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Winter Flounder Contaminant and Pathological Survey:

Narragansett Bay and Vicinity 65 pp

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Narragansett Bay Winter Flounder Macrophage Aggregate

Number Corrected for Age 15 pp

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Technical Report on Aging of Winter Flounder Otoliths from

Rhode Island 28 pp

Haas, R.E. (URI).

Narragansett Bay Estuary Program

Current Report

The Narragansett Bay Project

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FOREWORD

The United States Congress created the National Estuary Program in 1984, citing its concern for the "health and ecological integrity" of the nation's estuaries and estuarine resources. Narragansett Bay was selected for inclusion in the National Estuary Program in 1984 and designated an "estuary of national significance" in 1988. The Narragansett Bay Project (NBP) was established in 1985. Under the joint sponsorship of the U.S. Environmental Protection Agency and the Rhode Island Department of Environmental Management, the NBP's mandate is to direct a five-year program of research and planning focussed on managing Narragansett Bay and its resources for future generations. The NBP will develop a comprehensive management plan by December, 1991, which will recommend actions to improve and protect the Bay and its natural resources.

The NBP has established the following seven priority issues for Narragansett Bay:

* management of fisheries
* nutrients and potential for eutrophication
* impacts of toxic contaminants
* health and abundance of living resources
* health risk to consumers of contaminated seafood
* land-based impacts on water quality
* recreational uses

The NBP is taking an ecosystem/watershed approach to address these problems and has funded research that will help to improve our understanding of various aspects of these priority problems. The Project is also working to expand and coordinate existing programs among state agencies, governmental institutions, and academic researchers in order to apply research findings to the practical needs of managing the Bay and improving the environmental quality of its watershed.

This report represents the technical results of investigations performed for the Narragansett Bay Project. The information in the principal document has been funded wholly or in part by the United States Environmental Protection Agency through Cooperative Agreement #CX812768 to the Rhode Island Department of Environmental Management. The contributing study which appears as an addendum has been funded wholly or in part by the United States Environmental Protection Agency through Cooperative Agreement #CX812680 to the Rhode Island Department of Environmental Management. They have been subject to the Agency's and the Narragansett Bay Project's peer and administrative review and have been accepted for publication as technical reports by the Management Committee of the Narragansett Bay Project. The results and conclusions contained herein are those of the author(s), and do not necessarily represent the views or recommendations of the NBP. Final recommendations for management actions will be based upon the results of these and other investigations.

The interested reader is encouraged to refer to a related study: Haas, R.E., 1989. *Technical Report on Aging of Winter Flounder Otoliths from Rhode Island* . Narragansett Bay Project report # NBP-89-24A. Narragansett Bay Project, Providence, RI.

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List of Abbreviations and Symbols

See Table 2 for coded variables and units of measure used in analyses. Other abbreviations and symbols used in this study follow.

ANOVA	analysis of variance
α	alpha
QP	Quonochontaug Pond
c.v.	coefficient of variation
D	a fixed proportion of the mean (used in sample size determination)
F	variance ratio statistic
HMA	hepatic macrophage aggregate
Ho:	null hypothesis
μ	population mean
n	sample size
$1-\alpha$	the confidence coefficient
WN	Warwick Neck
r	simple correlation coefficient
r-square	coefficient of determination
root-M.S.E.	root mean squared error
$\sigma 2$	population variance
σ	population standard deviation
T	student's t statistic
WR	Whale Rock
x	sample mean
$z\alpha/2$	upper $\alpha/2$ point of the standard normal distribution

INTRODUCTION

Background

Diseases, organ and tissue anomalies, and contaminant levels in organs and tissues of marine organisms are recognized to be of value in the context of monitoring for pollution effects (Sindermann et al. 1980). However, the sources of variance in such survey data are numerous, and they have not always been adequately taken into account. An example of a fish disease survey which does consider sources of variance in the data is provided by Detlefsen et al. (1983). These investigators found that differences in the percentage infection rates of certain fish diseases varied by an order of magnitude due to seasonal variability. Other sources of variability included the variability in diagnosis and sampling procedures. Bucke (1985) has clearly recognized the above-mentioned variability and has recommended planned surveys with selected target species utilizing histological examination of certain internal organs as a more meaningful indication of the health status of a fish stock than epidermal anomalies. Recently, however, a method to monitor fish health using quantifiable collections of macrophages in the spleen and liver of higher teleosts has been suggested and tested (Wolke et al. 1985a; Wolke et al. 1985b; Blazer et al. 1987; NOAA 1987). This method lends itself to more careful statistical evaluation since such variables as age and season can be controlled by the investigator. Further, it is now recognized that certain hepatic lesions are indicative of contaminated environments and when carefully enumerated, decrease the problem of variability reported in earlier studies (Malins et al. 1984; Malins et al 1987). Some sampling design considerations for trend monitoring of contaminants have been developed (Jensen and Larsen 1981) with stratification by length being recommended in at least one instance..The intra-sampling variability of tissue metals in oysters has been assessed by Wright et al. (1985).

In summary, past disease and contaminant studies have recognized some of the sampling problems involved in careful monitoring. Our work attempts to utilize this

information and to expand upon it in order to minimize costs and to maximize effectiveness.

The specific objectives of this study were to establish (within available time and field constraints) a valid baseline and sampling protocol for hepatic macrophage aggregate (HMA) parameters, hepatic lesions, and contaminants in winter flounder (*Pseudopleuronectes americanus*) in Narragansett Bay and adjacent areas.

Figure 1 illustrates the three sites chosen for sampling winter flounder for this project. These three sites were selected with a view toward demonstrating an apriori assumed gradient extending in decreasing scale from Warwick Neck to Whale Rock to Quonochontaug Pond.

It was originally planned to sample the sites quarterly and to collect approximately 50 fish over an extended size range at each site during each sampling interval. Difficulties in collecting fish due to unavailability or to weather precluded this procedure. Table 1 illustrates the collection dates by sites and the numbers collected. It is clear from this table that only two seasons were effectively sampled, and that it was virtually impossible to sample intensively enough on one date to satisfy initial sample requirements. Table 2 provides a brief description of the variables used in the study and the units of measurement employed for them. This table includes coded variables which will be used through much of the report.

Procedure

The protocol used in this study included the following. Separate sections are presented for each of the major tasks presented below.

1) Sample collection, determination of length, weight, sex, and otolith removal. Remove samples of muscle tissue and liver for chemical analysis.
2) Perform gross examination of specimens for diseases and for parasites.
3) Perform microscopic examination of tissue sections.

4) Determine concentrations of contaminants of interest (see Chemical Analysis Section for details).
5) Examine statistically the relations between response variables and length and weight of the fish (see Regression Analysis Section for details).
6) Determine sample sizes required for future monitoring of contaminants in winter flounder (see Sample Size Section for details).
7) Determine the sources of variability in the response variables and make inferences concerning their relative importance (see Log Linear Model Section for details)
8) Make recommendations based on this study (see Recommendations Section for details).

SUMMARY

This study was directed primarily toward obtaining a better understanding of the probable effects of pollution on stocks of winter flounder in Narragansett Bay and vicinity. Specifically, we sampled more than 400 winter flounder from three sites, which were presumed to represent a pollution gradient. These three sites were Warwick Neck, Whale Rock in Narragansett Bay, and Quonochontaug Pond, a coastal lagoon in southern Rhode Island.

