metabolites in liver or bile were observed. However, relationships were generally not as strong as those with levels of contaminants in stomach contents, and for DDTs, the correlation was not statistically significant. This may be because animals tend to accumulate compounds such as DDTs and other pesticides over time, so their body burdens may not necessarily be correlated with contaminant levels in sediment from the areas where they are sampled.

Stomach Contents Taxonomy

Taxonomic analysis of stomach contents of winter flounder indicated that the types of food organisms varied considerably from site to site (Fig. 3). Because the invertebrates that serve as food sources for winter flounder and other benthic fish species vary in their ability to metabolize xenobiotic compounds (Varanasi et al. in press, Livingston 1991) and may contain different levels and forms of various toxicants, the type of food organism consumed could potentially have a significant effect on types and amounts of contaminants which fish are exposed to through their prey. Preliminary analyses suggested that concentrations of contaminants in stomach contents were much more highly correlated with contaminant levels in sediment than with the type of food organism that was consumed. The data available are quite limited, however, and further analyses are needed to evaluate the effect of diet on contaminant uptake in a comprehensive manner.

Hepatic Lesions

Hepatic Lesion Prevalences and Intersite Comparisons

Neoplasms. Several different types of neoplasms were observed in winter flounder from the Northeast Coast. Of the 41 neoplasms observed, 56% were hepatocellular in origin, 34% were cholangiocellular, and 10% were vascular. Types of neoplasms included hepatocellular adenomas (49%), cholangiomas (9%), hepatocellular carcinomas (14%), cholangiocellular carcinomas (22%), hemangiomas (8%), and hemangioendothelial sarcomas (3%). The prevalence of neoplasms in winter flounder (see Appendix Table A4) was significantly elevated in comparison with the Long Island Sound, Rocky Point reference site at 10 of the 22 sampling sites (Figs. 4-5). These sites included Hull Bay (8.3%, n=60), Deer Island (6.8%, n=147), Long Island (2.3%, n=42), and Mystic River (2.3%, n=86) in Boston Harbor (Fig. 4); Buzzards Bay (1.7%, n=60) (Fig. 4); Gravesend Bay (5.2%, n=58) and West Reach (3.6%, n=56) in Raritan Bay (Fig. 5); and Lloyd Point (2.3%, n=87) and New Haven (3.3%, n=30) in Long Island Sound (Fig. 5).

Foci of Cellular Alteration. Preneoplastic focal lesions observed included eosinophilic, basophilic, and clear cell foci of cellular alteration (FCA). The most common were basophilic foci, accounting for 64% of the preneoplastic lesions observed. Clear cell foci and eosinophilic foci accounted for 19 and 17%, respectively, of observed cases of preneoplastic lesions. Significantly elevated prevalences of FCA were found at three sampling sites: Buzzards Bay (6.7%, n=60) (Fig. 4), Deer Island in Boston Harbor (6.1%, n=147) (Fig. 4), and Gravesend in Raritan Bay (6.9%, n=58) (Fig. 5).

Proliferative Lesions. Non-neoplastic proliferative liver lesions observed included hepatocellular regeneration (36%), bile duct hyperplasia (38%), cholangiofibrosis (12%), and increased mitotic activity of hepatocytes or biliary epithelial cells (14%). Significantly elevated prevalences of non-neoplastic proliferative liver lesions were found at only two sites, Deer Island (11.6%, n=147) and Mystic River (5.8%, n=86) in Boston Harbor (Fig. 4).

Unique Degenerative Lesions. Unique degenerative or necrotic lesions observed in winter flounder included nuclear pleomorphism and spongiosis hepatis (also known as cystic

degeneration of parenchyma). Mild-to-moderate nuclear pleomorphism was noted at low prevalences at 16 of the 22 sampling sites, including the Rocky Point reference sites in Long Island Sound, but no significant intersite differences were found. Prevalences of spongiosis hepatis, on the other hand, were significantly elevated at six sampling sites: Hull Bay, Deer Island, Quincy Bay, and President Roads in Boston Harbor, the Folgers Point site in Salem Harbor (Fig. 4), and the New Haven site in Long Island Sound (Fig. 5).

Hydropic Vacuolation. Of all the toxicopathic lesions, hydropic vacuolation of hepatocytes and biliary epithelial cells was the most commonly observed, with elevated prevalences at 15 of the 22 sampling sites. These sites were Mystic River (48.8%, n=86), Deer Island (43.5%, n=147), President Roads (35.7%, n=42), Quincy Bay (33.9%, n=62), and Hull Bay (33.3%, n=60) in Boston Harbor (Fig. 4); West Reach (46%, n=56), Gravesend (43.1%, n=58), East Reach (33.3%, n=117), and Upper Bay (18.3%, n=60) in Raritan Bay (Fig. 5); Norwalk (41.3%, n=29), New Haven (36.7%, n=30), and Lloyd Point (10.3%, n=87) in Long Island Sound (Fig. 5); Folgers Point in Salem Harbor (23.3%, n=120); Black Point in Niantic Bay (14.8%, n=135) (Figs. 3-4); and Prudence Island in Narragansett Bay (9.6%, n=94) (Fig. 4).

Necrotic Lesions. Elevated prevalences of nonspecific necrotic lesions were also found at a number of sites, including: Quincy Bay (25.8%, n=62), President Roads (23.8%, n=42), Hull Bay (18.3%, n=60), and Mystic River (17.4%, n=86) in Boston Harbor (Fig. 4); Buzzards Bay (18.3%, n=60) (Fig. 4); West Reach (21.4%, n=56) and Gravesend (15.5%, n=58) in Raritan Bay (Fig. 5); and New Haven (20.0%, n=30) and Lloyd Point (13.8%, n=87) in Long Island Sound (Fig. 5).

Risk Factors Associated with Hepatic Lesion Occurrence in Individual Fish

Risk factors which were significantly ($p < 0.05$) associated with lesion occurrence and their corresponding calculated odds ratios are presented in Table 7. As the table shows, for all categories of hepatic lesions, excepting nuclear pleomorphism, the risk of lesion occurrence increased significantly with increasing fish age. In general, the gender of the animal examined did not have a significant influence, although females appeared to have a slightly decreased risk of

developing neoplasms and spongiosis hepatis. In males collected during spring NBSP sampling (n=378), the prevalences of neoplasms and spongiosis hepatis were 3.2% (n=378) and 1.9% (n=378), respectively. For females (n=916) collected during this period the prevalence of neoplasms was 1.13%, while the prevalence of spongiosis hepatis was 0.55%. Residence at a number of the sampling sites was associated with an increased risk of disease in comparison with the risk in fish from the reference site at Rocky Point in Long Island Sound. In general, the highest relative risks were computed for fish from sites in contaminated estuaries, such as the Hudson-Raritan Estuary or Boston Harbor. Fish from Deer Island and Hull Bay in Boston Harbor, and West Reach and Gravesend in Raritan Bay, were 4 to 21 times more likely to develop neoplasms than animals sampled from other areas. A four- to sevenfold increase in the risk of FCA was associated with residence at Deer Island and Mystic River in Boston Harbor, Buzzards Bay, and Gravesend in Raritan Bay. Fish from Mystic River and Deer Island in Boston Harbor were approximately 2 to 4 times as likely to develop proliferative liver lesions as animals from other sites, while fish from New Haven in Long Island Sound were 4 times as likely to develop nuclear pleomorphism, and fish from President Roads in Boston Harbor were 13 times as likely as reference animals to be affected with spongiosis hepatis. The risk of hydropic vacuolation was significantly increased at 10 of the 22 sampling sites: Mystic River, Deer Island, President Roads, Hull Bay, and Quincy Bay in Boston Harbor; East Reach and Gravesend in Raritan Bay; Norwalk and New Haven in Long Island Sound; and Folgers Point in Salem Harbor. The increase ranged from approximately 2.5-fold at Salem Harbor to over tenfold at Mystic River. Fish from Mystic River, Hull Bay, Quincy Bay and President Roads in Boston Harbor, and West Reach in Raritan Bay were 2 to 3 times more likely to be affected with nonspecific necrotic lesions than comparable fish from the reference site.

In female fish sampled in both winter and spring, the season of sampling had a significant impact on prevalences of two categories of lesions: hydropic vacuolation and nonspecific necrotic lesions (Table 8). Prevalences of both of these lesions were significantly elevated in spring in comparison to lesion prevalences in winter. In spring, hydropic vacuolation was found in 31%

and necrotic lesions in 13% of winter flounder sampled, while in winter, only 9% (hydropic vacuolation) and 6% (necrotic lesions) of flounder from the same sampling sites were affected. These differences were particularly notable at contaminated sites such as Deer Island and Mystic River in Boston Harbor.

Relationships Between Chemical Contaminants and Hepatic Lesion Prevalences

Of all the hepatic lesions monitored in this study, hydropic vacuolation showed the strongest and most consistent relationships with contaminants in tissue, stomach contents, and sediment (Tables 9-11). Hydropic vacuolation was associated with concentrations of LAHs, HAHs, total AHs, DDTs, and chlordanes in sediments. Similar relationships were found between concentrations of AHs, DDTs, and chlordanes in stomach contents and prevalences of this lesion. The risk of hydropic vacuolation was also increased at sites where flounder had elevated levels of FACs in bile and chlordanes and DDTs in liver tissue. However, no relationship was observed between hydropic vacuolation and PCB concentrations in sediment, stomach contents, or liver tissue.

Nonspecific necrotic lesions also showed positive associations with a number of classes of chemical contaminants. All classes of contaminants in sediment except for PCBs proved to be significant risk factors for necrotic lesions. In addition, necrotic lesions were associated with elevated levels of LAHs, DDTs, and chlordanes in stomach contents, and with elevated biliary FAC concentrations. No relationship was found, however, between necrotic lesion prevalences and any class of chlorinated hydrocarbons measured in winter flounder liver.

Prevalences of other categories of degenerative or regenerative hepatic lesions (i.e., spongiosis hepatis, nonneoplastic proliferative lesions, and nuclear pleomorphism) were less closely associated with concentrations of organic contaminants in sediments and fish than hydropic vacuolation and necrotic lesions. The prevalence of spongiosis hepatis was positively associated with concentrations of chlordanes in stomach contents (Table 10), but not with levels of contaminants in liver or bile. Non-neoplastic proliferative lesions showed no relationship with

sediment contaminant concentrations, but were positively associated with concentrations of chlordanes in stomach contents and liver. Nuclear pleomorphism showed no relationship to concentrations of any class of contaminants in sediment, stomach contents, bile, or liver tissue.

No significant positive associations were found between prevalences of neoplasms or FCA and levels of measured contaminants in sediment, liver tissue or bile (Tables 9 and 11). However, both neoplasms and FCA were positively associated with concentrations of LAHs and chlordanes in stomach contents (Table 10).

Renal Lesions

Renal Lesion Prevalences

Kidney lesions were observed less frequently than liver lesions in winter flounder, but elevated prevalences could be observed at some sites (Fig. 6, Appendix Table A5). Proliferative kidney lesions were observed at only four sites: Buzzards Bay (1.64%, n=61), Deer Island in Boston Harbor (0.83%, n=121), Folgers Point in Salem Harbor (1.65%, n=121) and East Reach in Raritan Bay (0.87%, n=115). Necrotic lesions were observed at six sites: Buzzards Bay (1.64%, n=61); Plymouth Entrance in Massachusetts Bay (1.67%, n=60); Deer Island (4.1%, n=121); East Boston (1.69%, n=59); and Hull Bay (1.67%, n=60) in Boston Harbor; and West Reach in Raritan Bay (1.8%, n=55). Sclerotic lesions were more common, with elevated prevalences at 11 of the 19 sites sampled. These sites were Buzzards Bay (4.9%, n=61); Clarks Point in New Bedford Harbor (1.8%, n=55); Deer Island (5.8%, n=121); Mystic River (1.7%, n=59); Hull Bay (3.33%, n=60); and Quincy Bay (6.8%, n=59) in Boston Harbor; Folgers Point in Salem Harbor (0.83%, n=121); Black Point in Niantic Bay (7.0%, n=86); West Reach (1.8%, n=55); Upper Bay (1.69%, n=59); and East Reach (1.74%, n=115) in Raritan Bay; and New Haven in Long Island Sound (13.8%, n=29).

Risk Factors Associated with Renal Lesion Occurrence in Individual Fish

An increased risk of the development of certain kidney lesions was associated with residence at several sites (Table 7). Fish from the Folgers Point site in Salem Harbor and the

Buzzards Bay site were about nine times more likely to have proliferative kidney lesions than fish of comparable age and gender from the comparison site at Rocky Point is Long Island Sound. Fish from the Deer Island site in Boston Harbor showed a tenfold increase in the risk of necrotic lesions. The risk of sclerotic lesions was 4 to 5 times greater in fish from the New Haven site in Long Island Sound and Black Point site in Niantic Bay. In addition, fish age was a significant risk factor for necrotic and sclerotic kidney lesions; and females were significantly less likely to be affected by necrotic renal lesions.

Relationships Between Chemical Contaminants and Renal Lesion Prevalences

In general, the classes of kidney lesions examined in this study showed little relationship with exposure to environmental contaminants (Tables 12-14). Concentrations of AHs in stomach contents were a significant risk factor for renal necrosis, but no relationships were found between necrotic lesions and contaminant levels in sediment, liver, or bile. Sclerotic and proliferative kidney lesions showed no relationship with any indicator of contaminant exposure measured in this study.

Fin Erosion

Fin erosion was diagnosed grossly in 12% of all winter flounder examined. The features necessary for a gross diagnosis of fin erosion included blunting or retraction of fin rays, fusion of fin rays, exposed fin rays, bent fins, loss of fin tissue, and thickening or scarring of connective tissue surrounding the fin tip. In some cases, these lesions were associated with hemorrhage or edema in fin tissue. Often one specimen would exhibit more than one feature at a time. Most common gross features were fin loss (29%), fin ray blunting and retraction (33%), and bent fins (30%).

In 15% of reported cases, fin erosion was observed in association with some type of infectious agent, most commonly with lymphocystis or trematode metacercariae, but generally the condition appeared to be idiopathic. Fins most frequently affected were the caudal fin (51% of lesions), dorsal fin (23%), and anal fin (26%).

