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Bioindicators of Contaminant Exposure, Liver Pathology, and Reproductive Development in Prespawning Female Winter Flounder *(Pleuronectes americanus)* from Urban and Nonurban Estuaries on the Northeast Atlantic Coast

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EXECUTIVE SUMMARY

Relationships between liver pathology and ovarian development, and exposure to xenobiotic compounds were evaluated in prespawning female winter flounder *(Pleuronectes americanus,* formerly *Pseudopleuronectes americanus)* sampled from 11 sites on the Northeast coast of the United States during the 1988 and 1989 spawning seasons. Three sites were located in Boston Harbor, Massachusetts, four sites were in Raritan Bay, New Jersey, and four sites were in nearby, less urbanized embayments. Sediments from these sites exhibited a wide range in concentrations of xenobiotic compounds (e.g. concentrations of polycyclic aromatic hydrocarbons (PAHs) ranged from 20 to 50,000 ng/g dry weight and concentrations of polychlorinated biphenyls (PCBs) ranged from 2 to 1,400 ng/g dry weight), with the sites in Boston Harbor and Raritan Bay the most heavily contaminated. The following parameters associated with ovarian development were measured: ovarian developmental stage, ovarian atresia, gonadosomatic index, plasma estradiol, fecundity, and egg weight. Contaminant exposure was assessed by measuring concentrations of fluorescent aromatic compounds (FACs) in the bile; hepatic aryl hydrocarbon hydroxylase (AHH) activity; concentrations of polychlorinated biphenyls (PCBs) in liver, ovary, and brain; and levels of xenobiotic-DNA adducts in liver tissue. Additionally, liver tissue was examined histologically for the presence of suspected toxicopathic lesions. In general, indicators of contaminant exposure were elevated and prevalences of suspected toxicopathic lesions were highest in fish from sites within Boston Harbor and Raritan Bay. Moreover, prevalences of two categories of lesions--hydropic vacuolation and biliary or hepatocellular proliferation--were positively correlated with concentrations of PCBs in tissue and FACs in bile. Hepatic AHH activity, however, was significantly depressed in reproductively active fish and showed little correlation with other indicators of contaminant exposure. Evidence of decreased egg weight and increased atresia in fish exposed to high levels of PCBs or PAHs was observed. However, contaminant exposure had no clear negative impact on gonadal recrudescence, gonadosomatic index, plasma estradiol concentrations, or fecundity in female winter flounder. These results are in contrast to results with

another pleuronectid species, English sole (*Pleuronectes vetulus*, formerly *Parophrys vetulus*), which shows inhibited gonadal development and lower plasma estradiol concentrations at contaminated sites in Puget Sound, Washington. The apparent difference between English sole and winter flounder in susceptibility to contaminant-induced reproductive dysfunction could be related to a number of factors, including possible interspecific differences in the activation and detoxication of contaminants. Additionally, English sole reside in contaminated estuaries throughout vitellogenesis and move offshore to spawn, while winter flounder often remain offshore for extended periods during early vitellogenesis and move into contaminated estuaries prior to spawning. Because of these contrasting migration patterns, both the duration and timing of exposure to contaminants during gonadal recrudescence may differ substantially in these two species.

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INTRODUCTION

Winter flounder *(Pleuronectes americanus,* formerly *Pseudopleuronectes americanus)* is an important commercial and recreational species on the Northeast coast. In recent years, however, there has been evidence of a decline in landings of winter flounder in the nearshore portion of its range (Witherell et al. 1990, Childs 1987, Smith 1989). Several factors may contribute to the apparent drop in winter flounder populations, including fishing pressure, variations in water temperature that could influence larval survival (Buckley et al. 1990, Jeffries and Terceiro 1985, Rogers 1976), changes in food supply (Laurence 1977), predation on larvae (Pearcy 1962), and destruction of larval habitat through dredging or other forms of disturbance (Monooch 1988, NOAA 1990). Additionally, because winter flounder often reside in highly contaminated urban estuaries, there has been concern that exposure to anthropogenic compounds, which may cause diseases or reproductive impairment, could be at least partially responsible for the decline in landings of this fish.

Studies conducted to date on the effects of contaminants on reproductive success in winter flounder have focused primarily on the later stages of the reproductive cycle, such as fertilization success, larval development or larval size, fecundity, and egg weight (e.g., Topp 1967, Smith and Cole 1979, Klein-MacPhee et al. 1984, Black et al. 1988, NOAA 1990). While no clear link has been established between exposure to environmental contaminants and egg viability in winter flounder, several studies present evidence of reduced egg or larval size in flounder from contaminated sites within Boston Harbor, Massachusetts and Narragansett Bay, Rhode Island (Black et al. 1988, NOAA 1990). In contrast, little is known about the impact of contaminant exposure on steroid metabolism or vitellogenesis in free-living winter flounder, in spite of the fact that recent field and laboratory studies (Singh and Singh 1987; Sivarajah et al. 1978a,b; Saxena and Garg 1978; Payne et al. 1978; Thomas 1988, 1989; Stein et al. 1991; Johnson et al. in press)

show that this phase of the reproductive cycle may be disrupted by exposure to xenobiotic compounds.

In the present study, gonadal development and plasma estradiol concentrations, as well as egg weight and fecundity were assessed in female winter flounder from sites along the northeast coast with a wide range of concentrations of contaminants in sediment. The objective was to determine whether flounder showing evidence of exposure to contaminants, particularly exposure to polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs), exhibited any signs of impairment in these reproductive parameters. Although a number of chemicals may alter reproductive function in fish (Lam 1983), in this study we chose to measure concentrations of PAHs and PCBs in flounder because

1) high sediment levels of both classes of compounds are found in urban areas along the northeast coast where winter flounder reside (Zdanowicz et al. 1986; NOAA 1988a,b; Johnson et al. 1992);

2) PAHs and PCBs are bioavailable to winter flounder from contaminated sediments (Zdanowicz et al. 1986, NOAA 1988b, Gronlund et al. 1991, Johnson et al. 1992); and

3) previous studies in both the field and the laboratory indicate that PAHs and PCBs have deleterious effects on the reproductive systems of teleost fish.

Both PAHs and PCBs or related chlorinated hydrocarbons have been associated with altered steroid levels in plasma (Sivarajah et al. 1978a; Singh and Singh 1987; Truscott et al. 1983; Thomas 1988, 1989; Stein et al. 1991; Johnson et al. in press) or inhibited gonadal development (Saxena and Garg 1978; Sivarajah et al. 1978b; Payne et al. 1978; Stott et al. 1983; Thomas 1988; 1989; Cross and Hose 1988; Sloof and De Zwart 1982; Johnson et al. 1988) in a variety of teleost species. Exposure to PAHs was assessed by measuring concentrations of fluorescent aromatic compounds (FACs) in the bile (Krahn et al. 1986), and exposure to PCBs was assessed by determining PCB levels in liver, ovary, and brain.

Several additional bioindicators of contaminant exposure were measured in conjunction with concentrations of FACs in bile and PCBs in tissues of winter flounder. Levels of xenobiotic-DNA

adducts in the liver were determined using the ^{32}P-postlabelling assay which measures the level of hydrophobic aromatic compounds bound to DNA (Varanasi et al. 1989a). Also, liver tissue was examined for toxicopathic lesions, so their relationship with other bioindicators of contaminant exposure and with reproductive indicators could be assessed. The relationship between hepatic lesions and reproductive development was of interest for two reasons: first, hepatic lesions serve as bioindicators of exposure to environmental contaminants; and second, it is possible that lesions could affect the reproductive process directly by interfering with the production of vitellogenin or other functions associated with gonadal development that are carried out by the liver. Relationships between liver lesions and other bioindicators of contaminant exposure were also examined, because, although the occurrence of neoplastic and nonneoplastic liver disease in winter flounder from contaminated areas on the northeast coast of the United States is well-documented (Murchelano and Wolke 1985, 1991; Moore 1991; Gronlund et al. 1991), there is little information on relationships between levels of specific contaminants in winter flounder tissues or other biological indicators of contaminant exposure (e.g., hepatic aryl hydrocarbon hydroxylase (AHH) activity or xenobiotic-DNA adducts) and the risk of disease occurrence.

Multivariate techniques were used to correlate indicators of exposure with indicators of reproductive development. Because biological factors such as fish age or condition may have a strong influence on reproductive development, and the timing of the reproductive cycle can vary clinally, variables accounting for these parameters were also included in analyses.

In addition to the indicators of exposure mentioned above, hepatic AHH activity was determined as a measurement of cytochrome P4501A (CYP1A). Increases in CYP1A are generally indicative of contaminant exposure in marine species (Collier and Varanasi 1991, Payne et al. 1987, Stegeman et al. 1988), and increased CYP1A has been positively correlated with reproductive dysfunction in some species (Spies and Rice 1988, Johnson et al. 1988). However, in certain teleosts, hepatic CYP1A is suppressed by estradiol (Snowberger and Stegeman 1987, Snowberger Gray et al. 1991, Forlin and Haux 1990, Pajor et al. 1990), diminishing its effectiveness as an indicator of contaminant exposure in reproductively active female fish. Hepatic

AHH activity was measured in this study so its utility as an indicator of contaminant exposure in reproductively active female winter flounder could be assessed.

MATERIALS AND METHODS

Fish Capture and Collection of Samples

Female winter flounder were collected by otter trawl from the sites shown in Figure 1. Latitudes and longitudes for each site are given in Table 1. Sampling was conducted in December 1988 and in October and December 1989, during the season when vitellogenesis normally occurs in this species (Burton and Idler 1984). Approximately 30 adult females (length $\geq$ 280 mm) were randomly selected for necropsy from each site at each sampling time. Females smaller than 280 mm were not included in the study to insure that sampled females were large enough and old enough to have reached sexual maturity, which in female winter flounder occurs at approximately 270-290 mm or 3 years of age (Klein-Macphee 1978).

Flounder were individually weighed and measured, otoliths were collected for age determination, and a one mL blood sample was taken with a heparinized syringe. Blood samples were subsequently centrifuged at 800 x g, and the plasma was stored at -20°C for later measurement of plasma estradiol concentrations. Ovaries were weighed and samples were taken for histological examination. Ovarian tissues samples were also collected and stored at -20°C for later determination of PCB concentrations. In selected vitellogenic females, one entire ovary was removed, slit lengthwise, and preserved in modified Gilson's fluid for determination of fecundity (Bagenal and Braum 1971). Livers were preserved in Dietrichs' fixative (Gray 1954) while ovaries were preserved in Davidsons' fixative (Mahoney 1973). A portion of the liver was also frozen immediately in liquid nitrogen, and stored at -80°C for later determination of AHH activity. Additionally, tissue samples from the liver and brain were collected and stored at -20°C for later determination of PCB concentrations. Bile was collected and stored at -20°C for measurement of FACs. Gonadosomatic index (GSI) was calculated according to the following formula:

GSI = [ovary weight (g)/gutted body weight (g)] x 100.

Because low body weight may be associated with suppressed ovarian development in adult female fish (Burton and Idler 1987), a condition factor was determined for all sampled animals so the influence of emaciation on ovarian development could be distinguished from any potential effects of contaminant exposure. Condition factor was calculated according to the following formula:

Condition factor = gutted body weight (g) /length^3 (cm).

Analyses of Tissues and Fluids

Tissues collected for histology were embedded in paraffin, sectioned, stained with hematoxylin and eosin (Luna 1968) and examined microscopically. Hepatic lesions were classified according to the criteria outlined in Myers et al. (1987, in press), then grouped into the following categories:

1) neoplasms (hepatocellular carcinoma, cholangiocellular carcinoma, adenoma, and cholangioma);

2) foci of cellular alteration (FCA) (eosinophilic foci, basophilic foci, clear cell foci);

3) hydropic vacuolation of hepatic or biliary cells (also described as RAM cells (Murchelano and Wolke 1985), atypical cellular vacuolation (Moore et al. 1989), and apotosis (Bodammer and Murchelano 1990)); and

4) proliferative lesions (hepatocellular or biliary regeneration or hyperplasia, cholangiofibrosis).

A complete description of the types of individual lesions included in these categories is given in Table 2.

Ovaries were classified into the following developmental stages (Table 3), using histological criteria modified from Wallace and Selman (1981): regressed (oocytes in the perinucleolar stage), previtellogenic (oocytes with cortical alveoli), and vitellogenic (oocytes with exogenous yolk

deposition in the cytoplasm). Spawning or spawned out females were not observed because sampling was conducted before the onset of spawning.

Ovaries were also examined histologically for follicular atresia and inflammatory lesions associated with oocyte resorption, including lymphoid or macrophage infiltrates. Atretic follicles were classified according to the scheme described in Hunter and Macewicz (1985) and Johnson et al. (1991) as alpha yolked atretic follicles, alpha nonyolked atretic follicles, or beta, gamma, or delta follicles (Table 4). Ovarian lesions were then divided into the following groups for statistical analysis: yolked atretic follicles (alpha stage); nonyolked atretic follicles (alpha stage); late stage atretic follicles (beta, delta, or gamma stage); and idiopathic ovarian inflammatory lesions (i.e., lymphoid or macrophage infiltrates not associated with parasitic infection). In addition, the actual proportion of yolked oocytes undergoing atresia in individual fish was determined by morphometric analysis for a subsample of approximately 10 females per site.

Concentrations of PCBs in liver and ovary were determined from approximately two gram samples, and in brain from approximately 0.2 g samples, according to the method described by MacLeod et al. (1985), with modifications as described in Stein et al. (1987). Briefly, tissue samples were ground with 10 g of silica, added to a column (270 x 23 mm) containing activated silica gel (Amicon Corp., Danvers, MA), and eluted with 50 mL pentane:methylene chloride (90:10, V/V). The eluant was concentrated and exchanged with 1 mL hexane before analysis by gas chromatography with electron capture detection. Biliary concentrations of FACs which fluoresce at benzo[a]pyrene (BaP) wavelengths were measured according to the method of Krahn et al. (1986). Hepatic microsomes were prepared and hepatic AHH activity was measured in duplicate using ^{14}C-BaP (80 µm) as a substrate as described by Collier et al. (1986). Levels of xenobiotic-DNA adducts in liver tissue were measured using the ^{32}P- postlabelling method as described in Varanasi et al. (1989a). Plasma estradiol levels were determined by radioimmunoassay as described by Sower and Schreck (1982).

Fecundity was determined gravimetrically using procedures described by Bagenal and Braum (1971). Preserved ovaries were allowed to remain in Gilson's fluid for at least 3 months to allow

eggs to harden and ovarian connective tissue to disintegrate. Preserved eggs were then washed with water, separated from ovarian connective tissue, filtered, and dried at 60° C for 24 hours. All eggs collected were weighed, then three random samples of 200 eggs each were weighed. Fecundity was determined according to the formula:

Fecundity = 2 [(total weight of eggs)(number of eggs in subsample)/mean weight of eggs in subsample].

Statistical Analyses

Hepatic and ovarian lesion prevalences were calculated for each sampling site and the G-statistic (Sokal and Rohlf 1981) was used to determine whether prevalences in winter from other sampling sites differed significantly from lesion prevalences in winter flounder from Niantic Bay, the site with the lowest levels of PAHs and PCBs in sediments. The significance level for these tests was set at $p \leq 0.05$.

Analysis of variance (Sokal and Rohlf 1981) was used to compare mean concentrations of estradiol in plasma and GSI at the sampling sites. Analysis of variance was also used to determine the effect of ovarian maturation stage on hepatic AHH activity, so any variation in AHH activity associated with the reproductive cycle could be separated from changes in AHH activity associated with contaminant exposure.

Regression analysis (Sokal and Rohlf 1981) was used to examine relationships between variables associated with contaminant exposure (i.e., concentrations of FACs in bile, hepatic DNA adducts, concentrations of PCBs in tissues, site of capture, and the presence of hepatic lesions) and variables associated with reproductive activity (i.e., GSI, plasma estradiol, fecundity, and egg weight). The effects of fish age, length, condition factor, year of sampling (1988 vs. 1989), season of sampling (October vs. December), and ovarian developmental stage were taken into account in these analyses. For analyses involving the effect of site of capture, Niantic Bay was chosen as the reference site, and other sites were evaluated relative to Niantic Bay. Regression models were fitted using stepwise linear regression, with the significance level for entry of

variables into the equation set at $p \leq 0.05$. Analyses were conducted using the Statview II* statistical package. Regression analysis was also used to assess the influence of fish age and sampling season on bioindicators of contaminant exposure. Spearman rank correlation, a nonparametric correlation technique (Sokal and Rohlf 1981), was used to examine relationships between liver lesion prevalences and other bioindicators of contaminants exposure.