Sampling was done during two seasons—winter and late spring. All organisms collected were carefully measured, weighed, and examined both grossly and histopathologically. Otoliths were removed for ageing, and organs and tissues (liver, spleen, and muscle) were removed for chemical analyses. Liver and muscle tissue were analyzed for PCBs, lead, cadmium, mercury, and arsenic. Specific histopathological conditions considered included the HMA (hepatic macrophage aggregates) parameter. These aggregates collect certain pigments which reflect pathological processes and tissue destruction.

A thorough statistical analysis of the available data was made with the primary goals of establishing certain baseline conditions for winter flounder monitoring and providing a rational basis for estimating sample sizes for future monitoring activities.

A multiple regression analysis was performed using the contaminants and the anomalous liver conditions as in dependent variables and fish length and fish weight as dependent variables. Weight was found to be somewhat more important than length in predicting PCBs and metal concentrations. However, both variables were significant statistically. The reverse was true for liver conditions where length seemed more significant than weight. However, in the samples analyzed, the reduction in variance achieved by sampling similar sized fish in terms of length and weight was not as great as that expected from a review of the literature.

Sample sizes required for detecting differences on the order of 25 percent in PCB and metal concentrations in muscle were relatively small—about 15 samples per site were estimated to be required for reasonable trend monitoring over time or space. Sample size was closely related to the variance of the observations.

The multidimensional contingency table analysis was made to ascertain the relative importance of sites, seasons, sex, and size of the fishes. It was found that sites and seasons were consistently the most important factors affecting sample variability This indicates that sampling for comparative purposes and monitoring should be done at the same site and season to maximize efficiency. Sex and size were much less important factors affecting the sampling strategy. Unfortunately, immediate ageing was not possible, limiting the significance of HMA data.

The correlation matrix among all variables provided many interesting possibilities for inferring possible relations among variables. It is evident that concentrations of some of the metals are highly correlated, and that the presence of neoplasms and macrophage aggregates seem to be associated with high levels of PCBs in the liver.

CONCLUSIONS

1) The relation between contaminants and anomalous liver conditions versus fish length and fish weight was demonstrated to be significant in all cases, except for As. However, other factors (site and season) were even more important sources of sample variability, and these must be accounted for in sampling designs.
2) Optimum sample sizes were calculated for a variety of contaminants and anomalous liver conditions under various assumptions concerning the magnitude fo the difference to be detected It was found that relatively few samples of muscle tissue (about 15) were necessary to detect 25 percent differences with 95 percent confidence at a power of 90 percent. However, larger samples were required for similar differences in macrophage aggregates.
3) None of the contaminants examined (PCBs, Pb, Cd, Hg, As) were found in unacceptably high amounts in the muscle tissues examined. The amounts found in the livers were consistently higher than muscle levels.
4) There were some interesting correlations found between the levels of contaminants in the liver and certain disease conditions.
5) A suitable baseline and sampling protocol for the determination of contaminants in winter flounder has been established.
6) A suitable baseline and sampling protocol for the determination of a sampling system using HMA parameter in Narragansett Bay winter flounder has been established.
7) Hepatic macrophage aggregate parameters, even uncorrected for age, are useful measures of winter flounder health and also reflect health of the fishes' environment.
8) Health of winter flounder in Narragansett Bay as measured by HMA and toxic changes appears related to the degree of environmental contamination.
9) Pre-neoplastic and neoplastic hepatic lesions involved less than 5 percent of the fish examined but were more common in fish from contaminated areas.

10) Anthropogenic pollution is adversely affecting the health of winter flounder in Narragansett Bay, as reflected in the HMA parameters.

RECOMMENDATIONS

1) It is recommended that the annual winter samples of similar size winter flounder be tested for metal, PCB, PAH, and HMA analysis from the Warwick Neck area and from other areas of possible concern for monitoring purposes, recognizing that sites and seasons are primary sources of sample variability.
2) It is recommended that sample sizes of about 15 fish per sample site are adequate for metal and PCB monitoring using the criteria indicated in the sample size table.
3) It is recommended that a similar survey of contaminants and diseases be made of quahogs (*Mercenaria mercenaria*) in Narragansett Bay, due to their high economic importance, their consumption by humans, and their immobility.
4) It is recommended that a careful study be made of the relations between neoplasms, macrophage aggregates, PCBs and PAHs in winter flounder, and other organisms of economic importance.
5) That the health of winter flounder be monitored at least annually using HMA from random sites to determine if the Bay contamination is decreasing or increasing.

CHEMICAL METHODS

Result and Discussion

The three selected sites, Warwick Neck (WN), Whale Rock (WR) and Quonochontaug Pond (QP) are characterized by a decreasing gradient of contaminant levels in both water and sediment. The site in WN is considered to be the most serious contamination area, because it receives large quantities of organic and inorganic pollutants from the heavily polluted area around the city of Providence. Quonochontaug Pond is considered to be he least contaminated area. It was chosen as the control site because no drainage from industrial and municipal effluents has been observed.

Adult winter flounder enter Narragansett Bay during autumn, and in winter they move into shallow coves to spawn. After spawning they return to the Bay, and by June have left the Bay and returned to the ocean. We might expect that the levels of the pollutants in the flounder would be higher in the spring season than in the winter season, because of the length of time spent in the Bay.

Concentration of Pollutants in the Liver

Tables 35 and 36 show the levels of the pollutants (μg/g, wet weight) in the liver of winter flounder collected from three selected sites around Narragansett Bay. For the calculations, each group of pooled (composite) liver samples is considered as one sample for determining the mean and standard deviation of the pollutants. Trends in the residue levels of PCBs, Cd, and Hg in the liver, can be observed. The highest levels are found in WN, while the lowest are found in.QP.

The concentration of PCBs (0.196-0.823 ppm) found in this study are greater than those found by Bulter and Schutzmann (1979) in livers of yellowtail flounder (0.13 ppm) and fourspot flounder (0.28 ppm) collected off the coast of the eastern United States and Canada. Greig et al. (1983) found the PCB residues to be 0.6-2.3 ppm in the livers of the windowpane flounder collected from Long Island Sound. Regardless of season, the

residue levels of PCBs are highest in WN (0.630-0.823 ppm) and lowest in QP (0.196-0.381 ppm).

Cadmiun (Cd) levels follow the same trend as PCBs in the three selected sites, with the highest in WN (0.274 and 0.289 ppm) and the lowest in QP (0.182 and 0.174 ppm). The levels of Cd from WR (0.194 ppm) are close to the levels of QP (0.182 ppm) in the winter season. Other studies examining Cd residues in fish liver showed 0.1-0.2 ppm for half of the 82 finfish species (Hall et al. 1978) and 0.08-0.68 ppm for windowpane flounder (Greig et al. 1983).