Fin Erosion Prevalences

Elevated prevalences of idiopathic fin erosion were found at 9 of 19 sampling sites where fin erosion prevalences were assessed (Fig. 7, Appendix Table A-6). These were Buzzards Bay (12.9%, n=62); Prudence Island in Narragansett Bay (8.1%, n=99); Deer Island (23.8%, n=151), Mystic River (31.1%, n=90), Hull Bay (10.0%, n=60), and Quincy Bay (21.9%, n=61) in Boston Harbor; Folgers Point in Salem Harbor (8.2%, n=122); and New Haven (36.7%, n=161) and Norwalk (20%, n=30) in Long Island Sound. The prevalence of fin erosion at the Rocky Point reference site in Long Island Sound was 3.3% (n=60).

Risk Factors Associated with Fin Erosion Occurrence in Individual Fish

Fin erosion was most likely to occur in fish from the Mystic River in Boston Harbor and the New Haven site in Long Island Sound, which showed 9 and 12 fold increases in relative risk, respectively (Table 7). Two to four-fold increases in the risk of fin erosion were found at Black Point in Niantic Bay, Deer Island and Quincy Bay in Boston Harbor, and Norwalk in Long Island Sound. In addition, the probability of fin erosion increased significantly with fish age.

Relationships Between Chemical Contaminants and Fin Erosion Prevalences

The prevalence of fin erosion was positively associated with levels of all classes of contaminants in sediment and stomach contents, with the exception of PCBs (Tables 12 and 13). A significant relationship was also observed between fin erosion prevalences and biliary FAC concentrations (Table 14). However, elevated levels of PCBs, DDTs, or chlordanes in flounder liver tissue were not associated with an increased risk of fin erosion (Table 14).

DISCUSSION

Patterns of Lesion Occurrence

Results of the present study indicate that winter flounder residing in contaminated areas are likely to exhibit certain pathological conditions, including fin erosion and lesions of the liver and

kidney, at prevalences that are significantly higher than those detected in animals collected from relatively uncontaminated reference sites. The sites with the highest prevalences of lesions were located within Boston Harbor, Raritan Bay, and Long Island Sound.

Hepatic lesions were probably the most reliable histological markers of contaminant exposure in winter flounder, which is consistent with current literature on histopathological biomarkers in fish (Hinton et al. 1992, Varanasi et al. in press). Hydropic vacuolation and nonspecific necrotic lesions appeared to be particularly useful for field assessment of anthropogenic stress in this species. Hydropic vacuolation was found in up to 50% and necrotic lesions in up to 25% of winter flounder collected from heavily contaminated sites in Boston Harbor, Raritan Bay, and Long Island Sound, but it was found in less than 5% of fish from minimally contaminated areas. Hydropic vacuolation has also been detected in rock sole *(Lepidopsetta bilineata)*, starry flounder *(Platichthys stellatus)*, and white croaker (Stehr 1990; Stehr et al. 1991; Myers et al. 1992; in press) from contaminated sites on the West Coast, and it appears to be associated with the development of cholangiocytic neoplasms (Harshbarger and Clark 1990). Consequently, it is of considerable value as a histopathologic biomarker. Similarly, hepatocellular necrosis and related lesions are well established as biomarkers of contaminant exposure, and they have been observed in both field and laboratory studies in a variety of fish species (Meyers and Hendricks 1985; Hinton et al. 1992). A variety of other hepatic lesions, including neoplasms, foci of cellular alteration, nonneoplastic proliferative lesions, and spongiosis hepatis, were also observed in flounder from these sites. Prevalences of these lesions were substantially lower (e.g., 2 to 10%) than prevalences of hydropic vacuolation and hepatocellular necrosis, but their pattern of distribution was reflective of an association with contaminant exposure, confirming their utility as histopathologic biomarkers.

Megalocytic hepatosis and nuclear pleomorphism, two closely related toxicopathic and presumably degenerative hepatic conditions described in English sole and highly correlated with contaminant exposure in that species (Myers et al. 1987, 1990, 1991, 1992, in press), showed no relationship with contaminant exposure in winter flounder. Megalocytic hepatosis was not

observed at all, while nuclear pleomorphism was found in less than 2% of winter flounder examined and did not occur at elevated prevalences in flounder from contaminated sites. Consequently, changes in nuclear size observed in this study probably represent the normal range of variation in nuclear size for this species and do not represent a lesion. As nuclear pleomorphism and megalocytic hepatosis are characteristic bioindicators of contaminant exposure in English sole, as well as in several other bottomfish species (Myers et al. 1987, in press), the absence of these two lesions in winter flounder is a striking finding.

In general, the types of lesions observed in winter flounder in the present study and their distribution among the common sampling sites are similar to the findings of other investigators (Murchelano and Wolke 1985, 1991; Moore 1991; Carr et al. 1991). At heavily contaminated sites within Boston Harbor, for example, Murchelano and Wolke (1985, 1991) and Moore (1991) report preneoplastic and neoplastic lesions in 10 to 15% and hydropic vacuolation in 56 to 74% of winter flounder sampled. In contrast, in flounder from minimally contaminated sites in Cape Cod Bay, Massachusetts Bay, or Georges Bank, neoplasms were not observed, while vacuolated cell lesions were found in less than 10% of fish examined (Moore 1991, Murchelano and Wolke 1991).

In addition to hepatic lesions, fin erosion and a variety of idiopathic lesions in the kidney were monitored in this study so their utility as biomarkers could be evaluated. Renal lesions occurred at relatively low prevalences in winter flounder, and they did not appear to be closely related to contaminant exposure. Fin erosion, on the other hand, was found in a surprisingly high proportion of winter flounder collected from contaminated areas in Boston Harbor and Long Island Sound. Elevated prevalences of fin erosion have been reported previously in winter flounder collected from Boston Harbor (Williams 1981) and Raritan Bay (Ziskowski and Murchelano 1975). However, it is uncertain whether xenobiotics are a principal or merely a contributing etiologic factor in the development of these lesions.

Toxicopathic Response to Contaminant Exposure in Winter Flounder

Although winter flounder exhibits a number of widely recognized toxicopathic liver lesions, in comparison with other teleost species which have been commonly used in carcinogenesis studies, its pathological response to environmental contaminants is quite distinctive. When exposed to carcinogenic compounds such as certain AHs, English sole and other widely studied species such as medaka, killifish, and rainbow trout characteristically respond in a manner that closely follows the mammalian pattern of histogenesis of neoplasia (Frith and Ward 1980, Couch and Harshbarger 1985, Myers et al. 1987, Myers et al. 1990). Early toxicopathic changes in this species typically include hepatocellular necrosis and megalocytosis in association with hepatocellular regeneration; preneoplastic focal lesions are common; and the majority of neoplasms observed in both field and laboratory studies are hepatocellular in origin (Myers et al. 1987, Myers et al 1990, Vogelbein et al. 1990, Metcalfe et al. 1988, Black et al. 1985, Hendricks et al. 1985, Schultz and Schultz 1984, Hawkins et al. 1990) . In contrast, the toxicopathic response exhibited by winter flounder is characterized by hydropic vacuolation of hepatocellular and biliary cells and biliary hyperplasia (this study, Moore 1991, Murchelano and Wolke 1985, 1991); a relatively low prevalence of altered foci ; and a predominance of cholangiocellular neoplasms over hepatocellular neoplasms. In recent field studies of winter flounder, for example, 42% to 90% of observed neoplasms were cholangiocellular (this study, Moore 1991, Murchelano and Wolke 1985, 1991). In contrast, cholangiocellular neoplasms account for only about 15% of liver neoplasms in English sole (Myers et al. 1987).

Several other teleost species, including starry flounder and white croaker sampled from contaminated sites on the West Coast as part of the NBSP (Stehr 1990, Stehr et al. 1991, Myers et al. 1992, in press), exhibit patterns of lesion occurrence similar to those observed in winter flounder. These findings suggest that toxicopathic response, and possibly histogenesis of neoplasia, may follow more than one pathway in teleost fish.

Biological Risk Factors Associated with Lesion Occurrence

Age

For all classes of hepatic lesions described in this study, other than hepatocellular nuclear pleomorphism, the risk of disease occurrence increased significantly with increasing age. A similar relationship between lesion prevalence and age has been documented in other studies on winter flounder. For example, Wolke et al. (1985) report that neoplasms were found only in winter flounder greater than 25 cm in length, while Murchelano and Wolke (1991) report no tumors in fish less than 320 mm in length. Moore (1991) reports that prevalences of biliary proliferation, and hydropic vacuolation and neoplastic lesions in winter flounder increased with increasing length or age, and no neoplasms were observed in winter flounder below 300 mm or 5 years of age. Positive relationships between prevalences of liver nodules, neoplasms, and other toxicopathic lesions and fish age have been noted in several other species as well, including English sole from Puget Sound (Rhodes et al. 1987; Barrick et al. 1985; Myers et al. 1990, 1991, 1992), ruffe *(Gymnocephalus cernuus)* from the Elbe estuary (Kranz and Peters 1985), and bream *(Abramis breama)* from contaminated European rivers (Sloof and DeZwart 1982).

Age was also a significant risk factor for renal lesions and fin erosion. There is little information available on the relationship between fish age and the occurrence of fin erosion, but for renal lesions this result is consistent with previous observations in other flatfish species. For example, in previous studies in English sole (McCain et al. 1982, Rhodes et al. 1987), sclerotic, necrotic, and proliferative renal lesions all showed a significantly increased risk attributable to fish age. In West Coast NBSP studies (Myers et al. 1992), the risk of one or more categories of renal lesions increased with age in English sole, starry flounder, white croaker, black croaker *(Cheilotrema saturnum)*, and flathead sole *(Hippoglossoides elassodon)*. The significance of age as a factor in renal necrotic lesions in fish is not understood, although the susceptibility of the kidney to such injury has been shown to increase with age in mammals as well as fish (Finn 1983). As for sclerotic lesions, the increase in mesangial matrix density that characterizes mesangiosclerosis or glomerular hyalinization, two of the most common forms of these lesions,

occurs as a cumulative nonspecific response to injury in a variety of chronic renal disease states (Schillings and Stekhoven 1980), as well as occurring as a normal part of the aging process in a number of vertebrates, including humans (Finn 1983).

Sampling Season

In addition to age, the risk of occurrence of certain lesion types appeared to be related to the season of sampling. Female winter flounder which were sampled at heavily contaminated sites in Boston Harbor and Raritan Bay during the winter spawning season were less likely to exhibit hydropic vacuolation and nonspecific necrotic lesions than females sampled in late spring after spawning was completed. This suggests that spawning populations may be partially composed of fish migrating from other less contaminated areas, that are not normally resident at the sampling sites at other times of the year. This theory is supported by studies on winter flounder migration patterns during the spawning season, which suggest that, for the most part, winter flounder are organized into distinct spawning groups that return to their respective spawning grounds year after year (Black et al. 1988, Saila 1961). Outside of the spawning season, however, many of these fish disperse to offshore waters and the stocks mix (Danila 1989). In Narragansett Bay, for example, certain subpopulations of fish congregate in highly contaminated estuaries of the upper bay to spawn, but at other periods these same animals may mingle with other populations in less polluted lower Narragansett Bay or offshore (Black et al. 1988). Consequently, fish captured at an area during the spawning season are not necessarily representative of the population that resides there during the rest of the year.

Gender

The data from the present study suggest that female winter flounder are slightly less likely than male winter flounder to develop certain lesions, including neoplasms, spongiosis hepatis, and necrotic lesions in the kidney. The finding that female winter flounder were less susceptible than males to hepatic neoplasms was somewhat surprising, as other studies have found little or no effect of gender on neoplasm prevalence in winter flounder (Murchelano and Wolke 1991, Moore 1991).

Nor did gender appear to have any effect on neoplasm prevalence in English sole (Rhodes et al. 1987; Myers et al. 1990, 1991, 1992). The differences in lesion prevalences observed in the present study between male and female flounder collected at the same time (i.e., during spring NBSP sampling, outside the spawning season) were relatively small, so it is possible that they represent a sampling anomaly rather than a true biological difference. However, true gender-associated differences in susceptibility to lesion development could arise as a result of the differences in the migratory behavior of male and female winter flounder. Studies of winter flounder migration patterns indicate that adult females are generally more mobile than males, often spending extended periods offshore to feed during summer months (Black et al. 1988, Valdez 1989, Danila 1989), in areas where contaminant exposure would be greatly reduced. Also, there is evidence that activities of xenobiotic metabolizing enzymes are affected differently by the reproductive cycle in males and females (Snowberger and Stegeman 1987, Snowberger-Gray et al. 1991) so there is the potential for gender-associated differences in the metabolism of toxic compounds and in the concentrations of toxic or carcinogenic intermediates produced. Consequently, it would be premature to draw any conclusions about the effect of gender on hepatic lesion susceptibility in winter flounder without additional research.

Gender had no apparent influence on proliferative or sclerotic renal lesions in winter flounder, but males appeared to have a higher risk of developing necrotic renal lesions. Increased risk of various types of kidney lesions in males were also reported for English sole (necrotic lesions), flathead sole (proliferative lesions), and hornyhead turbot (*Pleuronichthys verticalis* , proliferative lesions) (Myers et al. 1992). These results are in contrast to the studies of Rhodes et al (1987) which showed no influence of gender on renal lesions in English sole. It is not clear why such a relationship should exist, but its consistency across a variety of species suggests that it may have some biological foundation and consequently may merit further research.

Relationships Between Chemical Contaminants and Idiopathic Lesions

In addition to determining prevalences of suspected toxicopathic lesions at the sampling sites, we attempted to examine the relationships between prevalences of these lesions and specific

contaminants using logistic regression. As mentioned earlier, the analysis had certain limitations. For example, because of the time and cost involved in doing detailed chemical analyses, most classes of contaminants were measured in composite samples rather than in individual fish. The number of samples available for inclusion in analyses was relatively small, and it was not possible to examine relationships between contaminant levels and disease in individual fish. In addition, the high degree of intercorrelation among different classes of contaminants measured in tissue, stomach contents, and sediment made it difficult to determine the effects of specific compounds or groups of compounds on disease prevalence. However, this analysis was not intended to determine the precise chemical etiology of idiopathic disease in winter flounder. Rather, its objective was to provide preliminary information about the possible roles that selected organic contaminants might play in the development of disease conditions, thus providing direction for future research. With these considerations in mind, the discussion below attempts to interpret the significance of the associations identified between lesion types and parameters of contaminant exposure, especially in terms of their toxicologic significance.

