NATIONAL STATUS & TRENDS PROGRAM

Benthic Surveillance Project

SUMMARY REPORT
1984-86

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

September 1988

TABLE OF CONTENTS

I. Introduction

See integrated Summary Report

II. Methods

See integrated Summary Report

III. Results and Discussion

A. Physical and Chemical Characteristics of Sediments

1. Grain size and TOC
2. Coprostanol and Clostridium
3. Polycyclic aromatic hydrocarbons (PAHs)
4. Chlorinated hydrocarbons (PCBs and pesticides)
5. Metals
6. Principal components analysis

B. Chemicals in Fish

1. Stomach contents
2. Bile
3. Liver

C. Fish Pathobiology

1. Histopathology
2. Gross pathology
3. Length, age and gender characteristics
4. Stomach contents taxonomy

D. Relationships between Chemical Parameters

E. Relationships between Chemical and Biological Parameters

IV. Conclusions

See integrated Summary Report

Prepared by: V. Zdanowicz, Sandy Hook
D. Gadbois, Gloucester
M. Newman, Oxford

We gratefully acknowledge:

M. Cox, Sandy Hook, graphics
S. Fromm, Sandy Hook, SAS programming
R. Pikanowski, Sandy Hook, statistics support
A. Draxler, Sandy Hook, review and suggestions
J. O'Reilly, Sandy Hook, review and suggestions
W. Phoel, Sandy Hook, review and suggestions
R. Reid, Sandy Hook, review and suggestions
R. Murchelano, Woods Hole, review and suggestions

III. Results and Discussion - Northeast Region

Given the voluminous body of data generated between 1984 and 1986, the most concise evaluation of sites can be made using mean values of variables for comparison, as is done below. Many new variables (e.g. fraction of pesticides in total chlorinated hydrocarbons) may be created that are of interest in making various comparisons. Therefore, while this report addresses all aspects of the study, it is not an exhaustive treatment of the data.

A. Physical and Chemical Characteristics of Sediments

Data presented below are expressed as mean values for each of the twenty sites sampled during this study (study mean). Interannual differences in concentrations of various analytes were observed at several sites. Although these differences were statistically significant they were small in magnitude and probably due in part to changes in sediment sampling procedures. Variation in sampling occurred at twelve sites: Machias Bay, Frenchmans Bay, Penobscot Bay, Casco Bay, Merrimack River, Salem Harbor, Boston Harbor, Buzzards Bay, eastern and western Long Island Sound, Raritan Bay and Delaware Bay (Table 1). Changes consisted of station relocation after 1984 (e.g. Casco Bay), or sampling different numbers of stations in different years (e.g. Buzzards Bay).

Additionally, because of the large number of variables for correlation analysis, only the largest of the sometimes numerous statistically significant correlations between the variables discussed are presented. The statistical significance level is $\alpha = 0.05$.

1. Percentage fines and TOC

Between 1984 and 1986, percentages of fines and TOC were determined in sediments from twenty sites in the Northeast (Figure 1). Percentage fines refers to the weight percentage of sediment with particle size equal to or smaller than 63 μm in diameter, also called the silt-clay fraction, while TOC represents the weight percentage of total organic carbon. Sites were chosen to incorporate a range of environmental conditions, from well scoured, sandy areas with little or no anthropogenic inputs (e.g. eastern Long Island Sound and the mouth of the Merrimack River), to constricted, depositional areas with relatively high volumes of anthropogenic inputs (e.g. Boston Harbor).

Although the range of silt-clay levels was wide (Figure 2a), sediments at most sites contained 60% silt-clay or more. Only two sites had less than 35% silt-clay (Merrimack River, eastern Long Island Sound). Generally, sites having lower levels of silt-clay were the reference sites. Annual mean silt-clay levels were not significantly different within any site except Buzzards Bay and Casco Bay, where higher levels were found after 1984. Changes in Buzzards Bay levels can be attributed to variations in numbers of stations sampled between 1984-85 and 1986, while changes in Casco Bay levels were due to station relocation after 1984. Silt-clay was significantly correlated ($N=48, R \geq .63$) with aluminum, iron, TOC, nickel and thallium.

Percentage TOC in sediment also varied widely (Figure 2b). The distribution resembled that of silt-clay, as would be expected, since, in this study, the two parameters are highly correlated. Lower TOC levels were found at the sandier sites, while higher levels were found at the depositional sites. Differences in annual mean TOC levels were not statistically significant except at Casco Bay. This difference, however, is probably due to station relocation after 1984. Percentage TOC was significantly correlated ($N \geq 28$, $R \geq .70$) with lead, zinc, cadmium, copper, nickel, hepta- and octa-chlorobiphenyl, fluoranthene, benzo[a]anthracene, benzo[e]pyrene, benzo[a]pyrene and coprostanol (for which $N=12$). Although coprostanol data are available only for one year, the high correlation between TOC and coprostanol suggests that the TOC, at least in those sites where these substances were present in excess, is mostly sewage derived. The term sewage, in this report, refers both to the sources of the materials reaching sewage treatment plants, including urban runoff and domestic and industrial wastes, and to the effluents from those plants.

2. Coprostanol and Clostridium

Coprostanol measurements are available from 1984 only. Clostridium data are not available for the period 1984-1986.

Mean coprostanol levels (Figure 3) varied by an order of magnitude, with highest levels found in urbanized areas, such as Boston and Salem Harbors and Raritan Bay, where sewage volumes are considerable. Lowest levels (less than 1,000 ppb) were found at sites adjacent to rural areas. Sources of coprostanol include agricultural runoff and sewage. In the Northeast region, at sites with concentrations below approximately 1,000 ppb, it is apparent that both sources are important in determining concentrations in sediment, whereas, in sites with higher levels, sewage sources dominate. Coprostanol was correlated with levels of octachlorobiphenyl, dibenzo[a,h]anthracene, lead, cadmium and zinc in sediment, and levels of cadmium, lead, mercury, fluoranthene and pyrene in fish stomach contents. Correlations between coprostanol and these sediment contaminants are not surprising since their common origin is primarily sewage. Correlations between coprostanol and contaminants in stomach contents suggest that the origin of these compounds was the sediment, where the coprostanol was found. This is consistent with the observation that some species of demersal fish ingest sediment as they feed (see section D).

3. Polycyclic Aromatic Hydrocarbons

Mean total PAH levels (Figure 4) also varied over a wide range, with the highest levels found in the more urbanized areas. Comparison of mean PAH levels between 1984 and 1985 (1986 data not available in time for this report) showed differences in mean levels of fluoranthene, benzo[a]anthracene and pyrene, although total PAH levels were not different. These differences also can be related to changes in sampling between 1984 and 1985. Total PAH levels were highly correlated with the tri-, tetra-, penta- and hexa-chlorobiphenyls and several individual PAH compounds.

4. Chlorinated Hydrocarbons - Pesticides and PCBs

Except for the relatively high concentration in Boston Harbor, total pesticide levels did not vary widely (Figure 5a). Highest values were found in the urbanized areas (Raritan Bay, Salem Harbor), while low or trace levels were found elsewhere. Mean levels of total pesticides were not significantly different between 1984 and 1985. Although individual pesticide compounds were correlated with each other and with several PAH compounds, total pesticide levels were not highly correlated with other variables.

The range of levels of total PCBs (Figure 5b) was wider than that of total pesticides, more closely resembling the range of total PAHs. Highest total PCB levels were also found at urbanized sites. Total PCBs, like total pesticides, were not highly correlated with other compounds, although individual congeners were correlated with each other and with several PAH compounds.

The distribution of total chlorinated compounds (sum of all pesticide and PCB compounds, Figure 5c) was similar to distributions of total pesticides and total PCBs above. The distribution of the fraction of pesticides in total chlorinated compounds (Figure 5d) shows that higher proportions of pesticides were found at the watershed (rural) sites than the urbanized areas.

5. Metals

Concentrations of the major sediment elements, aluminum, silicon and iron (Figures 6a-c) depend mostly on silt-clay content and were within expected ranges for marine sediments (Riley and Chester, 1971). Differences in annual mean levels of these metals were related to sampling changes between years. These metals, along with TOC and percentage fines, were significantly intercorrelated.

Natural abundances of the group of fourteen elements measured in this study (Figures 6d-q) vary by two or three orders of magnitude (Riley and Chester, 1971). Clean, fine estuarine sediment contains approximately 0.2-0.3 mg/kg cadmium (Figure 6d) and approximately 120 mg/kg zinc (Figure 6m). Order of magnitude increases beyond natural (background) levels of cadmium were found in urbanized areas, while increases in zinc were smaller. In Salem Harbor, an increase of two orders of magnitude in chromium was found (Figure 6n). In general, highest increases above natural levels for all the trace metals were found at sites adjacent to urbanized sites, those sites that also had high levels of the other pollutants. Statistically significant annual variations in mean sediment concentrations were observed for all the trace metals, but were due to sampling differences, as described earlier.

Individual trace metals were highly intercorrelated and several were highly correlated with other contaminant variables, such as coprostanol and a variety of PAHs, pesticides and PCBs.

6. Principal Components Analysis of Sediment

Initially, this analysis was performed using all the sediment variables (except coprostanol) at once. The organic variables were found to be components of the TOC-contaminant factor derived in the first analysis described below. After this preliminary analysis, sediment variables were divided into two groups in order to maximize the ratio of observations to variables and minimize the effects of missing groups of measurements in some years.

The first group was comprised of TOC, percentage fines and seventeen metals, and spanned the years 1984-1986. A plot of the first two principal components (hereafter called factors), which accounted for 65% of the variance, is shown in Figure 7a. Factor 1 (X axis) can be considered a contaminant factor, as it is composed primarily of TOC and the contaminant metals, cadmium, copper, nickel, lead, zinc, thallium, arsenic, mercury, selenium and tin. Factor 2 (Y axis) can be considered a silt-clay factor, since the most important variables in this factor are percentage fines, iron and aluminum. At sites within the ellipse, there is a linear relationship between levels of fine sediment and levels of contaminants - clean, sandy stations (from sites such as Delaware Bay, eastern Long Island Sound, etc.) appear in the lower left corner, while clean, fine-grained stations (Frenchmans, Penobscot and Casco Bays) appear in the upper right. Stations to the right of the ellipse are contaminated, these sites containing higher levels of TOC and contaminants than would be expected for the levels of fine sediment present. These stations are from the Baltimore Harbor and Elizabeth River sites, in addition to sites in Boston Harbor, Raritan Bay and Salem Harbor.

The second group of sediment variables was comprised of TOC, percentage fines and forty-one hydrocarbons (not including coprostanol) and spanned the years 1984-1985. A plot of the first two principal components, which accounted for 60% of the variance, is given in Figure 7b. Factor 1 (X axis) can be considered as a PAH-PCB-TOC factor, and factor 2 (Y axis) a pesticide factor. Silt-clay was not an important variable in this data set. The relationship between PAH-PCB-TOC and pesticides is fairly linear (ellipse), even at stations from sites containing high levels of these compounds (upper right). Stations from cleaner, sandy sites are shown in the lower left portion of the plot. As PAH-PCB-TOC and pesticide concentrations increase, station characteristics vary from sandy and slightly contaminated (e.g. eastern Long Island Sound), to fine and slightly contaminated (e.g. Narragansett Bay), to fine and moderately contaminated (e.g. Raritan Bay), to fine and highly contaminated (e.g. Boston Harbor). Stations outside the ellipse contain a higher ratio of PAH-PCB-TOC to pesticides than stations inside the ellipse. These stations are primarily from the Boston and Salem harbor sites.

This analysis was subsequently re-run using only 1984 hydrocarbon data, in order to incorporate coprostanol measurements. Results were essentially unchanged, and it was found that coprostanol was a component of factor 1.

B. Chemicals in Fish

In order to compare levels of chemicals in fish tissues between sites, it is necessary to collect the same species at every site. However, because of the large latitudinal range in the northeast region, a suitable monitoring species was not available whose habitat spanned the entire area and, consequently, six species were used (Table 1). Although this enables comparisons between species, comparisons between sites are possible only where a single species was collected. Additionally, changes in sampling after 1984 resulted in the collection of different species at some sites. Therefore, for comparing sites, data for the following species and sites are depicted: longhorn sculpin (Myoxocephalus octodecemspinosus) from Machias Bay to Casco Bay, winter flounder (Pseudopleuronectes americanus) from Merrimack River to Great Bay, windowpane flounder (Scophthalmus aquosus) in Delaware Bay, spot (Leiostomus xanthurus) in upper and lower Chesapeake Bay and Atlantic croaker (Micropogon undulatus) in the Elizabeth River.

1. Stomach contents - PAHs, Chlorinated Hydrocarbons and Metals

Levels of total PAHs in stomach contents (Figure 8a) varied extensively, with highest values found in winter flounder from Boston Harbor and Raritan Bay, and spot from the upper Chesapeake Bay. Levels were generally lower than in sediment, suggesting these compounds are metabolized by prey and/or fish. Interannual differences were observed but are not surprising, considering that the composition of stomach contents can vary daily, if not more frequently.

Total pesticides, total PCBs and total chlorinated hydrocarbons (sum of all pesticide and PCB compounds) in stomach contents (Figure 8b-d) also varied widely and, in general, were higher than in sediment, suggesting that these compounds are accumulated by prey (and not metabolized rapidly by fish). Levels of these compounds were 2-5 fold higher in stomach contents than in sediment. At the Buzzards Bay and Merrimack River sites, stomach content levels of total PCBs and total chlorinated hydrocarbons were 10 fold (or more) greater. Highest levels of total PCBs and total chlorinated hydrocarbons were found in Quincy Bay, Raritan Bay, Buzzards Bay and Merrimack River, while highest levels of total pesticides were found in Raritan Bay, followed by Delaware Bay, western Long Island Sound, Boston Harbor, Quincy Bay and Salem Harbor. Interannual differences were observed in levels of these compounds, but their significance is more qualitative than quantitative because the frequency of variation in stomach contents (daily) is much greater than the frequency of observation (annual).

Differences were observed between the fraction of pesticides in stomach contents (Figure 8e) and sediment (Figure 5d) at some sites. Increases of 10% or more were found in upper Chesapeake Bay, Raritan Bay, eastern Long Island Sound, Salem Harbor and Machias Bay. Such increases suggest differential accumulation of pesticides over PCBs. Three different species of fish were collected at these sites, so it is difficult to determine whether these differences are species related or habitat related.