Lead (Pb) concentrations in liver vary with the sites where the fish were caught. WR has higher Pb residues (1.748 and 0.869 ppm) than WN (1.465 and 0.525 ppm) during both seasons. This variation may be because the levels of Pb which were present in the liver before migration to the WR could have been higher than the levels of those which migrated to WN. Higher contents of Pb in the sediment or water column may be another reason. No reliable information has been found to support this. In general, Pb residues in fish liver are 0.2-0.6 μg/g (Eisler 1981) for finfish and 0.4-0.8 ppm for windowpane flounder (Greig et al. 1983). Higher concentrations (2-10 μg/g) have been also detected in the liver of windowpane flounder in 58 out of 82 specimens found in industrialized areas (Hall et al. 1978).

Livers of winter flounder also show distinct area-dependent variability in Hg residues. The highest levels, 0.142 and 0.441 ppm, are found in WN area for winter and spring seasons, respectively (Tables 35 and 36). Hg residues in WR show levels close to that of WN during the spring season (0.131 ppm and 0.142 ppm, respectively).

Arsenic (As) residue levels show no consistency in area-dependent variability during the spring season. In WR, the As residues of the liver are highest (0.057 ppm), while lowest in WN (0.027 ppm) in the spring season. Small body size of winter flounder in WN during spring season may have contributed to the low levels of As residues.

Correlations can also be found between seasonal variations and levels of pollutants in the liver.

Concentration of Pollutants in the Muscle

Average residues of pollutants in selected muscle samples from each site during the two seasons are shown in Tables 37 and 38. The highest residues in muscle samples can be seen in the WN area during both seasons, except for arsenic residues. Arsenic residues in WR (0.021 ppm) are only slightly higher than that of WN (0.020 ppm) in spring season. The residue levels in muscle in the QP area are higher than those in WR, except that of Hg residues in WR (0.123 ppm), are less than in QP (0.155 ppm) in spring season.

The residue levels of liver in QP during winter were higher than that in WR, which is probably due to the higher mean length (36.2 cm) and weight (841.5 g) of the fish compared with that of WN (29.5 cm, 334.1 g) and WR (27.3 cm, 267.2 g).

According to the publication by Paulson and Brown in 1978, with one exception, PCB concentrations in fish samples from Rhode Island's fresh waters ranged from less than 10 to 419 ppb (wet weight) in 27 samples and marine waters ranged from less than 10 to 797 ppb (wet weight) in 4 samples. PCB residues (0.102-0.397 ppm) in fish muscle from this study can be seen to be in the range compared with the investigation above. These pollutant residues are not higher than the FDA tolerance level, 2 ppm, indicating that there is no serious hazard in respect to PCB contamination from Narragansett Bay. This is consistent with Paulson and Brown (1978).

Other Statistical Analyses

Other results were determined using the methods of regression, correlation, and multidimensional contingency table analysis. Table 10 illustrates the Pearson correlation coefficients and the probability of a greater value under the null hypothesis, and the sample sizes. Statistically significant correlations are frequently seen between the PCBs in the liver (PCBL) and NMA, TOTA1, MEANA1, and RAM. Other correlations also appear between the Hg in the liver (HgL) and NMA, TOTA1, and MEANA1. Only Pb in the liver (PbL)

shows a high statistical correlation between weight and length. Some interesting correlations appear between Cd in the muscle (CdM) and PCBs in the liver (PCBL), Pb in the liver (PbL), and Hg in the liver (HgL).

Contrasts between different seasons and stations were done using GLM procedure in SAS. From the analysis of the contrasts, it seems that PCBs in the liver differ among stations (Table 39), and there is no significant difference between the pollutants in the liver and QP versus WR and WN.

RECOMMENDATIONS

1) It is recommended that the water and sediment samples should also be collected from fish collecting sites for a background measure of the environmental burden in the area.
2) It is recommended that approximately similar size (weight, length, or age) of marine organisms be selected for the comparisons of the chemical variables.
3) All the pollutant residues in this fish species were lower than the FDA tolerance levels, it is recommended that emphasis be placed on the edible portion (muscle) on some other marine organisms used for human food consumption

MATERIALS AND METHODS

Sample Collections and Preparations

The winter flounder is the most abundant bottom-dwelling fish in Narragansett Bay (Jeffries and Johnson 1974; Oviatt and Nixon 1973). In 1980, the catch of winter flounder in Rhode Island totaled 8.5 million pounds and was valued at more than 2.6 million dollars (Rhode Island Department of Environmental Management 1982-83). Thus, this species is of importance in the study of residues of contaminants.

Fish captured were sent to Aquatic Pathology Laboratory, The University of Rhode Island, for dissection. Fish lengths, weights and ages determined from otoliths were measured and recorded. Internal and external examinations of the fish were made by researchers in the laboratory for gross and histopathologic examinations, as well as macrophage aggregate determinations. Individual fillets and/or pooled livers were dissected and labelled, then placed into polyethylene zip lock bags for heavy metal analysis or aluminum foil rinsed with hexane for PCB analysis. All the samples were kept frozen at -20°C in the Food Science and Nutrition Department's laboratory and thawed 24 hours at 4°C prior to analysis.

Chemical Analysis

Due to the limited weight of the livers, some livers from the same site were pooled (composited) and homogenized to reach a sufficient weight for analysis. In this study, a minimum of 3 grams and 5 grams of liver are necessary for the heavy metals and PCBs analyses, respectively.

PCBs (DeVault 1984)—

Liver and muscle were ground and extracted with pesticide-grade hexane and acetone (1:1) in a Soxhlet extractor for 16 hours. Solvent was evaporated by rotary vacuum to ca. 5 ml. The extract then was first cleaned up by alumina adsorption and then by Florsil for the elimination of lipid and

polar compounds. Elute was concentrated and diluted to a final 3 ml with hexane. The extracts were analyzed on a Tractor MT-200 Gas Chromatograph (GC) with the following instrument parameters and operating conditions.

Detector: $N1^{63}$ electron capture

Column: 1.5 percent SP-2250/1.9 percent SP-2401 on Supelcoport

Length: Diameter: 2.4 m, 3.175 mm (ID)

Injection, Column and Detector Temperature: 250, 200, and 270°C

Carrier Gas: 95 percent Argon/5 percent Methane

Flow Rate: 25 ml/min.

PCB concentrations were quantitated by comparing total area of peaks with that of standard Aroclor 1254 with a programming integrator.

Heavy Metals (AOAC 1984)—

Samples to be analyzed for Pb and Cd were dry-ashed at 500±25°C overnight. Then 2 ml HNO_3 was added and evaporated to dryness on a warm hot plate. Samples were then transferred to a furnace at 500°C to obtain practically C-free ash. Final volume was determined with 1 N HNO_3 by heating cautiously on a hot plate. Pb and Cd levels were analyzed on a Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer (AAS). H_2SO_4 and HNO_3 (1:1) were used to digest samples for Hg and As residues. A flask connected with circulating cold water was used for the extraction. No solid materials were apparent except for globules of fats. Deionized water was used to wash condenser and dilute to a final volume of 25 ml. Total Hg and As residues were analyzed in the Perkin-Elmer Model 5000 AAS connected with MHS-10 cold vapor equipment.

Reagent blanks were conducted through each batch of the chemicals used, which were prepared by methods identical to the samples. Other quality assurances (e.g., average spiked sample recoveries and direction limits) are shown below.