Hydropic Vacuolation

Of the lesions tested, hydropic vacuolation showed the strongest and most consistent relationships with levels of organic contaminants measured in sediment, stomach contents, and liver and bile. Concentrations of DDTs, chlordanes, and AHs (or metabolites) measured in all compartments were significant risk factors for this lesion. The observed relationships between tissue contaminant levels and the occurrence of hydropic vacuolation provide evidence of a chemical etiology for this lesion, and help to establish its validity as a biomarker of contaminant-induced stress. However, because concentrations of all three of these classes of organic contaminants in sediments were highly correlated and winter flounder are exposed to them simultaneously, it was difficult to determine the relative importance of DDTs, chlordanes, and AHs in the development of hydropic vacuolation. The problem is further compounded by the fact that in a recent laboratory study (Moore 1991), this lesion was not induced in winter flounder by intraperitoneal injection or dietary exposure of winter flounder to high levels of either chlordane or

the AH, benzo[a]pyrene (BaP). It is not clear why this was so, but is may be that exposure to the complex mixture of chemicals present in urban sediments is necessary for the generation of hydropic vacuolation. Alternatively, hydropic vacuolation may be caused by an unknown and unmeasured etiologic agent that covaries with the measured contaminants. Whatever the case, it would be premature to dismiss AHs, chlordanes, or DDTs as potential risk factors for this lesion without further investigation. This is particularly true as hydropic vacuolation has also been associated with exposure to organic contaminants in starry flounder and white croaker from contaminated sites sampled on the West Coast in the NBSP. In starry flounder, AHs, PCBs, and DDTs were all found to be significant risk factors for the development of this lesion, while in white croaker hydropic vacuolation was most closely associated with exposure to PCBs (Myers et al. 1992). Laboratory exposure studies with multiple species using intact sediments, organic extracts of urban sediments, and model compounds may be needed to determine how various classes of AHs and CHs, alone or in concert, may contribute to the development of this lesion.

Nonspecific Hepatocellular Necrosis

Nonspecific necrotic lesions were positively associated with AH concentrations in sediment and stomach contents, and with levels of FACs in bile. These consistent associations between AHs and necrotic lesion over several compartments strongly support AHs as a significant risk factor for hepatic necrosis. Moreover, laboratory data support the role of AHs as necrogenic agents in winter flounder (Moore 1991) and other fish species (Wyllie et al. 1980, Meyers and Hendricks 1985, Pitot 1988). Necrotic lesions were also positively associated with DDT and chlordane concentrations in sediments and stomach contents, but they showed little relationship with concentrations of these contaminants in liver tissue. As concentrations of chlordanes and DDTs in liver are a more reliable measure of chronic exposure to these compounds than concentrations in either sediment or stomach contents, the lack of relationship between concentrations of DDTs and chlordanes in liver and prevalences of necrotic lesions may be an indication that these contaminants are not major risk factors for the development of these lesions. However, laboratory exposure to chlordanes has induced necrotic lesions in winter flounder

(Moore 1991), and both chlordanes and DDTs have been shown to have necrogenic effects on the liver in mammals (Hodge et al. 1967; Murphy 1986). Consequently, they may be significant risk factors for the development of necrotic lesions if animals are exposed to sufficiently high doses of these compounds. Prevalences of necrotic lesions were not correlated with levels of PCBs in sediments or in stomach contents or liver tissue of winter flounder. In fact, in winter flounder from New Bedford Harbor, which had the highest concentrations of PCBs in sediment of all the Northeast sites, the prevalence of necrotic lesions was less than 4%. The lack of relationship between PCBs and idiopathic disease in winter flounder was a striking finding of this study and will be discussed in detail later in this report.

Neoplasms and FCA

In the present study, prevalences of neoplastic and preneoplastic lesions in winter flounder were not strongly associated with concentrations of organic contaminants in sediments, stomach contents, or biliary FACs. Those associations which were observed (i.e., with chlordanes and AHs) were relatively weak and, for no clear reason, occurred only with contaminant concentrations in stomach contents. However, AHs are well established as genotoxic carcinogens in mammals (Williams and Weisburger 1986), and there is substantial evidence that they act in the same way in fish (Varanasi et al. 1989d). AHs have been demonstrated to bind to hepatic DNA (Smolarek et al. 1987, Varanasi et al. 1986a,b, 1989b,c; Shugart et al. 1987), and administration of AHs in laboratory studies has led to the development of neoplastic or preneoplastic lesions in several fish species (Schiewe et al. 1991, Metcalfe et al. 1988, Black et al. 1985, Hendricks et al. 1985, Schultz and Schultz 1984, Hawkins et al. 1990). Moreover, several epidemiological studies, including histopathological field surveys of English sole in Puget Sound, show strong correlations between AH levels in sediments and metabolites of AHs in bile (Krahn et al. 1986) and prevalences of neoplasms and preneoplastic focal lesions (Malins et al. 1984; Landahl et al. 1990; Myers et al. 1990, 1991, 1992).

It is not entirely clear why correlations between AH exposure and FCA and neoplasms in winter flounder were not as strong as correlations observed in other flatfish species (e.g., English

sole) (Malins et al. 1984, 1985; Landahl et al. 1990; Myers et al. 1990). One explanation could be that the relatively low prevalences of neoplasms and FCA detected in this study made it difficult to establish a relationship between AH exposure and these lesions in winter flounder. Moreover, because AHs are largely metabolized and do not accumulate in fish tissue (Varanasi et al. 1989d, Stein et al. 1987), we currently have no reliable measure of chronic AH exposure. Levels of metabolites in bile are generally reliable measures of short-term exposure in other flatfish species (Krahn et al. 1986). However, biliary concentrations of FACs decline substantially within a few weeks after a single exposure to AHs (Collier and Varanasi 1991), so FAC levels are likely to be elevated only when animals are in or have recently left contaminated areas. For territorial species such as English sole, this does not present a problem, but in a more mobile species such as winter flounder, it may cause complications. The difficulty of obtaining an accurate assessment of chronic AH exposure in winter flounder may partially account for the relatively weak relationships observed in this study between neoplastic and preneoplastic lesions and AH exposure. It should also be kept in mind that there is a long latency period for the development of neoplasms and related lesions. Consequently, although environmental contaminants is likely to be critical factor in the etiology of these lesions, their occurrence may not be highly correlated with concentrations of contaminants in sediment or in fish tissues at the time the animals are collected, especially in a relatively mobile species such as winter flounder.

In summary, the long latency period for the development of neoplasms and related lesions, the lack of reliable measures of chronic exposure to potential environmental carcinogens, and the low prevalence of these lesions, may all have contributed to weak correlations between neoplasms and related lesions and potential etiologic agents such as AHs. However, because AHs are well established as carcinogens in several species of fish, they should not be dismissed as toxicologically significant risk factors for hepatic neoplasms and focal lesions in winter flounder.

The association between chlordane levels in stomach contents and neoplasms is too weak to be considered as evidence for involvement of chlordanes or other pesticides in the development of neoplasia in winter flounder, particularly as is it not consistent across all compartments (i.e.,

sediment, liver, and stomach contents). However, an association between chlordane and neoplasms is not inconsistent with current theories on carcinogenesis. While pesticides such as chlordanes and other chlorinated hydrocarbons show little evidence of genotoxicity and are generally not considered to be primary carcinogens (Pereira 1983, Rossi et al. 1983), they are classified as possible promoters that facilitate tumor development by cells which have been genetically altered by genotoxic compounds, such as certain AHs (O'Brien 1967). Moreover, hepatocarcinogenic or promotional effects of chlorinated pesticides have been found in fish (Halver 1967). In West Coast NBSP studies, one class of chlorinated pesticides, the DDTs, were identified as a strong risk factor for hepatic neoplasms in English sole (Myers et al. 1992). Consequently, chlordanes may play a role in the etiology and development of neoplasms in winter flounder, although it seems unlikely that they are the primary agents of initiation.

Proliferative Lesions

Proliferative lesions such as biliary and hepatocellular regeneration were significantly correlated with levels of chlordanes in both stomach contents and liver tissue. This is quite interesting, as laboratory exposure to chlordane induced both hepatocellular necrosis and lesions resembling biliary proliferation in winter flounder (Moore 1991). Aside from chlordanes, no other organic contaminants emerged as significant risk factors for proliferative lesions in the present study. However, in laboratory studies, these lesions have been induced in winter flounder by exposure to BaP (Moore 1991), as well as in other fish by exposure to hepatotoxic or hepatocarcinogenic compounds, including AHs (Schiewe et al. 1991, Hendricks et al. 1984, Nunez et al. 1991). As with neoplasms, the relatively low prevalence of proliferative lesions, coupled with the lack of a reliable chronic measurement of AH exposure, may have obscured the potential toxicological relationship between AHs and these lesions.

Spongiosis Hepatis

Aside from a relatively weak correlation with chlordane levels in stomach contents, prevalences of spongiosis hepatis showed no relationship with levels of measured organic contaminants in sediments, stomach contents, or tissues. Although this lesion has been observed

in laboratory exposures of medaka and sheepshead minnow to carcinogens (Hinton et al. 1984, 1988; Couch and Courtney 1987), and in winter flounder exposed to BaP in the laboratory (Moore 1991), it does not show strong evidence of a chemical etiology in winter flounder on the basis of the present study. Moreover, because of its low prevalence (< 5%) even in urban sites, it is unlikely to be useful as a biomarker of contaminant exposure in field studies.

Megalocytic Hepatosis/Nuclear Pleomorphism

Prevalences of nuclear pleomorphism in winter flounder were unrelated to contaminant concentrations in sediment, stomach contents, or liver, and, as noted previously, megalocytic hepatosis was not observed in flounder sampled as part of this study. Nuclear pleomorphism and megalocytic hepatosis are characteristic toxicopathic responses to AH exposure in English sole, as well as in several other bottomfish species (Myers et al. 1987, 1992, in press), and their rarity in winter flounder is an indication that this species may differ somewhat from other widely studied teleost fish in its pathological response to environmental contaminants.

Renal Lesions

In contrast to hepatic lesions, renal lesions in winter flounder were not highly correlated with concentrations of measured organic contaminants in fish or sediments. Of the three classes of lesions (proliferative, necrotic, and sclerotic) monitored in this study, only necrotic lesions showed any evidence of a relationship to contaminant exposure. Necrotic lesions were significantly elevated at Deer Island in Boston Harbor and showed significant positive correlations with concentrations of LAHs in sediment and stomach contents. Because of the relative lack of information on the effects of aromatic hydrocarbon exposure on the teleost kidney, the toxicological significance of this relationship is not clear. Although AHs are not widely regarded as nephrotoxicants in mammals (Hook and Hewitt 1986) or fish (Hinton and Lauren 1990), associations between AH exposure and renal lesions have been observed in other studies of bottomfish species. For example, in laboratory studies where English sole were exposed to BaP or an AH-enriched sediment extract, exposed fish showed a statistically higher incidence of tubular necrotic and proliferative lesions than controls (Schiewe et al. 1991). Moreover, in field-collected

English sole and starry flounder, there was some evidence of increased risk of renal disease associated with elevated biliary FAC levels or sediment AHs (Myers et al. 1992). In comparison to hepatic lesions, associations between contaminants and renal disease were relatively weak, but because of the consistency of these associations among several species, the kidney lesions, especially necrotic or degenerative conditions should not be dismissed as potential indicators of the effects of environmental contaminants.

In this study there was little evidence of a relationship between chlorinated hydrocarbons and kidney disease in winter flounder. This was not unexpected, as pesticides and other chlorinated aromatic compounds are not generally considered to be potent renal toxins in either mammals (Hook and Hewitt 1986) or fish (Pritchard and Renfro 1984). However, exposure to DDTs or PCBs was linked to an increased risk of renal disease in flathead sole, white croaker, and English sole collected from urban areas along the West Coast in NBSP samplings (Myers et al. 1992), and degenerative lesions have been induced in teleost kidney by exposure to Arochlor 1254 (Nestel and Budd 1975) and DDTs (King 1962). In view of this evidence, it would be premature to dismiss chlorinated hydrocarbons (CHs) as potential risk factors for renal disease in winter flounder, although it is possible that the lack of relationship between CHs and renal disease in winter flounder represents a true species difference in sensitivity to these compounds as renal toxicants.

Although renal lesions were not strongly associated with concentrations of organic contaminants in sediments and winter flounder, they did appear at elevated prevalences at certain industrialized sites. Several metals present in sediments, including copper and chromium, have been shown to produce degenerative changes in the kidneys of winter flounder and rainbow trout *(Onchorhynchus mykiss)* in laboratory exposure studies (Baker 1969, Van der Putte et al. 1981), and metals have been identified as potential risk factors for necrotic and sclerotic kidney lesions in white croaker, English sole, and flathead sole sampled from the West Coast during the NBSP (Myers et al. 1992). The possible significance of metals as renal toxicants in winter flounder will be investigated in future studies.

Fin Erosion

Fin erosion prevalences were relatively high at certain heavily contaminated sites such as Mystic River, Deer Island, and Quincy Bay in Boston Harbor. Moreover, prevalences of this condition appeared to be strongly correlated with contaminant concentrations in sediments and stomach contents, and there was also some evidence of an increased risk of fin erosion in winter flounder with elevated biliary FAC concentrations. Although fin erosion has been reported at elevated prevalences in winter flounder from Boston Harbor and Raritan Bay (Williams 1981, Ziskowski and Murchelano 1975), to our knowledge this is the first study showing a significant association between exposure to specific contaminants and the risk of fin erosion in winter flounder. There is some evidence for a chemical etiology for fin erosion in various fish species (Sindermann 1990). However, in a study examining the relationships between contaminants and fin erosion in English sole (L.D. Rhodes, unpubl. data. Department of Biological Structure, University of Washington, Seattle, WA 98195), no significant associations were found between the presence of fin erosion and any specific xenobiotic compounds in sediment. In English sole, where prevalences of fin erosion were generally less than 1%, this lesion appeared to have an inflammatory etiology, with a number of biotic or abiotic factors, including parasites or wounds as well as exposure to irritants in sediment potentially serving as initial causes of the inflammation. Exposure to contaminants is only one of the many factors that may be associated with the development of fin erosion, so further verification is needed to confirm its chemical etiology and to determine whether xenobiotic compounds are principal or merely contributing etiologic factors in its development. However, the results of this study, as well as similar findings of increased prevalences of fin erosion in other NBSP species (Cross 1985, McCain et al. 1992), suggest it may have some utility as an indicator of environmental quality.