Distributions of metals in stomach contents (Figure 8f-r) generally resembled those of sediment metals (Figure 6a-q). Levels of most metals in stomach contents were lower than sediment levels. This suggests that metals are metabolized by the prey, not accumulated. Selenium is one exception. Levels were higher in stomach contents than in sediment, suggesting accumulation by prey. Selenium stomach content levels, however, are well within the normal range found in marine fish tissues. Distributions of cadmium and arsenic, two of the more toxic metals, in general were similar to those of the other metals, but stomach content increases (beyond sediment levels) were found in certain sites (e.g. arsenic in Buzzards Bay, cadmium in Penobscot and Machias Bays), suggesting accumulation by prey at these sites.

2. Bile metabolites of PAHs

The metabolites of the naphthalenes include metabolites of all eleven low molecular weight PAHs measured (two- and three-ring compounds, also referred to as the petroleum derived fraction of PAHs). The metabolites of the benzopyrenes include metabolites of all seven higher molecular weight PAHs measured (compounds containing more than three aromatic rings, also referred to as the combustion derived fraction of PAHs).

The distribution of bile metabolites of petroleum PAHs is shown in Figure 9a. In general, levels in longhorn sculpin are lowest (≈20 ppm or less) and do not differ appreciably between sites. Levels in spot, windowpane flounder and croaker are comparable (≈80-130 ppm) and higher than sculpin levels. Winter flounder levels varied between low (≈30-40 ppm) levels and the maximum (≈255 ppm), with the highest levels found in Raritan and Narragansett Bays and Boston and Salem Harbors. These variations may be due to species differences, or they may be related to contaminant exposure. Figure 9b shows the distribution of petroleum PAHs in sediment. In general, the same trend is seen in sediment - lowest levels in sediments from sculpin and windowpane flounder sites, higher levels in sediment from spot sites, and a wide range of levels in winter flounder sites, where high and low levels of petroleum PAHs in sediment correspond with levels of metabolites fairly well.

The distribution of metabolites of combustion PAHs is shown in Figure 9c. In general, sculpin levels were lowest overall (and similar, site to site). Windowpane flounder levels were higher than sculpin levels, followed by spot and croaker. Levels in winter flounder, as with the petroleum PAHs, vary from near minimum to maximum, with highest levels found in Raritan and Narragansett Bays and Salem Harbor. Levels of total sediment combustion PAHs (Figure 9d) do not correspond to bile metabolite levels as well as in the case of petroleum compounds. Levels in sediment are all fairly low at all the sculpin, windowpane flounder and spot sites, but again show a wide range over the winter flounder sites.

3. Chlorinated Hydrocarbons and Metals in Liver

The range of total pesticides (Figure 10a) and total PCBs (Figure 10b) both varied by approximately an order of magnitude, while the range of total chlorinated compounds (Figure 10c) varied by two orders of magnitude. Highest mean levels of total PCBs and total chlorinated hydrocarbons were found at

sites adjacent to urbanized areas (e.g., Boston and Salem Harbors, Raritan Bay), and highest mean levels of total pesticides were found at rural (watershed) sites (e.g., Great Bay, Delaware Bay). From the viewpoint of species contamination, levels of these compounds in sculpin, spot and croaker were low and comparable. Winter flounder levels vary from near minimum to maximum, and windowpane flounder levels were near the middle of the range.

The fraction of pesticides in livers (Figure 10d) of species from watershed sites was greater (≥20%) than elsewhere (≤20%). Buzzards Bay fish had the smallest proportion of pesticides in liver tissue, possibly because the mixture of contaminants entering the bay is composed of a high proportion of PCBs, probably originating in New Bedford Harbor.

The compounds alpha-chlordane, trans-nonachlor and the tri- through hepta- chlorobiphenyls were all highly and significantly intercorrelated, consistent with similar geographic distributions of levels of these compounds in fish livers. Relationships with length or weight could not be explored since data are from composites of livers.

No significant differences between annual mean levels of total PCBs or total chlorinated compounds were found at any site. A significant increase in annual mean levels of total pesticides was found in Delaware Bay between 1984 and 1986, but mean lengths (Figure 10r) and weights (Figure 10s) of specimens varied during that period and may explain the increase. Rates of accumulation of these compounds are much greater than the corresponding depuration rates (due to lipid-aqueous partitioning), so increases in concentration would be expected to accompany length or weight increases.

Figures 10e-q show the distributions of trace metals in fish livers. Differences between sites due to different species are apparent, as are differences between sites due to habitat differences (e.g., winter flounder sites). Liver levels of metals were lower than sediment levels, except for selenium (all sites), arsenic in Buzzards Bay and cadmium in Penobscot and Machias Bays.

Interannual differences in mean levels of at least one metal were seen at all sites. Changes in mean lengths and/or weights of fish collected occurred in thirteen sites during the same period. Some of these differences in liver metal levels were due to sampling variations between years (different species sampled), others were due to sampling different size classes of the same species. Although statistically significant, all differences were small. The natural abundances of the group of metals determined in fish livers vary by as much as five orders of magnitude (Bowen, 1979). Also, many metals, like PAHs, are metabolized, so simple accumulation over time (as represented by length or weight) is not expected. Additionally, since body burdens of chemicals are influenced by the combined effects of all the chemical compounds to which an animal is exposed (i.e., the presence or absence of a certain compound may cause an increase or decrease in the level of another compound), elevated levels of metals in fish liver are not necessarily found in contaminated environments. Similarly, low levels of metals in fish livers are not necessarily found in clean environments.

Variations in liver metal levels due to sex were also explored. There was a significant difference between males and females in at least one metal at every site (except Casco Bay) over the period of the study. The greatest differences in liver metal levels associated with sex were found in iron levels, where livers of males (irrespective of species) had 2-3 times the iron content of livers of females. Where differences in length (due to sex) were significant (four sites), females were always larger. Significant differences in weight due to sex were found in two sites, females being heavier.

C. Fish Pathobiology

1. Histopathology

The number of fishes collected between 1984-1986 are shown below. Spot and croaker were collected only from Chesapeake Bay sites and will be discussed in conjunction with another report (Southeast and Gulf). Sculpin tissues were not examined in time for data analysis for this report.

	1984	1985	1986
Winter flounder	240	300	270
Windowpane flounder	90	188	68
Longhorn sculpin	0	180	90
Spot	54	60	60
Croaker	51	6	30

Liver: Seventy-seven types of liver lesions were found in flatfish. Of these, four lesions were chosen for detailed analysis, 1) ACV-atypical cell vacuolation ("RAM" cells, apoptosis), 2) biliary hyperplasia, 3) basophilic foci and 4) clear cell foci. Total neoplasms were also considered.

Kidney: Fifty-nine types of lesions were observed in flatfish. Two lesions were chosen for detailed analysis, 1) hyaline degeneration of tubular epithelium and 2) macrophage center proliferation.

Gill: Forty-two types of lesions were observed in flatfish. Of these, four lesions were chosen for detailed analysis, 1) papillary hyperplasia of filament epithelium, 2) bifurcation of lamellae, 3) thrombi in capillaries and 4) lamellar fusion.

The mean prevalence and standard deviation for these ten lesions for sites between Casco Bay, ME and Great Bay, NJ are shown in Figure 11a. These figures represent three years of data for six sites and two years of data for four sites (Casco Bay, Merrimack River, Raritan Bay and Great Bay). Total neoplasms are not included in this figure. Lesions with large standard deviations have highly variable prevalence and might prove fruitful for

further analysis. Figures 11b through 11j illustrate the mean and standard deviation of nine of the above lesions as they varied by site (gill lamellar fusion not shown). Lesions with small standard deviations are the best candidates for correlative studies, since there are small year to year variations in prevalence at any given site.

2. Gross pathology

Gross external lesions were encountered so infrequently, that they are not useful in a study containing so few samples per site. Gross internal lesions were also uncommon, except at the Boston Harbor site.

3. Length, age and gender characteristics

In spite of efforts to collect fish of a specific age, winter flounder specimens probably spanned four years in age. Based on lengths and the relation of length to age, and allowing for sexual dimorphism, as reported in the literature for different areas of northeast coastal waters (Table 2), the fish ranged in age from three to six years, with the majority aged four to five years. These differences were not considered in the data analyses.

4. Stomach contents taxonomy - not examined.

D. Relationships among Chemical Parameters

Numerous contaminants were measured in sediment, stomach contents, bile and liver, in an attempt to follow the paths of these compounds through various compartments of estuarine habitats. Various amounts of sediment were found in stomachs used for composites for stomach content analysis. Since the species of demersal fish used in this region ingest sediment when feeding, the degree of contamination of a site, as depicted by sediment contaminant concentrations, plays a part in determining the ration of pollutants in the diets of fish from these sites. Since benthic species comprise the majority of the diet of these fish, their response (e.g. accumulation or elimination) to sediment conditions also plays an important part in determining the pollutant ration of fish.

Knowledge of levels of contaminants and some of their metabolites in liver tissue and bile, in addition to pathological examination of tissues, allows an assessment of the response of these fish to sediment and dietary conditions in their habitats.

In order to compare dietary intake from site to site, it will be assumed that the feeding habits of all species used in this region are similar, that fish fed in the site where they were collected and that (persistent) compounds are similarly accumulable by all species in the food chain (in other words, if a PCB is bioaccumulated by a benthic species, it is also accumulated by a fish).

Conceptually, hypothetical distributions of contaminants in stomach contents might be presumed to resemble sediment distributions of those contaminants, at least qualitatively. This relationship, however, depends on

the following considerations. If stomach contents are comprised entirely of sediment, levels in stomach contents would be similar to sediment levels. However, if stomach contents are composed of sediment mixed with prey tissue, two interpretations of stomach content data are possible. First, if contaminant levels in prey tissue are less than levels in sediment, stomach content levels might be less than sediment levels. Compounds for which this is true are referred to in this report as non-persistent, as they are metabolized by prey and other species. That is, concentrations in tissues reach an "equilibrium" or "steady state" level determined partially by the amounts of these compounds ingested. They, therefore, do not accumulate (or biomagnify) indefinitely. Frequency of ingestion is also important, and, in the case of fish, is assumed to be daily. Thus, these compounds do not persist in the environment. Second, if contaminant levels in prey tissue are greater than the levels in the sediment, stomach content levels might be greater than sediment levels, depending on the proportions of sediment and prey tissue. These compounds are termed persistent in this report, as they are accumulated by prey and other species. That is, the rate of elimination of these compounds is negligible with respect to the rate of accumulation, so concentrations increase indefinitely with time, as represented by the length of the fish. Concentrations of these compounds attainable in tissues are determined by the frequency of their ingestion and by an animal's tolerance to them at elevated levels.

In general, levels of PAHs in stomach contents were similar to or less than levels in sediments. One rationale is that this is a result of, in the first instance, the presence of a considerable amount of sediment in the stomach, and, in the second, the presence of a greater proportion of food in the stomach. Levels in sandy sites, however, were greater in stomach contents than in sediment. This is consistent with the fact that PAHs reach some "steady state" or "equilibrium" concentration in food species (e.g. benthos), and indicates that prey items can apparently contribute significantly to stomach content PAH levels at sites where sediment PAH concentrations are very low, such as in sandy sites, where the steady state tissue concentration may be greater than the local sediment concentration.

While sediment PAH concentrations are expected to remain relatively constant over the years under normal conditions (no major perturbations), changes in stomach contents, and, therefore, in stomach content chemical concentrations, can occur daily, thus causing some doubt about the quantitative significance of annual changes. One might speculate that, unless the site is extremely patchy or the habitat actually changed from one year to the next (which appears unlikely based on sediment data), fish residing in a particular site consume a diet that is fairly consistent with respect to species composition, degree of contamination of prey tissue and degree of contamination of sediment. Still, changes in annual mean levels are difficult to interpret. They may simply reflect differences in the proportion of sediment in the gut at the time of sampling.

Levels of bile metabolites of PAHs give some indication of whether an animal has recently metabolized any PAH compounds, as metabolites appear in bile fluid within several hours of feeding. High levels would suggest that the intake of PAHs was considerable, which implies that the sediment or prey

contained high levels. High levels of metabolites were found only at the known polluted sites, but not consistently. One might expect the distribution of bile metabolites to resemble the distribution of sediment PAHs, that is, show high levels in bile of fish from contaminated sites and low levels in bile of fish from clean sites. Two conditions must exist, however, before this can occur, the sediment in the site must contain PAHs and the fish collected must have ingested the sediment within hours of sampling (fish feed daily, but stomachs can be empty twelve hours after feeding, Cite, 1999). Since field sampling activities were heavily influenced by vessel work schedules, it is possible that bile metabolite levels from a highly contaminated site were low simply because of the time of sampling. That is, sampling well after feeding, when PAH metabolite levels in bile were much lower than levels in specimens sampled optimally in time. High levels of bile metabolites should not be found in fish from clean sites, since sediment levels of PAHs are relatively low at those sites. Under ideal capture conditions, then, a single-species distribution would show high levels of bile metabolites at contaminated sites and low levels at clean sites. Under less than ideal conditions, low levels would be found at clean sites and, possibly, at contaminated sites inopportunely sampled (but high levels would not be found at clean sites).

Distributions of chlorinated hydrocarbons (total pesticides, total PCBs and total chlorinated hydrocarbons) were qualitatively similar in stomach contents and in sediments. However, levels of these compounds were higher in stomach contents than in sediment, generally 2-5 fold (≈100 fold in eastern LIS). This occurs because prey species accumulate these compounds, making them available to higher predators. Levels of several individual pesticide and PCB compounds in liver (trans-nonachlor, dieldrin, p,p'-DDE, o,p'-DDD, p,p'-DDD, Mirex, and the tri- through heptachlorobiphenyls) were significantly correlated with the corresponding compounds in either sediment or stomach contents.

Liver concentrations of chlorinated hydrocarbons were higher than concentrations in stomach contents (where levels were higher than in sediment). Total pesticide levels were generally 20-50 fold higher in liver than in sediment, while levels of total PCBs and total chlorinated hydrocarbons were generally 10-30 fold higher in liver than in sediment. This strongly supports the contention that bioaccumulation of these persistent synthetic compounds is occurring through the food chain. Compared to the fraction of pesticides in sediments, the fraction of pesticides in livers was greater at all sites except Delaware Bay. For example, the fractions of pesticides in sediments from upper and lower Chesapeake Bay (sites CU and CL, Figure 5d), were approximately 15% and 10%, respectively. The fractions of pesticides in livers of spot from those sites were approximately 28% and 30%, increases of 13 and 20 percentage points. In general, liver levels were 5-20 percentage points higher than sediment levels. This differential uptake suggests that pesticide compounds are more persistent in the environment than PCBs, that is, pesticides are metabolized to a lesser extent than PCBs.