Quality assurance of the chemical pollutants examined

	PCBs	Pb	Cd	Hg	As
Blanks	< 0.001*	< 0.1	< 0.01	< 0.02	< 0.02
Recovery	90 ± 5%	93 ± 2%	108 ± 5%	98 ± 10%	100 ± 12%
Detection limit	0.01**	0.1	0.01	0.02	0.01

* μg/ml

** μg/g, wet weight basis

PATHOLOGICAL METHODS

The primary objective of the Narragansett Bay Project entitled *Winter Flounder Contaminant and Pathological Survey* was to assess the health of winter flounder (*Pseudopleuronectes americanus*) as it relates to environmental and tissue burdens of specific pollutants. To assess the effects of environmental degradation (increased environmental pollutant levels), fish were collected at three sites varying in their degree of impaction. These sites were:

1) near the mouth of the Providence River (Warwick Neck; heavily impacted)
2) near the mouth of the west passage (Whale Rock; moderately impacted)
3) removed form the Bay (Quonochontaug Pond; slightly impacted)

(VanVleet and Quinn 1978; Olsen and Lee 1979; Hoffman 1987). Fish were sampled in the winter and spring of 1987.

Health of the fish was assessed by means of hepatic macrophage aggregate (HMA) parameter quantification and the presence of liver lesions. The former methodology has been described for winter flounder and largemouth bass (*Micropterus salmoides*) and has been adopted as a monitoring system by the National Status and Trends Programs of the National Marine Fisheries Service (Wolke 1985a and b; Blazer et al 1987; NOAA 1984). Recent field studies have suggested that the presence of certain hepatic lesions are more indicative of degraded environments than are lesions of other organs. These lesions include hepatic vacuolar cells, atypical foci of cellular alteration and frank neoplasia (Murchelano and Wolke, 1985; Malins et al. 1984).

In addition, tissue burdens of PCB, mercury, arsenic, lead, and cadmium were determined for liver and muscle in subsets of fish from the three sites.

The design of the project allows for a number of interesting comparisons to be made among site, pollutant, and fish which include the following:

1) Health of fish as a function of site, season, and sex

   a) hepatic macrophage aggregate parameters (HMA) vs. site, all fish

      i number/sq. mm. (NMA)

      ii percent area occupied (TOTA1)

      iii mean area sq. mm. (MEANA1)

   b) HMA vs. site vs. sex

   c) HMA vs. site vs. season

   d) hepatic vacuolated cells vs. site, all fish

   e) hepatic vacuolated cells vs. site vs. season

   f) pre-neoplastic lesions vs. site, all fish

   g) pre-neoplastic lesions vs. site vs. season

2) Health of fish as a function of tissue pollutant burden

   a) HMA vs. PCB, Hg, As, Pb, and Cd

   b) hepatic vacuolated cells vs. each pollutant

   c) pre-neoplastic lesions vs. each pollutant

3) Tissue pollutant burdens as a function of site.

The comparisons allow assessment of winter flounder health and its relationship to the degree of environmental contamination and/or tissue pollutant burden.

Results

Health of winter flounder assessed by means of HMA and liver lesions as a function of site, season, and sex reveal significant differences.

All mean hepatic macrophage aggregate parameters were significantly increased in all fish from Warwick Neck (WN), the site of severest contamination, when compared to all fish from Whale Rock (WR), or Quonochontaug Pond (QP)(Table 27). Fish livers from QP had a significantly greater number of aggregates when compared to those from WR, but no differences were noted in percent area or mean size of aggregates. This study and previous studies have shown that numbers of HMAs are related to fish age (length)

(Blazer et al. 1987; Brown and George 1985). Fish from QP were far larger (35.5 cm) than fish from WR (24.3 cm) thus explaining the increase in aggregate number. When an attempt was made to correct for size and season (spring) comparing all fish between 31 and 40 centimeters, the percent area and number per square millimeter revealed little difference between the QP and WR sites (Table 30). This phenomenon underlies the importance of comparing fish of the same age when using the monitoring system.

No differences were noted between sexes by site for all fish. However, a significant difference was noted between males and females from WN for all parameters. Percent area and number per square millimeter were increased threefold, and mean size by 0.00014 square millimeter in male fish from WN (Table 29). No differences were noted between and winter and spring samples within or among sites (Table 28).

Vacuolated hepatic cell numbers revealed a gradation related to the degree of environmental contamination and similar to those present among hepatic aggregates. Differences among sites were significant at the 1 percent level (Table 27). Forty-eight livers (34.7 percent, n = 138) from WN had vacuolated cells; 20 (11 percent, n = 179) from WR, and one 1 (1 percent, n = 97) from QP (Table 27). Livers of fish from QP had the fewest of these cells per unit area (0.07/4HPF), while those from WN the most (1.89/HPF). Those from WR were intermediate in number (0.26/4HPF). However, unlike the hepatic aggregates, numbers of livers containing vacuolated cells varied seasonally and were significantly higher in the winter sample of fish (49) than the spring sample (20).

Pre-neoplastic and neoplastic lesions were few in number at all sites. The total number of hepatocellular carcinomas was 2 (1.4 percent of all liver lesions), and both were from the site of greatest contamination, Warwick Neck. The total number of pre-neoplastic lesions (foci of cellular alteration) was 13, and their distribution was 6 at WN, 4 at WR, and 3 at QP.

Fish from WN had higher hepatic burdens of all contaminants with the exception of lead which was equal to or slightly higher in livers of fish from WR (Table 31). Health of winter flounder assessed by HMA and hepatic vacuolar cells as a function of pollutant burden revealed few correlations. Of the hepatic aggregate parameters number/square millimeter was correlated with muscle PCB levels and weakly correlated with liver cadmium. Percent area was also weakly correlated with both liver and muscle PCB burdens while mean area was correlated with liver PCB. Hepatic vacuolar cells were strongly correlated with muscle PCB levels (Table 10).

STATISTICAL METHODS

Regression and Correlation Analysis

Background—The samples of winter flounder collected from the three stations were subjected to multiple regression analysis of the form:

$$\log (y+1) = a + b_1 \log x_1 + b_2 \log x_2 \qquad (1)$$

where y is the concentration of a particular contaminant —such as PCB, lead, mercury, etc.—x_1 is the length of the fish, and x_2 is the weight of the fish. The age of the fish, although desirable, was not determined for an adequate sample to be included in the analysis. In order to linearize a relation of the form $y = a\ (\text{length})^b$ and in an attempt to homogenize variances, the dependent variables as well as the independent variables were transformed to natural logarithms before the analysis was performed. The specific transform for the dependent variables was ln (x+1) in order to avoid attempting to take logarithms of zeros. The purposes of the multiple regression analysis were to determine the principal variable(s) affecting the concentrations of contaminants in winter flounder. This procedure was considered important initially in calculating required sample sizes for monitoring purposes. For example, if there is a very strong relation between a particular contaminant and the size (weight or length) of a fish, then it is possible to minimize sample variance (and sample size) by restricting comparative contaminant analyses to a particular size group.