PCBs and Idiopathic Disease in Winter Flounder

In this study PCBs did not emerge as significant risk factors for the development of any pathological condition in winter flounder. The lack of a relationship between concentrations of

PCBs in tissue and sediments and prevalences of idiopathic disease in winter flounder was somewhat unexpected. In a similar study in which PCB concentrations in tissues of individual female winter flounder were measured and correlated with lesion occurrence (Johnson et al. 1992), PCB concentrations were positively correlated with prevalences of both hydropic vacuolation and biliary proliferation. Moreover, in starry flounder and white croaker examined as part of the West Coast NBSP, PCBs were found to be significant risk factors for hydropic vacuolation and other degenerative lesions (Myers et al. 1992), as well as contributory risk factors for the development of neoplasms. In the above-mentioned studies, however, fish were captured from sites where PCBs were present in combination with other contaminants such as AHs, DDTs, or pesticides. In the present study, fish were also collected from Clark's Point in New Bedford Harbor, a site where concentrations of PCBs in sediments and in winter flounder liver (approximately 3000 ng/g dry weight and 39,000 ng/g, respectively) were much higher than at any of the other Northeast Coast sampling sites, but levels of other contaminants in sediments and fish were moderate to low (Brown et al. in prep.). At the New Bedford site, then, PCBs were the major etiologic agent to which winter flounder were exposed, and under these conditions fish did not exhibit significantly elevated prevalences of any category of hepatic lesion, compared to flounder from the Rocky Point, Long Island Sound reference site. The prevalence of hydropic vacuolation, for example, was less than 6% in flounder from the PCB-contaminated New Bedford site, while prevalences of this lesion in flounder from several contaminated sites in Boston Harbor and Raritan Bay, where AHs and CHs co-occur in sediments, were over 40%. These findings suggest that while PCBs may contribute to the development of hydropic vacuolation, neoplasms, and other hepatic lesions in winter flounder by acting in concert with other contaminants such as AHs, they are not likely to be the primary etiologic agents.

It is not surprising that winter flounder from the New Bedford site which were exposed to PCBs alone did not exhibit high prevalences of neoplasms or preneoplastic lesions, because chlorinated hydrocarbons such as PCBs are not generally considered to be primary carcinogens in either fish or mammals (Silberhorn et al. 1990), although they may promote neoplasm

development if animals are also exposed to genotoxic compounds such as certain AHs (Shelton et al. 1984, Safe 1989, Hendricks et al. 1990, Silberhorn et al. 1990). On the other hand, the finding that winter flounder from the New Bedford site did not develop degenerative or regenerative lesions, such as biliary hyperplasia and hydropic vacuolation, was unexpected, as exposure to PCBs has been associated with biliary proliferative lesions in mammals (Kimbrough et al. 1972, Kimbrough and Linder 1974), and with hydropic vacuolation in winter flounder in previous field studies (Johnson et al. 1992). However, the results of the present study suggest that if PCBs do contribute to the development of hydropic vacuolation, they do so in concert with other contaminants such as AHs.

In summary, using data gathered during the Northeast Coast NBSP, several potential chemical risk factors (e.g., AHs, DDTs, and chlordanes) were identified for idiopathic disease in winter flounder. Moreover, these data suggested that exposure to PCBs alone did not play a major role in lesion initiation, although it is possible that selected toxic PCB congeners (Safe 1984) or PCBs in combination with other contaminants could have an influence on lesion development. Because different classes of contaminants generally occurred together in sediments, and animals were exposed to a mixture of agents simultaneously, it was difficult to evaluate the roles that specific toxicants might play in the etiology of pollution-related disease in winter flounder on the basis of epizootiological data alone. However, the correlational evidence generated by the Northeast Coast NBSP provides valuable insights into involvement of chemical contaminants in lesion development in winter flounder, and it may be used to formulate definitive cause-and-effect studies of disease initiation and progression with potential etiologic agents present in urban environments.

CONCLUSIONS

1. Overall, hepatic lesions proved to be extremely reliable histological markers of contaminant exposure in winter flounder. Hydropic vacuolation and nonspecific necrotic lesions showed particular utility as indicators of anthropogenic stress. Both lesions occurred at high

prevalences in winter flounder from heavily contaminated sites and showed a clear gradient in prevalence from heavily to minimally contaminated sites. In addition, they were closely correlated with levels of several classes of organic contaminants in fish tissue and fluids, sediments, and stomach contents.

2. Hepatic neoplasms, foci of cellular alteration, and nonneoplastic proliferative lesions such as biliary hyperplasia were also found in winter flounder from several heavily contaminated sites, but these lesions were not as highly correlated with chemical contaminant levels as might be expected in view of laboratory and field studies in other teleost fish linking environmental carcinogens to the development of neoplasms. Such relationships may have been difficult to identify in winter flounder for several reasons, including: 1) the relatively low prevalence of neoplasms and FCA (e.g., 2-10%) in this species; 2) the long latency period for the development of these lesions; 3) the fact that winter flounder is a relatively mobile species; and 4) the lack of a reliable measure of chronic exposure to certain classes of environmental carcinogens, such as AHs.

3. Nuclear pleomorphism and megalocytic hepatosis, two closely related toxicopathic and presumably degenerative hepatic conditions described in English sole and highly correlated with contaminant exposure in that species, either did not occur or were found only at low prevalences in winter flounder and did not show any relationship with indicators of contaminant exposure.

4. Those compounds clearly identified as risk factors for pathological conditions in winter flounder were AHs, DDTs, and chlordanes. However, because these three classes of contaminants generally occurred together in sediments, and animals were exposed to a mixture of agents simultaneously, it was difficult to evaluate the relative importance of these three classes of contaminants in the development of pollution-associated disease in winter flounder.

5. In addition to chemical contaminants, certain biological factors, particularly age and sampling season, had a significant influence on disease prevalence and should be accounted for in analyses examining relationships between contaminant exposure and disease.

6. PCBs did not emerge as significant risk factors for any pathological condition observed in winter flounder. Not only did PCB levels in sediment, stomach contents, and liver fail to

correlate with lesion prevalences, but perhaps more importantly, fish sampled from one site (New Bedford Harbor) that had high levels of PCBs in sediments but relatively low levels of other contaminants had low prevalences of all pathological conditions monitored in this study. While this does not eliminate the possibility that PCBs may play a promotional role in the development of neoplastic lesions, or function as toxicants in concert with other compounds such as AHs, it does suggest that they are not the primary etiologic agents for either neoplastic lesions or regenerative and degenerative conditions in winter flounder.

7. Renal lesions were not highly correlated with levels of organic contaminants in sediments, stomach contents, bile or liver of winter flounder. Of the three classes of lesions monitored in this study (proliferative, necrotic, and sclerotic), only necrotic lesions showed any evidence of a relationship to contaminant exposure.

8. Fin erosion prevalences were relatively high at certain heavily contaminated sites especially within Boston Harbor, and were significantly correlated with contaminant concentrations in sediments and stomach contents, and with biliary FAC concentrations. Because biotic factors such as fungal or bacterial pathogens as well as xenobiotic contaminants may be associated with the development of fin erosion, it is not as specific a biomarker as other toxicopathic lesions, but data from this study indicate that it may be a useful indicator of contaminant exposure in winter flounder.

9. The toxicopathic response typically exhibited by winter flounder exposed to organic contaminants (e.g., hydropic vacuolation of hepatocellular and biliary cells and biliary hyperplasia, and a predominance of cholangiocellular neoplasms over hepatocellular neoplasms) is quite different from patterns of lesion occurrence associated with the histogenesis of neoplasia in mammals and fish species such as medaka *(Oryzias latipes),* rainbow trout, and English sole. The types of lesions found in winter flounder in contaminated areas have also been noted in other bottomfish (e.g., starry flounder and white croaker) at contaminated sites on the West Coast sampled in the NBSP. These species differences suggest that toxicopathic response, and possibly histogenesis of neoplasia, may follow more than one pathway in teleost fish.

10. Although the present analysis identified several chemical risk factors for disease in winter flounder, the relative importance of different types of contaminants in the development of specific lesion types in winter flounder is not clear. The associations between organic contaminants and disease conditions observed in winter flounder examined in the NBSP may provide the foundation for future cause-and-effect studies which can examine in a more systematic fashion the roles that specific organic contaminants, alone or in combination, may play in the etiology of pollution-associated disease in winter flounder.

ACKNOWLEDGMENTS

We thank Tracy Collier and John Stein of the Environmental Conservation Division and Doug Wolfe, Donna Turgeon, Carol-Ann Manen, Harris White, and Jawed Hameedi of NOAA National Ocean Services for reviewing the manuscript, and Ms. Sharon Giese for editorial assistance. These studies were partially supported by the Office of Ocean Resources Conservation and Assessment (NOAA National Ocean Services) as part of the National Status and Trends Program, and by the NOAA Coastal Ocean Program.

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TABLES

Table 1. East Coast sites sampled in 1987, 1988, or 1989 as part of the National Benthic Surveillance Project (NBSP Cycle 5 1988, NBSP Cycle 6 1989), or in related studies in Long Island Sound (LIS intensive 1987) and Boston Harbor (BH 1988).

State	Site name	Site abbrev.	Lat.	Long.	Years sampled: LIS intensive 1987 (spring)	NBSP cycle 5 1988 (spring)	BH 1988 (winter)	NBSP cycle 6 1989 (spring)	Types of samples[1]
MA	Salem Harbor, Folgers Point	SALFP	42°32.2'	70°49.6'		x		x	c
MA	Boston Harbor, Deer Island	BOSDI	42°19.9'	70°58.1'			x	x	c
MA	Boston Harbor, Quincy Bay	BOSQB	42°18.4'	70°58.4'				x	c
MA	Boston Harbor, Mystic River	BOSMR	42°23.2'	71°03.2'			x	x	c
MA	Boston Harbor, Hull Bay	BOSHB	42°17.1'	70°54.4'				x	c
MA	Boston Harbor, President Roads	BOSPR	42° 20.0'	70° 59.0'			x		p
MA	Massachusetts Bay, outside Boston	MASBS	42°20.5'	70°46.0'			x		p
MA	Massachusetts Bay, Plymouth Entrance	MASPE	41°59.3'	70°37.6'			x	x	c
MA	New Bedford Harbor, Clarks Point	NWBCP	41°35.0'	70°53.5'		x		x	c
MA	Buzzards Bay	BUZWI	41°35.0'	70°45.0'		x			c
RI	Narragansett Bay, Prudence Island	NARPI	41°40.4'	71°21.2'		x	x		c
CT	Niantic Bay, Black Point	NIABP	41°17.2'	72°11.2'	x		x	x	c
CT	Long Island Sound, New Haven	LISNH	41°15.3'	72°54.8'	x				c
CT	Long Island Sound, Norwalk	LISNO	41°02.3'	73°28.2'	x				c
CT	Long Island Sound, Bridgeport	LISBR	41°14.9'	70°28.0'			x		p
CT	Long Island Sound, Rocky Point	LISRP	41°08.7'	72°24.7		x		x	c
NY	Long Island Sound, Lloyd Point	LISLP	40°58.0'	73°28.9'	x	x		x	c
NJ	Raritan Bay, East Reach	RARER	40°29.5'	74°05.4'		x		x	c
NJ	Raritan Bay, West Reach	RARWR	40°30.4	74°10.2'		x		x	c
NJ	Raritan Bay, Gravesend	RARGB	40°35.4'	74°01.6'				x	c
NJ	Raritan Bay, Upper Bay	RARUB	40°39.4'	74°02.8'				x	c
NJ	Great Bay, Intracoastal Waterway	GRTIW	39°25.8'	74°25.1'		x			c

[1] c = complete set of samples (sediment, stomach contents, and liver for chemistry; liver and kidney for pathology); p = pathology only

Table 2. Abbreviated names and full descriptions of disease categories utilized in documenting pathological conditions in winter flounder.

Abbreviation	Full description
Histologically diagnosable liver lesions	
Neoplasms	epithelial neoplasms (liver cell adenoma, cholangioma, hepatocellular carcinoma, cholangiocellular carcinoma, mixed hepatobiliary carcinoma, biliary cystadenoma, biliary cystadenocarcinoma, pancreatic acinar cell adenoma, pancreatic ductal adenoma, pancreatic cystadenoma, pancreatic acinar cell carcinoma, pancreatic adenocarcinoma); and mesenchymal neoplasms (hemangioma, hemangioendothelioma, hemangioentothelial sarcoma, fibroma, fibrosarcoma, hemangiopericytoma, neurilemmoma, neurofibroma, neurofibrosarcoma
Foci of cellular alteration (FCA)	putative preneoplastic focal lesions (foci of cellular alteration, including clear cell focus, eosinophilic focus, basophilic cell focus, and hyperplastic hepatocellular regeneration)
Proliferative lesions	proliferative non-neoplastic lesions (hepatocellular or biliary regeneration, biliary hyperplasia, papillary biliary hyperplasia, cystic biliary hyperplasia, cholangiofibrosis/adenofibrosis, increased hepatocellular mitotic activity)
Hydropic vacuolation	hepatocellular or biliary hydropic vacuolation (syn RAM cell areas, atypical cellular vacuolation)
Spongiosis hepatis	spongiosis hepatis or cystic degeneration of hepatocellular parenchyma
Megalocytic hepatosis/nuclear pleomorphism	increased hepatocellular and nuclear diameter and nuclear nyperchromasia, often accompanied by cytoplasmic changes including hyalinization (megalocytic hepatosis); enlarged or pleomorphic hepatocellular nuclei, unaccompanied by changes in hepatocellular size (nuclear pleomorphism)
Necrotic lesions	nonspecific hepatocellular or biliary necrotic/degenerative conditions including coagulative necrosis, hydropic degeneration, hyalinization, liquefactive necrosis, pyknosis, karyorrhexis, eosinophilic change, and hemorrhagic necrosis
Histologically diagnosable kidney lesions	
Proliferative lesions	non-neoplastic proliferative lesions, including hemopoietic tissue hyperplasia or proliferation, eruythroblastic hyperplasia or proliferation, glomerular hypercullularity, thickened, proliferative Bowman's capsule, tubular hyperplasia or proliferation, increased mitotic activity of tubules or hemopoietic tissue, and tubular regeneration
Necrotic lesions	necrotic lesions, including mesangiolysis, glomerular degeneration, tubular necrosis/degeneration, coagulative necrosis, hyalinization, or hydropic degeneration or tubular epithelium, hemopoietic tissue atrophy or hypoplasia, degeneration of Bowman's capsule, glomerular hyalinization, microvacuolar degeneration of tubular epithelium, erythrocyte necrosis of hemopoietic tissue, exfoliation of tubular epithelium, pyknosis of tubular epithelium or other nephron elements, cellular debris in tubular lumina, atypical cytoplasmic vacuolation of tubular epithelium or other nephron elements
Sclerotic lesions	sclerotic lesions including mesangiosclerosis, hypermembranous tubules (thickened basal lamina), hypermembranous glomerular tuft (thickened peripheral basal lamina), calcification, glomerulosclerosis, peritubular fibrosis, and fibrosis/fibroplasia of the interstitium
Grossly diagnosable lesions	
Fin erosion	fin erosion and associated lesions, including loss of fin, hemorrhagic fin tissue, fused rays, bent fin, exposed fin rays, fin ray blunting or retraction, and reflection of fin tissue back onto body surface

Table 3. Organic compounds measured during NBSP Cycles 5-6 (1988 and 1989). Low and high molecular weight aromatic hydrocarbons (LAHs and HAHs) and chlorinated hydocarbons (DDTs, PCBs, chlordanes, and dieldrin) were measured in sediment and fish stomach contents, and chlorinated hydrocarbons were also measured in fish livers.