Logistic regression (Breslow and Day 1980) was used to assess the relationships between ovarian recrudescence and ovarian atresia and indicators of contaminant exposure (i.e., site of capture, bile FACs, hepatic DNA adducts, tissue PCBs, and the presence of hepatic lesions). This statistical method is similar to multiple regression but is suitable for use with binomially distributed outcome data (e.g., data denoting whether or not ovarian development is occurring, or whether ovarian lesions are present or absent). Fish age, length, and condition factor were included in all analyses so the influence of these factors could be accounted for. As in the multiple regression analyses, when the effect of site of capture was examined, Niantic Bay was chosen as the reference site and the effects of other sites were evaluated relative to Niantic Bay. The EGRET statistical package was employed to fit the logistic regression models using a procedure similar to stepwise regression (Draper and Smith 1980). Variables were discarded from the models if their entry failed to produce a change in scaled deviance that was statistically significant ($p \leq 0.05$).

RESULTS

Fish Size, Age, and Condition

Mean length, age, and condition factor of fish captured at the sampling sites are shown in Figure 2; mean values of age, length, and condition factor broken down by site, year, and sampling season are given in Appendix Table A-1. While the range in these parameters (e.g. 1-6 cm, 1-2 years) was not great, significant differences were found between the sampling sites, and between fish sampled from different geographical areas and during different years (Fig. 2,

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Table 5). In general, fish from north of Cape Cod were larger and had higher condition indices than fish from south of Cape Cod. In addition, there were size and age differences among fish collected from various sites. Fish from Deer Island, Mystic River, and Quincy Bay were significantly older and larger than those at the Niantic reference site, while fish from Gravesend were younger and smaller. There also appeared to be some year-to-year variation in fish condition; condition indices were significantly lower in the 1989 than in the 1988 sampling (Appendix Table A-1).

Bioindicators of Contaminant Exposure

Biliary Fluorescent Aromatic Compounds

Of all the fish sampled, those from Mystic River had the highest overall levels of FACs in bile (Fig. 3, Appendix Table A-2). Over the 2 years of sampling, mean concentrations of FACs-BaP in bile ranged from 1,500 to 14,000 ng/g. In both October and December of 1989, FACs-BaP concentrations in flounder from Mystic River were roughly the same, with FAC-BaP concentrations ranging from approximately 1,500 to 12,000 ng/g, and means of 4,000 to 5,000 ng/g. However, in December 1988, bile FAC concentrations were much higher, exceeding 10,000 ng/g in 58% of fish sampled. The reason for the exceptionally high FAC-BaP concentrations observed in 1988 is unknown. In addition to Mystic River, FAC concentrations (mean ± SD) were also elevated at the Massachusetts Bay outside Boston Harbor (2,900 ± 3,000 ng/g) and Old Orchard Shoals within Raritan Bay (4,100 ± 2,000 ng/g). However, at other sites within Boston Harbor and Raritan Bay FAC concentrations were generally less than 2,000 ng/g and were not statistically different from concentrations at the Niantic Bay or Duxbury reference sites. Apart from the unusually high FAC concentrations in flounder sampled from Mystic River in 1988, sampling season appeared to have little impact on FACs concentrations (Table 6); the grand mean concentrations were 1,400 ± 2,000 ng/g in December (n = 145), and 1,400 ± 1,300 ng/g in October (n = 65).

Tissue PCBs

Mean concentrations of PCBs in winter flounder ovaries ranged from approximately 300 ng/g wet weight at Duxbury to approximately 500 ng/g wet weight at the Boston Harbor and Raritan Bay sites (Fig. 4a). Concentrations of PCBs in ovary tissue were significantly elevated in comparison to Niantic Bay levels in flounder from four sites: Mystic River and Deer Island in Boston Harbor, and Sandy Hook Channel and Shrewsbury River in Raritan Bay. Mean concentrations of PCBs in liver of fish from Boston Harbor were about 1,000 ng/g wet weight or less at all three sampling sites (i.e., Mystic River, Deer Island, and Quincy Bay), and were not significantly different from liver PCB concentrations in fish from Niantic Bay (Fig. 4b). In Raritan Bay, on the other hand, liver PCB concentrations were significantly elevated at both Sandy Hook Channel and Shrewsbury River (Fig. 4b), the same sites with elevated concentrations of PCBs in ovaries. In brain tissue, mean concentrations of PCBs ranged from a low of 530 ng/g at Deer Island to a high of 1,000 ng/g at Gravesend Bay (Fig. 4c), but intersite differences were not statistically significant. Concentrations of PCBs in brains of fish from Duxbury and Niantic Bay (720 and 740 ng/g, respectively) were comparable to those in fish from heavily contaminated sites in Boston Harbor and Raritan Bay. Concentrations of PCBs in winter flounder tissues listed by site, year, and sampling season are given in Appendix Table A-2. Wet weight concentrations of PCBs in tissues are approximately 1/5 of concentrations expressed in dry weight.

Concentrations of PCBs in liver, ovary, and brain were positively correlated: for liver vs. ovary, $r^2 = 0.107$, $n = 70$, $p = 0.006$; for brain vs. liver, $r^2 = 0.068$, $n = 70$, $p = 0.03$; for brain vs. ovary, $r^2 = 0.14$, $n = 79$, $p = 0.007$. However, relative concentrations of PCBs in various tissue compartments appeared to be affected by the sampling season and reproductive stage of winter flounder (Table 6, Appendix Table A-2). Ovarian PCB concentrations were significantly higher ($p = 0.0018$) in fish sampled in December than in fish sampled from the same areas in October (360 ± 200 ng/g wet weight ($n = 54$) and 250 ± 140 ng/g wet weight ($n = 52$), respectively) and were positively correlated with gonadosomatic index ($p = 0.001$, $r^2 = 0.231$, $n = 105$). Liver PCB concentrations showed the opposite pattern; they were significantly ($p = 0.0002$)

lower in fish sampled in December than in fish sampled in October (1,300 ± 750 ng/g wet weight, n = 66, and 550 ± 400 ng/g wet weight, n = 19, respectively), and tended to decline as GSI increased (for log[liver PCB] vs. GSI, $r^2 = 0.09$, $p = 0.012$, $n = 70$). Brain PCB concentrations, in contrast, did not change significantly during the sampling season. The mean PCB concentration in October was 760 ± 700 ng/g wet weight (n = 82), while in December it was 860 ± 540 ng/g wet weight (n = 82). Brain PCB concentrations were not significantly correlated with GSI.

Hepatic Xenobiotic-DNA Adducts

Like concentrations of PCBs in tissues and FACs in bile, levels of xenobiotic-DNA adducts in liver were generally elevated in winter flounder from within Boston Harbor and Raritan Bay (Fig. 5, Appendix Table A-2). Xenobiotic-adduct levels were significantly higher at Deer Island and Mystic River in Boston Harbor, and at Sandy Hook Channel in Raritan Bay than at the Niantic Bay reference site. For flounder from Deer Island and Mystic River, the mean level of DNA adducts in liver tissue was approximately 50 pmol/mol bases, and levels of DNA adducts in liver tissue of fish from Sandy Hook Channel were also relatively high (38 pmol/mol bases). In contrast, DNA adduct levels in flounder from other sampling sites were generally less than 20 pmol/mol bases. Concentrations of xenobiotic-DNA adducts in liver were also positively correlated with concentrations of PCBs in tissues and FACs in bile. For FAC-BaP vs. DNA adducts, $r^2 = 0.27$, $n = 37$; and for ovarian PCBs vs. DNA adducts $r^2 = 0.22$, $n = 22$, and for liver PCBs vs. DNA adducts, $r^2 = 0.34$, $n = 19$. Levels of xenobiotic-DNA adducts in liver remained relatively stable throughout the sampling season (Table 6). In October, the mean concentrations was 34 ± 7.0 pmol/mol bases (n = 13), while in December it was 29 ± 21 (n = 40) pmol/mol bases.

Hepatic AHH Activity

Hepatic AHH activity was not significantly elevated in flounder from sites within Boston Harbor or Raritan Bay in comparison to flounder from the Niantic Bay reference site, in spite of the relatively high concentrations of sediment-associated contaminants in Boston Harbor and Raritan Bay (Fig. 6). In fact, in some cases AHH activity was significantly lower (analysis of

variance (ANOVA), $p \leq 0.05$) in flounder from heavily contaminated sites. Activity levels varied considerably, ranging from 61 to 780 pmol/mg/min in fish from Mystic River, 210 to 730 pmol/mg/min in fish from Deer Island, and from 100 to 750 pg/mg/min in fish from the Raritan Bay sampling sites. In fish from Niantic Bay, one of the least contaminated of the sampling sites, AHH activity ranged from 460 pmol/mg/min to 1,100 pmol/mg/min. Moreover, hepatic AHH activity was not positively correlated with other bioindicators of contaminant exposure. However, hepatic AHH activity was closely related to the stage of ovarian developmental stage in sampled animals (Table 6, Fig. 7). In flounder from Boston Harbor and Raritan Bay as well as in flounder from the less contaminated embayments, hepatic AHH activity was significantly lower (ANOVA, $p = 0.001$) in vitellogenic flounder (220 ± 330, $n = 250$) than in flounder that were nonvitellogenic (980 ± 620, $n = 29$) (Fig. 5). Even in October, when animals were in early stages of vitellogenesis, differences in AHH activity were marked. For vitellogenic fish sampled at this time, mean AHH activity was 480 ± 400 pmol/mg/min ($n = 55$), while in nonvitellogenic fish it was $1,500 \pm 800$ pmol/mg/min ($n = 13$). Moreover, AHH activity was negatively correlated with both concentrations of estradiol in plasma ($r^2 = 0.312$, $p = 0.0001$, $n = 312$, log-transformed AHH) and GSI ($r^2 = 0.437$, $p = 0.0001$, $n = 342$, log-transformed AHH), declining as estradiol levels and ovary weight increased. Because of the effect of the reproductive cycle on hepatic CYP1A in female winter flounder, it was not used as an exposure indicator in subsequent analyses of relationships between contaminant exposure and ovarian development.

Fish Pathology

Neoplastic lesions were relatively rare in the winter flounder sampled in this study (Fig. 8, Appendix Table A-3). Of the 586 fish examined, only one fish from the Mystic River had a neoplasm. Foci of cellular alteration were more prevalent, affecting 3% of all fish examined. Of the 16 fish affected, 63% were from Boston Harbor and Raritan Bay, but 37% were from the moderately to minimally contaminated sites such as Niantic Bay, Connecticut; Duxbury Bay, Massachusetts; and Narragansett Bay, Rhode Island. The two other categories of hepatic lesions,

hydropic vacuolation and proliferative lesions (predominantly biliary proliferation), were more common, affecting 22 and 5% of fish, respectively. Prevalences of proliferative lesions were significantly elevated at the Deer Island, Mystic River, and Quincy Bay sites in Boston Harbor, and at the Shrewsbury River, Sandy Hook Channel, and Old Orchard Shoals sites in Raritan Bay (Fig. 6). Prevalences of hydropic vacuolation were significantly elevated at all sampling sites within Boston Harbor and Raritan Bay, and at Narragansett Bay. Highest prevalences (>40%) were found at Mystic River, Old Orchard Shoals, and Sandy Hook Channel.

Neoplastic and putatively preneoplastic lesions showed no relationship with tissue contaminant concentration, partly because they were observed in so few animals (Table 7). However, concentrations of FACs-BaP in bile were positively correlated (Spearman-Rank correlation coefficient, $p \leq 0.05$) with prevalences of both hydropic vacuolation (rho = 0.497, n = 21) and proliferative liver lesions (rho = 0.565, n = 21). Concentrations of PCBs in liver were also positively correlated with both of these lesions (for hydropic vacuolation, rho = 0.870, n = 21; for proliferative lesions, rho = 0.660, n = 15). Proliferative liver lesion prevalences were positively correlated with ovarian PCB concentrations (rho = 0.647, n = 15) and levels of DNA adducts in liver (rho = 0.717, n = 9).

In general, prevalences of hepatic lesions were somewhat higher in October than in December (Appendix Table A-3). Prevalences of FCA, proliferative lesions, and hydropic vacuolation were 4.5, 8 and 40%, respectively, in October (n = 224), while in December, prevalences were 1.6, 3, and 20% (n = 362).

Reproductive Parameters

Ovarian Development

Assessment of ovarian development in winter flounder showed that although gonadal recrudescence was well under way in most females sampled, some adult females had not entered vitellogenesis (Fig. 9, Appendix Table A-4). The majority of these animals were from sites north of Raritan Bay. In Raritan Bay over 99% of sampled females were maturing, while only 77%

were maturing from sites north of Raritan Bay. Furthermore, the prevalence of inhibited ovarian development showed no clear relationship to contaminant levels at the sampling sites; females from the Raritan Bay sites, which had relatively high contaminant levels, showed no impairment of ovarian development, and fish from Mystic River, which was the most contaminated of all sites sampled, showed prevalences of ovarian development comparable to those at minimally contaminated sites north of Cape Cod. Logistic regression analysis (Fig. 10) revealed that the most important factor influencing ovarian development was fish age. In addition, fish captured north of Cape Cod were less likely to be vitellogenic than animals captured farther south. However, none of the measured indicators of contaminant exposure were related to a decreased probability of ovarian development. Neither sampling year nor sampling season (i.e., October vs. December) significantly influenced the stage of ovarian development. Apparently, by October most females that were going to undergo gonadal development that season had entered vitellogenesis.

Plasma Estradiol and GSI

Significant intersite differences in GSI and plasma estradiol concentrations in female winter flounder were observed (Fig. 11 a,b); however, these reproductive indicators were not depressed in fish from contaminated sites. Moreover, no negative relationships could be found between either GSI or estradiol and any indicator of contaminant exposure that was measured. Similarly, the presence of hepatic lesions had no significant effect on either plasma estradiol concentrations or GSI (Table 8). Both plasma estradiol concentrations and GSI were significantly higher in fish sampled in December than in fish sampled in October (Table 8).

Fecundity and Egg Weight

No significant intersite differences in fecundity were found in winter flounder (Fig. 12a, Appendix Table A-4), and fecundity showed no relationship with concentrations of FACs in bile (Table 8). Fecundity tended to be lower in flounder with the highest concentrations of PCB in ovary and brain, but these relationships were not statistically significant after the influence of fish

length on fecundity had been taken into account (Table 8). Mean individual egg weight was somewhat lower in fish from Deer Island and Mystic River than in fish from other sites, but the differences were not significant (Fig. 12b, Appendix Table A-4). However, a significant negative correlation was found between egg weight and biliary FACs, which remained significant even after biological factors had been taken into account (Table 8). The effects of sampling year and sampling season could not be evaluated because data were collected only in December 1989.

Ovarian Lesions

Ovarian lesions were found in substantial proportions of both vitellogenic and nonvitellogenic winter flounder (Fig. 13 a,b). Prevalences of ovarian lesions were similar for flounder at all sites sampled, except for fish from Mystic River, which showed significantly higher prevalences of atresia in both vitellogenic and nonvitellogenic females. However, the morphometrically-determined percentage of yolked oocytes undergoing atresia was approximately the same at all sites sampled, ranging from 5.4 to 6.6%. Logistic regression analysis (Table 9) indicated that high concentrations of PCBs in the liver were associated with a significantly increased probability of atresia of yolked oocytes in vitellogenic females ($p = 0.026$, 6% of variation explained), but the actual percentage of atretic oocytes within ovaries of individual females was not correlated with any of the indicators of contaminant exposure. In nonvitellogenic females, atresia of nonyolked oocytes was significantly more common (logistic regression, $p = 0.042$, 4% of variation explained) in fish with elevated biliary FAC concentrations. No correlations were found between ovarian or brain PCB concentrations, hepatic lesions, or xenobiotic-DNA adducts and the probability of ovarian atresia. In vitellogenic fish, GSI was found to be negatively associated with atresia of yolked oocytes ($p = 0.016$, 1% of variation explained), nonyolked oocytes ($p = 0.001$, 8% of variation explained), and ovarian inflammatory lesions ($p = 0.011$, 2% of variation explained). The negative correlation between GSI and the presence of ovarian lesions may be an indication that lesions are more likely to develop during early stages of ovarian development.