Distributions of metals in sediment and stomach contents were qualitatively similar, but distributions of liver metals did not resemble either sediment or stomach content distributions, i.e., concentrations of

metals in livers were not highly correlated with the corresponding metals in either sediment or stomach contents. Most metals are not persistent (as defined above) in the environment, so their liver concentrations cannot be directly related to habitat characteristics, unlike persistent compounds. Metal levels do not increase or decrease only in relation to sediment contamination. Stomach content and liver levels are ultimately linked to habitat characteristics, but, the mechanisms resulting in the observed liver concentrations are poorly understood. Consequently, response parameters have not been derived that easily express tissue levels in terms of sediment contaminants.

For example, for eight of eleven trace metals, overall mean sediment levels were higher than both stomach content and liver levels. These were silver, cadmium, chromium, copper, nickel, lead, zinc and tin. Mercury was higher in stomach contents than in sediments, suggesting bioaccumulation by the benthos, but liver levels were lower than both stomach content and sediment levels. Arsenic and selenium levels showed an increase from sediment to stomach contents to liver, indicating bioaccumulation, but levels of these elements in fish livers are naturally greater than levels in marine sediment. (This is only one instance of natural biomagnification.) Liver chemical burdens represent time-integrated responses by an animal to its habitat. In three of the most highly polluted sites in the region (i.e., Boston Harbor, Quincy and Raritan Bays - winter flounder sites), while levels of mercury, one of the more persistent pollutant metals, appear elevated, most metal levels are unremarkable. Iron levels are elevated in livers of these fish, possibly indicating some similarity in physiological response. However, arsenic levels are high in Raritan Bay fish livers, but low in Boston Harbor and Quincy Bay fish livers, indicating differences in physiological response.

E. Relationships between Chemical and Biological Parameters

Ideally, one could take the results of the histopathologic survey and compare them with the results of the chemical analyses to explore possible causal relationships between lesion prevalence and contaminant levels; however, this has proven to be unrealistic. Some of the reasons for this are: 1) age differences, mostly between sites, but also between years at the same sites; 2) sex differences which caused markedly skewed sex ratios (usually favoring females) in several samples; and 3) many of the measured values for some variables used in the analyses were zero. When these factors are compounded by the relatively small number of sites at which multi-year data exist for even the most intensely sampled species in the northeast (winter flounder), this results in analyzing distributions which may significantly depart from normal. Of the lesions chosen for analysis, only three, atypical cytoplasmic vacuolation of hepatocyte (Figure 11b), hyaline degeneration of renal tubular epithelium (Figure 11f), and bifurcation of gill lamellae (Figure 11i), had distributions approaching normal after the arcsin transformation was applied to the data (Figures 12a-c). However, these three may be the most significant of those studied with respect to their possible induction by environmental contaminants.

1. Lesion prevalence vs. sediment chemical factors

In spite of the problems discussed above, an attempt was made to use the data to explore causal relationships between levels of contaminants in sediments and observed histologic lesions. Lesions chosen for analysis were those which showed a marked difference in prevalence between the most contaminated site and the least contaminated site. Twenty-one liver, twenty-one kidney and six gill lesions were selected. The distributions of the mean prevalence (two or three years data) of these lesions among the sampling sites were then examined graphically. A subset of the lesions was then selected; these were re-examined to evaluate the normalcy of their distributions. Lesions with approximately normal distributions were then evaluated using multiple regression against several different groups of sediment contaminants, i.e. metals, pesticides, PCBs and PAHs. Because lesion prevalence actually represents a ratio (the proportion of a 30-fish sample affected) the arcsin transformation was used. In some cases contaminant data were subjected to a log [log(1+x)] transformation, as this might better represent a dose-response relationship.

The relationships between these lesions, sediment contaminants and sampling sites are shown in Figures 13a-c. Figures 13d-g represent other lesion-contaminant-site relationships which were less closely correlated or which had non-normal distributions of lesion prevalence. Some probably significant lesions (such as basophilic foci, which are thought to be pre-neoplastic) had such a low frequency of occurrence that no quantitative evaluation could be made. The six sites for which multi-year data were available were too few to use non-parametric statistical analysis.

Neoplastic lesions are not illustrated. A total of sixteen benign and malignant neoplasms were found in winter flounder livers. Twelve (75%) of these were found in fish from Boston Harbor, with one each from Merrimack River, Buzzards Bay, Raritan Bay and Great Bay. In addition, nine benign gill neoplasms were observed in winter flounder, five from Boston harbor and four from Salem Harbor. Because of the extreme "clumping" exhibited by these lesions, nothing could be gained by further attempts at analysis.

Biliary hyperplasia occurred at a very low prevalence and only at the most contaminated sites. While the relationships between these two types of lesions (neoplastic, biliary) and gross contamination are obvious, their distributions were too restricted (neoplasms at only two of the sites which were monitored for three years; biliary hyperplasia at only three sites) to statistically identify any specific compound or group of compounds as causative. Table 3 is a list of all neoplastic lesions encountered.

Two other lesions are noteworthy. Cytoplasmic "droplets" in hepatocyte were observed in liver tissue of fish from several sites. This lesion appears associated only with samples not optimally fixed during field collection, so it should probably be considered an artifact, at least in the winter flounder from this study. Another liver lesion often noted was non-uniform hepatocellular vacuolation. This lesion appears to occur in greater prevalence at less contaminated sites and may be negatively correlated with contaminant levels.

2. Lesions vs. bile metabolites - see 4.

3. Lesions vs. liver chemistry - see 4.

4. Lesions vs. stomach contents chemistry

Tissue levels of contaminants which bioaccumulate can vary significantly in magnitude among individual fish. Relationships between tissue levels of contaminants and lesion prevalence should probably be examined only for the individual animals whose tissues were analyzed. This was not done because of lack of time and because the samples would be only one-third the size of the already small 30 fish per site.

Of the numerous lesions observed in winter flounder between 1984 and 1986, approximately ten appear useful as indicators of environmental degradation. Of these, most occur so infrequently that data are inadequate to make a statistically strong inference for causation by particular environmental contaminants. Of those lesions which occur with sufficient frequency and whose distributions can be normalized, the data suggest causation by certain classes of contaminants. For other lesions, whose distributions could not be readily normalized, correlations with certain contaminants are suggested when data are viewed graphically. With increased sample sizes, or with multi-year data from additional sites, some of these may yet prove useful. While not definitive, monitoring results suggest that more intensive sampling for specific conditions might result in sufficient data to make a strong case for causal relationships between specific lesions and particular anthropogenic hydrocarbons. Results also indicate combinations of contaminants which could be used experimentally to attempt controlled induction of certain lesions.

When interpreting results of monitoring in the Northeast, it is important to note that the winter flounder appears to thrive in contaminated environments. Catch per unit effort has been as great or greater at the Boston Harbor site - the most highly contaminated of any benthic surveillance site in the nation - as it has for any site in the Northeast. While it is true that some species of flatfish have shown a propensity to develop malignant neoplasms in response to certain environmental contaminants, it is also true that they are able to survive for many years in such environments. Indeed, adjunct studies utilizing winter flounder from contaminated environments in the Northeast have indicated that these fish may have enhanced resistance to infectious disease. So, while this species may provide an excellent model for environmentally induced carcinogenesis, it may be a poor one for use as an early-warning organism. For that purpose a benthic species that cannot survive the levels of contaminants found at the worst sites would be more useful.

In this preliminary analysis of the large quantity of data available from three years of monitoring, the frequency of occurrence of each lesion at each site (prevalence), or the arcsin transformation of this number, was used. Another index available, however, which might prove more useful, is the severity score (1-9) that is recorded for each lesion. This number was not used initially because it was not routinely entered in the database created

for examining the initial results of the study. The severity scores, either alone or factored into the prevalence data, would provide an additional database for analysis that would have the advantage of containing greater information than only the prevalence data, which merely indicates the presence or absence of a lesion in an individual fish. It would also provide a continuous variable for statistical analysis, rather than the ratio represented by prevalence.

REFERENCES

Bowen, H.J.M., 1979. Environmental Chemistry of the Elements, Academic Press, London

Klein-MacPhee, G., 1978. Synopsis of Biological Data for the Winter Flounder, Pseudopleuronectes americanus (Walbaum), FAO Fisheries Synopsis No. 117, NOAA Technical Report NMFS Circular 414. 43pp.

Riley, J.P. and R. Chester, 1971. Introduction to Marine Chemistry, Academic Press, New York

Table 1. Sampling summary, 1984 - 1986

SITE	YEAR	SPECIES	SED STA #
Machias Bay			
MB	1984	-	1
	1985	LS	1,2,3,4
	1986	LS	1,3,4
Frenchmans Bay			
FB	1985	LS	1,2,3,4
	1986	-	1,2,3
Penobscot Bay			
PB	1985	LS	1,2,3,4,5
	1986	LS	1,3,4
Casco Bay			
CB	1984	WF	5,14,15
	1985	LS	1,2,3,4,5
	1986	LS	1,2,4
Merrimack River			
MR	1984	WF	2,3
	1985	WF	1,2,3
Salem Harbor			
SH	1984	WF	1,2,3
	1985	WF	1,2,3,4
	1986	WF	1,2,3
Quincy Bay			
QB	1986	WF	1,2,3
Boston Harbor			
BH	1984	WF	1,2,3
	1985	WF	2,3,4,5
	1986	WF	1,2,3
Buzzards Bay			
BB	1984	WF	1,2,3,4,5
	1985	WF	1,2,3,4,5
	1986	WF	1,3,4,5
Narragansett Bay			
NB	1984	WF	1,2,3,4
	1985	WF	1,2,3,4
	1986	WF	1,2,3,4
Eastern LIS			
EL	1984	WF	1,2,3
	1985	WF	1,2,3,5
	1986	WF	1,2,3
Western LIS			
WL	1984	WF	1,2
	1985	WF	1,2,3,4
	1986	WF	1,2,4
Raritan Bay			
RB	1984	-	1,2,3,5
	1985	WF	1,2,3,4,5
	1986	WF	1,2,3,4
Great Bay			
GB	1985	WF	1,2,3,4
	1986	WF	1,2,3
Delaware Bay			
DB	1984	WP	1,9,11
	1985	* WP,SF	1,2,15,16
	1986	WP	1,15,16
Baltimore Harbor			
BA	1986	-	1,2,3
Upper Chesapeake			
CU	1985	SP	1,2,3,4,5
	1986	SP	1,2,3,4,5
Mid Chesapeake			
CM	1985	-	1,2,3,4
	1986	-	1,2,3,4
Lower Chesapeake			
CL	1984	CR	1,2,3
	1985	SP	1,2,3
	1986	SP	1,2,3
Elizabeth River			
ER	1986	CR	1,2,3

LS = longhorn sculpin; WF = winter flounder; WP = windowpane flounder
SF = summer flounder; SP = spot; CR = Atlantic croaker
* WP analyzed for metals, SF analyzed for hydrocarbons

Table 2. Age (years) at length (mm) - winter flounder

SITE	SEX	AGE						
		1	2	3	4	5	6	7
Casco Bay	M							
	F							
Merrimack River	M							
	F							
Salem Harbor	M							
	F							
Boston Harbor	M							
	F			290	341	376	401	418
Buzzards Bay	M			252	301	340	370	393
	F			294	350	390	418	438
Narragansett Bay	M		+/-180	+/-220	+/-250	+/-295	+/-300	
	F		+/-200	+/-280	+/-300	+/-350	+/-275	
Eastern LIS	M	106-116	174-194	225-253	263-286			
	F	106-116	182-211	254-282	305-324			
Western LIS	M	102-107	158-164	187-202	227-232			
	F	102-107	165-172	209-220	255-290			

Table 3. Neoplastic lesions in Northeast region winter flounder

Merrimack River	1984	Liver	Hemangiopericytoma
Salem Harbor	1984	Gill	Osteoma
		Gill	Capillary Hemangioma
		Gill	Capillary Hemangioma
		Gill	Capillary Hemangioma
	1985	Liver	Cholangioma
Boston Harbor	1984	Liver	Cholangiocellular Carcinoma
		Liver	Hepatocellular Carcinoma
		Liver	Cholangioma
		Gill	Capillary Hemangioma
		Gill	Capillary Hemangioma
		Gill	Hemangiopericytoma
	1985	Liver	Adenoma
		Liver	Cholangiocellular Carcinoma
	1986	Liver	Cholangiocellular Carcinoma
		Liver	Cholangiocellular Carcinoma
		Liver	Cholangiocellular Carcinoma
		Liver	Cholangiocellular Carcinoma
		Liver	Cholangiocellular Carcinoma
		Liver	Fibrosarcoma
		Liver	Carcinoma
		Liver	Sarcoma
		Liver	Biliary Cystadenocarcinoma
		Gill	Capillary Hemangioma
		Gill	Hemartoma
Quincy Bay	1986	Liver	Cholangiocellular Carcinoma
Buzzards Bay	1986	Liver	Adenoma
Narragansett Bay	1984	Gill	Osteoma
Western LIS	1984	Gill	Capillary Hemangioma
	1985	Liver	Adenoma
Raritan Bay	1986	Liver	Cholangiocellular Carcinoma
		Liver	Cholangiocellular Carcinoma
Great Bay	1985	Liver	Adenoma
		Liver	Adenoma