Type	Chemical
Low molecular weight aromatic hydrocarbons (LAHs)	naphthalene
	2-methylnaphthalene
	1-methylnaphthalene
	biphenyl
	2,6-dimethylnaphthalene
	acenapthene
	acenapthylene
	2,3,5-trimethylnaphthalene
	fluorene
	phenanthrene
	anthracene
	1-methylphenanthrene
High molecular weight aromatic hydrocarbons (HAHs)	fluoranthene
	pyrene
	benz[a]anthracene
	chrysene
	benzo[b]fluoranthene
	benzo[k]fluoranthene
	benzo[e]pyrene
	benzo[a]pyrene
	perylene
	ideno[1,2,3-cd]pyrene
	dibenz[a,h]anthracene
	benzo[ghi]perylene
DDTs	o,p'-DDE
	p,p'-DDE
	o,p'-DDD
	p,p'-DDD
	o,p'-DDT
	p,p'-DDT
PCBs	trichlorobiphenyls
	tetrachlorobiphenyls
	pentachlorobiphenyls
	hexachlorobiphenyls
	heptachlorobiphenyls
	octachlorobiphenyls
	nonachlorobiphenyls
	decachlorobiphenyls
Chlordanes	alpha-chlordane
	trans-nonachlor

Table 4. Ranking of sampling sites by concentration of chemical contaminants in sediment. Total AHs = total aromatic hydrocarbons; HAHs = high molecular weight aromatic hydrocarbons; LAHs = low molecular weight aromatic hydrocarbons; DDTs = DDT and its derivatives; PCBs = polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific compounds measured in each class are listed in Table 3.

Site name	Total AHs	LAHs	HAHs	DDTS	PCBs	Chlordanes
Boston Harbor, Mystic River	1	1	1	1	2	1
Boston Harbor, Quincy Bay	2	2	2	3	3	2
Raritan Bay, Gravesend	3	10	3	6	6	11
Raritan Bay, East Reach	4	4	5	2	4	4
Raritan Bay, West Reach	5	11	4	4	5	12
Boston Harbor, Deer Island	6	5	7	15	7	8
Salem Harbor, Folgers Point	7	3	8	5	9	3
Raritan Bay, Upper Bay	8	12	6	12	13	14
Long Island Sound, Lloyd Point	9	6	9	8	11	5
Long Island Sound, New Haven	10	7	11	11	10	6
Boston Harbor, Hull Bay	11	14	10	13	14	13
Narragansett Bay, Prudence Island	12	9	12	9	8	10
Long Island Sound, Norwalk	13	8	14	10	12	7
New Bedford Harbor, Clarks Point	14	13	13	7	1	9
Niantic Bay, Black Point	15	16	16	16	16	18
Great Bay, Intracoastal Waterway	16	15	15	14	15	16
Buzzards Bay	17	17	17	17	17	19
Massachusetts Bay, Plymouth Entrance	18	18	18	19	19	15
Long Island Sound, Rocky Point	19	19	19	18	18	17

Table 5. Spearman-Rank correlation coeffecients (Spearman's rho) for different classes of contaminants in sediment from the East Coast of the United States. Bold type indicates that correlation coeffecients are statistically significant at $p < 0.05$. A total of 19 sites were sampled. Some sites were sampled more than once (i.e., in more than one year), and each sampling was considered as a separate data point for statistical analyses, resulting in $n = 24$ for all classes of contaminants. Total AHs = total aromatic hydrocarbons; HAHs = high molecular weight aromatic hydrocarbons; LAHs = low molecular weight aromatic hydrocarbons; DDTs = total DDTs and DDT derivatives; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific compounds measured in each of these categories are listed in Table 3.

	Total AHs	LAHs	HAHs	DDTs	PCBs	Chlordanes
Total AHs	1	**0.99**	**0.99**	**0.72**	**0.71**	**0.88**
LAHs		1	**0.96**	**0.71**	**0.70**	**0.86**
HAHs			1	**0.74**	**0.71**	**0.88**
DDTs				1	**0.79**	**0.87**
PCBs					1	**0.82**
Chlordanes						1

Table 6. Interdepartmental matrix of correlation coeffecient (Spearman's rho) between classes of contaminants in sediment, bile, liver, and stomach contents of winter flounder. A total of 19 sites were sampled, and some sites were sampled more than once (i.e., in more than one year). Each sampling was considered a separate data point for statistical analyses, resulting in n = 24 for sediment, stomach contents, tissue, and bile. All classes of contaminants were measured in sediment and stomach contents. Metabolites of aromatic hydrocarbons were measured in bile at benzo[a]pyrene (BaP) and napthalene (NPH) wavelengths. All other classes of compounds were measured in liver tissue. Correlations in bold type are statistically significant ($p \leq 0.05$).*

Chemical class	Sediment vs. stomach contents	Stomach contents vs. tissue or bile	Sediment vs. tissue or bile
LAHs (n=24)	**0.82**	**0.56** (BaP), **0.79** (NPH)	**0.52** (BaP), **0.72** (NPH)
HAHs (n=24)	**0.78**	**0.53** (BaP), **0.65** (NPH)	**0.45** (BaP), **0.68** (NPH)
Total AHs (n=24)	**0.84**	**0.55** (BaP), **0.70** (NPH)	**0.48** (BaP), **0.69** (NPH)
DDTs (n=24)	**0.43**	**0.83**	0.21
PCBs (n=24)	**0.58**	**0.76**	**0.50**
Chlordanes (n=24)	**0.48**	**0.77**	0.37

*LAHs = low molecular weight aromatic hydrocarbons; HAHs = high molecular weight aromatic hydrocarbons ; Total AHs = total aromatic hydrocarbons; DDTs = total DDTs and DDT derivatives; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane + trans-nonachlor. Specific compounds determined in each of these classes are listed in Table 3.

Table 7. Calculated odds ratios for significant ($p < 0.05$) risk factors for 6 categories of hepatic lesions, 3 categories of renal lesions, and fin erosion. Odds ratios for the site of capture are interpreted relative to the reference site at Rocky Point in Long Island Sound. Odds ratios for age (in years) represent the effect of each additional year of age on the odds of disease occurrence. GM = grand mean.

Lesion	Risk factor	Odds ratio
Hepatic lesions		
Neoplasms	Age	1.988
GM = 5.773E-04	Female	.1928
	Raritan Bay, Gravesend	21.46
	Boston Harbor, Deer Island	3.887
	Raritan Bay, West Reach	8.973
	Boston Harbor, Hull Bay	5.081
Foci of cellular alteration	Age	1.373
GM = 2.572E-03	Raritan Bay, Gravesend	7.708
	Boston Harbor, Deer Island	3.500
	Boston Harbor, Mystic River	4.368
	Buzzards Bay	3.828
Proliferative lesions	Age	1.673
GM = 1.275E-03	Boston Harbor, Deer Island	3.939
	Boston Harbor, Mystic River	1.673
Cystic degeneration	Age	2.244
GM = 3.837E-02	Boston Harbor, President Roads	13.06
	Female	0.1582
Nuclear pleomorphism	Long Island Sound, New Haven	4.883
GM = 1.463E-02		
Hydropic vacuolation	Age	1.345
GM = 2.940E-01	Boston Harbor, Mystic River	10.56
	Raritan Bay, East Reach	6.594
	Boston Harbor, Deer Island	5.403
	Long Island Sound, Norwalk	6.752
	Long Island Sound, New Haven	5.620
	Raritan Bay, Gravesend	8.962
	Salem Harbor, Folgers Point	2.481
	Boston Harbor, President Roads	4.336
	Boston Harbor, Hull Bay	3.875
	Boston Harbor, Quincy Bay	4.387
Necrosis	Age	1.184
GM = 4.681E-02	Boston Harbor, Mystic River	2.176
	Boston Harbor, Hull Bay	2.010
	Boston Harbor, Quincy Bay	3.258
	Boston Harbor, President Roads	2.607
	Raritan Bay, West Reach	2.826

Table 7. Continued.

Lesion	Risk factor	Odds ratio
Kidney lesions		
Proliferative lesions GM = 1.838E-03	Salem Harbor, Folgers Point Buzzard Bay	9.143 9.067
Necrotic lesions GM = 3.566E-03	Age Boston Harbor, Deer Island Female	1.786 10.04 0.2252
Sclerotic lesions GM = 1.28E-03	Age Long Island Sound, New Haven Niantic Bay, Black Point	1.839 4.331 4.831
Fin lesions		
Fin erosion GM = 1.489E-02	Age Boston Harbor, Mystic River Long Island Sound, New Haven Boston Harbor, Deer Island Boston Harbor, Quincy Bay Long Island Sound, Norwalk Niantic Bay, Black Point	1.379 8.690 11.89 2.884 3.547 3.752 2.505

Table 8. Liver lesion prevalences (%) in female winter flounder sampled in winter and spring. Asterisk (*) indicates that prevalence for fish collected in winter is significantly different from prevalence for fish collected at the same site or sites in spring.

	Hydropic vacuolation		Necrotic lesions	
Site name	Spring	Winter	Spring	Winter
Massachusetts Bay, Plymouth Entrance	0% (n=25)	0% (n=22)	16% (n=25)	5% (n=22)
Narragansett Bay, Prudence Island	4% (n=51)	10% (n=39)	2% (n=51)	3% (n=39)
Boston Harbor, Deer Island	47% (n=100)	11%* (n=27)	10% (n=100)	0%* (n=27)
Boston Harbor, Mystic River	59% (n=37)	20%* (n=30)	27% (n=37)	30% (n=30)
Niantic Bay, Black Point	20% (n=81)	4%* (n=69)	6% (n=81)	0%* (n=69)
Total	30% (n=294)	9%* (n=187)	10% (n=294)	6%* (n=187)

Table 9. Chemical compounds or classes of compounds in sediment showing significant positive relationships (logistic regression, $p < 0.05$) with selected categories of idiopathic liver lesions. Analyses were performed separately for each major class of contaminants, while adjusting for the possible influences of gender and age. Percentages indicate percent of total variation in lesion prevalence explained. A total of 19 sites were sampled, and some sites were sampled more than once. Each sampling was considered a separate data point for statistical analyses, resulting in n = 24.

Chemical* class	n	Neoplasms	FCA	Cystic degeneration	Nuclear pleomorphism	Hydropic vacuolation	Proliferative lesions	Necrotic lesions
LAHs	24	ns	ns	ns	ns	(+) p=0.001 22%	ns	(+) p=0.001 16%
HAHs	24	ns	ns	ns	ns	(+) p=0.001 29%	ns	(+) p=0.001 20%
Total AHs	24	ns	ns	ns	ns	(+) p=0.001 28%	ns	(+) p=0.001 19%
PCBs	24	ns	ns	ns	ns	ns	ns	ns
DDTs	24	ns	ns	ns	ns	(+) p=0.001 18%	ns	(+) p=0.001 19%
Chlordanes	24	ns	ns	ns	ns	(+) p=0.001 30%	ns	(+) p=0.001 24%

*LAHs = low molecular weight aromatic hydrocarbons; HAHs = high molecular weight aromatic hydrocarbons ; TAHs = total aromatic hydrocarbons; DDTs = total DDTs and DDT derivatives; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific compounds determined in each of these classes are listed in Table 3.

Table 10. Chemical compounds or classes of compounds in winter flounder stomach contents showing significant positive relationships (logistic regression, $p < 0.05$) with selected categories of idiopathic liver lesions. Analyses were performed separately for each major class of contaminants, while adjusting for the possible influences of gender and age. Percentages indicate percent of total variation in lesion prevalence explained. A total of 19 sites were sampled, and some sites were sampled more than once. Each sampling was considered a separate data point for statistical analyses, resulting in n = 24.

Chemical* class	n	Neoplasms	FCA	Cystic degeneration	Nuclear pleomorphism	Hydropic vacuolation	Proliferative lesions	Necrotic lesions
LAHs	24	(+) p=0.050 7%	(+) p=0.009 22%	ns	ns	(+) p=0.001 27%	ns	(+) p=0.002 9%
HAHs	24	ns	ns	ns	ns	(+) p=0.001 29%	ns	ns
Total AHs	24	ns	(+) p=0.043 8%	ns	ns	(+) p=0.001 15%	ns	ns
PCBs	24	ns	ns	ns	ns	ns	ns	ns
DDTs	24	ns	ns	ns	ns	(+) p=0.001 18%	ns	(+) p=0.001 19%
Chlordanes	24	(+) p=0.031 22%	ns	(+) p=0.018 47%	ns	(+) p=0.001 43%	(+) p=0.006 15%	(+) p=0.014 8%

*LAHs = low molecular weight aromatic hydrocarbons; HAHs = high molecular weight aromatic hydrocarbons ; TAHs = total aromatic hydrocarbons; DDTs = total DDTs and DDT derivatives; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific compounds determined in each of these classes are listed in Table 3.

Table 11. Chemical compounds or classes of compounds in winter flounder bile and liver tissue showing significant positive relationships (logistic regression, $p < 0.05$) with selected categories of idiopathic liver lesions. Analyses were performed separately for each major class of contaminants, while adjusting for the possible influences of gender and age. Percentages indicate percent of total variation in lesion prevalence explained. A total of 19 sites were sampled, and some sites were sampled more than once. Each sampling was considered a separate data point for statistical analyses, resulting in n = 24.

Chemical* class	n	Neoplasms	FCA	Cystic degeneration	Nuclear pleomorphism	Hydropic vacuolation	Proliferative lesions	Necrotic lesions
FACs-L	24	ns	ns	ns	ns	(+) p=0.001 16%	ns	(+) p=0.001 36%
FACs-H	24	ns	ns	ns	ns	(+) p=0.001 14%	ns	(+) p=0.001 32%
PCBs	24	ns	ns	ns	ns	ns	ns	ns
DDTs	24	ns	ns	ns	ns	(+) p=0.001 7%	ns	ns
Chlordanes	24	ns	ns	ns	ns	(+) p=0.001 16%	(+) p=0.023 9%	ns

*FACs-H = aromatic compounds in bile fluorescing at BaP (high molecular weight) wavelengths; FACs-L = aromatic compounds in bile fluorescing at napthalene (low molecular weight) wavelengths; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific PCB congeners measured are listed in Table 3.