DISCUSSION

Bioindicators of Contaminant Exposure

In general, concentrations of PCBs in tissues and FACs in bile tended to be higher in winter flounder from contaminated sites in Boston Harbor and Raritan Bay than in flounder from other embayments, although exceptions were found for some tissue contaminants at selected sampling times. Moreover, levels of contaminants in bile and tissues were generally correlated with other biological indicators of exposure such as xenobiotic-DNA adducts in liver and the presence of hepatic lesions. The only measure that showed no relationship with sediment contaminant levels or concentrations of contaminants in tissues or fluids of winter flounder was hepatic AHH activity. Hepatic AHH activity was significantly depressed in gonadally maturing female winter flounder, even in animals collected in October which were in early stages of vitellogenesis, presumably because of the strong suppressive influence of estradiol on CYP1A in female winter flounder (Snowberger Gray et al. 1991). These findings suggest that hepatic AHH activity is not a reliable indicator of contaminant exposure in vitellogenic winter flounder and would dictate the employment of other measurements to assess chemical contamination in flounder undergoing reproductive development.

Results show that levels of selected contaminants in tissues and bile of winter flounder varied with the time of sampling. This was particularly noticeable with levels of PCBs in ovary and liver. Between October and December, PCB concentrations in liver declined, while concentrations in ovaries increased. This suggests mobilization of PCBs from liver to ovaries in conjunction with the transfer of lipids to the developing gonads. This same phenomenon has been described in other fish species (Pizza and O'Conner 1983). Levels of PCBs in brain, however, remained constant throughout the reproductive season. This suggests that levels of PCBs in brain may be a more stable measure of the body burden of PCBs than levels in liver or ovary. At the same time, however, brain PCB concentrations showed little correlation with liver pathology, although both ovarian and liver PCB concentrations were positively correlated with prevalences of hydropic

vacuolation and proliferative liver lesions. Moreover, intersite differences in brain PCB concentrations were less marked than differences in liver or ovary PCB concentrations. We are continuing to evaluate the utility of brain PCB concentrations as an indicator of long-term PCB exposure and to examine relationships between pathological effects in fish and PCB concentrations in different tissue compartments.

Fish Pathology

Results of the present study indicate that winter flounder residing in contaminated areas are likely to exhibit a variety of suspected toxicopathic liver lesions, including unique degenerative or proliferative conditions. Both the proliferative lesions and hydropic vacuolation were significantly elevated in vitellogenic flounder from sites within Boston Harbor and Raritan Bay in comparison to prevalences in flounder from reference sites. Of all toxicant-associated lesions, the most commonly observed was hydropic vacuolation of hepatocytes and biliary epithelial cells, which was found in fish from all 11 sampling sites at prevalences ranging from 3 to 60%. In addition to winter flounder, hydropic vacuolation has been observed in several other flatfish species in association with exposure to contaminants (Stehr 1990, Stehr et al. 1991, Myers et al. in press). Hepatic neoplasms were rarely observed in winter flounder sampled in this study; only one case was found in a fish from the Mystic River site in Boston Harbor. Foci of cellular alteration (FCA), which are putatively preneoplastic lesions (Frith and Ward 1980, Squire and Levitt 1975), were found in fish from several sites within and in the vicinity of Boston Harbor and Raritan Bay at prevalences ranging from 2 to 13%. However, they were also found at low prevalences at some of the less-contaminated embayments, including Duxbury Bay, Narragansett Bay, and Niantic Bay.

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). However, prevalences of most lesions in the present study were somewhat lower than those reported in other studies, in which both male and female winter flounder were sampled and fish were collected outside of the

reproductive season. For example, hydropic vacuolation, the most common toxicant-associated lesion found in the present study, were observed in 23 to 46% of fish examined from sites within Boston Harbor. In contrast, other investigators (Murchelano and Wolke 1985, 1991; Moore 1991; Carr et al. 1991) report prevalences of hydropic vacuolation from 50 to 74% in Boston Harbor winter flounder. In the present study, biliary proliferation was observed in 7 to 12% of Boston Harbor winter flounder, while Moore (1991) reports prevalences of 25%. Prevalences of neoplastic and preneoplastic lesions were also somewhat higher, and preneoplastic lesions were less uniformly distributed in other investigations than in the present study. For example, Wolke et al. (1985) and Murchelano and Wolke (1985, 1991) examined winter flounder from several sites along the northeast coast, and observed preneoplastic and neoplastic lesions in 10-15% of winter flounder sampled at sites within Boston Harbor, approximately 10% of winter flounder from New Haven, and 3% of flounder from Narragansett Bay. In contrast, no neoplasms were found in 93 winter flounder from central and eastern Long Island Sound, Casco Bay in Maine, and George's Bank. Moore (1991) observed neoplasms in approximately 10% of Deer Island winter flounder, but saw none in fish from Cape Cod Bay, Massachusetts Bay, or Georges Bank.

It is not entirely clear why neoplasm prevalences were so low in winter flounder sampled in this study. However, preliminary data from samplings of winter flounder conducted in spring of 1988 and 1989 in conjunction with NOAA's National Benthic Surveillance Project (NBSP) show that winter flounder which were sampled at heavily contaminated sites in Boston Harbor and Raritan Bay during the spring exhibited higher prevalences of neoplasms and other toxicopathic lesions, including hydropic vacuolation, than females sampled in the present study during the winter spawning season (Johnson et al. 1992). This suggests that the population of flounder sampled in winter for the present study may have included fish that were not normally resident at the sampling sites during the year, but had migrated to these sites from other, less contaminated areas to spawn. 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, Black et al. 1988). Consequently, all fish captured in an area during the reproductive season do not necessarily remain there during the rest of the year, although a large proportion may be permanent residents.

Additionally, because only female flounder were sampled in the present study, it is possible that the lower prevalences of neoplasms in this sampling could be due to gender-associated differences in disease susceptibility. Our data from the NBSP sampling during 1988 and 1989 (Johnson et al. 1992) do suggest that female winter flounder are slightly less likely than male winter flounder to develop neoplasms and cystic degeneration. This finding is 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). Similarly, Rhodes et al. (1987) found that gender did not appear to have any effect on neoplasm prevalence in English sole (*Pleuronectes vetulus*, formerly *Parophrys vetulus*). Because the gender-related differences in prevalences for winter flounder sampled in the NBSP were relatively small (e.g., 1% vs. 3%), the possibility that they simply represent sampling error cannot be discounted. 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.

In addition to showing elevated prevalences at contaminated sites, hydropic vacuolation and proliferative lesions were positively correlated with levels of FACs in bile and PCBs in tissues of winter flounder. Proliferative lesions were also positively correlated with levels of DNA-xenobiotic adducts in liver. Because both of these lesion types appear to be strongly linked to exposure to multiple classes of chemical contaminants in winter flounder, they represent reliable bioindicators of contaminant exposure. The relationship between PAHs and PCBs and presumably degenerative and regenerative lesions such as hydropic vacuolation and biliary proliferation is not surprising, as a number aromatic and chlorinated hydrocarbons have been shown to have necrogenic effects on the liver (Hodge et al. 1967, Murphy 1986). However, it is difficult to identify the precise etiology of either of these lesion types because of their apparent

association with both types of contaminants, namely PAHs and PCBs, which generally co-occur in urban sediments. Laboratory exposure studies with winter flounder may help to clarify the relative roles of PAHs and CHs in the development of lesions such as hydropic vacuolation and biliary proliferation.

Unlike hydropic vacuolation and proliferative lesions, neoplasms and preneoplastic focal lesions were not significantly correlated with bioindicators of contaminant exposure. As noted above, neoplasm prevalences were too low for their relationship with measures of contaminant exposure to be evaluated effectively. However, the lack of correlation between the focal lesions and indicators of PAH exposure (i.e., concentrations of biliary FACs and xenobiotic-DNA adducts in liver) was somewhat surprising, because PAHs are well established as genotoxic carcinogens in mammals (Williams and Weisburger 1986), and administration of PAHs in laboratory studies has led to the development of 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 PAH levels in bile and sediments and prevalences of neoplasms and preneoplastic focal lesions (Malins et al. 1984, 1985, Landahl et al. 1990, Myers et al. 1990).

It is not entirely clear why correlations between PAH exposure and prevalences of FCA in winter flounder were not as strong as correlations observed, for example, in English sole, but several factors may have been involved. First, because winter flounder are relatively mobile, it may have been difficult to obtain an accurate measure of chronic PAH exposure in this species. Levels of metabolites in bile are generally reliable measures of short-term exposure to PAHs (Krahn et al. 1986), but because PAHs are rapidly metabolized (Varanasi et al. 1989b, Stein et al. 1987), biliary FAC levels are unlikely to remain elevated for long periods of time if animals leave contaminated areas. In a species such as winter flounder, which may undergo extensive seasonal migrations, biliary FAC concentrations at the time of capture may not necessarily be an accurate reflection of the level of PAH exposure over the animal's lifetime. Levels of xenobiotic-DNA

adducts in liver (Varanasi et al. 1989a) may provide a more reliable measure of chronic PAH exposure than biliary FAC concentrations. However, they were measured in relatively few animals in this study, and perhaps for this reason they showed no clear relationship with prevalences of FCA or neoplasms in winter flounder.

Another factor that must be considered in evaluating the relationships between chemical contaminants and neoplastic and preneoplastic lesions in winter flounder is that winter flounder may differ somewhat from English sole and other teleost species that have been used as models in carcinogenesis in their pathological response to environmental contaminants. Patterns of liver lesion occurrence in winter flounder from Boston Harbor and Raritan Bay are quite different, for example, from those observed in English sole from urban areas in Puget Sound. Neoplasm prevalences in winter flounder are relatively low (e.g., 7% in sexually mature winter flounder from Deer Island Flats (Moore 1991) compared to prevalences of 36% in sexually mature English sole from a similarly contaminated site in the Duwamish Waterway in Puget Sound, WA (Johnson et al. 1988)). Moreover, a higher proportion of the neoplasms in winter flounder are cholangiocellular in origin than in English sole. In winter flounder, cholangiocellular neoplasms make up 42 to 90% of all liver neoplasms observed (Moore 1991; Murchelano and Wolke 1985, 1991), while in English sole they account for only about 15% of liver neoplasms (Myers et al. 1987). Hepatocellular neoplasms, which are typically associated with PAH exposure in both field (Landahl et al. 1990, Myers et al. 1990, Vogelbein et al. 1990) and laboratory studies (Metcalfe et al. 1988, Black et al. 1985, Hendricks et al. 1985, Schultz and Schultz 1984, Hawkins et al. 1990) with English sole and other fish species, appear to occur less frequently and compose a lower proportion of the neoplasms detected in winter flounder. Putatively preneoplastic focal hepatic lesions, which are commonly observed in English sole (Myers et al. 1987), are far less frequently detected in winter flounder (Moore 1991).

The early degenerative and proliferative changes found in the livers of winter flounder and English sole after exposure to toxicants are also markedly different. In winter flounder, the most common early toxicopathic lesion is hydropic vacuolation (Murchelano and Wolke, 1985, Moore

et al. 1989), a condition which is not found in English sole (Myers et al. in press). Instead, English sole develop a unique degenerative condition, megalocytic hepatosis (Myers et al. 1987), which is only rarely observed in winter flounder (Johnson et al. 1992). Because of these differences in patterns of lesion occurrence, it has been suggested (Moore 1991) that the histogenesis of hepatic neoplasms, as well as the roles that various classes of contaminants play in their development, may be somewhat different in winter flounder than in species such as English sole or killifish, which have comparatively high prevalences of hepatocellular neoplasms in PAH-contaminated areas.

Additionally, it should be remembered that only a limited number of samples were available for the analyses of relationships between contaminant exposure and lesion prevalences, and the number of neoplastic and preneoplastic lesions was relatively low at all sites sampled. With a larger data base a more comprehensive assessment of the relationships between contaminants and neoplastic and preneoplastic lesion in winter flounder may be possible.

Reproductive Success

The results of this study suggest that winter flounder sampled from contaminated estuaries may exhibit declines in egg weight and an increased probability of ovarian atresia, although the magnitude of these effects appears to be relatively small. These findings corroborate reports by other researchers of reduced egg or larval size in winter flounder from contaminated sites in Boston Harbor and Long Island Sound (Black et al. 1988, NOAA 1990). Contaminant-associated declines in egg weight have also been noted in other species, including white croaker (*Genyonemus lineatus*) (Cross and Hose 1988) and kelp bass (*Paralabrax clathratus*) (Cross and Hose 1989) from southern California, and striped bass (*Morone saxatilis*) from the San Francisco area (Whipple et al. 1987a). Atresia of both immature and vitellogenic oocytes in conjunction with exposure to aromatic and chlorinated organic compounds has been reported in several other fish species as well (Saxena and Garg 1978, Cross et al. 1984, Nagler et al. 1986, Whipple et al. 1987b, Cross and Hose 1988).

The present results show, however, that mean steroid hormone levels, ovarian development, and fecundity were not depressed in winter flounder from heavily contaminated sites in Boston Harbor and Raritan Bay. Moreover, on an individual fish basis, these reproductive parameters showed little correlation with any of the indicators of contaminant exposure which we measured, even though concentrations of PCBs in tissues and FACs in bile were elevated in a relatively large proportion of sampled animals. In fact, Raritan Bay flounder, which exhibited high frequencies of hepatic lesions as well as elevated levels of PCBs in tissues and FACs in bile, showed the greatest degree of reproductive development of all groups of animals sampled. Variations in steroid hormone levels, ovarian growth, and fecundity were most closely associated with biological or ecological factors, such as fish age or size, sampling time, and the geographical area from which the fish were sampled. Perhaps the most marked influence was the clinal difference in the timing and probability of gonadal maturation between fish from areas north and south of Cape Cod. This geographical variation in the timing of reproduction has also been noted by other investigators (Pearcy 1962).

The finding that exposure to marine pollutants such as PAHs and PCBs, which were used as general indicators of anthropogenic contamination, had little apparent effect on ovarian development or steroid levels in winter flounder was quite surprising. Alterations in both of these reproductive parameters have been described in other fish species collected from areas contaminated with these types of xenobiotic compounds (Johnson et al. 1988, Stott et al. 1983, Munkittrick et al. 1991). The reasons for the apparent resistance of winter flounder to contaminant-associated reproductive impairment are not clear. However, a number of factors could influence its susceptibility to the effects of reproductive toxicants in the environment, including the level of exposure, the duration and timing of exposure, and species-specific pathways of uptake, metabolism, and detoxication of contaminants.

In the present study, the results show that a substantial proportion of gravid female winter flounder sampled had high levels of contaminants in their tissues and body fluids, at least at the time of collection. Some of these contaminants, such as PCBs, are not extensively metabolized or

eliminated, and tend to build up in tissues over time (Lieb et al. 1974, Pizza and O'Conner 1983, Stein et al. 1987). Consequently, there is a high probability that PCBs which were present in winter flounder tissues at the time of collection were in the body at similar concentrations prior to and throughout gonadal development. If winter flounder from contaminated areas on the northeast coast have elevated levels of PCBs in the body throughout ovarian recrudescence, yet show no signs of reproductive dysfunction, it would appear that PCBs have little effect on early reproductive development in winter flounder.

The finding that winter flounder with high concentrations of PCBs in tissue do not show altered ovarian development is not entirely unexpected, because in English sole, little relationship was found between tissue PCB levels and this particular type of reproductive dysfunction (Johnson et al. 1988). Moreover, although other fish species with tissue PCB concentrations comparable to those measured in winter flounder (e.g., ovarian PCB concentrations of 200 to 300 ng/g wet weight and above) have exhibited various types of reproductive impairment in field studies (Cross et al. 1984, Cross and Hose 1989, von Westernhagen et al. 1981), the types of impairment reported in these studies, such as impaired spawning or decreased larval viability, involve relatively late stages of the reproductive cycle. In contrast, inhibited ovarian development and altered plasma steroid levels manifest themselves during early gonadal recrudescence. While further research is needed, our observations on both winter flounder and English sole suggest that PCBs may be more toxic during later stages of reproductive development (e.g., affect egg and larval viability), than during early vitellogenesis in fish.

Like winter flounder with elevated tissue PCB concentrations, flounder with elevated biliary FAC concentrations showed no discernible impairment in gonadal development or alteration in plasma estradiol levels. This was surprising, because PAH exposure appears to be closely correlated with impairment of the early phases of the reproductive cycle in English sole. For example, English sole from PAH-contaminated areas showed impaired gonadal development, decreased estradiol levels in plasma, and reduced ovarian estradiol production. These types of impairment were statistically correlated with elevated concentrations of FACs in bile (Johnson et al.