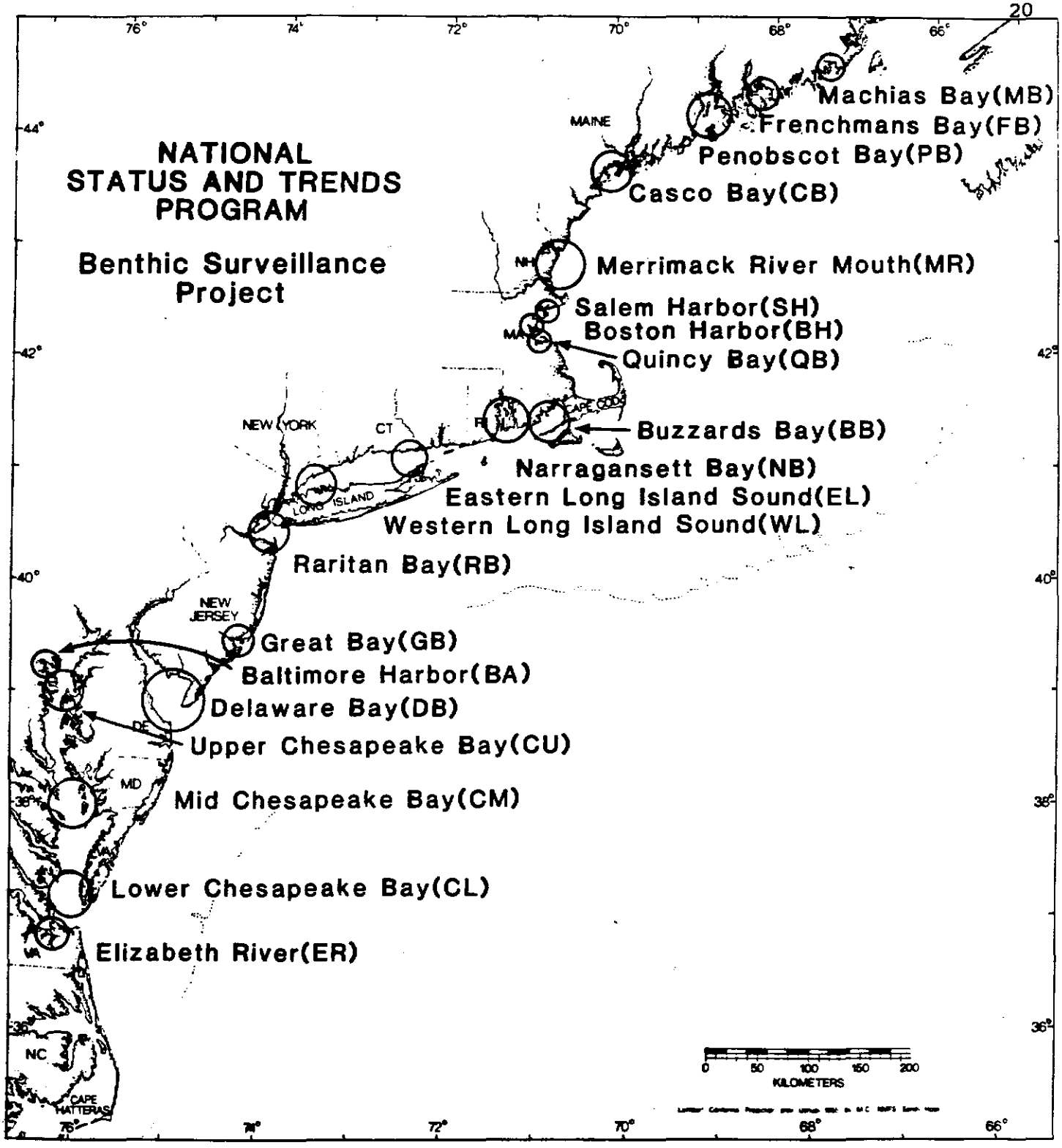

Figure 1. Northeast region sites sampled between 1984 and 1986.

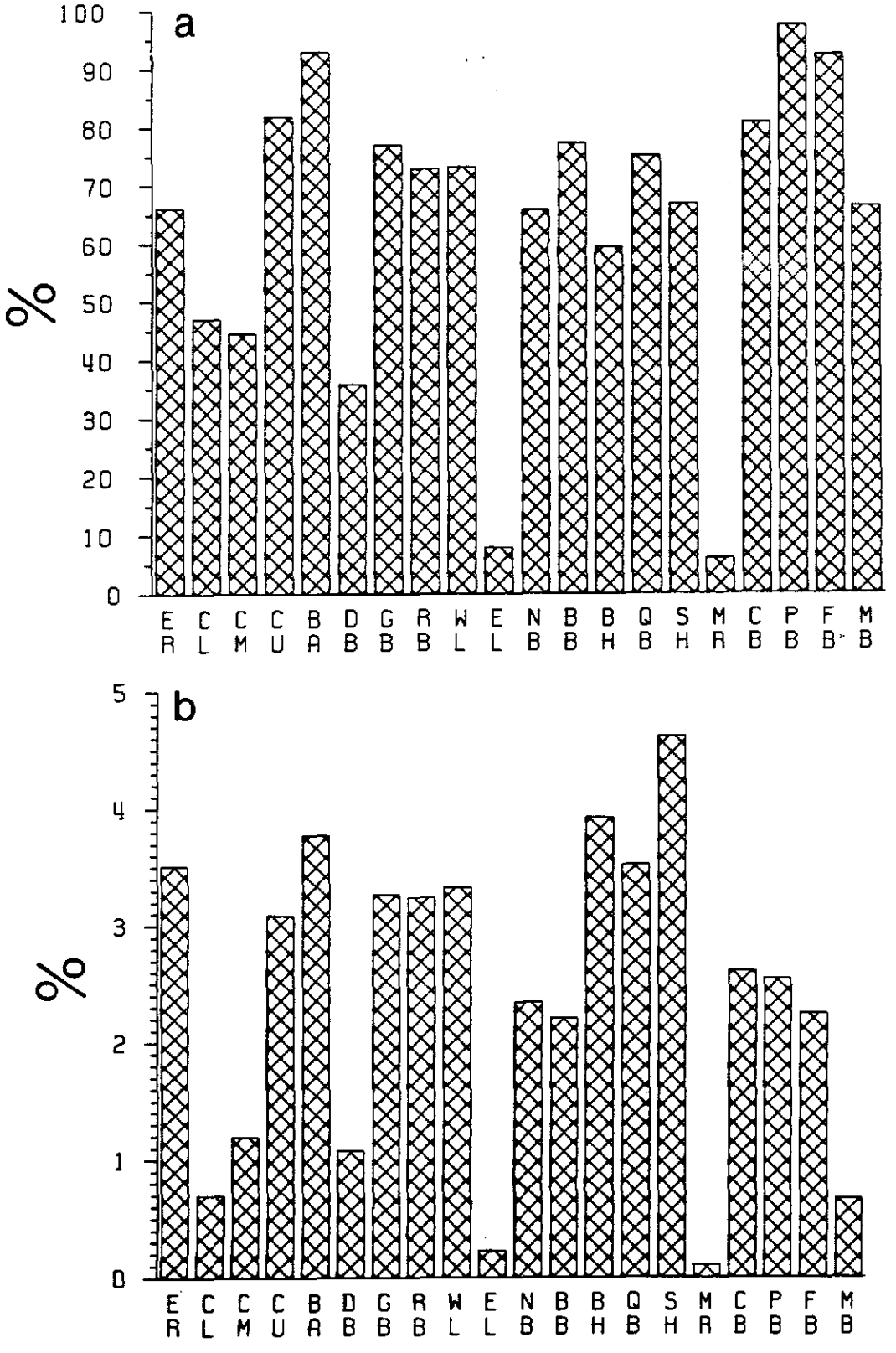

Figure 2. Sediment: a) percent fines (silt-clay), b) percent TOC (total organic carbon)