Results—Tables 3 through 9 illustrate the statistically-significant relationships obtained between various dependent variables with length and weight as independent variables with $\alpha = 0.05$. The significant relations include PCBs, Cd, Hg, NMA, TOTA1, and MEANA1, in the liver and independent variables in length and weight. PCBs, Pb, Cd, Hg, as well as As in the muscle, also provided statistically significant multiple regressions against length and weight.

The fit of the multiple regression equations were not particularly good even though statistical significance of the regression equation was demonstrated. Note the relatively low R-square (coefficient of determination) values, the very high coefficients of variability of the contaminants, and the high root mean square error terms. The amount of variability in the analytical results was particularly high and contributed considerably to the "noise" in their analyses. In general, it appeared that weight was consistently a slightly better independent variable than length for PCBs and all elemental analyses. However, length seemed slightly better with respect to macrophage aggregates as the response variable. The reasons for this difference are not known at present, nor can they be objectively determined on the basis of available samples.

From the results of these preliminary analyses, if only one independent variable is used, it is suggested that weight be utilized in place of length. A small saving in sample size may be possible by analyzing samples within similar weight groups. However, this does not appear to be as substantial as has been reported elsewhere. Indeed, as will be demonstrated in the generalized linear models analysis, length groups, hence weight, were frequently non-significant contributors to the total variability accounted for in the generalized linear model. It is believed that if a greater range of sizes had been available for analysis, the affect of size (length or weight) would have been more important.

Table 10 illustrates the Pearson correlation coefficients which are statistically significant at the 95 percent confidence level, the probability of a greater value under the null hypothesis that the correlation is zero, and the sample sizes.

Some interesting correlations include: cadmium in the muscle (CDM) with PCBs in the liver (PCBL), lead in the liver (PBL), mercury in the liver (HGL); number of macrophage aggregates (NMA) with mercury in liver (PBL), PCBs in muscle (PCBM); mean area of macrophage aggregates (MEANA1) with PCBs in liver (PCBL), and arsenic in muscle (ASM); level of RAM cells with PBCs in liver, PCBs in muscle and arsenic in muscle. There are certainly other correlations which also may have interesting further

implications. However, it should be recognized that significant correlations do not imply any causality, and that statistically significant correlations based on large samples may not have much biological relevance.

Sample Size Estimates

Background—Estimation of field population parameters (including levels of incidence of contaminants or diseases) is a vital part of many monitoring and management activities, such as the Narragansett Bay Project. It is obvious to most people that the greater the sample size, the more reliable the estimates become. However, the cost per unit sampled is often very substantial in the case of certain contaminants (i.e., PCBs) and certain disease conditions involving extensive histopathology. Therefore, it is clearly unwise to process unnecessarily large samples, and to have a scientifically sound procedure for determining sample sizes required to meet previously defined monitoring requirements.

This material is provided to help determine in advance the smallest sample size that would produce a desired reliability of the estimate. Such a sample is sometimes called an optimum sample size. There are various formulas and methods available for sample size estimation, and these depend on the way one choses to define reliability.

Our definition of reliability is provided as follows: If one takes a sample of size n from a distribution having mean μ and variance σ^2, then according to the central limit theorem, for a sufficiently large sample size, the following probabilistic statement holds.

$$P\left(x - z_{\alpha/2}\frac{\sigma}{\sqrt{n}} < \mu < x + z_{\alpha/2}\frac{\sigma}{\sqrt{n}}\right) \sim 1 - \alpha \qquad (2)$$

where :

x = sample mean

$1-\alpha$ = the confidence coefficient

$z_{\alpha/2}$ = the upper $\alpha/2$ point of the standard normal distribution.

For $(1-\alpha) = 0.95$, $z_{\alpha/2} = 1.96$. The relation in Equation (2) states that the confidence interval for μ :

$$x \pm z_{\alpha/2} \frac{\sigma}{\sqrt{n}} \quad (3)$$

will include the mean μ with probability approximately equal to $(1 - \alpha)$ regardless of the form of the parent distribution provided that it has a finite second moment.

There are now three quantities: n,. $(1 - \alpha)$, and the length of the interval given in Equation 3. One must decide on the value of two of these quantities, and a value for the third, say n, which is a sample size estimate, can be determined. The value of $(1 - \alpha)$ is usually set at the 0.95 or perhaps 0.99 levels. A common and straightforward way to decide upon the interval (Equation 4) is to set its half-length equal to a fixed proportion D of the mean μ and solve for μ.

From:

$$z_{\alpha/2} \frac{\sigma}{\sqrt{n}} = D\mu, \quad (4)$$

we obtain the general formula:

$$n = (\frac{z\alpha/2}{D})^2 \frac{\sigma^2}{\mu^2} \quad (5)$$

This is the formula given by Southwood (1972) p. 19. This equation, Equation (5), has been utilized in the computation of the estimated required sample sizes for various elemental contaminants and liver anomalies under various constraints. The sample means and standard deviations are derived from Table 11, which describes the overall values for both statistics. D was varied, with chosen values of 0.10, 0.25 and 0.40. The value of α was maintained at 0.05 so that $z_{\alpha/2}$ was equal to 1.96 in all cases. The estimated required sample sizes are shown in Table 12 for the above conditions. Clearly, it would be possible to change α and the value of D easily if this were desired. Table 12 illustrates that the number of samples required to detect reasonable (25 percent) differences in the mean values of the metals Pb, Cd, Hg, and As are relatively small—about 15. On the other hand, differences of 0.1 (i.e., 10 percent differences) in the mean require up to six times more

samples. Also, it is evident that small differences in some variables are difficult to detect without very large samples. This is due to the larger variance in this material.

Multidimensional Contingency Table Analysis

Only a brief introduction to this complex subject will be provided herein. The interested reader is referred to suitable texts such as Feinberg (1980) for details. This method has much in common with regression analysis and factorial analysis of variance. As with the above two techniques, the procedure involves selecting from a sequence of linear models that model with the smallest number of parameters that fit well. For each model, the parameters are estimated by maximum likelihood, and all frequencies predicted by the model are calculated. The fit of the model is then tested.

Consider a simple two-dimensional example to provide a few details. Assume a table with I rows and J columns is used to determine whether or not two categorical variables are related. The null hypothesis specifies a model for cell probabilities P_{ij} of the form:

$$P_{ij} = P_{i+} P_{+j}$$

where i = 1, ..., I and j = 1, ..., J. The corresponding expression for the expected cell frequencies m_{ij} is:

$$m_{ij} = N p_{i+} p_{+j}$$

The replacement of a subscript by the symbol + denotes that the frequencies have been summed over that index. The above is a multiplicative model which may be transformed to a logarithmic model by taking logarithms. That is:

$$\log m_{ij} = \log N + \log P_{i+} + \log P_{+j}$$

Using other notation, a more general log-linear model may be written as:

$$\log m_{ij} = u + u_{1(i)} + u_{2(i)} + u_{12(ij)} \qquad (6)$$

when i = 1, ..., I; j = 1, ..., J. In Equation (1) the grand mean term u is an average of the log-expected frequencies over all cells: That is:

$$u = \frac{1}{IJ} \sum_{i=1}^{I} \sum_{j=1}^{J} \log m_{ij}$$

whereas the main effect terms $u_{1(i)}$ and $u_{2(i)}$ and the interaction term $u_{12(ij)}$ are deviations, as follows:

$$u_{1(i)} = \frac{1}{J} \sum_{j=1}^{I} (\log m_{ij} - u) \quad i = 1, ..., I$$

$$u_{2(j)} = \frac{1}{I} \sum_{i=1}^{I} (\log m_{ij} - u) \quad ij = 1, ..., J$$

$$u_{12(ij)} = \log m_{ij} - u - u_{1(i)} - u_{2(j)} \quad i = 1, ..., I; j = 2, ..., J.$$