1988, in press). Moreover, in laboratory studies, gravid English sole exposed to extracts of creosote-contaminated sediments showed reduced plasma estradiol concentrations and an increased rate of excretion of metabolites of estradiol (Stein et al. 1991). Preliminary studies indicate that rock sole and flathead sole show similar changes in plasma estradiol concentrations and ovarian estradiol production when exposed to PAHs (Johnson et al. in press). In contrast, winter flounder from sites within Boston Harbor had biliary FAC levels two to three times as high as those associated with reproductive impairment in English sole, but they exhibited no adverse effects on ovarian development.

Unlike PCBs, however, biliary FAC concentrations are an indicator of relatively short-term exposure; so, as noted earlier, high FAC concentrations at the time of sampling are not necessarily an indication of chronic exposure to PAHs. Previous studies show that after a single exposure to PAHs, biliary concentrations of FACs decline substantially within 3 weeks (Collier and Varanasi 1991). Consequently, seasonal migrations or similar factors affecting the duration and timing of short-term exposure to PAHs could have a marked impact on tissue concentrations of PAHs and their metabolites. Studies of migration patterns of winter flounder during the reproductive season (Black et al. 1988, Valdez 1989, Danila 1989) indicate that while female flounder may move into highly contaminated urban estuaries in the winter to spawn, in summer they spend extended periods in less contaminated areas offshore (Valdez 1989, Danila 1989). Because of these migration patterns, flounder captured within Boston Harbor or Raritan Bay in late fall or early winter may not be resident at these PAH-contaminated sites during critical early stages of vitellogenesis, and so they may not be exposed to appreciable PAHs when contaminant-associated alterations in steroid production or metabolism could have a significant impact on ovarian development. In contrast, English sole, which show a high prevalence of inhibited ovarian development in association with PAH exposure, remain in contaminated estuaries for most of their life cycle, including the period when they are undergoing vitellogenesis, and migrate to deeper, less contaminated areas offshore for a brief period in winter to spawn (Johnson et al. 1991, Day 1976). Laboratory exposure studies with English sole and winter flounder are necessary to test the

hypothesis that limited PAH exposure at critical phases of the reproductive cycle could at least partially account for the apparent resistance of winter flounder to early reproductive impairment.

Finally, it is possible that winter flounder may be less sensitive than English sole to the effects of reproductive toxicants because of species-specific differences in contaminant metabolism in recrudescent females. Interspecific differences in toxicant metabolism do exist in flatfish species; starry flounder *(Platichthys stellatus)*, for example, appear to have an greater ability to conjugate and detoxicate PAH metabolites than English sole (Varanasi et al. 1986, 1987; Collier et al. in press). Moreover, starry flounder, somewhat like winter flounder, exhibit a substantially lower prevalence of hepatocellular neoplasms than English sole exposed to similar levels of contaminants at polluted sites (Myers and Rhodes 1988, Myers et al. in press). These findings, combined with the fact that PAHs are strongly correlated with the prevalence of neoplasms in English sole and other fish species (Myers et al. 1990), suggest that differences in toxicant metabolism may affect susceptibility to liver disease. It is possible, therefore, that differences in toxicant metabolism may affect susceptibility to reproductive impairment as well, although this is yet to be established.

In addition to exhibiting differences in their pathologic responses to contaminants, winter flounder and English sole also show differences in the impact of the reproductive cycle on CYP1A dependent activity. In English sole, hepatic AHH activity changes relatively little until just before spawning (Johnson et al. 1988). However, in winter flounder, as this and other investigations show (Snowberger and Stegeman 1987, Snowberger Gray et al. 1991), CYP1A-dependent activity declines markedly in early vitellogenesis. Changes in activity of xenobiotic metabolizing enzymes during this phase of the reproductive cycle may alter the disposition of toxicants in the liver and in extrahepatic tissues such as ovary and, consequently, alter the effects of exposure to PAHs and similar compounds on winter flounder.

Further study is clearly needed to establish the effects of behavioral and physiological factors on the fate and effects of PAHs and similar compounds in winter flounder. Most critical are laboratory studies examining the impact of reproductive toxins on all phases of the reproductive cycle, to determine whether the apparent resistance of winter flounder to the effects of these

compounds is due to a lower sensitivity to the chemicals, or to migration patterns that limit exposure at critical phases of the reproductive cycle. Additionally, it should be kept in mind that in the natural environment winter flounder is simultaneously exposed to a wide array of different classes of contaminants, which could have widely divergent effects on reproductive function. In fact, certain compounds, including the heavy metal cadmium, appear to stimulate rather than inhibit reproductive development (Thomas 1989). Interpretation of the present findings may be complicated by interactive effects of these various chemicals. Such questions can best be clarified through laboratory studies.

In summary, the present results indicate that, in general, winter flounder from heavily contaminated sites within Boston Harbor and Raritan Bay had higher concentrations of PCBs in tissues, FACs in bile, and xenobiotic-DNA adducts in liver, as well as higher prevalences of toxicopathic liver lesions, than winter flounder from nearby, less urbanized embayments. However, ovarian growth and estradiol concentrations in plasma were not depressed in flounder from the contaminated areas, or in flounder with high concentrations of PCBs in tissues or FACs in bile. It is not clear why these contaminants, which have been shown to cause reproductive impairment in other fish species, have little apparent effect on gonadal development in winter flounder. However, migratory patterns that limit exposure to known reproductive toxicants (e.g., PAHs) at critical phases of the reproductive cycle, and differences in metabolism of or sensitivity to these toxicants, could be contributing factors.

It should be noted that although winter flounder appear to be relatively resistant to the effects of reproductive toxicants during early phases of gonadal development, this does not rule out the possibility that exposure to environmental contaminants may lead to impairment in later phases of the winter flounder reproductive cycle. Because winter flounder often spawn in contaminated inshore estuaries, and have demersal eggs, there is a particular risk that egg and larval development may be affected by contact with contaminated sediments. In fact, preliminary studies indicate that environmental disturbances, such as dredging, or exposure to contaminants in sediments or

sewage effluents may decrease hatching success and impair the growth and development of winter flounder larvae (Weis et al. 1989, Monooch 1988, NOAA 1990).

The contrasting effects of exposure to contaminants on ovarian development in winter flounder and English sole point to the need for both field and laboratory studies on a variety of fish species to increase our understanding of the behavioral and physiological characteristics that influence an animal's sensitivity to toxicants which may affect reproductive processes. Such research is extremely important to evaluate the potential impact of degradation of marine estuaries on marine fish populations (Chambers in press). Studies that will enhance our ability to identify species which may be at risk for contaminant-induced reproductive impairment are critical to successful conservation and management of marine fish populations.

CONCLUSIONS

1. In general, winter flounder from sites within Boston Harbor and Raritan Bay showed greater evidence of contaminant exposure than winter flounder from nearby, less urbanized embayments, based on levels of FACs in bile, concentrations of PCBs in tissues, levels of xenobiotic-DNA adducts in the liver, and prevalences of hepatic lesions.

2. Hepatic AHH activity was significantly depressed in gonadally maturing female winter flounder, even in animals collected in October which were in early stages of vitellogenesis. Because of the strong influence of reproductive status on AHH activity, it showed little correlation with other indicators of contaminant exposure and was not elevated in winter flounder from Boston Harbor or Raritan Bay sites. This finding suggests that hepatic AHH activity is not a reliable indicator of contaminant exposure in vitellogenic winter flounder.

3. Significant relationships were observed between several classes of idiopathic liver lesions in winter flounder and indicators of exposure to contaminants in the environment. Pathological conditions were most likely to occur in fish from sites in urban estuaries where concentrations of aromatic and chlorinated hydrocarbons in sediment were high. Moreover, prevalences of two of these conditions (hydropic vacuolation and proliferative liver lesions) showed significant

associations with bioindicators of contaminant exposure, such as PCBs in tissues, biliary FACs, and levels of xenobiotic-DNA adducts in liver. Because different classes of contaminants tend to occur together in sediments, and animals are exposed to a mixture of agents simultaneously, it is difficult to evaluate the roles that these compounds might play in the etiology of liver disease on the basis of epidemiological data alone. However, the associations between tissue contaminant levels and liver pathology observed in this study provide evidence for the involvement of environmental contaminants in the development of hepatic lesions, and clearly demonstrate the utility of these lesions as bioindicators of contaminant exposure in feral fish.

4. Winter flounder from Boston Harbor and Raritan Bay showed little evidence of contaminant-associated reproductive impairment. Ovarian growth and estradiol concentrations in plasma were not depressed in flounder from the contaminated areas or in flounder with high concentrations of PCBs in tissues or FACs in bile. It is not clear why these contaminants, which have been shown to cause reproductive impairment in other fish species, have little apparent effect on gonadal development in winter flounder. However, migratory patterns that limit exposure to known reproductive toxicants (e.g., PAHs) at critical phases of the reproductive cycle, and differences in metabolism of or sensitivity to these toxicants, could be contributing factors.

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TABLES

Table 1. East Coast sites sampled in 1988 and 1989 as part of winter flounder reproductive studies.

State	Site name	Latitude	Longitude	Years sampled		
				December 1988	October 1989	December 1989
MA	Deer Island, Boston Harbor	42°19.9'N	70°58.1'W	x	x	x
MA	Quincy Bay, Boston Harbor	42°18.4'N	70°58.4'W		x	
MA	Mystic River, Boston Harbor	42°23.2'N	71°03.2'W	x	x	x
MA	Massachusetts Bay	42°20.5'N	70°46.0'W	x		
MA	Duxbury Bay	41°59.3'N	70°37.6'W	x	x	
RI	Narragansett Bay	41°40.4'N	71°21.2'W	x		x
CT	Niantic Bay	41°17.2'N	72°11.2'W	x	x	x
NJ	Gravesend, Raritan Bay	40°35.4'N	74°01.6'W			x
NJ	Shrewsbury River, Raritan Bay	40°26.0'N	74°00.0'W		x	x
NJ	Sandy Hook, Raritan Bay	40°27.1'N	74°02.0'W		x	
NJ	Old Orchard Shoals, Raritan Bay	40°31.0'N	74°09.0'W		x	x

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)

Table 3. Classification scheme for ovarian developmental stages, based on histological criteria.

Stage	Features
Regressed	Primary oocytes or a mixture of primary and secondary oocytes present; secondary oocytes may be beginning to enlarge but are not vacuolated.
Previtellogenic	Oocytes with clear peripheral vacuoles (cortical alveoli); zona radiata present.
Vitellogenic	Yolked oocytes present
Spawning	Yolk globules coalescing; hydrated oocytes present; post-ovulatory follicles visible.
Spawned out	Many post-ovulatory follicles present; yolked oocytes undergoing resorption; inflammatory infiltrate, beta or gamma atretic follicles and/or macrophage aggregates generally present.

Table 4. Classification scheme for stages of follicular atresia, based on histological criteria.

Stage	Features
Alpha atresia, yolked oocytes	During this phase, the oocytes are being resorbed, leaving only follicular cells. The nucleus and yolk globules disintegrate, the zona radiata dissolves, and granulosa cells enlarge and invade the disintegrating oocyte, absorbing both the yolk and basophilic cytoplasm. The process is complete when all yolk and basophilic cytoplasm are gone.
Alpha atresia, nonyolked oocytes	Similar to alpha atresia of yolked oocytes, except that no yolk, only basophilic cytoplasm, is present.
Beta atresia	The atretic follicle appears as a compact structure composed of numerous disorganized granulosa cells surrounded by a thin thecal and blood vessel layer.
Gamma atresia	The atretic follicle is smaller than a beta follicle and contains light-yellowish flocculent material. Nuclei are irregularly shaped, and the follicle is still surrounded by a thecal and blood vessel layer.
Delta atresia	Follicles are small structures composed of 2-20 granulosa cells in the ovarian connective tissue. The follicles are not surrounded by thecal cells or blood vessels, and the granulosa cells contain dark-yellow finely granulated pigment.

Table 5. Relationships between site of capture, season of capture (October vs. December), and year of capture (1988 vs. 1989) and length (mm), age, and condition factor in winter flounder. Data were analyzed using stepwise multiple regression, with $p \leq 0.05$ as the significance level.

			Independent variable												
Dependent variable	df	r2	Mystic River	Deer Island	Mass. Bay	Quincy Bay	Dux-bury	Narra-gansett	Shrews-Bury	Sandy Hook	Orchard Shoals	Grave-send	North of Cape Cod	Sampled 1989	Sampled Dec.
Age	590	0.20	(+) p<0.001	(+) p<0.001	ns	(+) p<0.001	ns	(-) p<0.002	ns	ns	ns	(-) p<0.001	ns	ns	ns
Length	590	0.20	(+) p<0.002	(+)	ns	(+)	ns	ns	ns	ns	ns	(-) p<0.001	(+) p<0.021	ns	ns
Condition factor	590	0.34	ns	(+) p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	(+) p<0.001	(-) p<0.001	ns

Table 6. Relationships between site of capture, season of capture (October vs. December), year of capture (1988 vs. 1989), and age of sampled animals and bioindicators of contaminant exposure (biliary fluorescent aromatic compounds (FACs), xenobiotic-DNA adducts in liver, hepatic aryl hydrocarbon hydroxylase (AHH) activity, and concentrations of polychlorinated biphenyls (PCBs) in brain, liver, and ovary) in winter flounder. Data were analyzed using stepwise multiple regression, with $p \leq 0.05$ as the significance level.

			Independent variable												
Dependent variable	df	Regression r^2	Mystic River	Deer Island	Mass. Bay	Quincy Bay	Dux-bury	Narra-gansett	Shrews-Bury	Sandy Hook	Orchard Shoals	Grave-send	Age	Sampled 1989	Sampled Dec.
FACs	268	0.42 p=0.0001	(+) p<0.001	ns	ns	ns	ns	ns	ns	ns	(+) p<0.03	ns	(+) p<0.03	(-) p<0.001	ns
DNA adducts	52	0.47 p=0.0001	(+) p<0.001	(+) p<0.002	ns	ns	ns	ns	ns	(+) p<0.009	ns	ns	ns	ns	ns
Hepatic AHH	342	0.28 p=0.0001	ns	ns	ns	ns	ns	(-) p<0.001	(-) p<0.001	ns	(-) p<0.01	(-) p<0.001	ns	(+) p<0.001	ns
Brain PCBs	164	0.04 p=0.0039	(+) p<0.004	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Liver PCBs	84	0.50 p=0.0001	ns	ns	ns	ns	(-) p<0.004	ns	(+) p<0.001	(+) p<0.001	ns	ns	ns	ns	(-) p<0.001
Ovarian PCBs	105	0.44 p=0.0001	(+) p<0.001	(+) p<0.001	ns	(+) p<0.01	ns	ns	(+) p<0.001	(+) p<0.001	(+) p<0.001	ns	ns	ns	(+) p<0.001

Table 7. Spearman rank correlations between lesion prevalences and mean concentrations of selected contaminants in tissues and bile of winter flounder sampled from sites along the northeast Atlantic coast of the United States. FACs = fluorescent aromatic hydrocarbons; PCBs = polychlorinated biphenyls; AHH = aryl hydrocarbon hydroxylase.

Lesion category	Biliary FACs	Ovarian PCBs	Liver PCBs	Brain PCBs	DNA adducts	AHH activity
Neoplasms	ns[a] (n=21)	ns (n=15)	ns (n=15)	ns (n=21)	ns (n=9)	ns (n=21)
Foci of cellular alteration	ns (n=21)	ns (n=15)	ns (n=15)	ns (n=21)	ns (n=9)	ns (n=21)
Hydropic vacuolation	0.497 p=0.0264 (n=21)	ns (n=15)	0.870 p=0.0011 (n=15)	ns (n=21)	ns (n=9)	ns (n=21)
Proliferative lesions	0.565 p=0.0115 (n=21)	0.647 p=.0155 (n=15)	0.660 p=0.0136 (n=15)	ns (n=21)	0.717 p=0.0426 (n=9)	ns (n=21)
Necrotic lesions	ns (n=21)	ns (n=15)	ns (n=15)	ns (n=21)	ns (n=9)	ns (n=21)

[a]not significant ($p \leq 0.05$).