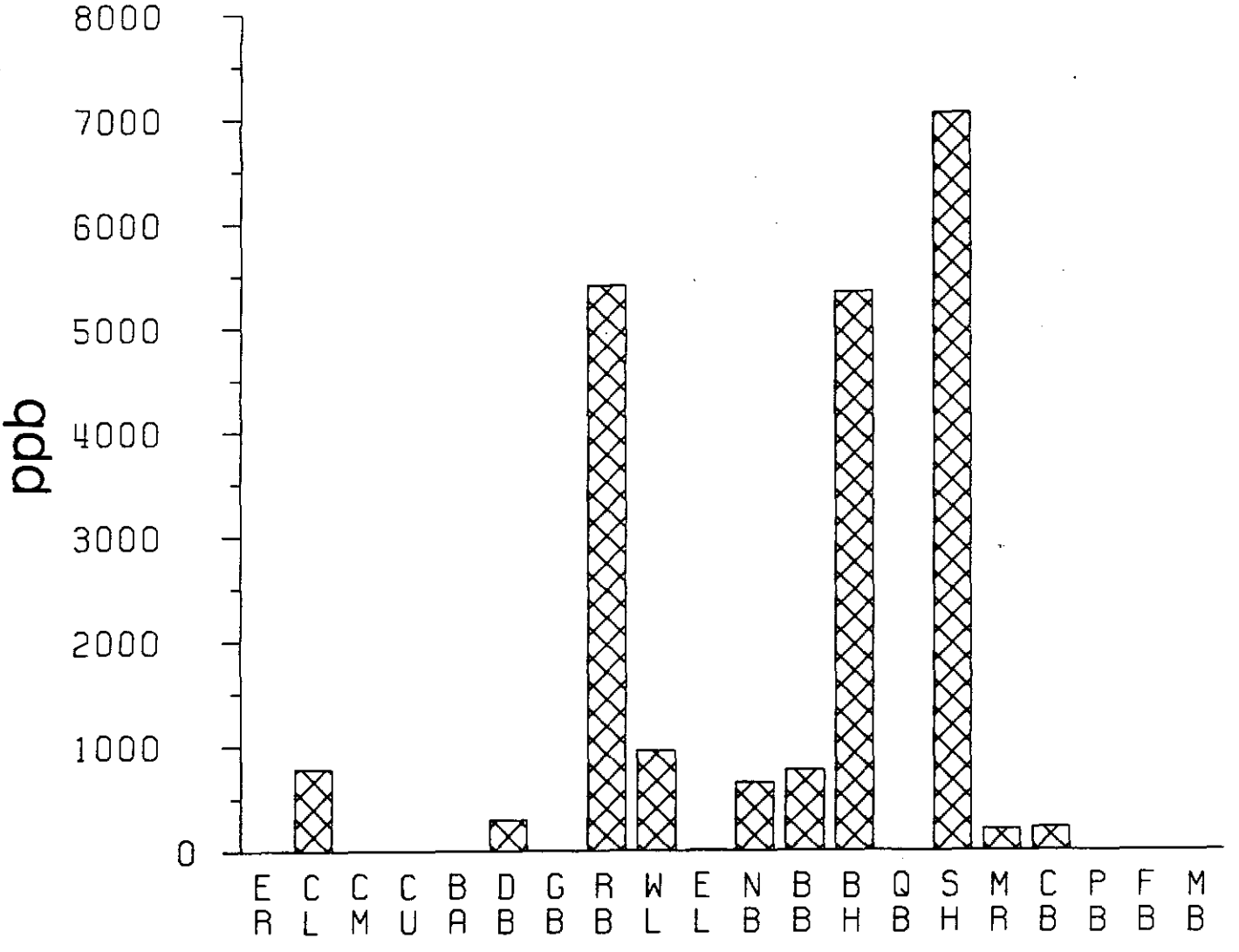

Figure 3. Sediment: coprostanol

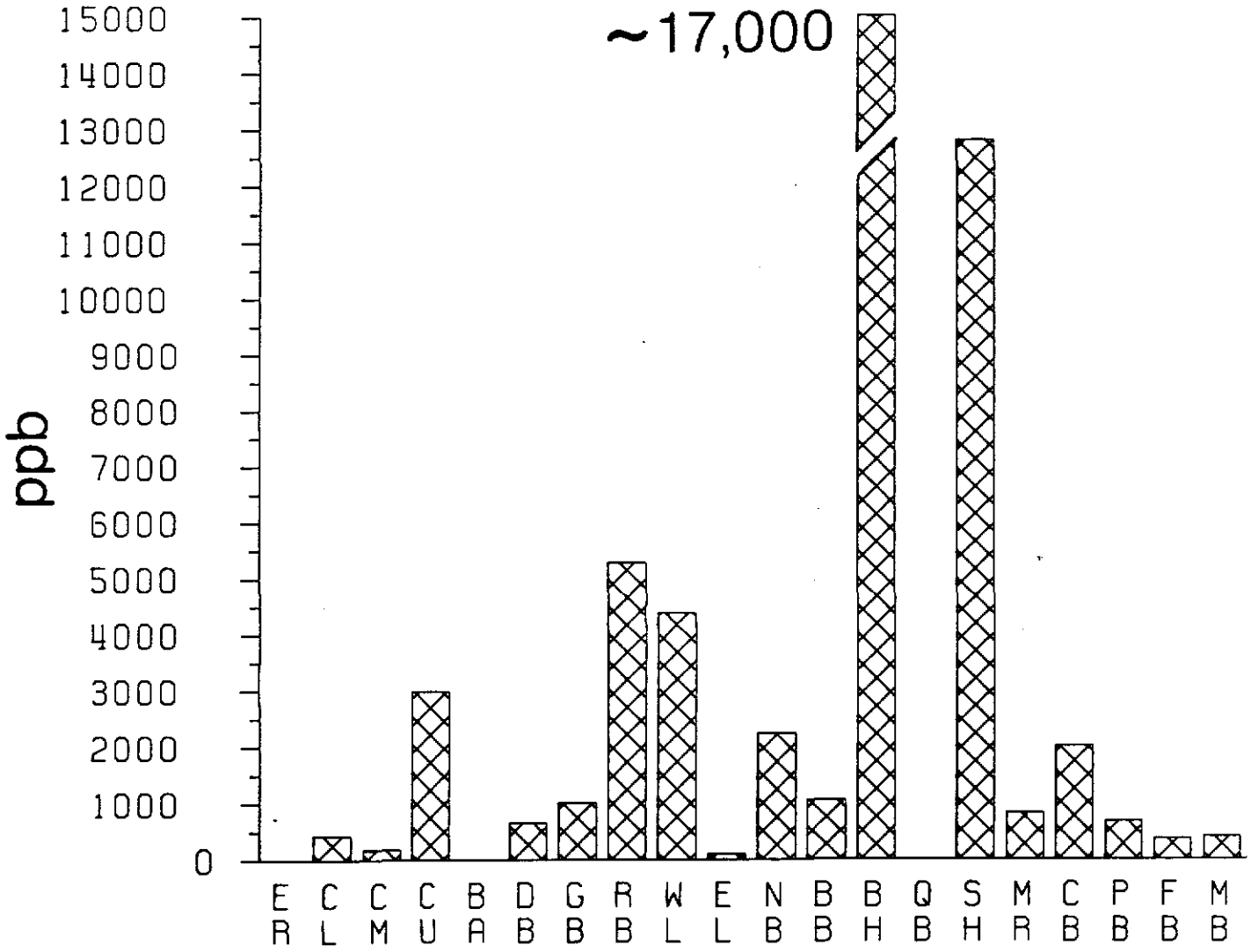

Figure 4. Sediment: total PAHs

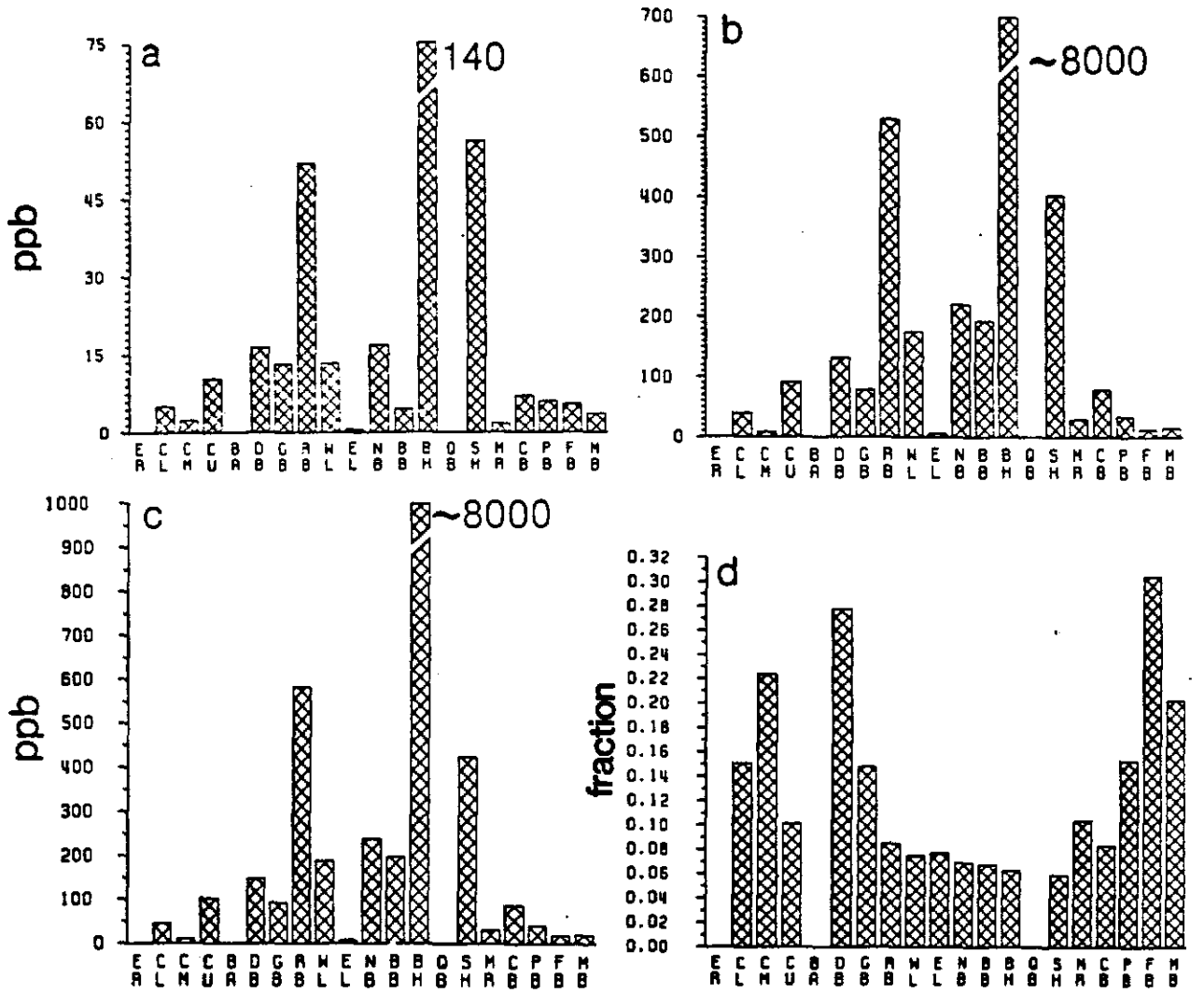

Figure 5. Sediment: a) total pesticides, b) total PCBs, c) total chlorinated hydrocarbons, d) fraction pesticides (a/c)

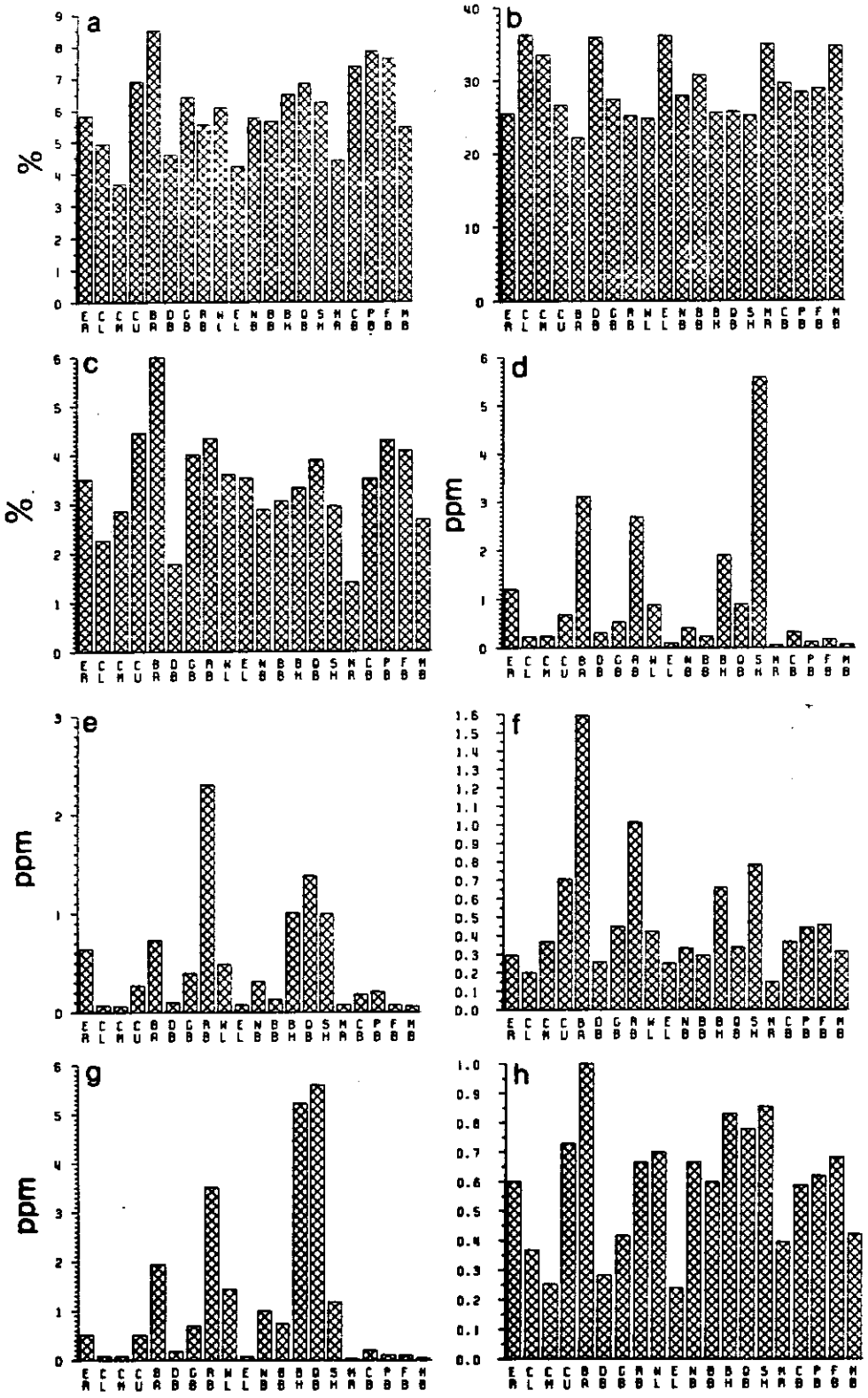

Figure 6. Sediment: a) aluminum, b) silicon, c) iron, d) cadmium, e) mercury, f) selenium, g) silver, h) thallium

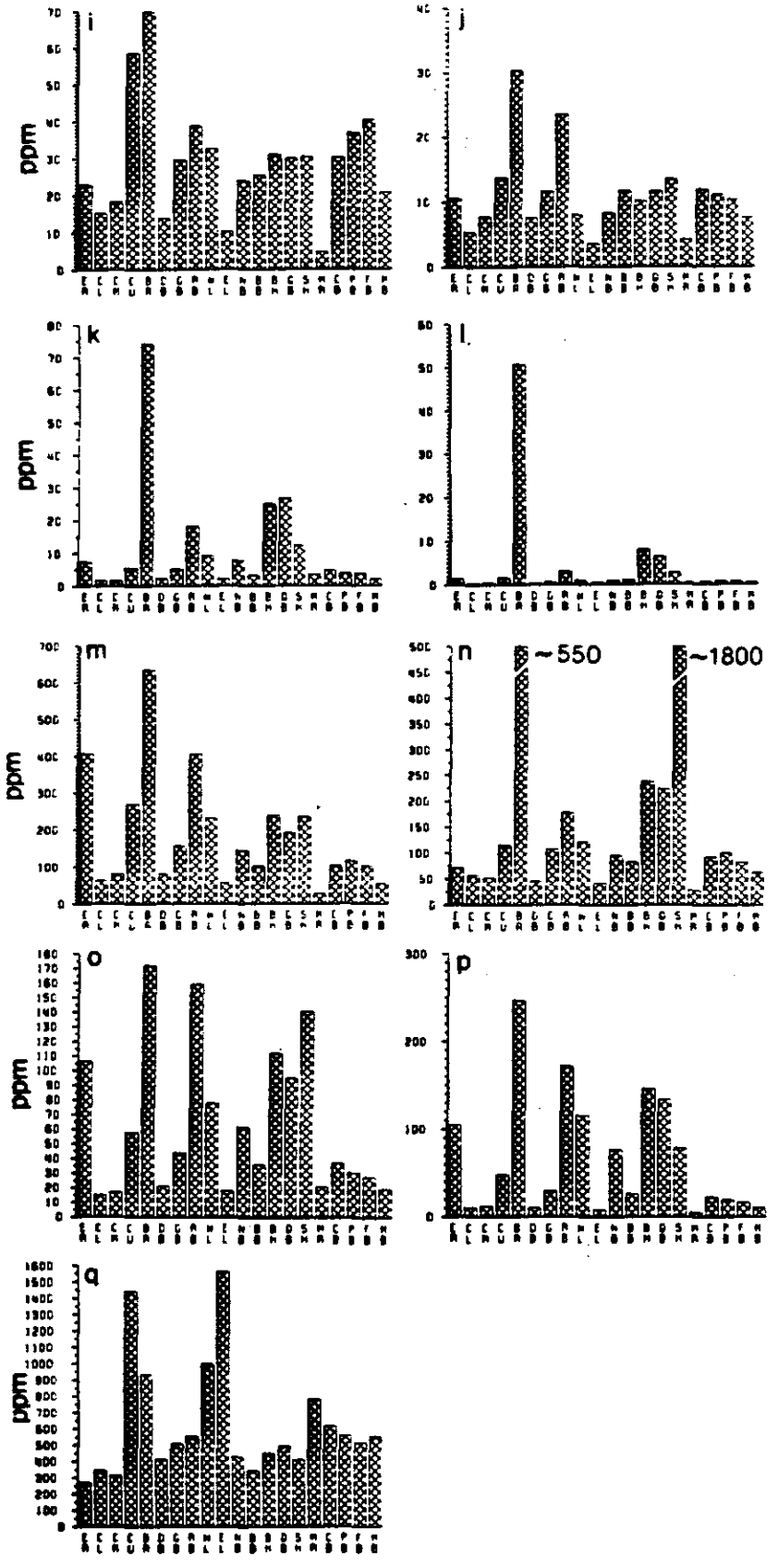

Figure 6. (continued): i) nickel, j) arsenic, k) tin, l) antimony, m) zinc, n) chromium, o) lead, p) copper, q) manganese

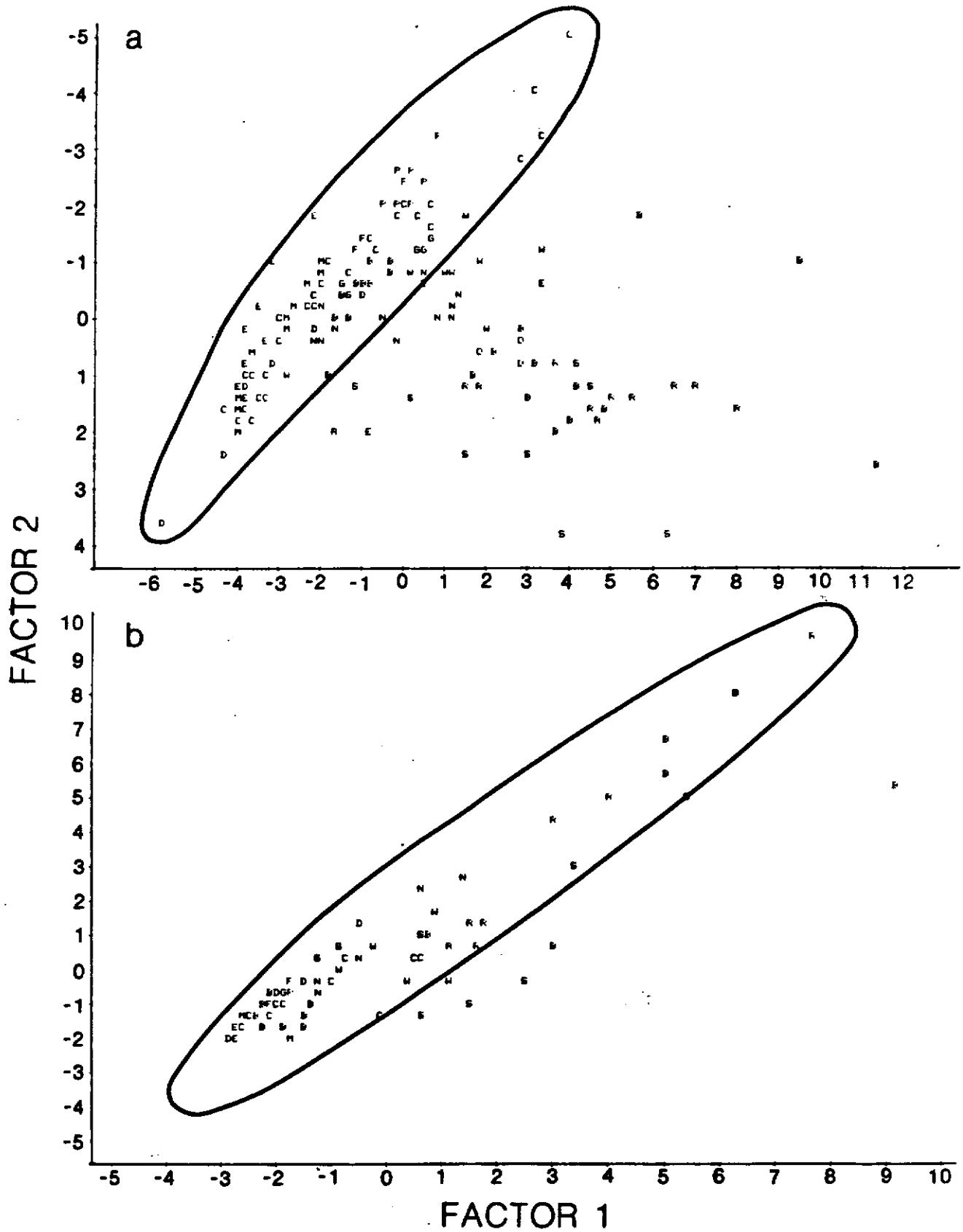

Figure 7. Sediment principal components: a) fine sediment (Y), contaminant metals (X); b) pesticides (Y), PAH+PCB+TOC (X)