Table 12. Chemical compounds or classes of compounds in sediment showing significant relationships (logistic regression, $p < 0.05$) with selected categories of idiopathic kidney lesions and fin erosion in winter flounder. Analyses were performed separately for each major class of contaminants, while adjusting for the possible influences of gender and age. Table indicates direction of correlation, p value, and percent of total variation in lesion prevalence explained. A total of 19 sites were sampled, and some sites were sampled more than once (i.e., in more than one year). Each sampling was considered a separate data point for statistical analyses, resulting in n = 24.

Chemical* class	n	Proliferative lesions	Necrotic lesions	Sclerotic lesions	Fin erosion
LAHs	24	ns	ns	ns	(+) p=0.001 14%
HAHs	24	ns	ns	ns	(+) p=0.001 15%
Total AHs	24	ns	ns	ns	(+) p=0.001 15%
DDTs	24	ns	ns	ns	(+) p=0.025 3%
PCBs	24	ns	ns	ns	ns
Chlordanes	24	ns	ns	ns	(+) p=0.001 14%

*LAHs = low molecular weight aromatic hydrocarbons; HAHs = high molecular weight aromatic hydrocarbons ; Total AHs = total aromatic hydrocarbons; DDTs = total DDTs and DDT derivatives; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific compounds determined in each of these classes are listed in Table 3.

Table 13. Chemical compounds or classes of compounds in winter flounder stomach contents showing significant relationships (logistic regression, $p < 0.05$) with selected categories of idiopathic kidney and fin lesions. Analyses were performed separately for each major class of contaminants, while adjusting for the possible influences of gender and age. Table reports direction of correlation, p value, and percent of total variation in lesion prevalence explained. A total of 19 sites were sampled, and some sites were sampled more than once (i.e., in more than one year). Each sampling was considered a separate data point for statistical analyses, resulting in n = 24.

Chemical* class	n	Proliferative lesions	Necrotic lesions	Sclerotic lesions	Fin erosion
LAHs	24	ns	(+) p=0.05 11%	ns	(+) p=0.001 15%
HAHs	24	ns	ns	ns	(+) p=0.014 5%
Total AHs	24	ns	ns	ns	(+) p=0.004 5%
DDTs	24	ns	ns	ns	(+) p=0.025 3%
PCBs	24	ns	ns	ns	ns
Chlordanes	24	ns	ns	ns	(+) p=0.007 16%

*LAHs = low molecular weight aromatic hydrocarbons; HAHs = high molecular weight aromatic hydrocarbons; Total AHs = total aromatic hydrocarbons; DDTs = total DDTs and DDT derivatives; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific compounds determined in each of these classes are listed in Table 3.

Table 14. Chemical compounds or classes of compounds in winter flounder liver tissue and bile showing significant positive relationships (logistic regression, $p < 0.05$) with selected categories of idiopathic kidney and fin lesions. Analyses were performed separately for each major class of contaminants, while adjusting for the possible influences of gender and age. Table reports direction of correlation, p value, and percent of total variation in lesion prevalence explained. A total of 19 sites were sampled, and some sites were sampled more than once (i.e., in more than one year). Each sampling was considered a separate data point for statistical analyses, resulting in n = 24.

Chemical* class	n	Proliferative lesions	Necrotic lesions	Sclerotic lesions	Fin erosion
Bile FACs-H	24	ns	ns	ns	(+) p=0.001 7%
Bile FACs-L	24	ns	ns	ns	(+) p=0.001 9%
DDTs	24	ns	ns	ns	ns
PCBs	24	ns	ns	ns	ns
Chlordanes	24	ns	ns	ns	ns

*FACs-H = aromatic compounds in bile fluorescing at BaP (high molecular weight) wavelengths; FACs-L = aromatic compounds in bile fluorescing at NPH (low molecular weight) wavelengths; PCBs = total polychlorinated biphenyls; Chlordanes = alpha-chlordane and trans-nonachlor. Specific PCB congeners measured are listed in Table 3.

FIGURES

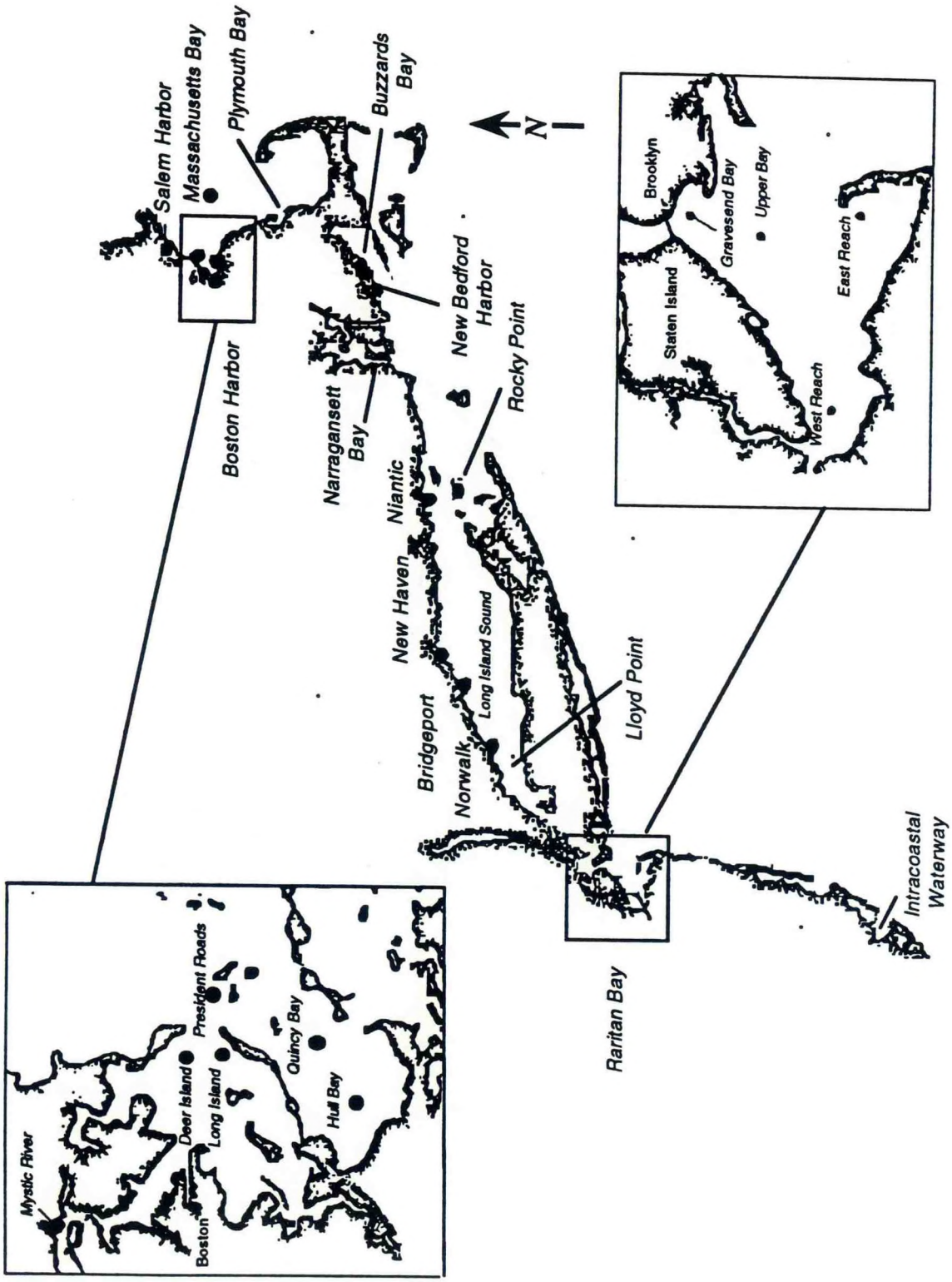

Figure 1. Map of East Coast showing sampling sites.

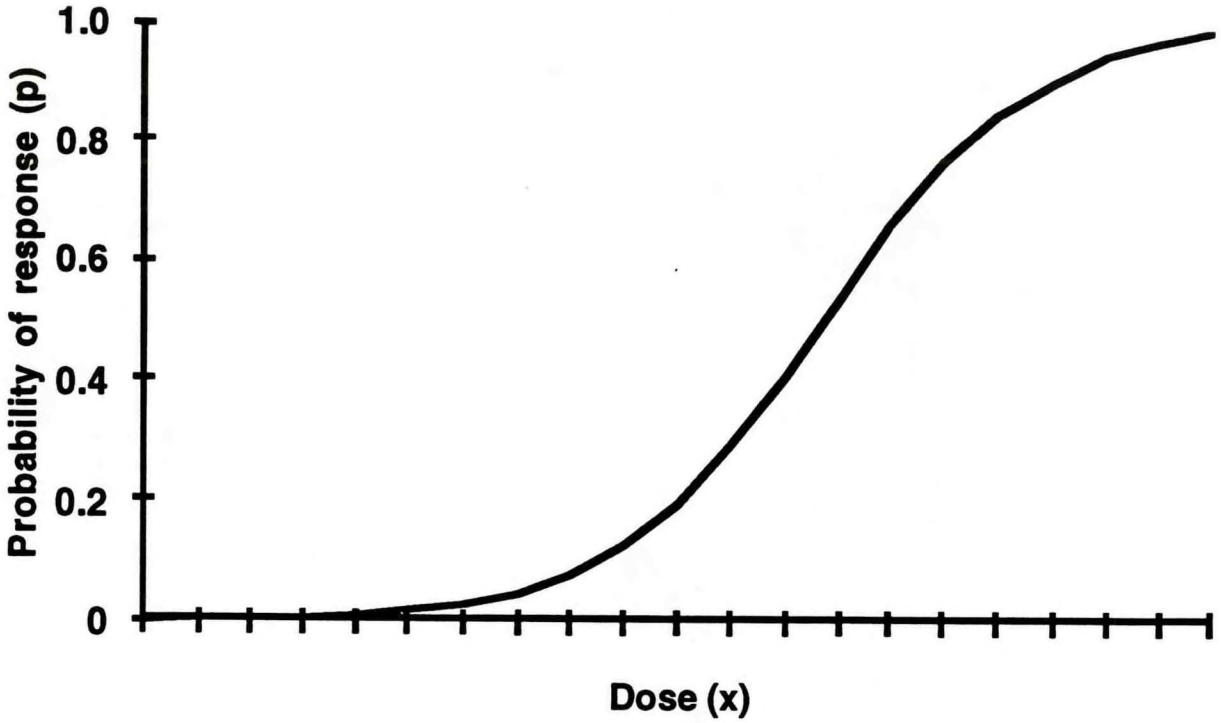

Figure 2. General shape of logistic function, $p = 1/(1 + e^{-x})$. Adapted from Schlesselman (1982).

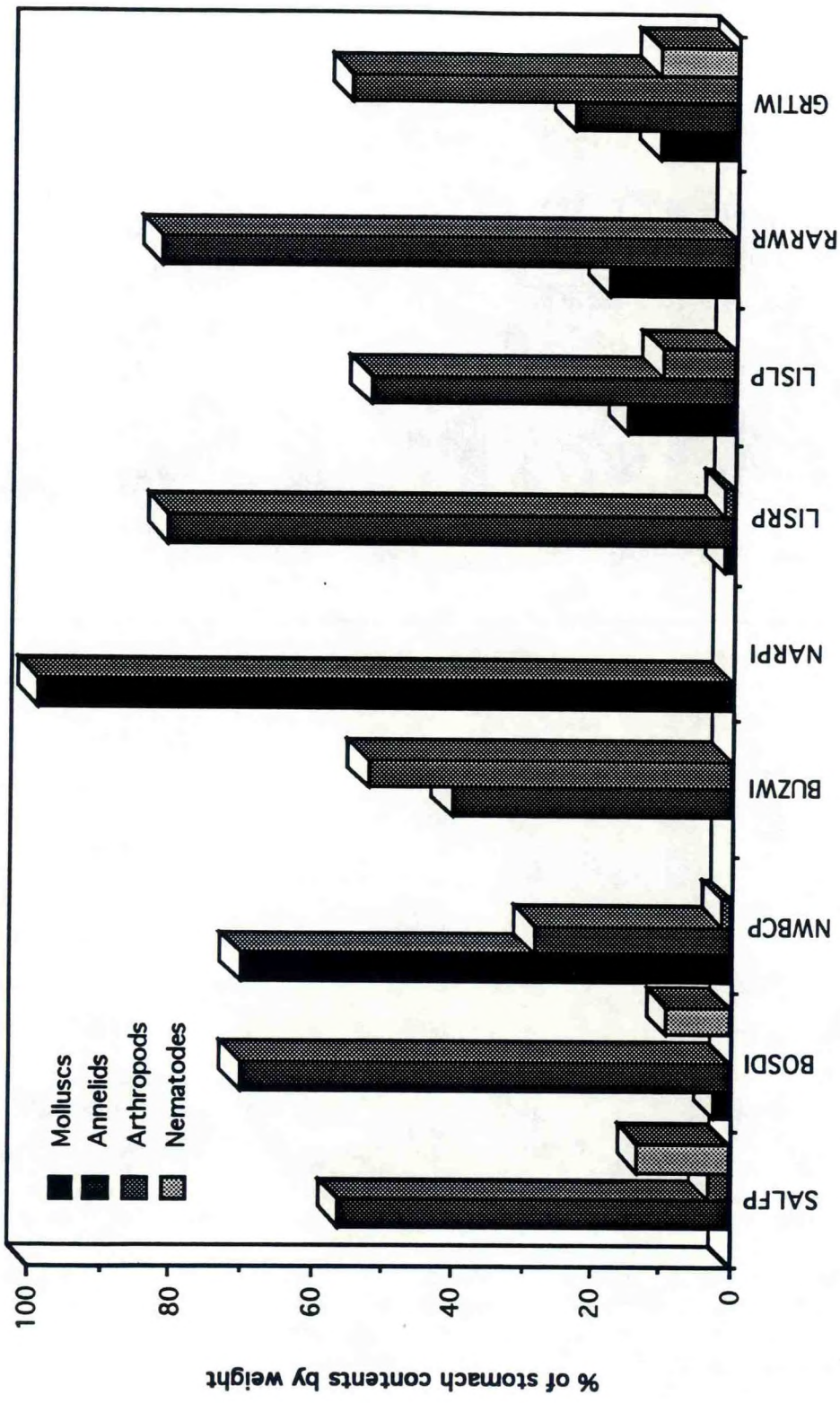

Figure 3. Taxonomic composition (% by weight) of stomach contents of winter flounder from selected East Coast sampling sites. Samples were collected during Cycle 5 (1988). Site abbreviations are defined in Table 1.