Table 8. Relationships between gonadosomatic index (GSI), plasma estradiol concentration, fecundity, and egg weight and selected biological variables (age, length, condition factor, geographical area collected (north of Cape Cod vs. south of Cape Cod), season collected (October vs. December), and ovarian developmental stage (vitellogenic vs. nonvitellogenic)), and indicators of contaminant exposure (i.e., polychlorinated biphenyl (PCB) concentrations in brain, ovary, and liver, levels of fluorescent aromatic compounds (FACs) in bile, levels of xenobiotic-DNA adducts in liver, and the presence of suspected toxicopathic liver lesions) in winter flounder from the northeast Atlantic coast. Data were analyzed using stepwise multiple regression, with $p \leq 0.05$ as significance level for entry of variables into the model. [a,b]

				Independent variables (significant at $p \leq 0.05$)				
Dependent variable	df	F-Test	r^2	Biliary FACs	Length	N. of Cape Cod	Sampled December	Vitellogenic
Plasma estradiol	551	p=0.0001	0.70	ns[c]	(+) p=0.0001	(-) p=0.0001	ns	(+) p=0.0001
GSI	567	p=0.0001	0.83	ns	(+) p=0.0001	(-) p=0.0001	(+) p=0.0001	(+) p=0.0001
Fecundity	53	p=0.0001	0.47	ns	(+) p=0.0001	ns	nd[d]	nd
Egg weight	43	p=0.05	0.08	(-) p=0.05	ns	ns	nd	nd

[a]Only those variable that showed significant relationships with one or more reproductive parameters are included in the table. Relationships between PCB concentrations in brain, liver, and ovary, xenobiotic DNA-adducts in liver, condition factor, age and liver lesions were not statistically significant.

[b]Suspected toxicopathic liver lesion include one or more of the following: neoplasms, foci of cellular alteration, hydropic vacuolation, or proliferative liver lesions including bile duct hyperplasia, hepatocellular regeneration, and chlolangiofibrosis.

[c]not statistically signficant, multiple regression, $p \leq 0.05$.

[d]not determined. All fish in which egg weight and fecundity were measured were collected in December and were vitellogenic.

Table 9. Relationships between indicators of contaminant exposure (biliary fluorescent aromatic compounds (FACs); concentrations of polychlorinated biphenyls (PCBs) in brain, liver and ovary; and xenobiotic-DNA adducts in liver), gonadosomatic index (GSI), and condition factor and selected categories of ovarian lesions in vitellogenic and non-vitellogenic female winter flounder. Data were analyzed using logistic regression, with $p \leq 0.05$ as the significance level.

	Vitellogenic fish				Nonvitellogenic fish		
Chemical class	n	Atresia of yolked oocytes	Atresia of nonyolked oocytes	Inflammatory lesions	n	Atresia of nonyolked oocytes	Inflammatory lesions
Biliary FACs	187	ns	ns	ns	69	(+) p=0.042 4%	ns
Brain PCBs	127	ns	ns	ns	30	ns	ns
Liver PCBs	70	(+) p=0.026 6%	ns	ns	12	ns	ns
Ovary PCBs	93	ns	ns	ns	8	ns	ns
DNA adducts	43	ns	ns	ns	8	ns	ns
GSI	466	(-) p=0.016 1%	(-) p=0.001 8%	(-) p=0.011 2%	98	ns	ns
Condition factor	466	ns	ns	ns	98	ns	ns

FIGURES

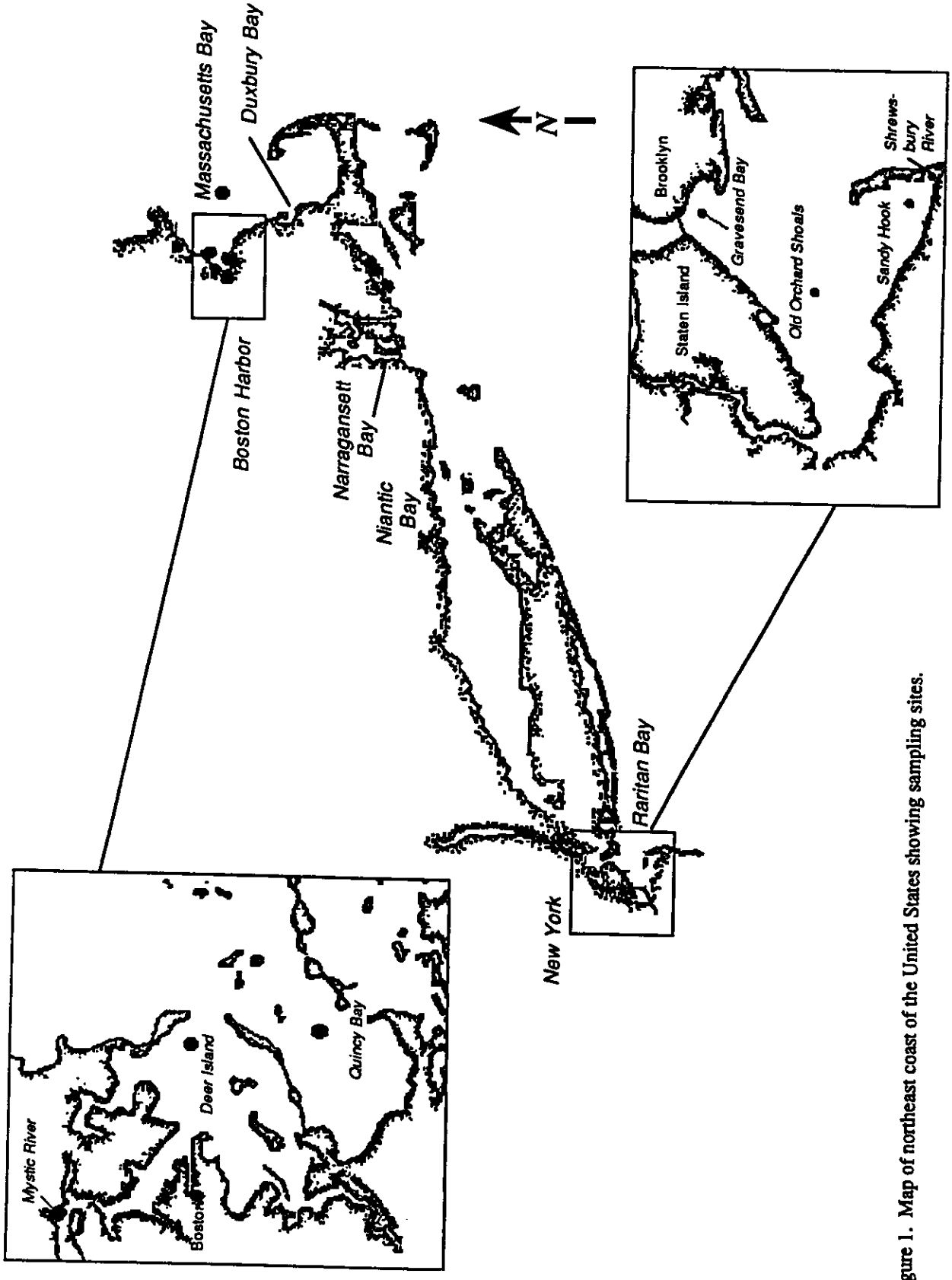

Figure 1. Map of northeast coast of the United States showing sampling sites.

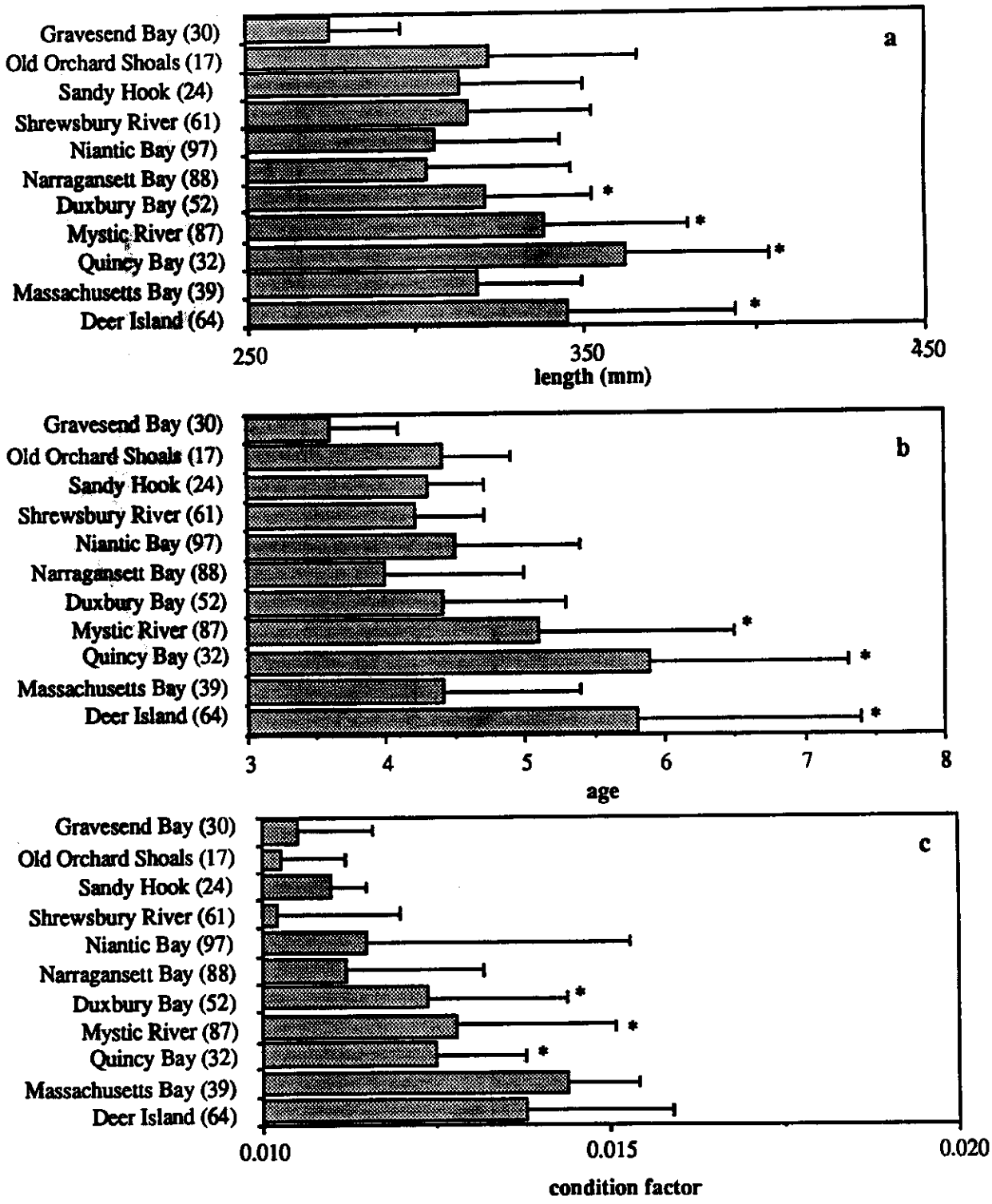

Figure 2. Mean values (± SD) of (a) fish length (mm), (b) fish age, (c) and condition factor in female winter flounder from sampling sites along the northeast coast of the United States. Numbers of animals collected per site are in parentheses. Asterisk (*) indicates value is significantly higher (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) then value at the Niantic Bay reference site.

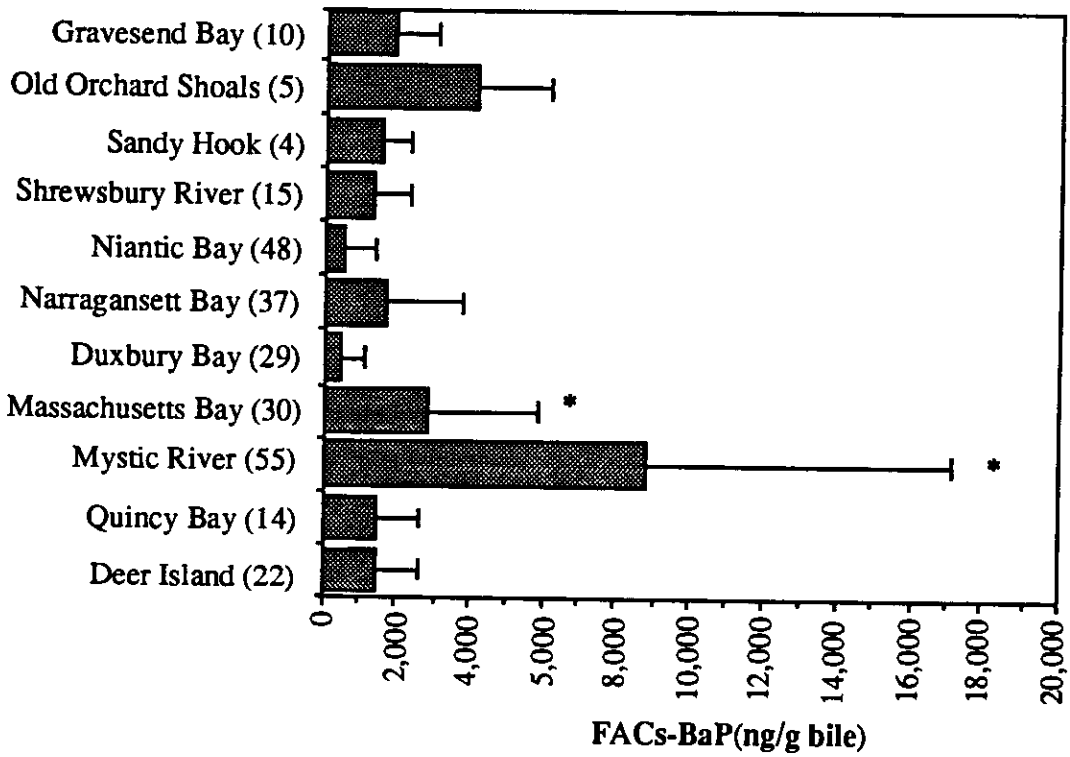

Figure 3. Mean biliary FAC levels (ng/g bile) in winter flounder at sampling sites along the northeast coast of the United States. Number of animals captured per site are in parentheses. Asterisk (*) indicates value is significantly higher (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) than value at the Niantic Bay reference site.

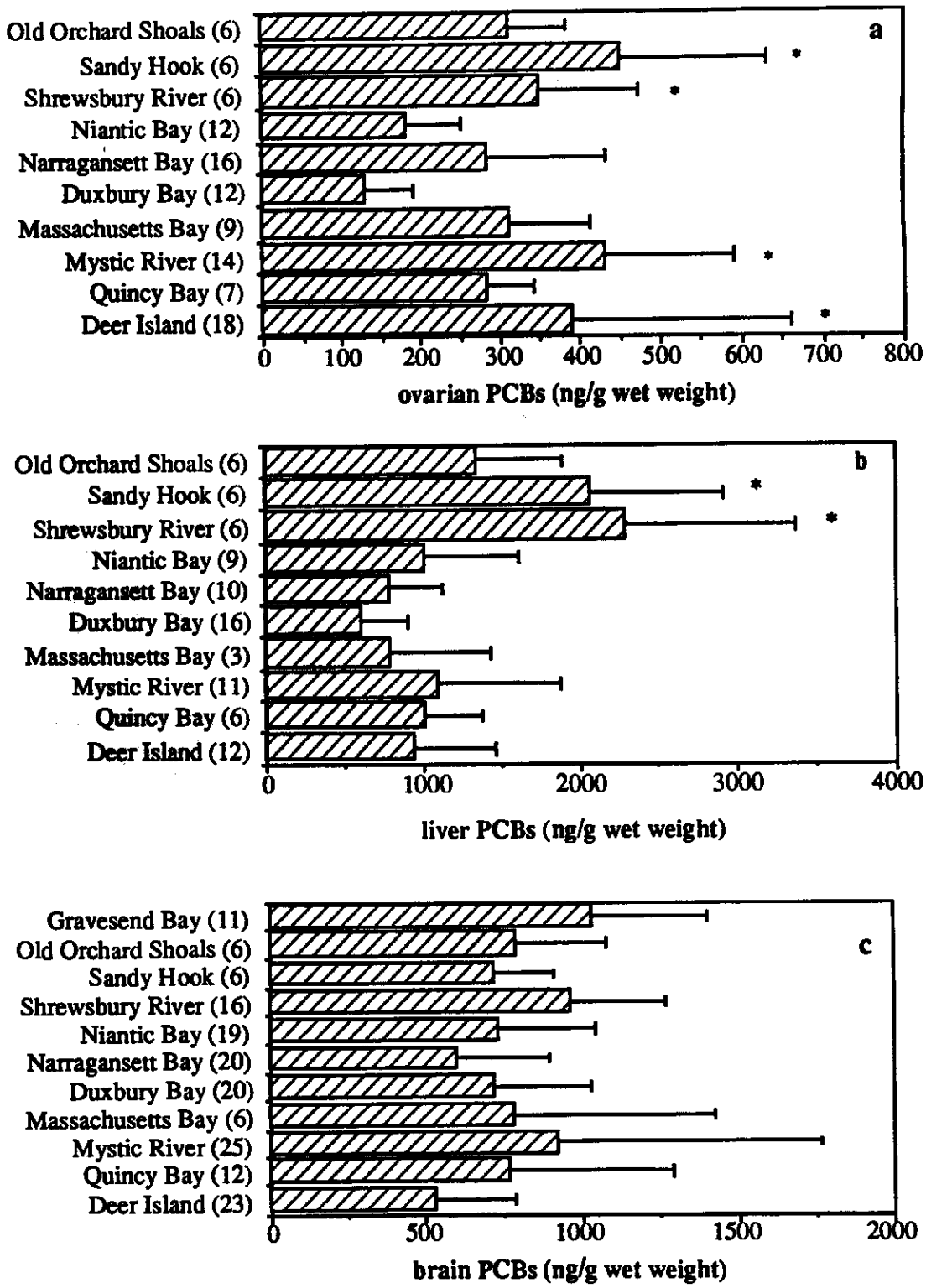

Figure 4. Mean concentrations (ng/g wet weight) of PCBs in (a) ovary, (b) liver, and (c) brain of winter flounder from sampling sites along the northeast coast of the United States. Number of animals sampled per site are in parentheses. Asterisk (*) indicates value is significantly higher (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) than value at the Niantic Bay reference site.