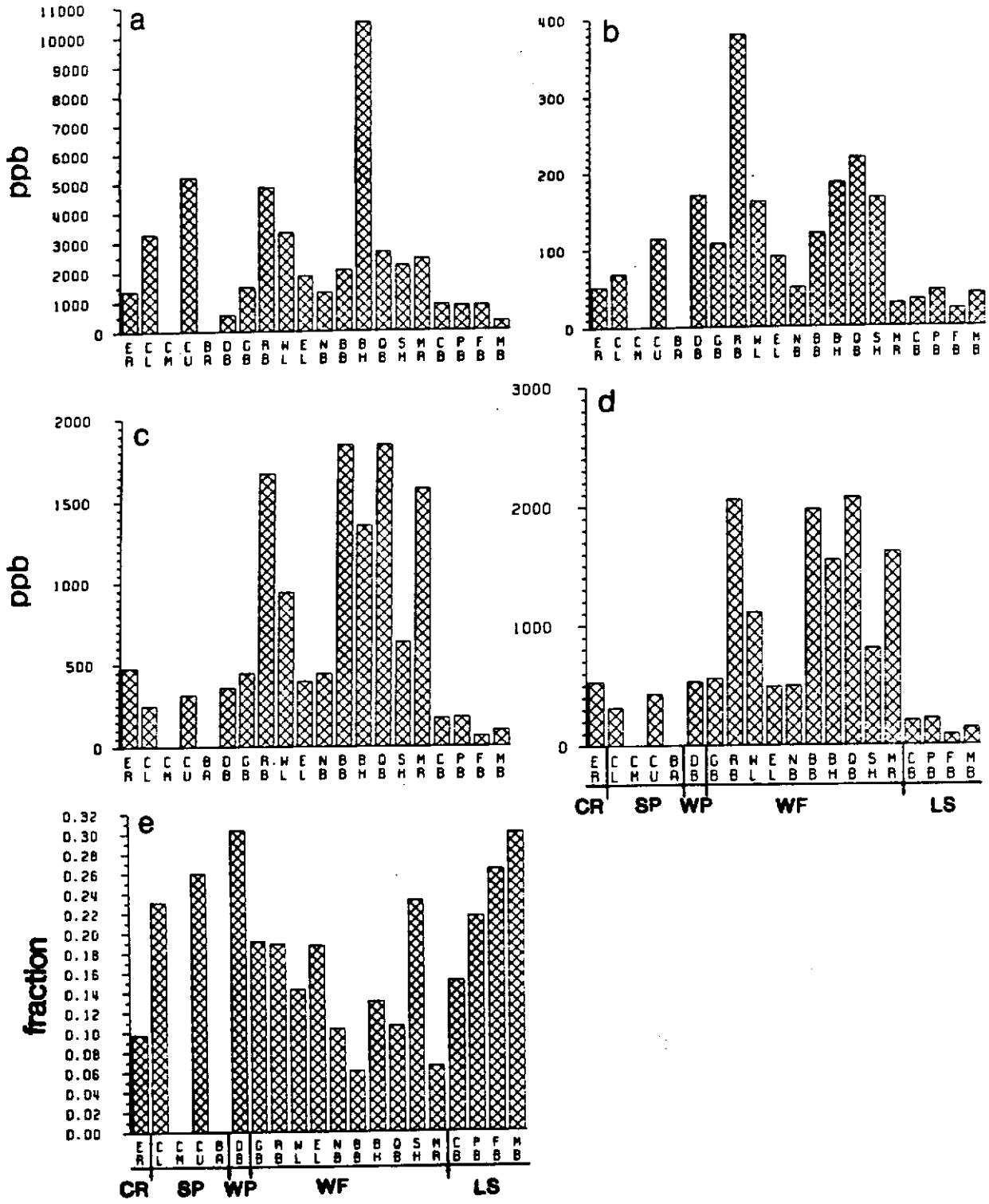

Figure 8. Stomach contents: a) total PAHs, b) total pesticides, c) total PCBs, d) total chlorinated hydrocarbons, e) fraction pesticides (b/d)

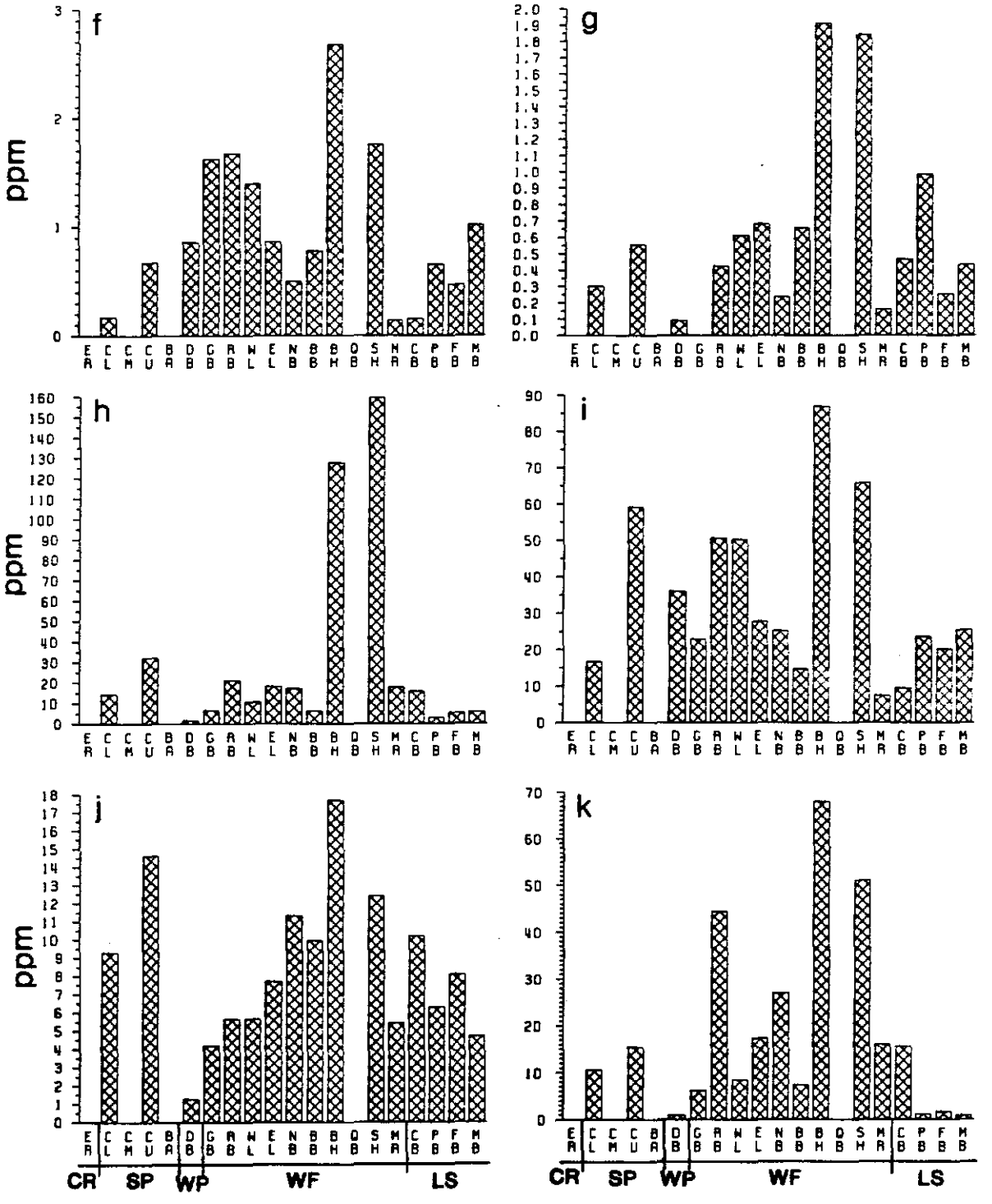

Figure 8. (continued): f) silver, g) cadmium, h) chromium, i) copper, j) nickel, k) lead

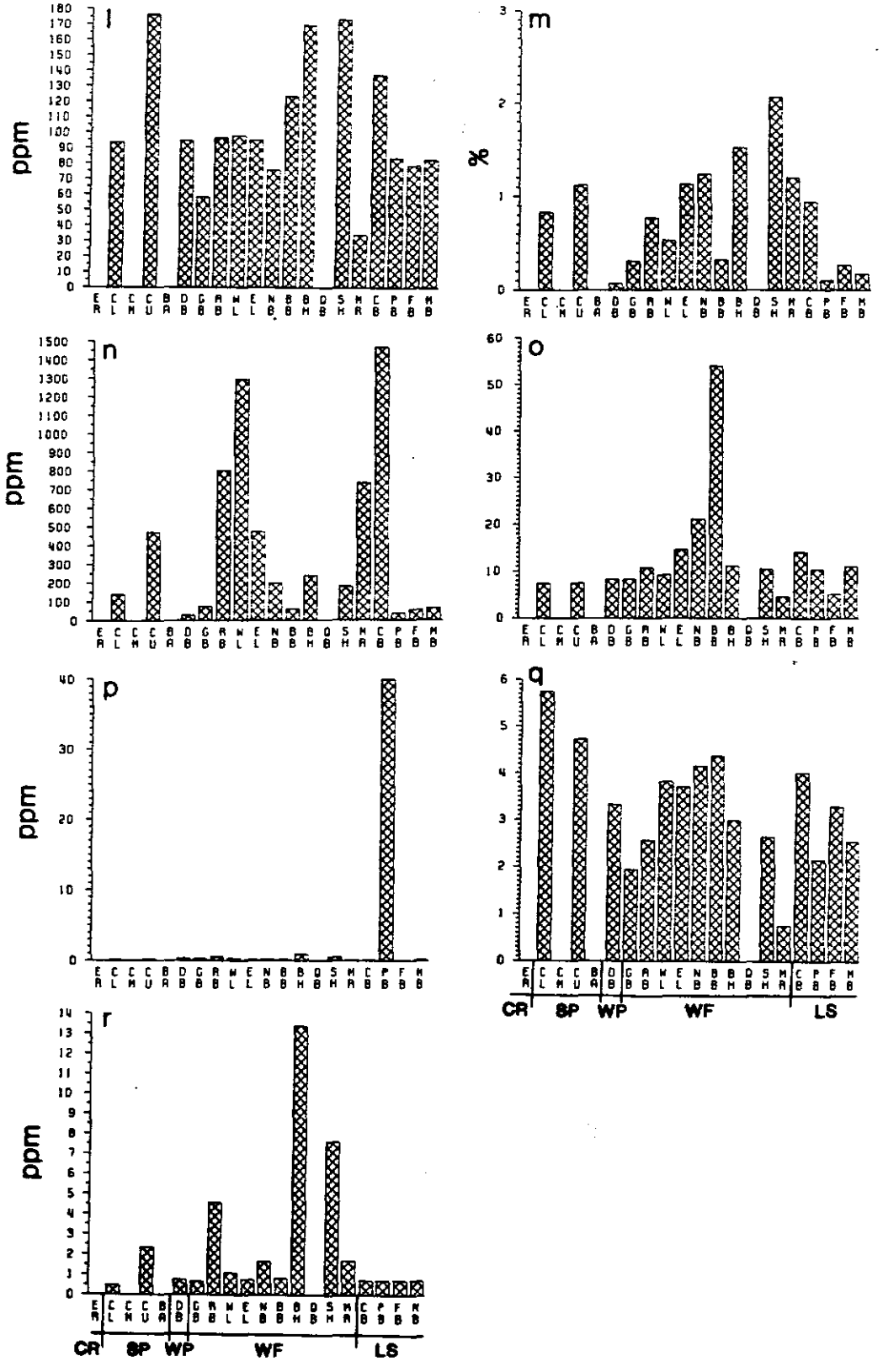

Figure 8. (continued): l) zinc, m) iron, n) manganese, o) arsenic, p) mercury, q) selenium, r) tin

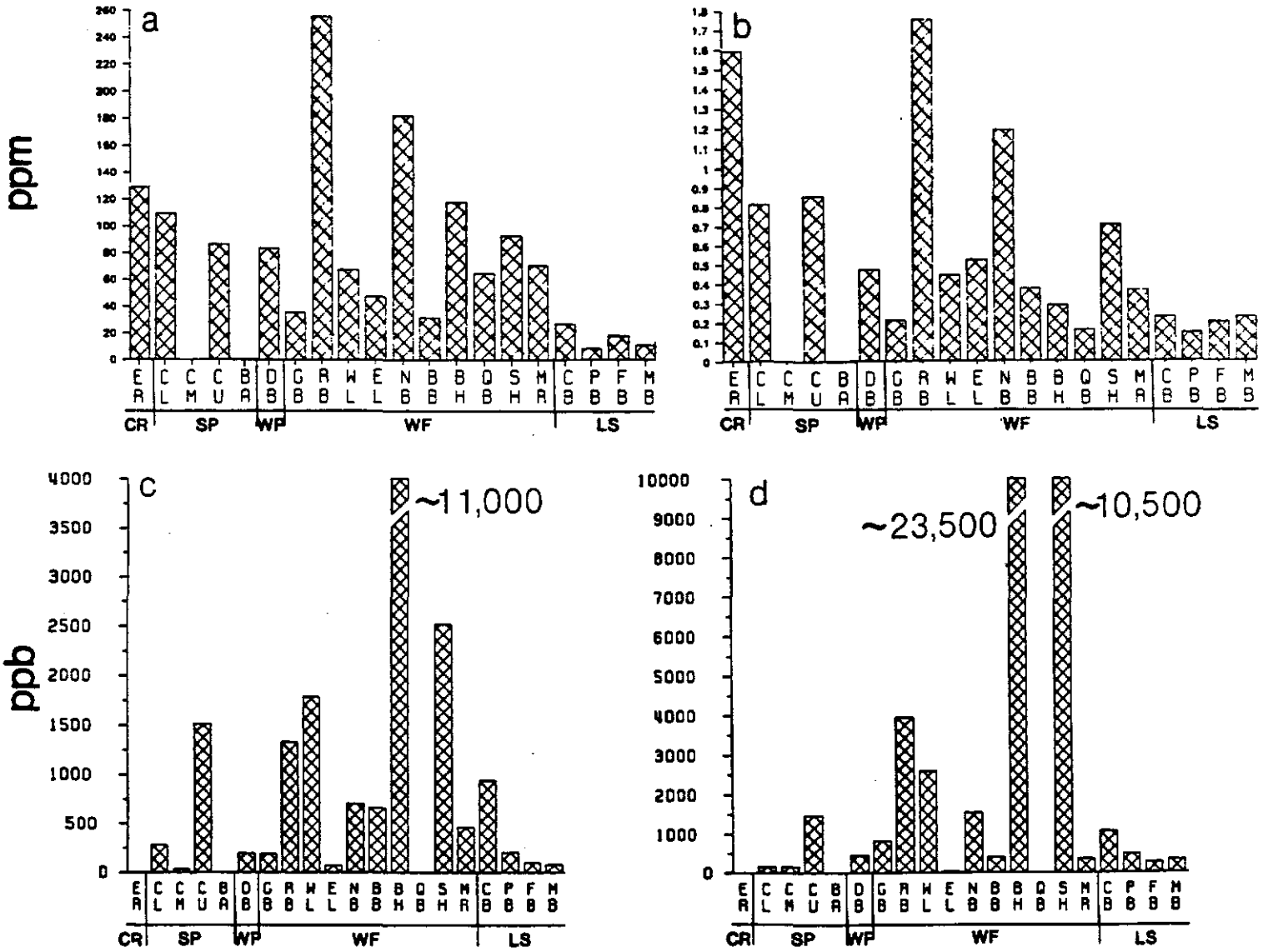

Figure 9. Bile metabolites of a) petroleum PAHs, b) combustion PAHs. Sediment concentrations of c) total petroleum PAHs, d) total combustion PAHs