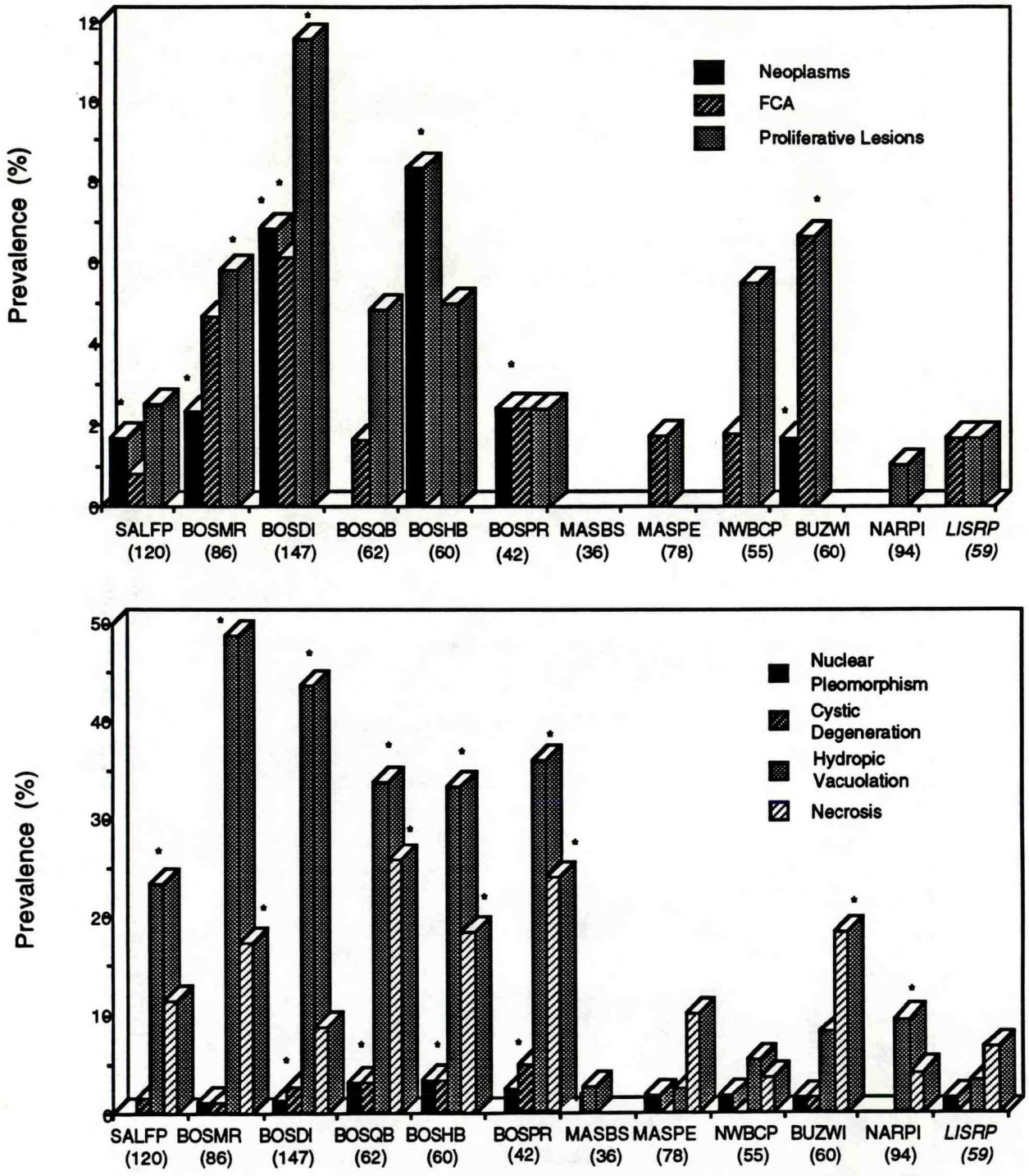

Figure 4. Prevalences of hepatic lesions in winter flounder from the northern East Coast. Asterisk (*) indicates prevalence is significantly higher (G-statistic, $p < 0.05$) than prevalence at Rocky Point reference site in Long Island Sound (in italics). FCA = foci of cellular alteration. Site abbreviations are defined in Table 1. For actual prevalence values, see Appendix Table A-3.

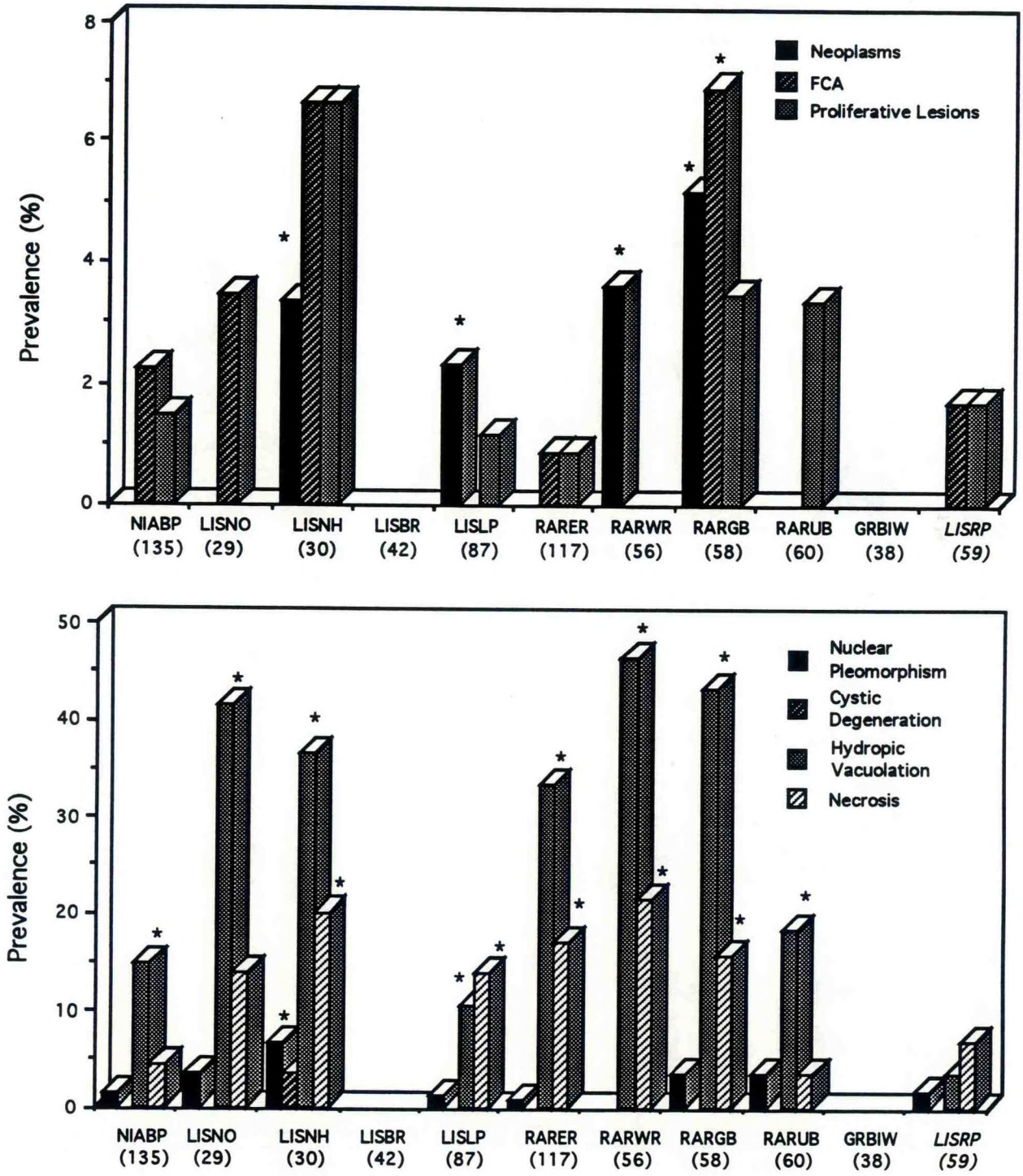

Figure 5. Prevalences of hepatic lesions in winter flounder from the southern East Coast. Asterisk (*) indicates prevalence is significantly higher (G-statistic, $p < 0.05$) than prevalence at Rocky Point reference site in Long Island Sound. FCA = foci of cellular alteration. Site abbreviations are defined in Table 1. For actual prevalence values, see Appendix Table A-3.

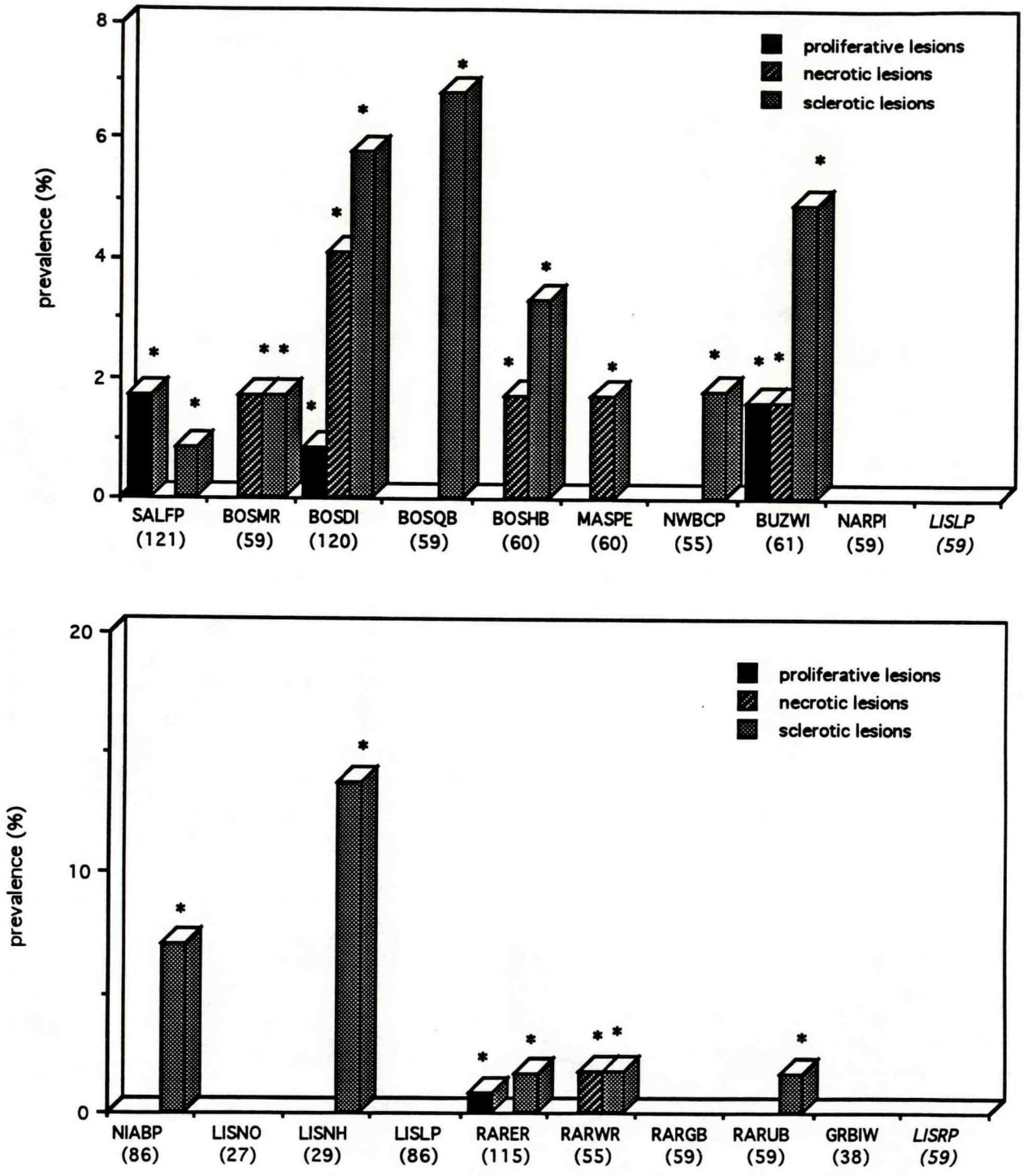

Figure 6. Prevalences of kidney lesions in winter flounder from the East Coast. Asterisk (*) indicates prevalence is significantly higher (G-statistic, $p < 0.05$) than prevalence at Rocky Point reference site in Long Island Sound. Site abbreviations are defined in Table 1. For actual prevalence values, see Appendix Table A-4.