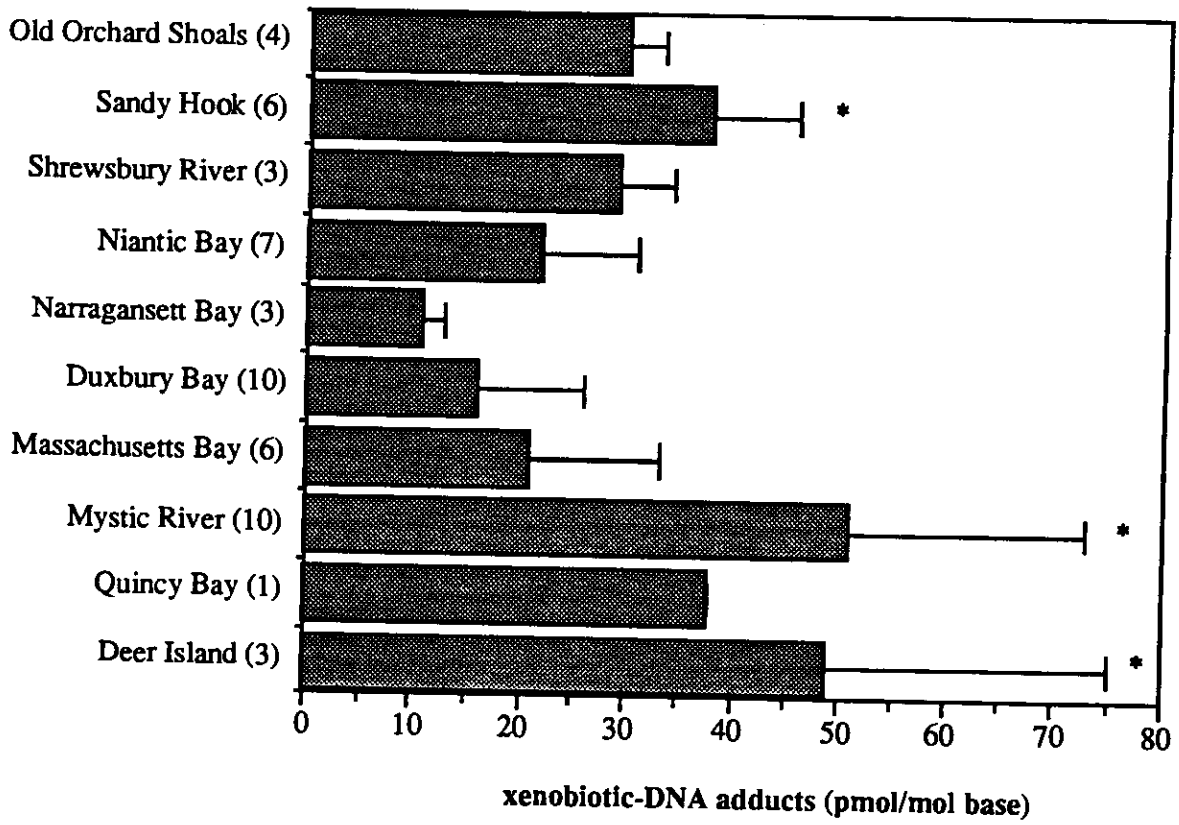

Figure 5. Mean level (pmol/mol base) of xenobiotic-DNA adducts in liver of winter flounder from sampling sites along the northeast coast of the United States. Numbers of animals sampled per site are in parentheses. Asterisk (*) indicates value is significantly higher (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) than value at Niantic Bay reference site.

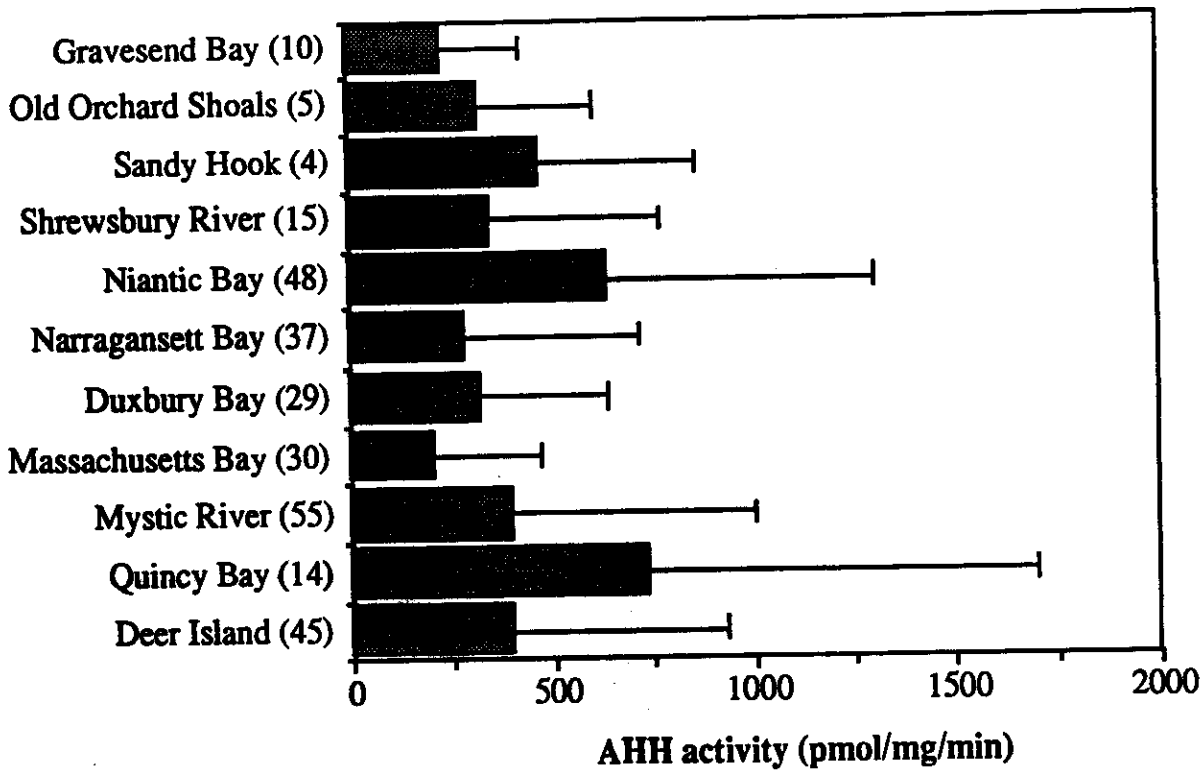

Figure 6. Mean hepatic AHH activity (pmol/mg/min) in winter flounder at sampling sites along the northeast coast of the United States. Numbers of animals sampled per site are in parentheses. None of the sampling sites showed significantly higher AHH activity (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) than the Niantic Bay reference site.

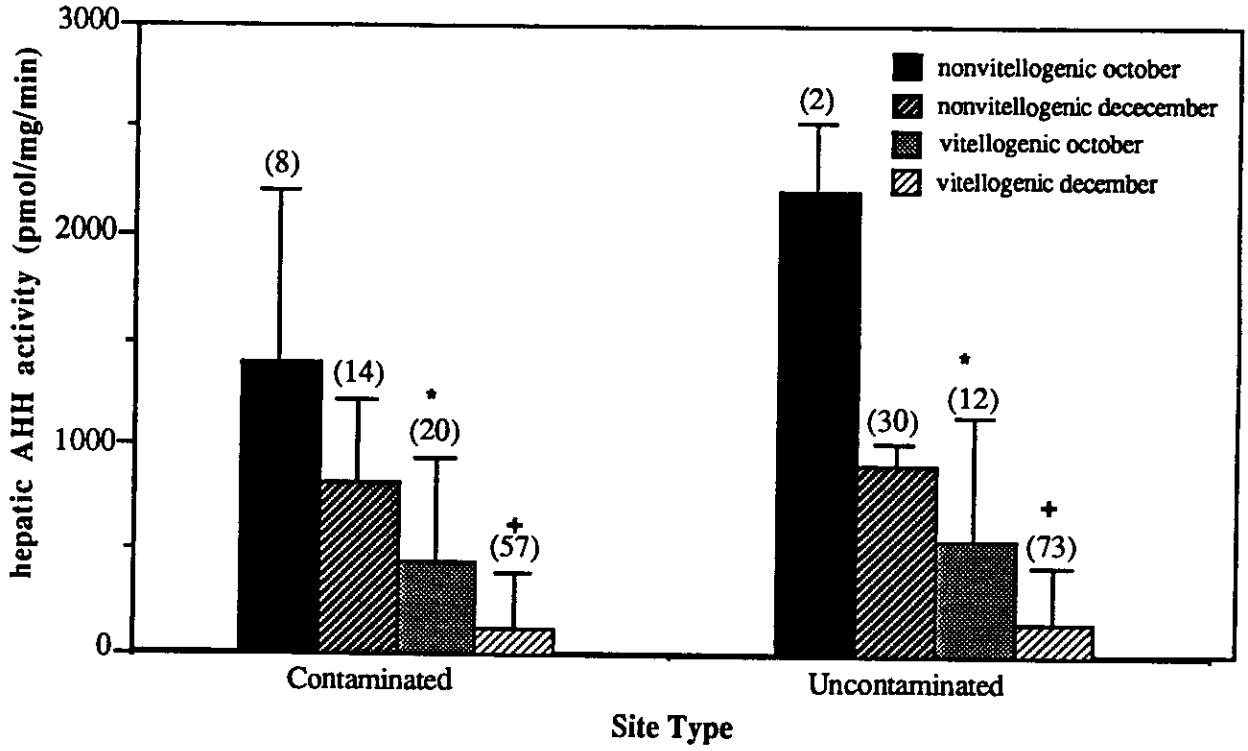

Figure 7. Mean hepatic AHH activity (pmol/mg/min) in vitellogenic and nonvitellogenic winter flounder collected in October and December from contaminated and relatively uncontaminated embayments along the northeast Atlantic Coast of the United States. Contaminated sites include all sampling sites within Boston Harbor and Raritan Bay. Uncontaminated sites include Duxbury Bay, Niantic Bay, Narragansett Bay, and Massachusetts Bay. Numbers of animals sampled per group are in parentheses. For both groups of sites, AHH activity is significantly lower (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) in vitellogenic flounder than in non-vitellogenic flounder, as indicated by (*) for October samples and (+) for December samples. However, no significant differences were found between contaminated and uncontaminated sites.

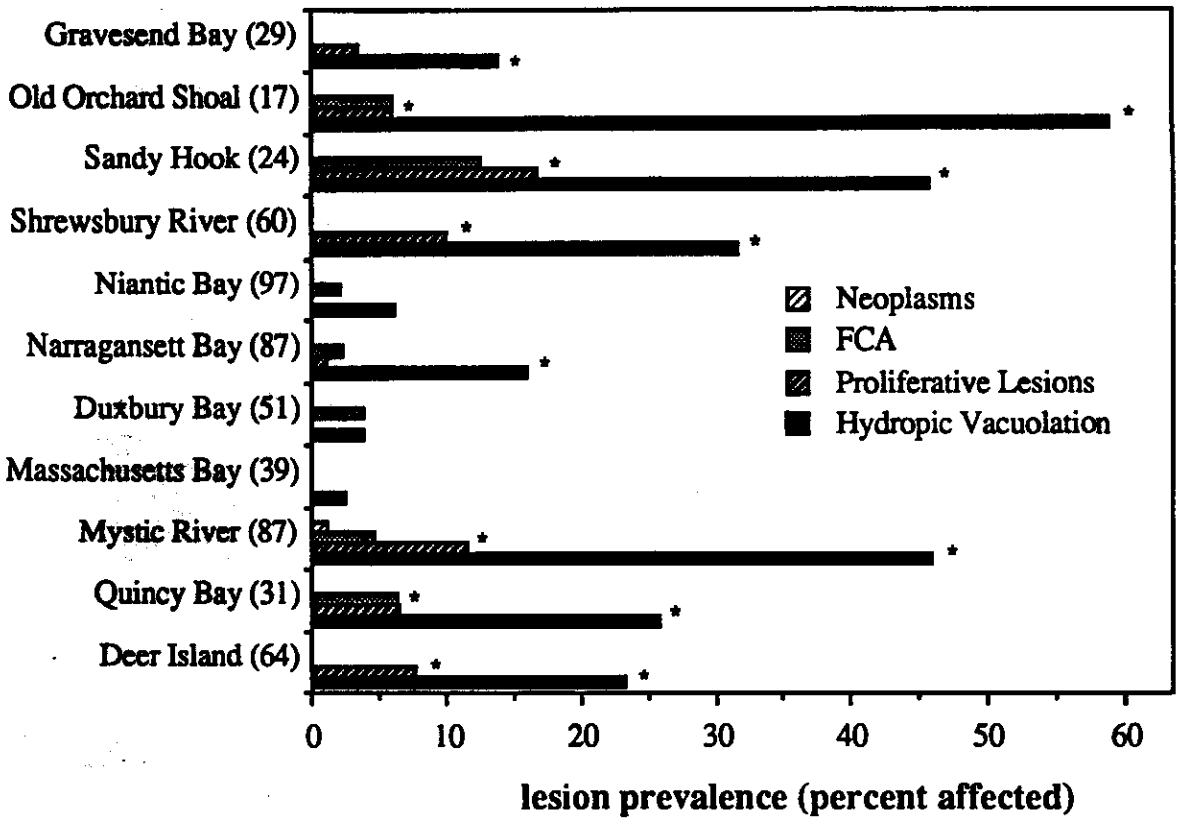

Figure 8. Prevalences of selected toxicopathic liver lesions in winter flounder from sampling sites along the northeast Atlantic coast of the United States. FCA = foci of cellular alteration. Numbers of animals sampled per site are in parentheses. Asterisk (*) indicates that the prevalence is significantly higher (G-statistic, $p \leq 0.05$) than prevalence at Niantic Bay reference site.