As in the analysis of variance, the deviation terms sum to zero. That is:

$$u_{1(+)} = u_{2(+)} = u_{12(+j)} = u_{12(i+)} = 0.$$

There are 18 possible models for a three-way multidimensional contingency table analysis and 113 for a four-way classification. Clearly, one must be somewhat parsimonious in the choice of models. The "best" model (parsimonious model) was developed the following way. A first hypothesis was tested, including all the four factors to be tested (season, station, length class, and sex) and all the possible two way, three way, and four way interactions. Non-significant interactions and non-significant main factors not involved in any significant interaction were developed from the model. The remaining was then considered the "best" model." This procedure was repeated for the dependent variables: PCBM, PBM, CDM, HGM, ASM, NMA, TOTA1, MEANA1 and RAM. The initial four dimensional model involves two seasons, three stations, two sexes, and four length classes. For the variables selected with contaminant levels in the liver, PCBL, PBL, HGL, CDL, and ASL, only two main factors (season and station) were included in the initial model since some of the data corresponded to a pooled value from more than one specimen, and the fish pooled not always had the same sex or belonged to the same length class.

Tables 13 to 26 illustrate the "best" model for each one of the variables considered. The tables correspond to the output from SAS using PROC GLM. The values for the SS,

F value were PR >F for each one of the effects included in the model correspond to model type III (see manual reference). In all cases, the model provides a significant fit.

The effects of SEASON and STATION were generally significant with the exception of STATION being non-significant in the case of mercury in muscle (Table 21) and SEASON being non-significant in the case of cadmium in liver (Table 15), arsenic in the muscle (Table 22). The interaction between SEASON and STATION seemed more independent in the case of muscle tissue than in the case of liver.

Tables 23 to 26 deal with anomalous liver conditions as the dependent variable. In the case of these dependent variables—NMA, TOTA1, MEANA1, and RAM—it was found that STATION effects were consistently significant, but that SEASON and the interaction between SEASON and STATION were variable. This may have been due to high variability in the dependent variable

Relationship of Pollutant Residues vs. Site, Season, LClass, and Sex

The following tables show the interactions between levels of pollutant residues in the liver and muscle versus various season, station, length class, and sex. An analysis of variance (ANOVA) method was applied in these two tables, and a logarithmic model was developed in the multi-dimensional contingency table analysis. Two seasons, three sites, four length classes, and sex are included as the four factors to be tested, and each pollutant in the liver and muscle was selected as the dependent variable for the interactions. Only the individual liver samples were chosen for this factorial analysis of variance, for the data from the pooled liver samples may not really reflect the value corresponding to the main independent variables.

Highly significantly differences ($P < 0.01$) could be generally observed between seasons and pollutant residues in the muscle and liver, except for cadmium and arsenic in the liver and arsenic in the muscle. The effects of stations and pollutant residues in the muscle and liver were mostly significant, but non-significant in the liver for cadmium and in the muscle for mercury. For the independent variable of length class, it seems only lead

levels in the muscle and liver show consistent significance. None of the interactions between the length class and pollutant residues in the liver and muscle were sufficient, except for PCB residues in the liver. However, only PCB residues in the liver have significant differences between male and female fish. None of the heavy metal residues in the fish tissues (muscle or liver) and sex were significantly associated.

ANOVA for pollutant residues in the liver using transformed date
(number of observations = 105)

	Seasons	Stations	LCLASS	Sex
PCBL	0.0009* 11.75**	0.0001 27.56	0.025 3.85	0.006 7.90
PbL	0.0001 30.53	0.0001 10.09	0.018 4.18	NS
CdL	NS	NS	NS	NS
HgL	0.0001 25.86	0.007 5.26	NS	NS
AsL	NS	0.002 6.83	NS	NS

*Probability > F value **F values

ANOVA for pollutant residues in the muscle using transformed data
(number of observations = 84)

	Seasons	Stations	LCLASS	Sex
PCBM	0.0001* 32.93**	0.0001 19.34	NS	NS
PbM	0.008 3.13	0.0001 10.78	0.025 3.34	NS
CdM	0.0001 40.86	0.0001 11.26	0.0001 9.80	NS
HgM	0.0001 18.53	NS	NS	NS
AsM	NS	0.0001 11.56	NS	NS

*Probability > F value **F values

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Figure 1. Location of three selected sites for winter flounder collected around Narragansett Bay, Rhode Island, during 1986-87.