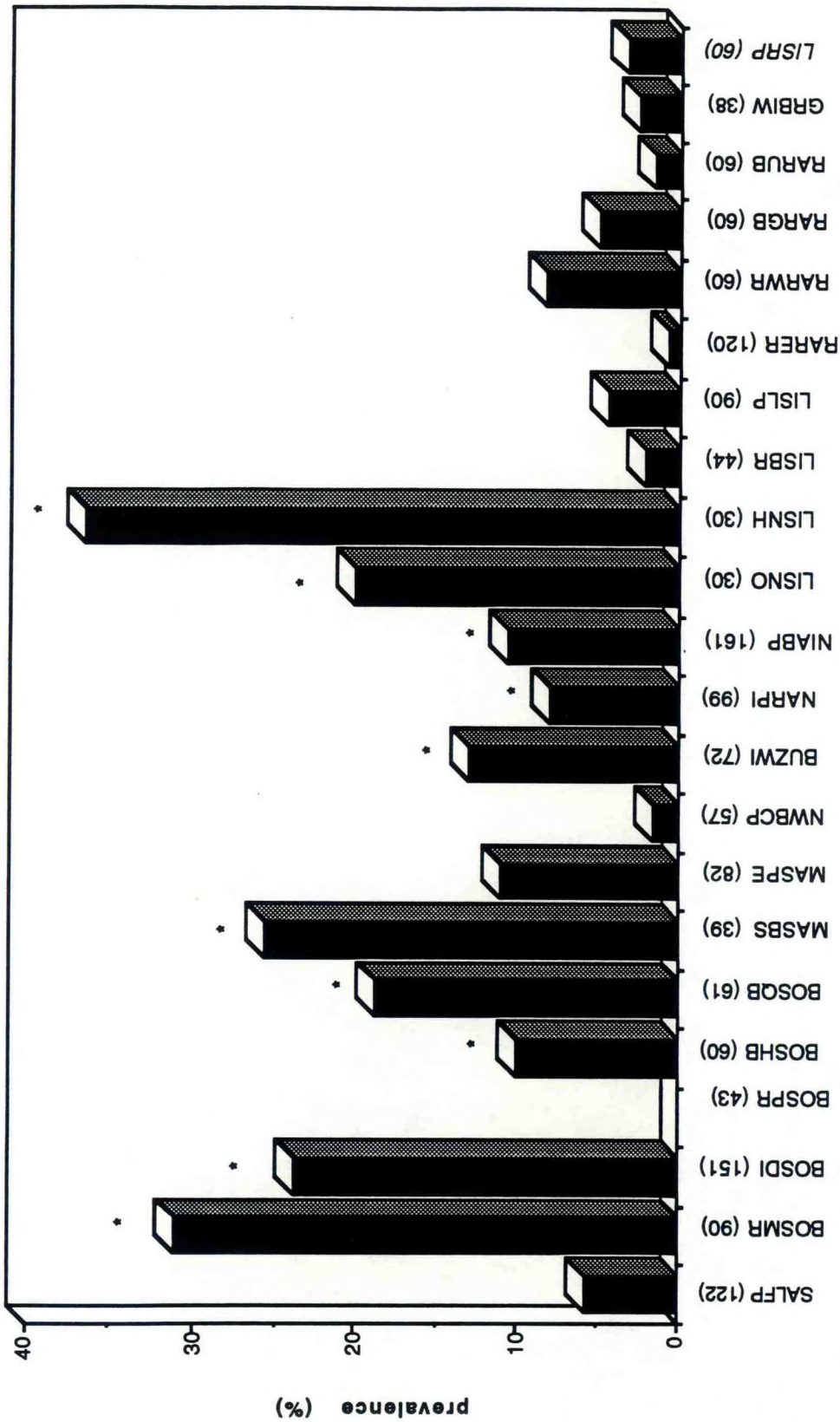

Figure 7. Prevalences of fin erosion in winter flounder from the East Coast. Asterisk (*) indicates prevalence is significantly higher (G-statistic, $p < 0.05$) than prevalence at Rocky Point reference site in Long Island Sound (in italics). Site abbreviations are defined in Table 1. For actual prevalence values, see Appendix Table A-5.

APPENDIX

Appendix Table A1. Mean lengths, weight, ages (± SD), and sex ratios of winter flounder at Northeast Coast sampling sites. Values in bold type are significantly different ($p \leq 0.05$) from values at the Rocky Point, Long Island Sound reference site, as determined by ANOVA for length, weight, and age and the Herogeneity G-Test for gender ratio.

Site name	n	Length (mm)	Weight (g)	Age	Gender ratio (M:F)
Salem Harbor, Folgers Point	122	**337 ± 58**	**407 ± 244**	**5.0 ± 1.8**	30:70
Boston Harbor, Mystic River	90	**313 ± 54**	**454 ± 240**	**4.3 ± 1.8**	26:74
Boston Harbor, Deer Island	151	**361 ± 43**	**529 ± 224**	**5.8 ± 1.4**	**16:84**
Boston Harbor, Quincy Bay	61	**327 ± 66**	**448 ± 212**	**4.7 ± 2.2**	**58:42**
Boston Harbor, Hull Bay	60	**338 ± 37**	**472 ± 156**	**5.1 ± 1.2**	**77:23**
Boston Harbor, President Roads	43	**357 ± 51**	**679 ± 322**	**5.6 ± 1.7**	**0:100**
Massachusetts Bay, outside Boston	39	**318 ± 31**	**474 ± 149**	**4.4 ± 1.0**	**0:100**
Massachusetts Bay, Plymouth Entrance	82	**405 ± 41**	**390 ± 163**	**3.9 ± 1.1**	**43:57**
New Bedford Harbor, Clarks Point	57	**337 ± 57**	**412 ± 203**	**5.0 ± 1.3**	25:75
Buzzards Bay	62	**391 ± 46**	**471 ± 162**	**6.2 ± 1.0**	**8:92**
Narragansett Bay, Prudence Island	99	270 ± 39	**212 ± 170**	3.4 ± 0.8	**8:92**
Niantic Bay, Black Point	161	**308 ± 42**	**363± 156**	**4.5 ± 0.9**	**5:95**
Long Island Sound, Norwalk	30	256 ± 43	183 ± 95	**4.5 ± 1.4**	**57:43**
Long Island Sound, New Haven	30	**291 ± 70**	**295 ± 198**	**4.6 ± 2.8**	23:77
Long Island Sound, Bridgeport	43	268 ± 44	**262 ± 159**	**3.8 ± 1.0**	**0:100**
Long Island Sound, Rocky Point (REF)	*60*	*261 ± 19*	*134 ± 33*	*3.1 ± 0.8*	*32:68*
Long Island Sound, Lloyd Point	90	248 ± 27	127 ± 67	**4.2 ± 1.7**	39:61
Raritan Bay, East Reach	120	272 ± 20	**190 ± 81**	**3.7 ± 0.5**	37:63
Raritan Bay, West Reach	60	**325 ± 51**	**400 ± 132**	**4.3 ± 0.5**	**20:80**
Raritan Bay, Gravesend	60	**308 ± 38**	**351 ± 142**	**4.1 ± 0.4**	**10:90**
Raritan Bay, Upper Bay	60	246 ± 19	179 ± 49	3.2 ± 0.4	**6:94**
Great Bay, Intracoastal Waterway	38	**281 ± 33**	**209 ± 66**	3.3 ± 0.8	**11:89**

Appendix Table A2. Age/length regression equations for winter flounder captured in the National Benthic Surveillance Project, Northeast Coast, Cycles 5 and 6 (1988 and 1989).

Geographic area	Site numbers	Sex	n	Age(y)/length(x) equation
Great Bay	2410,2420	M	14	$y = 5.135 - 0.037x + (1.13E\text{-}4)x^2$
		M	14	$y = 5.135 - 0.037x + (1.13E\text{-}4)x^2$
		all	58	$y = 40.021 - 0.32x + 0.001x^2$
Raritan Bay	2550 - 2559	M	39	$y = 0.0188x - 1.258$
		F	29	$y = .0123x + 0.2323$
		all	58	$y = 0.0156x - 0.5307$
Long Island Sound Area[1]	2630 - 2740	M	89	$y = 0.0224x - 1.6535$
		F	316	$y = 0.0224X - 2.3683$
		all	405	$y = 0.0204x - 1.6691$
Massachusetts Bay/Boston Harbor[2,3]	2800-2880	all	119	$y = 30.956 - 0.187x + (3.192E\text{-}4)x^2$

[1]Includes Niantic Bay, Narragansett Bay, and New Bedford Bay.

[2]Includes Massachusetts Bay, Plymouth Entrance, all Boston Harbor sites, and Salem Harbor.

[3]The number of males was insufficient to calculate a separate regression equation, so the equation for all animals was used for both males and females.

Appendix Table A3. Classes of food organisms (percent by weight) in stomach contents of winter flounder from selected NBSP sampling sites. Samples were collected during Cycle 5 (1988).

Site Name	Molluscs %	Annelids %	Arthropods %	Nematodes %	Debris %
Salem Harbor, Folgers Point	0	56	3	13	28
Boston Harbor, Deer Island	2	70	0	9	19
New Bedford Harbor, Clarks Point	70	28	1	0	0
Buzzards Bay	0	40	52	0	8
Narragansett Bay, Prudence Island	99	0	0	0	1
Long Island Sound, Rocky Point (REF)	1	81	1	0	17
Long Island Sound, Lloyd Point	15	52	10	0	22
Raritan Bay, West Reach	18	82	0	0	0
Great Bay, Intracoastal Waterway	11	23	55	11	1

Appendix Table A4. Liver lesion prevalences (%) in winter flounder from sampling sites along the Northeast Coast. Fish were collected during intensive studies in Long Island Sound (1987), Boston Harbor reproductive studies (1988), and Cycles 5 and 6 of the National Benthic Surveillance Project (1988 and 1989). Prevalences in bold type are significantly higher than those at the reference site at Rocky Point, Long Island Sound. n = total number of fish sampled at a site. The site may have been sampled one or more years.

Site name	n	Neoplasms	FCA*	Proliferative lesions	NP*	Spongiosis Hepatis	Hydropic vacuolation	Necrotic lesions
Salem Harbor, Folgers Point	120	**1.67**	0.83	2.50	0.00	1.67	**23.33**	11.67
Boston Harbor, Mystic River	86	**2.33**	4.65	**5.81**	1.16	1.16	**48.84**	**17.44**
Boston Harbor, Deer Island	147	**6.80**	**6.12**	**11.56**	1.36	**2.72**	**43.54**	8.84
Boston Harbor, Quincy Bay	62	0.00	1.61	4.84	3.23	**3.23**	**33.87**	**25.81**
Boston Harbor, Hull Bay	60	**8.33**	0.00	5.00	3.33	**3.33**	**33.33**	**18.33**
Boston Harbor, President Roads	42	**2.38**	2.38	2.38	2.38	**4.76**	**35.71**	**23.81**
Massachusetts Bay, outside Boston	36	0.00	0.00	0.00	0.00	0.00	2.78	0.00
Massachusetts Bay, Plymouth Entrance	78	0.00	1.28	0.00	1.72	0.00	2.56	10.26
New Bedford Harbor, Clarks Point	55	0.00	1.82	5.45	1.82	0.00	5.45	3.64
Buzzards Bay	60	**1.67**	**6.67**	0.00	1.67	1.67	8.33	**18.33**
Narragansett Bay, Prudence Island	94	0.00	0.00	1.06	0.00	0.00	**9.57**	4.26
Niantic Bay, Black Point	135	0.00	2.22	1.48	1.48	0.00	**14.81**	4.44
Long Island Sound, Norwalk	29	0.00	3.45	0.00	3.45	0.00	**41.38**	13.79
Long Island Sound, New Haven	30	**3.33**	6.67	6.67	6.67	**3.33**	**36.67**	**20.00**
Long Island Sound, Bridgeport	42	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Long Island Sound, Rocky Point (REF)	59	0.00	1.69	1.69	1.69	0.00	3.39	6.78
Long Island Sound, Lloyd Point	87	**2.30**	0.00	0.00	1.15	0.00	**10.34**	**13.79**
Raritan Bay, East Reach	117	0.00	0.85	0.00	0.85	0.00	**33.33**	**17.09**
Raritan Bay, West Reach	56	**3.57**	0.00	1.79	0.00	0.00	**46.43**	**21.43**
Raritan Bay, Gravesend	58	**5.17**	**6.90**	1.72	3.45	0.00	**43.10**	**15.52**
Raritan Bay, Upper Bay	60	0.00	0.00	0.00	3.33	0.00	**18.33**	3.33
Great Bay, Intracoastal Waterway	38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	1551	1.89	2.22	2.81	1.50	0.98	23.84	11.63

*FCA = foci of cellular alteration; NP = nuclear pleomorphism

Appendix Table A5. Kidney lesion prevalences (%) in winter flounder, from samping sites along the Northeast Coast. Fish were collected during Long Island Sound intensive studies (1987) and Cycles 5 and 6 of the National Benthic Surveillance Project (1988 and 1989). Kidney lesions were not monitored during Boston Harbor reproductive studies. Prevalences in bold type are significantly higher than those at the Rocky Point reference site in Long Island Sound. n = total number of animals sampled per site. The site may have been sampled one or more years.

Site name	n	Proliferative lesions	Necrotic lesions	Sclerotic lesions
Salem Harbor, Folgers Point	121	**1.65**	0	**0.83**
Boston Harbor, Mystic River	59	0	**1.69**	**1.69**
Boston Harbor, Deer Island	120	**0.83**	**4.13**	**5.79**
Boston Harbor, Hull Bay	60	0	**1.67**	**3.33**
Boston Harbor, Quincy Bay	59	0	0	**6.78**
Massachusetts Bay, Plymouth Entrance	60	0	**1.67**	0
New Bedford Harbor, Clarks Point	55	0	0	**1.82**
Buzzards Bay	61	**1.64**	**1.64**	**4.92**
Narragansett Bay, Prudence Island	59	0	0	0
Niantic Bay, Black Point	86	0	0	**6.98**
Long Island Sound, Norwalk	27	0	0	0
Long Island Sound, New Haven	29	0	0	**13.79**
Long Island Sound, Rocky Point (REF)	59	0	0	0
Long Island Sound, Lloyd Point	86	0	0	0
Raritan Bay, East Reach	115	**0.87**	0	**1.74**
Raritan Bay, West Reach	55	0	**1.82**	**1.82**
Raritan Bay, Gravesend	59	0	0	0
Raritan Bay, Upper Bay	59	0	0	**1.69**
Great Bay, Intracoastal Waterway	38	0	0	0
Total	1268	0.39	0.79	2.60

Appendix Table A6. Prevalences (%) of idiopathic fin erosion in winter flounder from sampling sites along the Northeast Coast. Fish were collected during Long Island Sound intensive studies (1987), Boston Harbor reproductive studies (1988), and Cycles 5 and 6 of the National Benthic Surveillance Project (1988 and 1989). Prevalences in bold type are significantly higher than those at the Rocky Point reference site in Long Island Sound. n = number of animals sampled per site. The sites may have been sampled one or more years.

Site name	n	% Fin erosion
Salem Harbor, Folgers Point	122	5.74
Boston Harbor, Mystic River	90	**31.11**
Boston Harbor, Deer Island	151	**23.84**
Boston Harbor, President Roads	43	0.00
Boston Harbor, Hull Bay	60	**10.00**
Boston Harbor, Quincy Bay	61	**18.75**
Massachusetts Bay, outside Boston	39	**25.60**
Massachusetts Bay, Plymouth Entrance	82	**10.98**
New Bedford Harbor, Clarks Point	57	1.75
Buzzards Bay	62	**12.90**
Narragansett Bay, Prudence Island	99	**8.08**
Niantic Bay, Black Point	161	**10.56**
Long Island Sound, Norwalk	30	**20.00**
Long Island Sound, New Haven	30	**36.67**
Long Island Sound, Bridgeport	44	2.27
Long Island Sound, Rocky Point (REF)	60	3.33
Long Island Sound, Lloyd Point	90	4.44
Raritan Bay, East Reach	120	0.83
Raritan Bay, West Reach	60	8.33
Raritan Bay, Gravesend	60	5.00
Raritan Bay, Upper Bay	60	1.64
Great Bay, Intracoastal Waterway	38	2.63
Total	1475	10.92

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