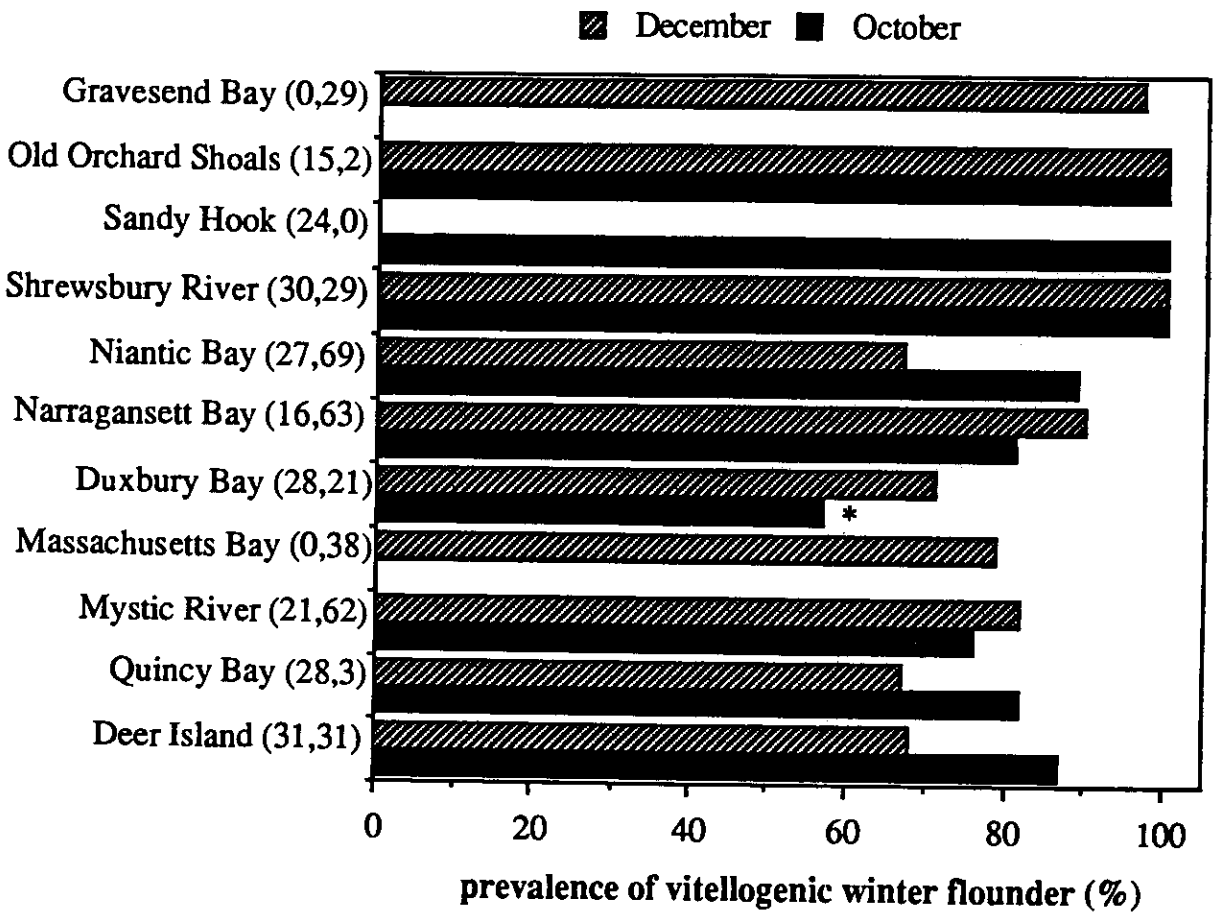

Figure 9. Prevalences of vitellogenic female winter flounder from sampling sites along the northeast coast of the United States. Numbers of animals sampled per site in October and December, respectively, are in parentheses. Asterisk (*) indicates that the prevalence is significantly lower (G-statistic, $p \leq 0.05$) than prevalence at Niantic Bay reference site.

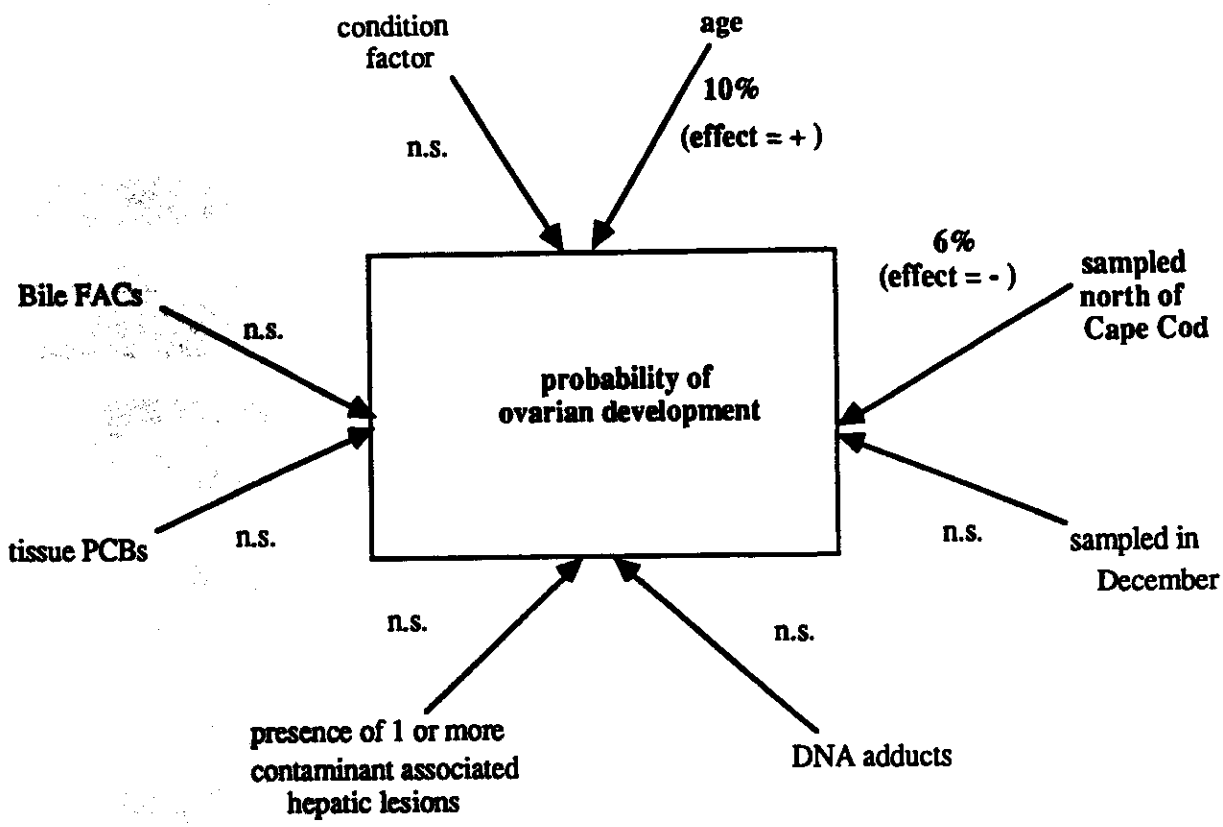

Figure 10. Logistic regression model relating indicators of contaminant exposure to the probability of ovarian development in winter flounder, while adjusting for the influence of fish age, condition factor, and geographical variation. Age and area captured (i.e. north of Cape Cod vs south of Cape Cod) account for 16% of the observed variability in ovarian development; none of the indicators of contaminant exposure had a significant impact on the probability of ovarian develoment. % = % of variation explained; n.s. = effect not significant at $p \leq 0.05$; asterisk = effect significant at $p < 0.05$).

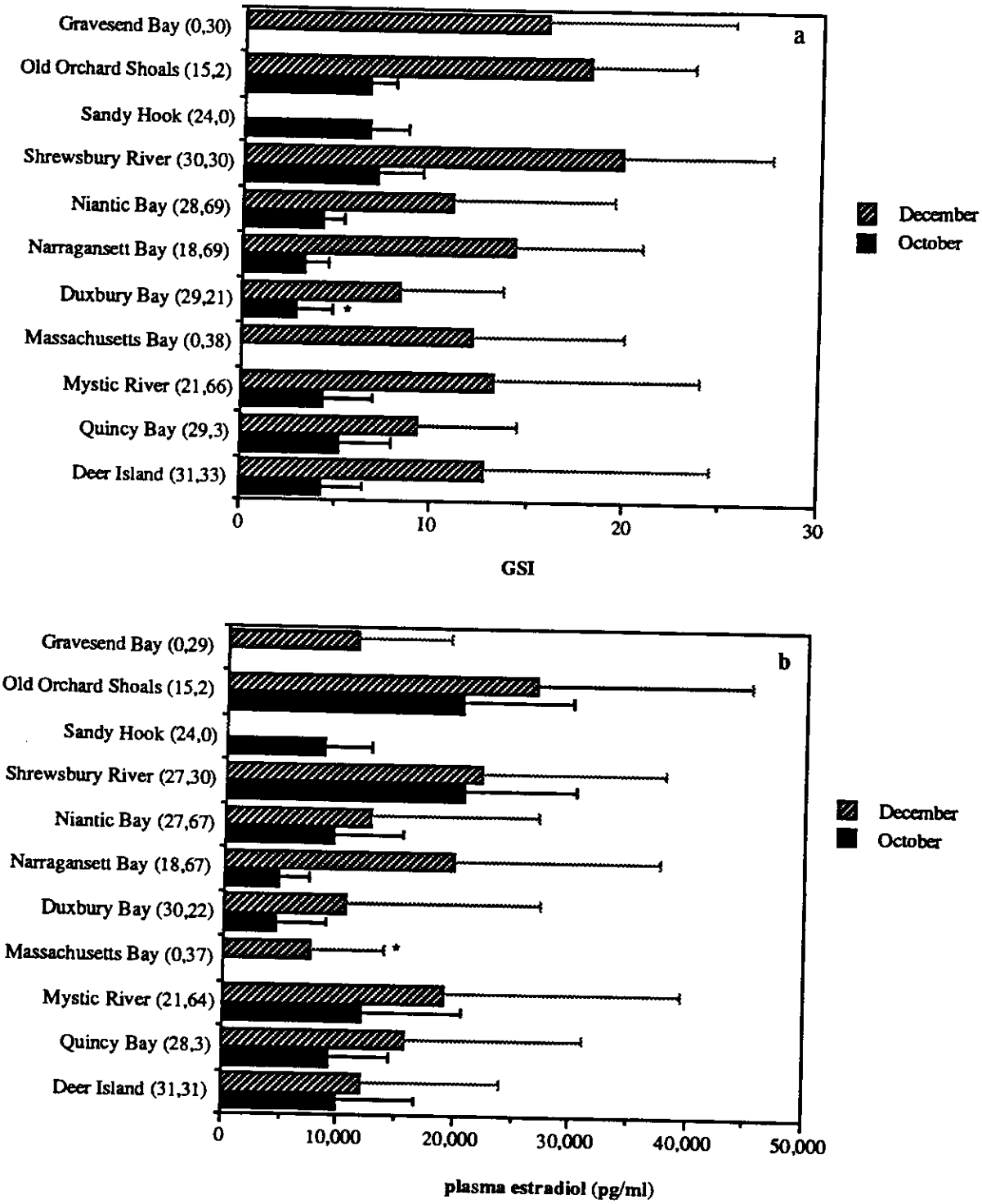

Figure 11. (a) Mean gonadosomatic index and (b) mean plasma estradiol concentrations (pg/ml) in female winter flounder sampled in October and December from sampling sites along the northeast Atlantic coast of the United States. Numbers of animals sampled per site in October and December, respectively, are given in parentheses. Asterisk (*) indicates value is significantly lower (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) than Niantic Bay reference site.

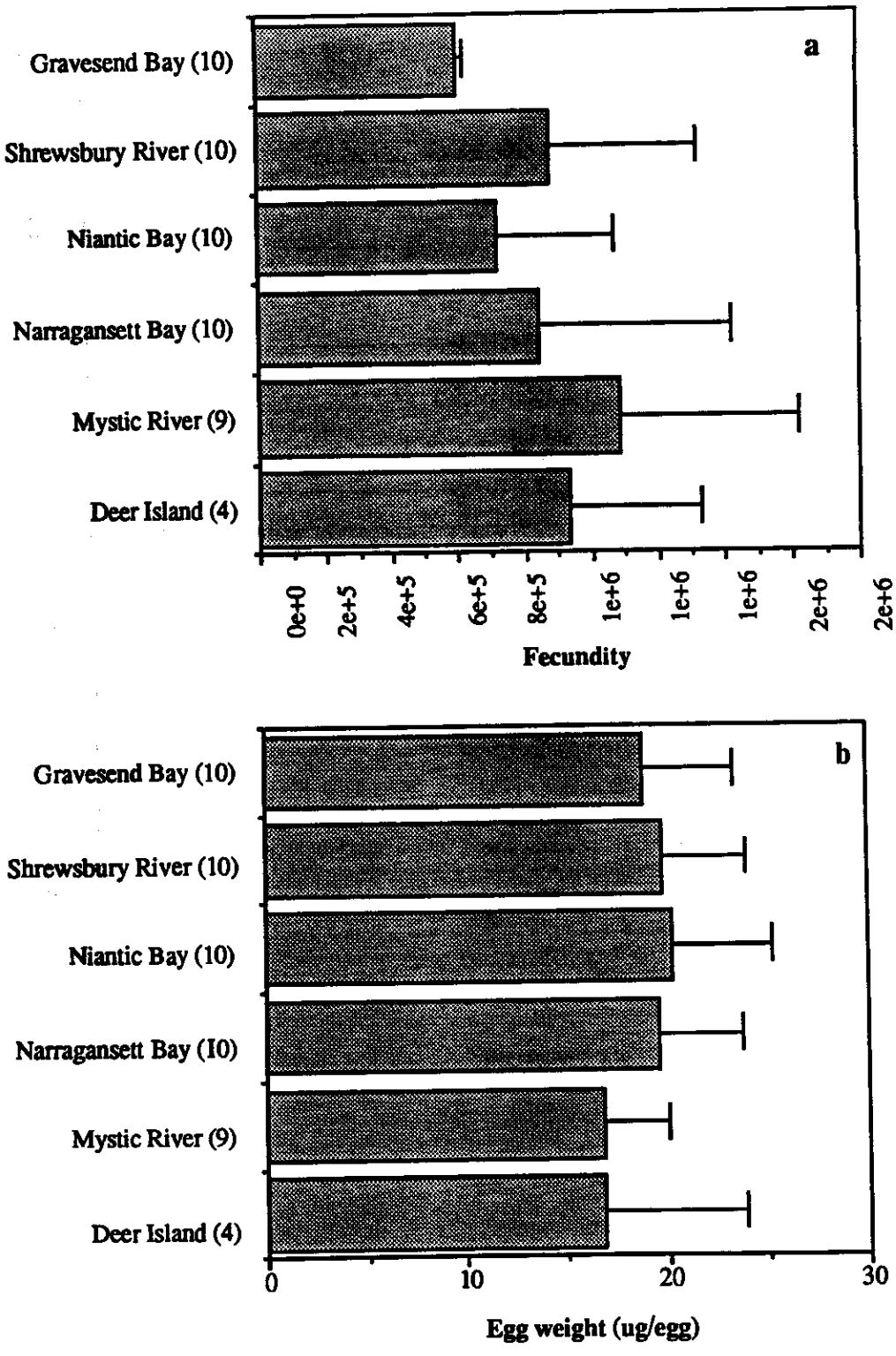

Figure 12. (a) Mean fecundity and (b) egg weight (ug/g) in female winter flounder from sampling sites along the northeast coast of the United States. Numbers of animals sampled per site are in parentheses. No significant intersite differences (ANOVA, Fisher's LSD multiple range test, $p \leq 0.05$) in fecundity or egg weight were observed.