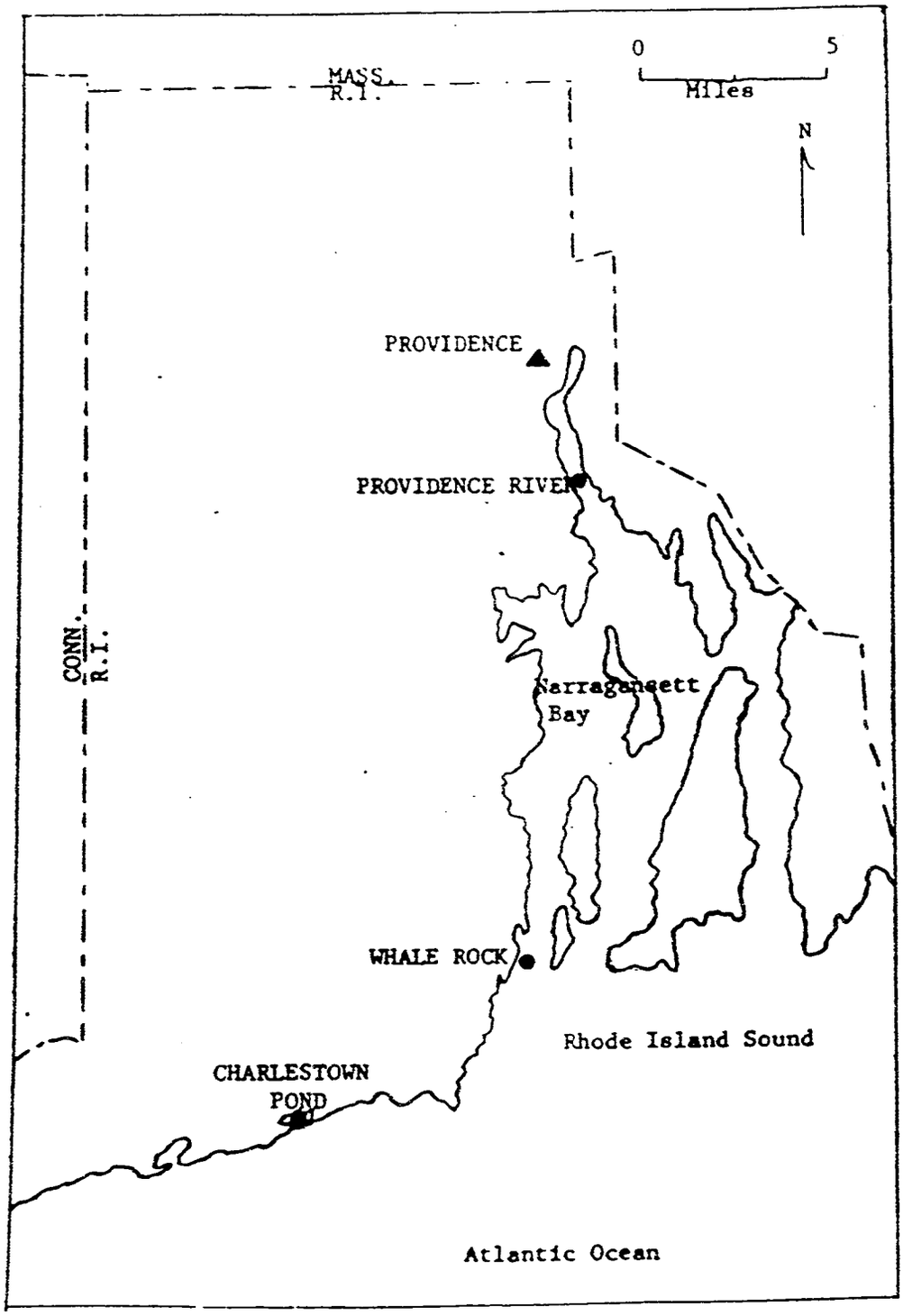

Table 1. Dates, sites, and number of winter flounder samples collected by the comparative Aquatic Pathology Lab, FAVS, The University of Rhode Island, Kingston

Date	Sites	Sample Codes	Number of Fish Collected
11/26/86	QP*	Q612-Q639	28
12/9/86	WR**	Q640-Q659	20
]12/10/86	WN***	Q662-Q665	4
12/12/86	WN	Q668-Q705	38
12/16/86	WR	Q706-Q723	18
12/22/86	WR	Q724-Q741	18
12/29/86	WR	Q743-Q766	24
1/6/87	WR	R1-R5	5
2/12/87	WN	R33-R65	33

Winter season total number of fish collected: 188

Date	Sites	Sample Codes	Number of Fish Collected
5/19/87	WR	R187-R213	27
5/27/87	WR	R218-R284	67
6/2/87	QP	R285-R327	43
6/5/87	QP	R328-R353	26
6/9/87	WN	R356-R418	63

Spring season total number of fish collected: 226

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* Quonochontaug Pond

** Whale Rock

*** Warwick Neck

Table 2. List of coded variables, sample size, definition and units of measure employed in this study

Coded Variable	Sample Size	Definition and Units
WEIGHT	378	individual weight (grams)
LENGTH	379	total length (millimeters)
LCLASS	379	arbitrary decision of length into 10 cm size classes
PCBL	175	polychlorinated biphenyls in liver (µg/g wet weight)
PBL	175	lead in liver (µg/g wet weight)
CDL	175	cadmium in liver (µg/g wet weight)
HGL	175	mercury in liver (µg/g wet weight)
ASL	175	arsenic in liver (µg/g wet weight)
PCBM	81	polychlorinated biphenyls in muscle (µg/g wet weight)
PBM	81	lead in muscle (µg/g wet weight)
CDM	81	cadmium in muscle (µg/g wet weight)
HGM	81	mercury in muscle (µg/g wet weight)
ASM	379	arsenic in muscle (µg/g wet weight)
NMA	379	number of macrophage aggregates in liver/square mm
TOTA1	379	total area of macrophage aggregates/square mm (percent)
MEANA1	379	mean area of macrophage aggregates (TOTA1/NMA/)
RAM	379	number/4 HPF (vacuolated cell count)

Table 3. Multiple regression of PCBs in liver on length and weight, with the independent variables transformed to natural logarithms and the dependent variable transformed by ln (y+1)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	0.3330	0.1665	4.231	0.0172
Error	102	4.0133	0.0393		
Total	104	4.3463			

Summary Statistics

Root M.S.E.	0.1984
Mean	0.3159
C.V.	62.7931
R-Square	0.0766
Adj. R-Square	0.0585

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t for HO: Parameter=0	Prob>|t|
Intercept	1	-2.1816	1.1434	-1.908	0.0592
Length	1	1.6191	0.6292	2.573	0.0115
Weight	1	-0.5.082	0.1805	-2.816	0.0058

Table 4. Multiple regression of Pb in liver on length and weight (transformation similar to Table 3)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	10.1775	5.0887	32.634	0.0001
Error	102	15.9053	0.1559		
Total	104	26.0828			

Summary Statistics

Root M.S.E.	0.3949
Mean	0.7123
C.V.	55.4409
R-Square	0.3902
Adj. R-Square	0.3782

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t for HO: Parameter=0	Prob>|t|
Intercept	1	8.0405	2.2763	3.532	0.0006
Length	1	-1.7502	1.2525	-1.397	0.1654
Weight	1	-0.1677	0.3593	-0.467	0.6417

Table 5. Multiple regression of Cd in liver on length and weight (transformation similar to Table 3)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	0.0130	0.0065	0.388	0.6794
Error	102	1.7155	0.0168		
Total	3104	1.7286			

Summary Statistics

Root M.S.E.	0.1297
Mean	0.1892
C.V.	68.5550
R-Square	0.0075
Adj. R-Square	-0.0119

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t for HO: Parameter=0	Prob>|t|
Intercept	1	0.5529	0.7476	0.740	0.4612
Length	1	-0.1240	0.4113	-0.301	0.7637
Weight	1	0.0122	0.1180	0.103	0.9179

Table 6. Multiple regression of Hg in liver on length and weight (transformation similar to Table 3)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	0.1190	0.0595	4.751	0.0107
Error	102	1.2770	0.0125		
Total	104	1.3960			

Summary Statistics

Root M.S.E.	0.1119
Mean	0.1504
C.V.	74.3746
R-Square	0.0852
Adj. R-Square	0.0673

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t for HO: Parameter=0	Prob>|t|
Intercept	1	-1.8374	0.6450	-2.849	0.0053
Length	1	1.06418	0.3549	2.998	0.0034
Weight	1	-0.2804	0.1018	-2.754	0.0070

Table 7. Multiple regression of NMA (number of macrophage aggregates per square mm) in liver on length and weight (transformation similar to Table 3)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	55.1922	27.5961	29.468	0.0001
Error	410	383.9591	0.9365		
Total	412	439.1513			

Summary Statistics

Root M.S.E.	0.9677
Mean	0.7256
C.V.	133.3675
R-Square	0.1257
Adj. R-Square	0.1214

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t for HO: Parameter=0	Prob>|t|
Intercept	1	-12.3747	1.9908	-6.216	0.0001
Length	1	7.1085	1.2034	5.907	0.0001
Weight	1	-1.8753	0.3661	-5.122	0.0001

Table 8. Multiple regression of TOTA1 (total area of macrophage aggregates per square mm) in liver on length and weight (transformation similar to Table 3)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	853.3898	446.6949	33.996	0.0001
Error	408	5145.9801	12.5512		
Total	410	5999.3699			