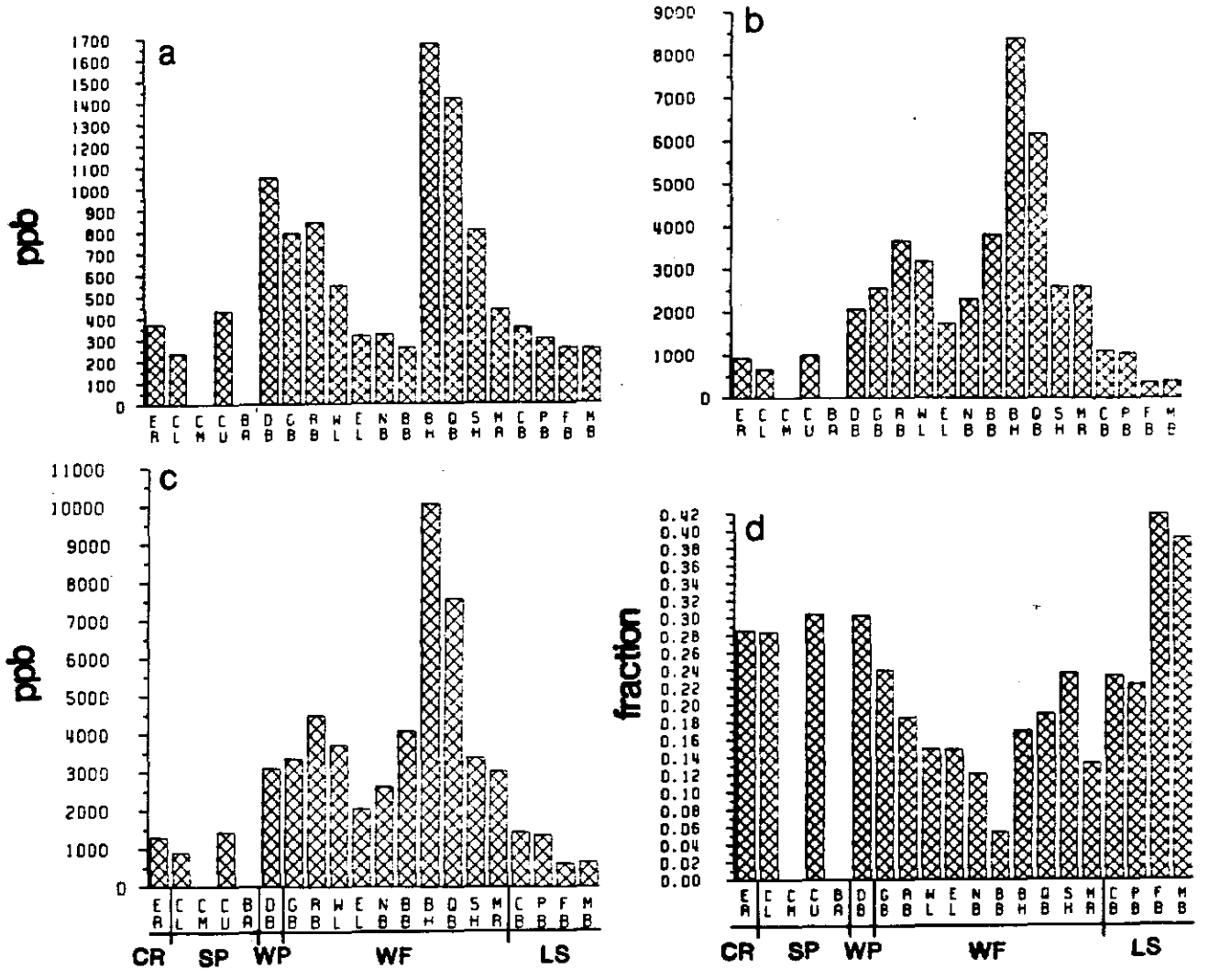

Figure 10. Liver: a) total pesticides, b) total PCBs, c) total chlorinated hydrocarbons, d) fraction pesticides (a/c)

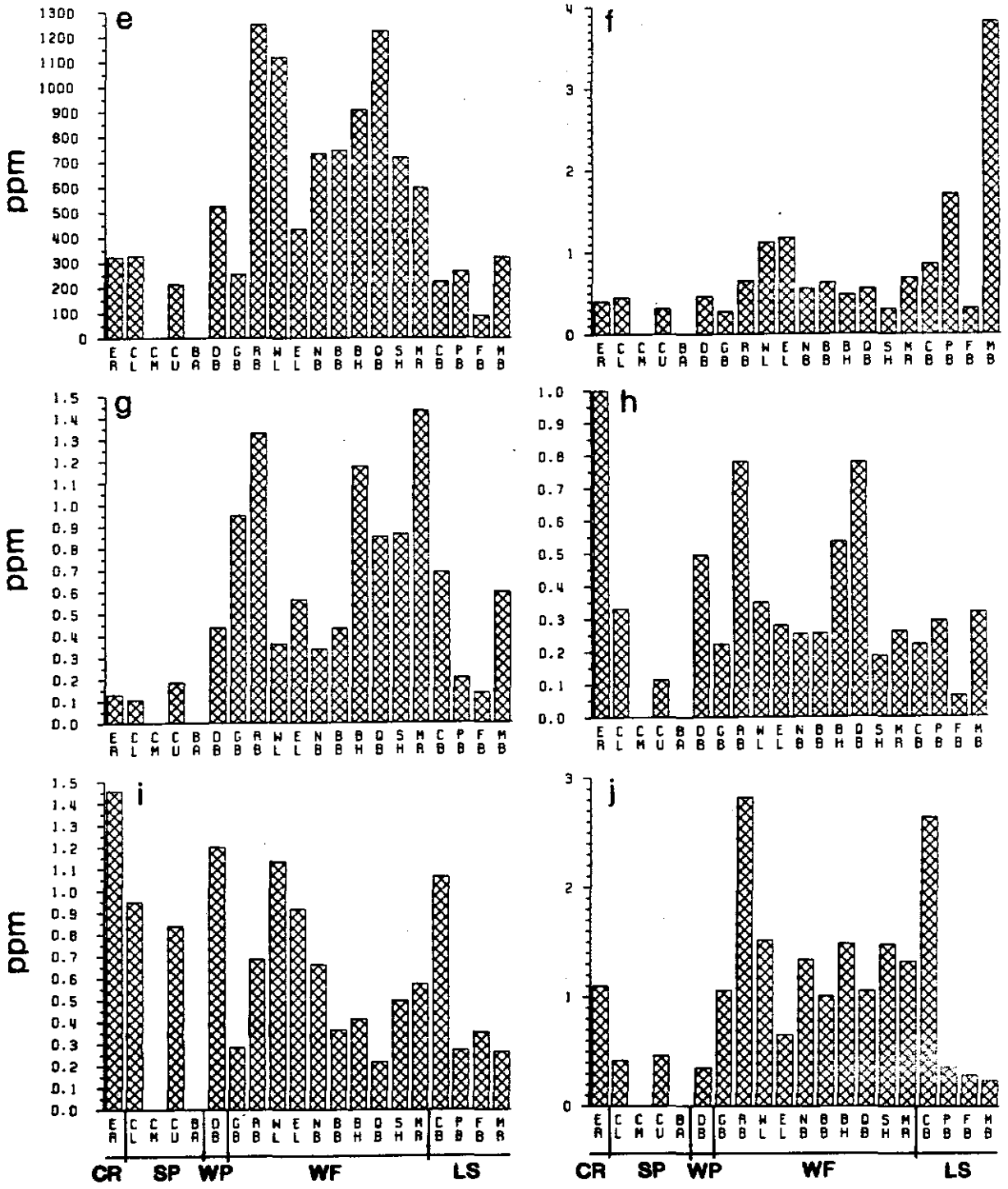

Figure 10. (continued): e) iron, f) cadmium, g) silver, h) mercury, i) nickel, j) lead

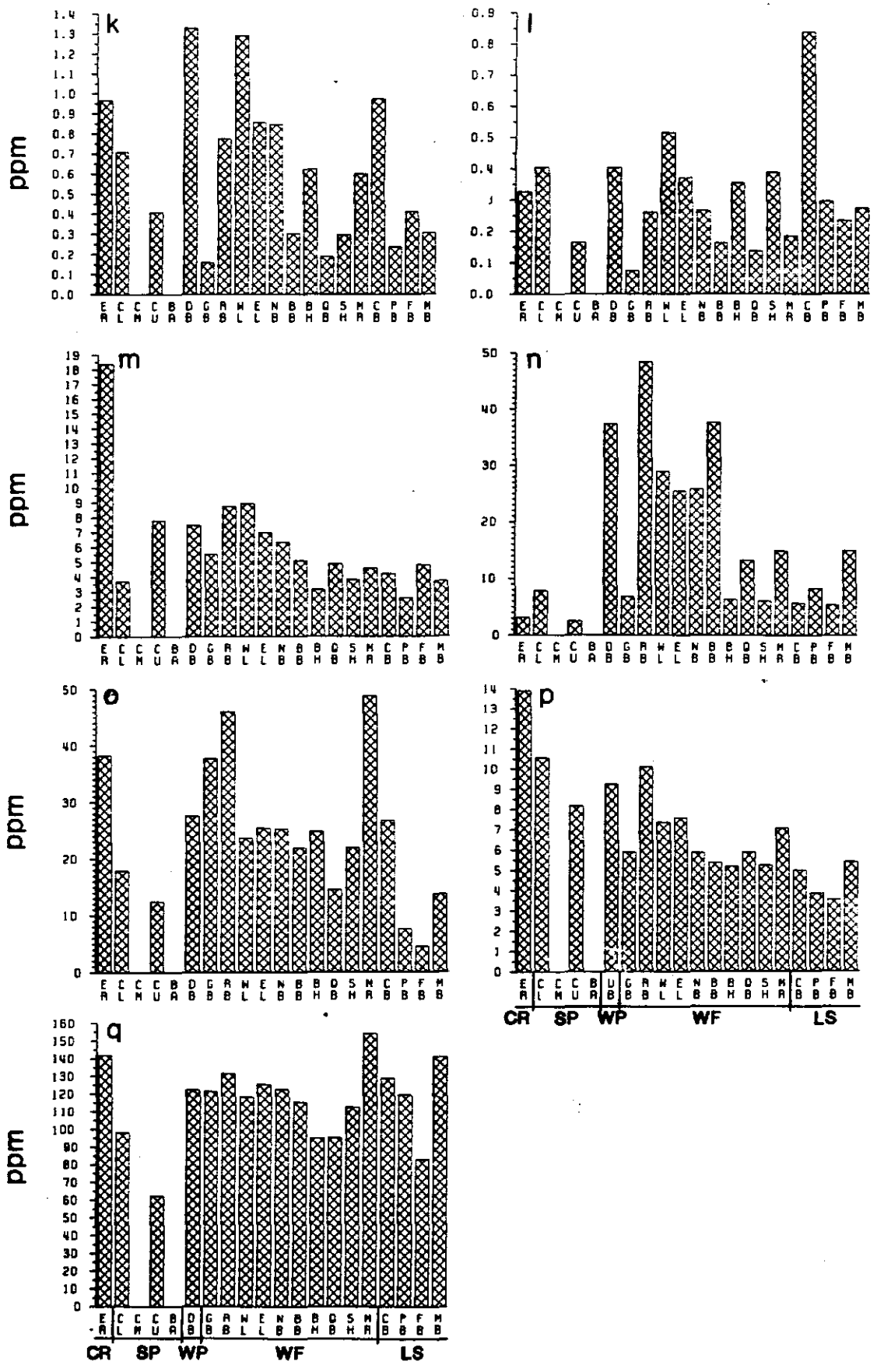

Figure 10. (continued): k) tin, l) chromium, m) manganese, n) arsenic, o) copper, p) selenium, q) zinc