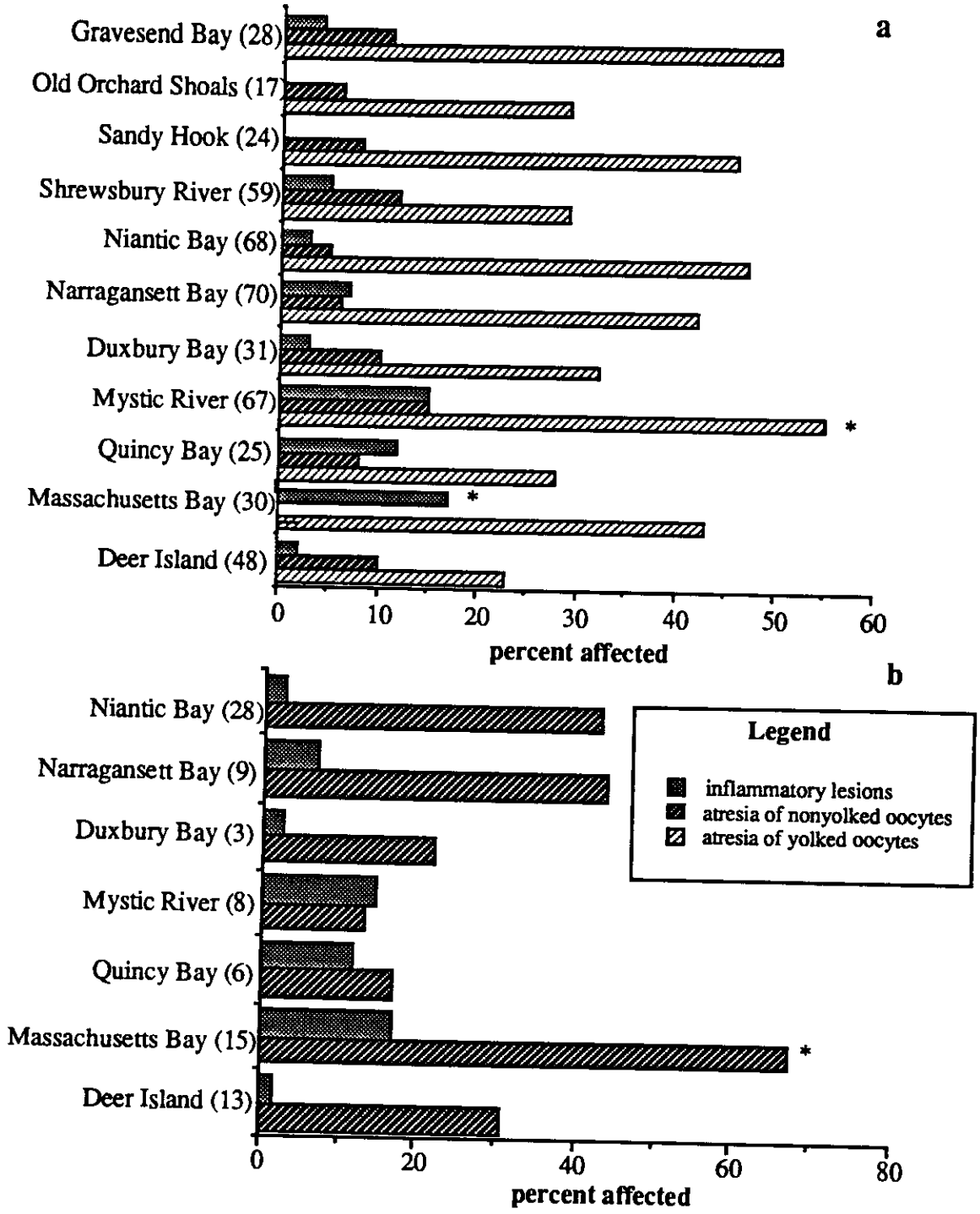

Figure 13. Prevalence of ovarian lesions at northeast coast sampling site in (a) vitellogenic and (b) nonvitellogenic winter flounder. Numbers of animals sampled per site are in parentheses. Prevalences designated by asterisk (*) are significantly different from overall lesion prevalence (in vitellogenic fish, 50% for atresia of yolked oocytes, 7% for atresia of nonyolked oocytes, and 7% for inflammatory lesions; in vitellogenic fish, 37% for atresia of nonyolked oocytes and 5% for inflammatory lesions (G-statistic, $p \leq 0.05$).

APPENDIX

Table A-1. Mean fish length (mm), age, and condition (± SD) in female winter flounder from Boston Harbor, Raritan Bay, and adjacent embayments. Numbers of animals sampled per site are in parentheses. Values in bold type are significantly different ($p \leq 0.05$, ANOVA and Fisher's LSD multiple range test) from the Niantic Bay reference site (in italics).

Site	Length (mm)	Condition factor	Age
December 1988			
Deer Island	**345 ± 58 (26)**	**0.0150 ± 0.0013 (26)**	**5.3 ± 1.8 (26)**
Mystic River	**343 ± 38 (30)**	**0.0149 ± 0.0013 (30)**	**5.2 ± 1.3 (30)**
Massachusetts Bay	318 ± 31 (39)	**0.0144 ± 0.0001 (39)**	4.4 ± 1.0 (39)
Duxbury Bay	325 ± 28 (22)	**0.0140 ± 0.0010 (22)**	4.4 ± 0.9 (22)
Narragansett Bay	302 ± 48 (36)	0.0124 ± 0.0018 (36)	4.0 ± 1.1 (36)
Niantic Bay (ref)	*312 ± 35 (43)*	*0.0123 ± 0.0018 (43)*	*4.6 ± 0.9 (43)*
	p=0.0001	p=0.0001	p=0.0001
October 1989			
Deer Island	**347 ± 38 (31)**	**0.0132 ± 0.0023 (31)**	**5.4 ± 1.2 (31)**
Quincy Bay	**363 ± 42 (29)**	**0.0125 ± 0.0012 (29)**	**5.9 ± 1.4 (29)**
Mystic River	326 ± 39 (21)	**0.0121 ± 0.0012 (21)**	4.6 ± 1.3 (21)
Duxbury Bay	318 ± 33 (30)	**0.0113 ± 0.0018 (30)**	4.3 ± 0.9 (30)
Narragansett Bay	313 ± 35 (28)	0.0101 ± 0.0007 (18)	4.2 ± 0.8 (18)
Shrewsbury	317 ± 35 (32)	0.0107 ± 0.0008 (30)	4.3 ± 0.5 (30)
Sandy Hook	313 ± 37 (24)	0.0110 ± 0.0005 (24)	4.3 ± 0.4 (24)
Old Orchard Shoals	321 ± 46 (15)	0.0103 ± 0.0009 (15)	4.3 ± 0.5 (15)
Niantic Bay (ref)	*305 ± 27 (28)*	*0.0104 ± 0.0008 (28)*	*4.4 ± 0.7 (28)*
	p=0.0001	p=0.0001	p=0.0001
December 1989			
Deer Island	**331 ± 60 (7)**	0.0124 ± 0.0011 (7)	4.6 ± 1.9 (7)
Mystic River	**342 ± 46 (36)**	0.0115 ± 0.0021 (36)	**5.2 ± 1.5 (36)**
Narragansett Bay	298 ± 41 (34)	0.0106 ± 0.0020 (34)	3.9 ± 0.9 (24)
Shrewsbury	313 ± 38 (31)	0.0098 ± 0.0023 (31)	4.2 ± 0.5 (31)
Gravesend	**275 ± 21 (30)**	0.010 5± 0.0011 (30)	**3.6 ± 0.5 (30)**
Old Orchard Shoals	326 ± 37 (2)	0.0107 (2)	4.5 ± 0.7 (2)
Niantic Bay (ref)	*297 ± 47 (26)*	*0.0114 ± 0.0067 (26)*	*4.4 ± 1.2 (26)*
	p=0.0001	p=0.2699	p=0.0001

Table A-2. Indicators of contaminant exposure (± standard deviation) in female winter flounder from Boston Harbor, Raritan Bay, and adjacent embayments. Mean values in bold type are significantly different ($p \leq 0.05$, ANOVA and Fisher's LSD multiple range test) from Niantic reference site (in italics). AHH = aryl hydrocarbon hydroxylase, FACs = fluorescent aromatic compounds; PCBs = polychlorinated biphenyls, nd = not determined.

Site	Hepatic AHH activity (pmol/mg/min)	DNA adducts (pmol/g)	Biliary FACs (ng/g)	Brain PCBS (ng/g wet wt)	Ovarian PCBs (ng/g wet wt)	Liver PCBs (ng/g wet wt)
December 1988						
Deer Island	210 ± 70 (n=26)	49 ± 15 (n=3)	2080 ± 580 (n=8)	680 ± 150 (n=6)	520 ± 80 (n=11)	356 ± 94 (n=3)
Mystic River	60 ± 20 (n=29)	51 ± 7 (n=10)	14200 ± 2000 (n=24)	1480 ± 650 (n=6)	490 ± 60 (n=8)	770 ± 256 (n=5)
Massachusetts Bay	210 ± 40 (n=39)	21 ± 5 (n=6)	2900 ± 540 (n=30)	1030 ± =240 (n=6)	310 ± 30 (n=9)	773 ± 376 (n=3)
Duxbury Bay	330 ± 70 (n=22)	16 ± 3 (n=10)	480 ± 180 (n=13)	670 ± 200 (n=3)	160 ± 30 (n=6)	465 ± 42 (n=3)
Narragansett Bay	140 ± 40 (n=36)	11 ± 1 (n=3)	1580 ± 480 (n=22)	500 ± 100 (n=3)	360 ± 40 (n=10)	412 ± 80 (n=3)
Niantic Bay (ref)	*460 ± 70 (n=42)*	*22 ± 3 (n=7)*	*680 ± 200 (n=28)*	*530 ± 100 (n=3)*	*220 ± 20 (n=7)*	*313 ± 39 (n=2)*
	p=0.0001	p=0.0001	p=0.0001	p=0.3372	p=0.0004	p=0.5711
October 1989						
Deer Island	730 ± 190 (n=13)	nd	1100 ± 150 (n=10)	480 ± 50 (n=13)	180 ± 20 (n=7)	1149 ± 138 (n=9)
Quincy Bay	960 ± 340 (n=9)	nd	1400 ± 270 (n=11)	630 ± = 70 (n=11)	290 ± 30 (n=6)	1018 ± 143 (n=6)
Mystic River	590 ± 180 (n=9)	nd	5000 ± 780 (n=10)	1500 ± 570 (n=10)	350 ± 40 (n=6)	1456 ± 339 (n=6)
Duxbury Bay	290 ± 90 (n=6	nd	560 ± 130 (n=16)	730 ± 80 (n=17)	100 ± 10 (n=6)	639 ± 83 (n=13)
Narragansett Bay	740 ± 180 (n=7)	nd	1700± 450 (n=7)	820 ± 110 (n=7)	140± 20 (n=6)	956± 95 (n=7)
Shrewsbury	750 ± 170 (n=6)	29 + 3 (n=3)	2300 + 340 (n=6)	750 ± 90 (n=6)	350 ± 50 (n=6)	2336 ± 435 (n=6)
Sandy Hook Channel	466 ± 159 (n=6)	38 + 3 (n=6)	1600 + 370 (n=4)	720 ± 80 (n=6)	440 ± 70 (n=6)	2080 ± 344 (n=6)
Old Orchard Shoals	320 ± 118 (n=6)	30 + 1 (n=4)	4100 + 900 (n=5)	790 ± 120 (n=6)	310 ± 30 (n=6)	1337 ± 226 (n=6)
Niantic Bay (ref)	*1100 ± 280 (n=8)*	*nd*	*660 ± 180 (n=6)*	*510 ± 90 (n=6)*	*110 ± 10 (n=5)*	*1228 ± 185 (n=7)*
	p=0.2726	p=0.1084	p=0.0001	p=0.0918	p=0.0001	p=0.0001
December 1989						
Deer Island	520 ± 180 (n=6)	nd	1070 + 310 (n=4)	480 ± 40 (n=4)	nd	nd
Mystic River	780 ± 170 (n=21)	nd	4600 + 660 (n=21)	590 ± 40 (n=9)	nd	nd
Narragansett Bay	470 ± 170 (n=13)	nd	1800 + 880 (n=8)	490 ± 90 (n=10)	nd	nd
Shrewsbury	100 ± 50 (n=6)	nd	630 + 120 (n=9)	1100 ± 90 (n=10)	nd	nd
Gravesend	230 ± 60 (n=11)	nd	1900 + 360 (n=10)	1000 ± 110 (n=10)	nd	nd
Niantic Bay (ref)	*910 ± 230 (n=15)*	*nd*	*230 + 30 (n=14)*	*930 ± 90 (n=10)*	*nd*	*nd*
	p=0.017		p=0.0001	p=0.0001		

Table A-3. Liver pathology in female winter flounder from Boston Harbor, Raritan Bay, and adjacent embayments. Values in bold type are significantly different ($p \leq 0.05$, G-statistic) from Niantic reference site (in italics).

Site	n	Neoplasms	Foci of cellular alteration	Hydropic vacuolation	Proliferative lesions
December 1988					
Deer Island	26	0	0	**12**	**8**
Mystic River	39	**3**	**3**	**20**	**13**
Massachusetts Bay	30	0	0	3	0
Duxbury Bay	22	0	0	0	0
Narragansett Bay	36	0	0	**11**	0
Niantic Bay (ref)	*43*	*0*	*0*	*0*	*0*
October 1989					
Deer Island	31	0	0	**29**	6
Quincy Bay	28	0	4	**25**	7
Mystic River	21	0	0	**52**	**10**
Duxbury Bay	29	0	7	7	0
Narragansett Bay	18	0	6	17	6
Shrewsbury	30	0	0	**43**	**20**
Sandy Hook	24	0	**13**	**46**	**17**
Old Orchard Shoals	15	0	7	**67**	7
Niantic Bay (ref)	*28*	*0*	*7*	*18*	*0*
December 1989					
Deer Island	7	0	0	**43**	**14**
Mystic River	36	0	**8**	**64**	**11**
Narragansett Bay	33	0	**3**	**21**	0
Shrewsbury	30	0	0	**20**	0
Old Orchard Shoals	2	0	0	0	0
Gravesend Bay	29	0	0	14	3
Niantic Bay (ref)	*26*	*0*	*0*	*4*	*0*

Table A-4. Indicators of reproductive activity (± standard deviation) in female winter flounder from Boston Harbor, Raritan Bay, and adjacent embayments. Values in bold type are significantly different ($p \leq 0.05$) from those at the Niantic Bay reference site (in italics). Intersite differences in GSI, estradiol, fecundity, and egg weight were evaluated using ANOVA and Fisher's LSD multiple range test. Intersite differences in % vitellognenic females and % vitellogenic females with atresia were evaluated using the G-statistic. Numbers of animals sampled per site are in parentheses. nd = not determined

Site	Gonadosomatic index	%Vitellogenic females	% Females with atresia	Estradiol (pg/ml)	Fecundity (egg number)	Egg weight (ug/egg)
December 1988						
Deer Island	13.8 ± 2.4 (26)	75 (26)	12 (17)	10700 ± 9200 (26)	nd	nd
Mystic River	15.1 ± 1.2 (30)	100 (30)	23 (26)	19000 ± 18000 (30)	nd	nd
Massachusetts Bay	9.4 ± 0.9 (39)	79 (39)	33 (30)	7600 ± 6500 (39)	nd	nd
Duxbury Bay	8.3 ± 1.1 (22)	71 (22)	13 (15)	10500 ± 16900(22)	nd	nd
Narragansett Bay	14.4 ± 1.1 (36)	91 (36)	26 (31)	21000 ± 21000 (36)	nd	nd
Niantic Bay (ref)	*10.7 ± 1.4 (43)*	*56 (43)*	*13 (24)*	*13300 ± 16800 (43)*	*nd*	*nd*
	p=0.0046	p=0.0006	p=0.1148	p=0.0044		
October 1989						
Deer Island	4.3 ± 2.2 (31)	87 (31)	15 (27)	10000 ± 6800 (31)	nd	nd
Quincy Bay	5.2 ± 2.8 (29)	82 (28)	17 (23)	9300 ± 5100 (28)	nd	nd
Mystic River	4.3 ± 2.6 (21)	76 (21)	57 (16)	12100 ± 8500 (21)	nd	nd
Duxbury Bay	2.8 ± 2.0 (29)	57 (28)	38 (16)	4500 ± 4400 (30)	nd	nd
Narragansett Bay	3.4 ± 1.1 (18)	81 (17)	8 (13)	4700 ± 2700 (18)	nd	nd
Shrewsbury	7.0 ± 2.4 (30)	100 (30)	13 (30)	20500 ± 6800 (28)	nd	nd
Sandy Hook	6.6 ± 2.8 (24)	100 (24)	38 (24)	8500 ± 4100 (24)	nd	nd
Old Orchard Shoals	6.6 ± 2.8 (15)	100 (15)	20 (15)	28300 ± 9700(15)	nd	nd
Niantic Bay (ref)	*4.2 ± 1.2 (28)*	*89 (27)*	*38 (24)*	*9400 ± 6000 (27)*	*nd*	*nd*
	p=0.0001	p=0.0001	p=0.0301	p=0.0001		
December 1989						
Deer Island	8.9 ± 8.2 (7)	57 (7)	25 (4)	15900 ± 20400 (7)	464000 ± 100000	16.8 ± 3.6
Mystic River	9.7 ± 8.1 (36)	71 (34)	50 (24)	14700 ± 8500 (34)	545000 ± 87500	16.8 ± 1.1
Narragansett Bay	14.2 ± 6.4 (33)	86 (29)	16 (25)	18800 ± 12300 (30)	422000 ± 91000	19.6 ± 1.3
Shrewsbury	19.7 ± 7.9 (30)	100 (29)	10 (29)	22100 ± 15800 (30)	438000 ± 70000	19.8 ± 1.3
Gravesend	15.8 ± 4.7 (30)	97 (29)	38 (29)	11400± 8000 (29)	303000 ± 32000	18.9 ± 1.4
Old Orchard Shoals	18.0 ± 5.5 (2)	100 (2)	0 (2)	27000 ± 18400 (2)	nd	nd
Niantic Bay (ref)	*11.5 ± 6.9 (26)*	*77 (26)*	*10 (20)*	*11300 ± 10400 (26)*	*359000 ± 56000*	*20.2 ± 1.6*
	p=0.0001	p=0.0038	p=0.0167	p=0.05	p=0.2476	p=0.5373