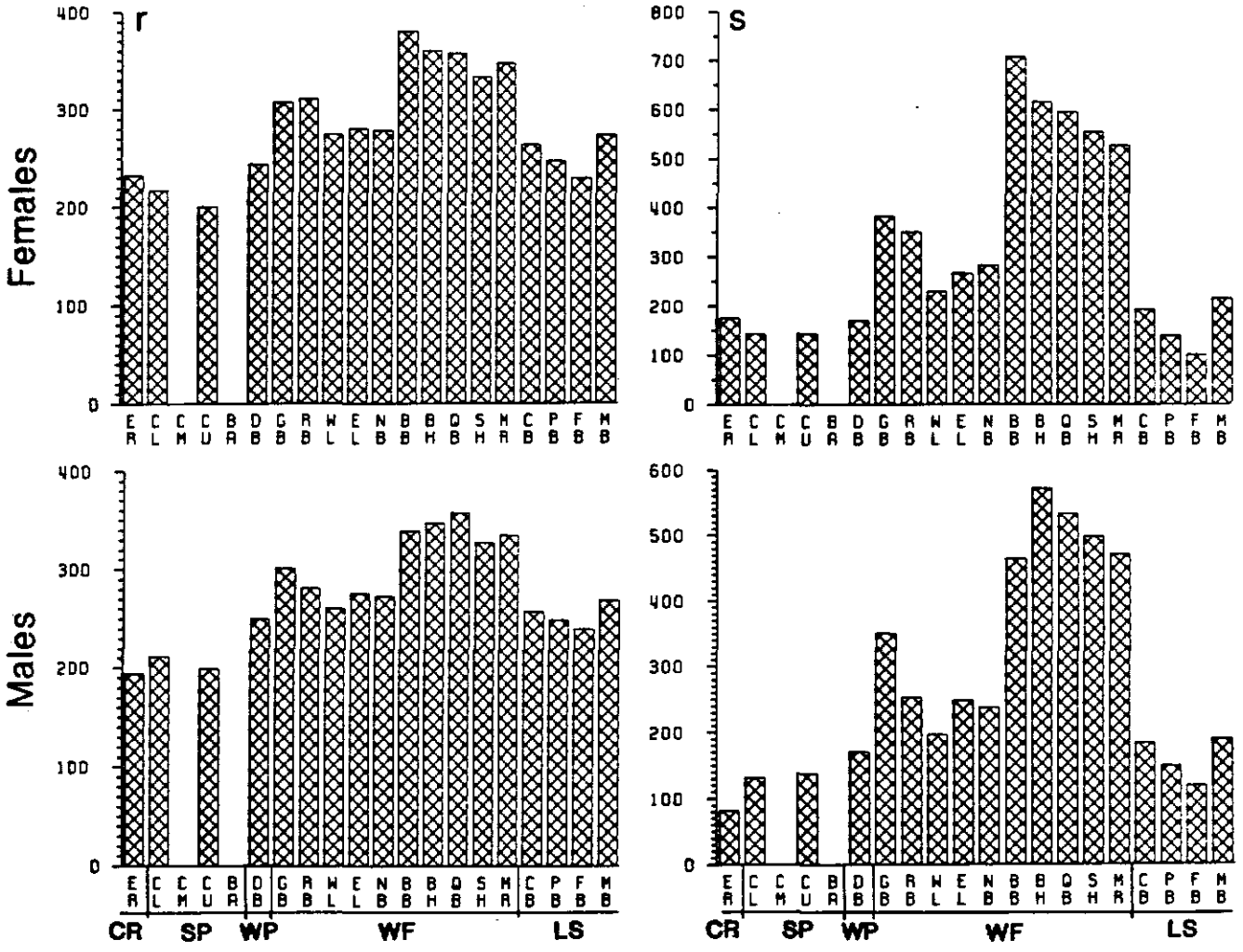

Figure 10. (continued): r) length (mm), s) weight (g)

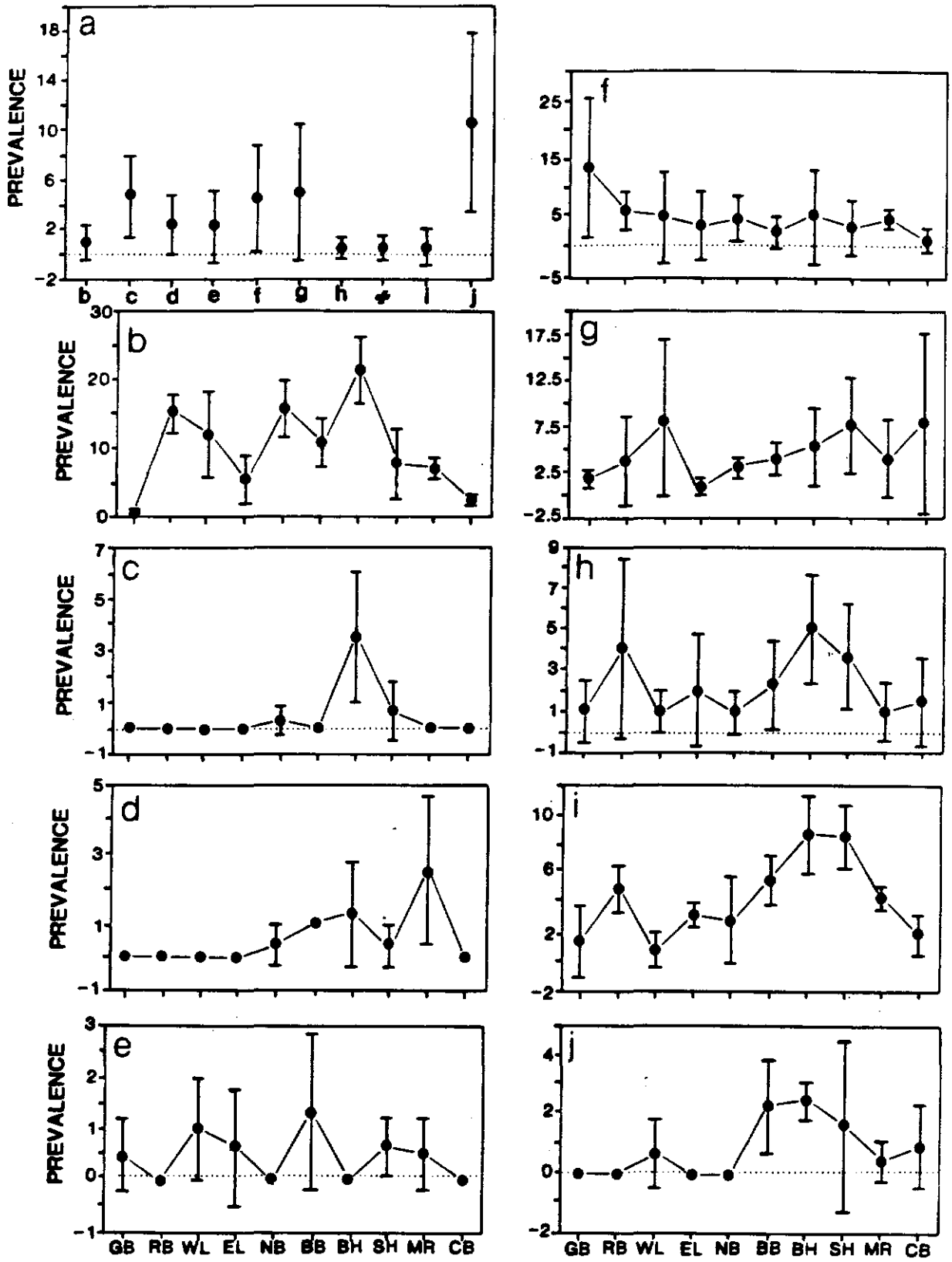

Figure 11. Lesions: a) mean prevalence of lesions b-j.
Liver: b) atypical cell vacuolation, c) biliary hyperplasia, d) basophilic foci, e) clear cell foci.
Kidney: f) hyaline degeneration of tubular epithelium, g) macrophage center proliferation.
Gill: h) papillary hyperplasia of filament epithelium, i) bifurcation of lamellae, j) thrombi in capillaries
- gill lamellar fusion

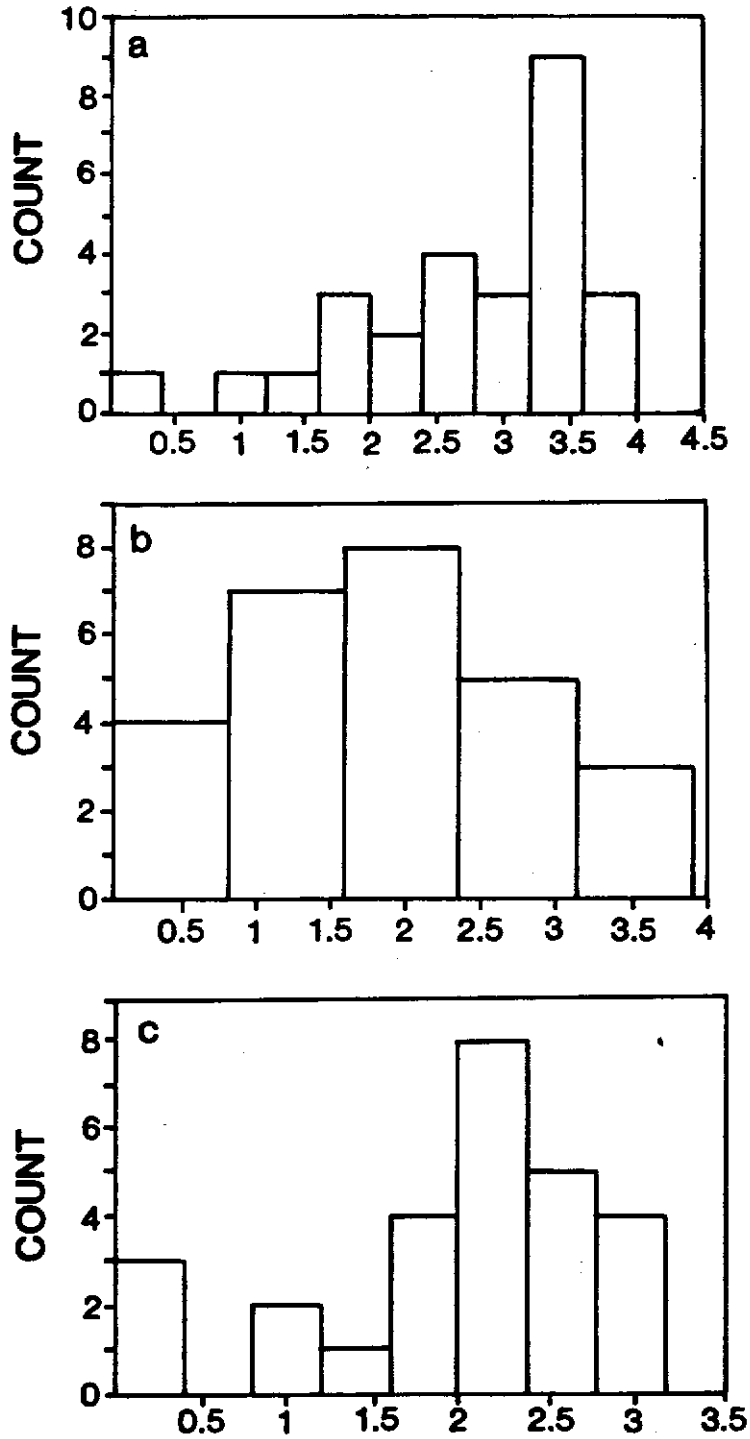

Figure 12. Lesions: arcsin transformation of a) atypical cytoplasmic vacuolation of hepatocyte, b) hyaline degeneration of renal tubular epithelium, c) bifurcation of gill lamellae

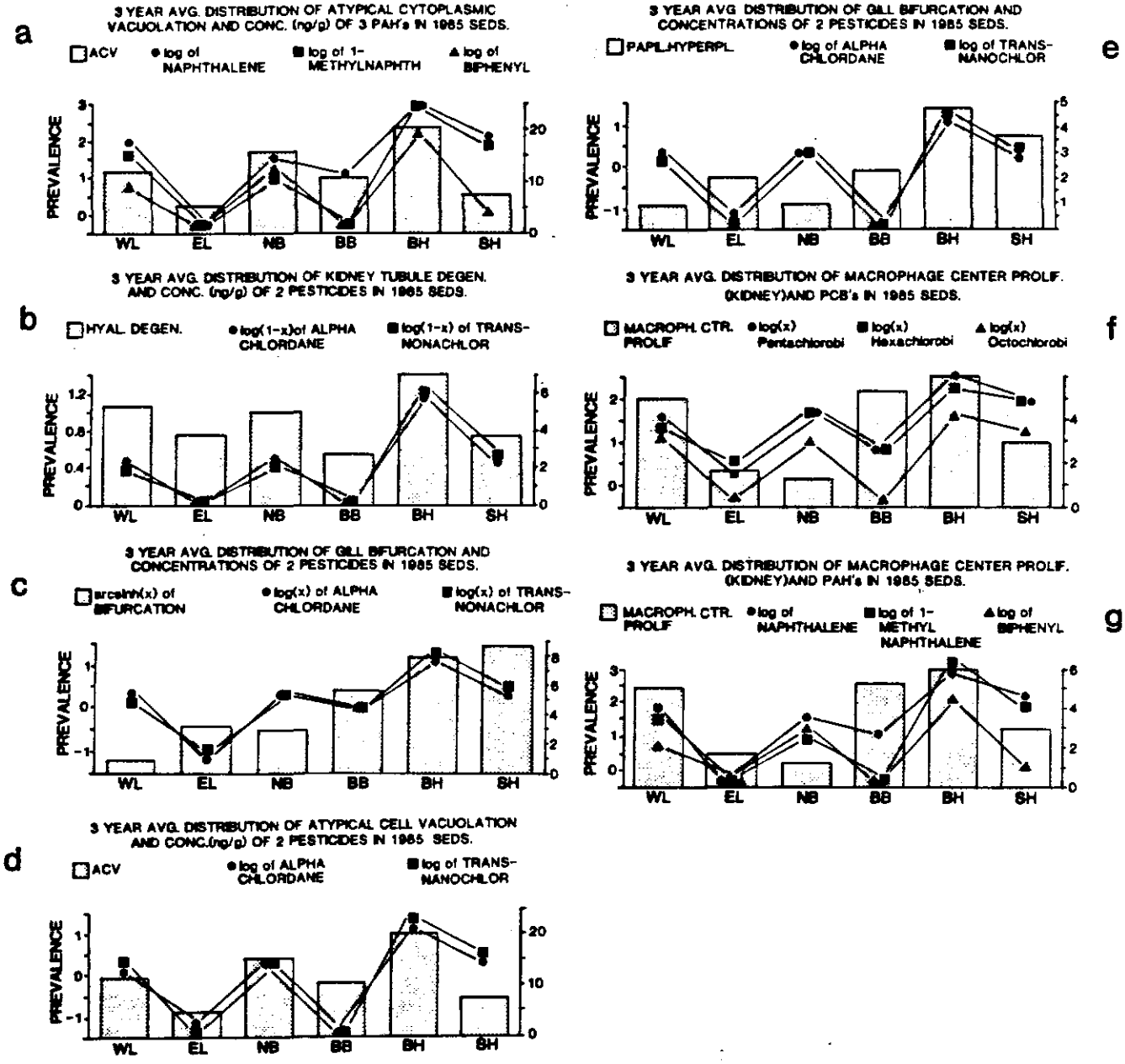

Figure 13. Lesions: graphic overlays of selected contaminants and lesion types by site, a) liver atypical liver cell vacuolation, PAHs, b) kidney tubular degeneration, pesticides, c) gill bifurcation, pesticides, d) liver atypical cell vacuolation, pesticides, e) gill papillary hyperplasia, pesticides, f) kidney macrophage center proliferation, PCBs, g) kidney macrophage center proliferation, PAHs