Summary Statistics

Root M.S.E.	3.5428
Mean	2.9571
C.V.	119.8039
R-Square	0.1422
Adj. R-Square	0.1381

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t for HQ: Parameter=0	Prob>|t|
Intercept	1	-46.0087	7.2883	-6.5313	0.0001
Length	1	26.0729	4.4055	5.918	0.0001
Weight	1	-6.7149	1.3403	-5.010	0.0001

Table 9. Multiple regression of MEANA1 (mean area of macrophage aggregates) in liver on length and weight (transformation similar to Table 3)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	2	509.8036	254.9018	33.372	0.0001
Error	410	3131.6358	7.6381		
Total	412	3641.4394			

Summary Statistics

Root M.S.E.	2.7637
Mean	2.3240
C.V.	118.9187
R-Square	0.1400
Adj. R-Square	0.1358

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t for HO: Parameter=0	Prob>|t|
Intercept	1	-34.6779	5.6856	-6.099	0.0001
Length	1	19.5421	3.4368	5.686	0.0001
Weight	1	-4.9792	1.0456	-4.762	0.0001

Table 10. Correlation half-matric of variables used in this study. Three values: the Pearson coefficient, the probability of a greater value under a null hypothesis of Ho: rho = 0, and the number of observations are provided for each paried comparison by rows respectively.

MEANA1		WEIGHT	LENGTH	PCBL	PBL	CDL	HOL	ASL	PCBM	PBM	CDM	HGM	ASM	NMA	TOTA1	MEANA1	RAM
0.19 0.00 413	WEIGHT	1.00 0.00 413	0.90 0.00 413	NS	-0.45 0.02 105	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.15 0.00 413	0.19 0.00 413	NS
0.27 0.00 414	LENGTH	0.90 0.00 413	1.00 0.00 414	NS	-0.53 0.00 105	NS	NS	NS	NS	NS	NS	NS	NS	0.18 0.00 414	0.22 0.00 414	0.27 0.00 414	NS
0.47 0.00 105	PCBL	NS	NS	1.00 0.00 105	0.31 0.00 105	0.31 0.00 105	0.20 0.04 105	0.20 0.00 105	NS	NS	0.50 0.00 33	NS	NS	NS	NS	0.47 0.00 105	0.22 0.02 105
NS	PBL	-0.45 0.02 105	-0.53 0.00 105	0.31 0.00 105	1.00 0.00 105	0.50 0.00 105	-0.25 0.00 105	NS	NS	NS	-0.51 0.00 33	NS	NS	NS	NS	NS	NS
NS	CDL	NS	NS	0.31 0.00 105	0.50 0.00 105	1.00 0.00 105	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NS	HOL	NS	NS	0.20 0.04 105	-0.25 0.00 105	NS	1.00 0.00 105	0.72 0.00 105	NS	NS	0.54 0.00 33	NS	NS	0.24 0.01 105	NS	NS	NS
NS	ASL	NS	NS	0.20 0.00 105	NS	NS	0.72 0.00 105	1.00 0.00 105	NS	NS	NS	NS	0.55 0.00 33	NS	NS	NS	NS
NS	PCBM	NS	NS	NS	NS	NS	NS	NS	1.00 0.00 84	NS	NS	0.38 0.00 84	0.22 0.04 84	0.23 0.03 84	NS	NS	0.38 0.00 84
NS	PBM	NS	NS	NS	NS	NS	NS	NS	NS	1.00 0.00 84	0.67 0.00 84	NS	NS	NS	NS	NS	NS
NS	CDM	NS	NS	0.50 0.00 33	-0.51 0.00 33	NS	0.54 0.00 33	NS	NS	0.67 0.00 84	1.00 0.00 84	NS	NS	NS	NS	NS	NS
NS	HGM	NS	NS	NS	NS	NS	NS	NS	0.38 0.00 84	NS	NS	1.00 0.00 84	NS	NS	NS	NS	NS
0.26 0.02 84	ASM	NS	NS	NS	NS	NS	NS	0.55 0.00 33	0.22 0.04 84	NS	NS	NS	1.00 0.00 84	NS	NS	0.26 0.02 84	0.24 0.03 84
0.48 0.00 414	NMA	NS	0.18 0.00 414	NS	NS	NS	0.24 0.01 105	NS	0.23 0.03 84	NS	NS	NS	NS	1.00 0.00 414	0.83 0.00 414	0.48 0.00 414	0.25 0.00 414
0.63 0.00 414	TOTA1	0.15 0.00 413	0.22 0.00 414	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.83 0.00 414	1.00 0.00 414	0.63 0.00 414	0.23 0.00 414
1.00 0.00 414	MEANA1	0.19 0.00 413	0.27 0.00 414	0.47 0.00 105	NS	NS	NS	NS	NS	NS	NS	NS	0.26 0.02 84	0.48 0.00 414	0.63 0.00 414	1.00 0.00 414	0.13 0.01 414
0.13 0.01 414	RAM	NS	NS	0.22 0.02 105	NS	NS	NS	NS	0.38 0.00 84	NS	NS	NS	0.24 0.03 84	0.25 0.00 414	0.23 0.00 414	0.13 0.01 414	1.00 0.00 414

Table 11. Summary statistics for all variables used in the analyses. All metals and PCB are expressed in parts per million, weight in grams, and length in centimeters.

Variable	N	Mean	Std. Dev.	Minimum	Maximum
WEIGHT	413	373.6	284.896	10	1563
LENGTH	414	28.6	6.872	10	44
PCBL	175	0.511	0.374	0.007	3.556
PBL	175	1.056	1.266	0.127	9.091
CDL	175	0.274	0.217	0.044	1.864
HGL	175	0.263	0.211	0.009	1.568
ASL	175	0.048	0.119	0.006	1.467
PCBM	84	0.193	0.129	0.033	0.827
PBM	84	0.579	0.171	0.217	1.031
CDM	84	0.175	0.076	0.066	0.365
HGM	84	0.151	0.075	0.022	0.522
ASM	84	0.019	0.009	0.006	0.043
NMA	414	3.031	6.075	0	41
TOTA1	414	1634.1	4197.6	0	40623
MEANA1	414	202.4	349.9	0	2422.9
RAM	414	0.76	2.53	0	26

Table 12. Optimum sample sizes (n) for a = 0.05 and for three values of D, the fixed proportion of the mean, for some contaminants found in winter flounder samples from Narragansett Bay and vicinity

	D Values		
Contaminant	0.1	0.25	0.40
PCBL	164	26	10
PBL	308	49	19
CDL	266	44	17
HGL	243	39	15
ASL	1,291	206	81
PCBM	171	27	11
PBM	34	10	10
CDM	72	12	10
HGM	96	15	10
ASM	86	13	10
NMA*	132	21	10
TOTA1	2,239	358	140
MEANA1	1,128	180	70
RAM*	505	81	32

*sample sizes for these conditions were based on an assumed Poisson distribution

Table 13. ANOVA for PCBs in liver (PCBL) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR

DEPENDENT VARIABLE: PCBL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	3	2.7674	0.9225	32.75	0.0001
ERROR	171	4.8166	0.0282		
CORRECTED TOTAL	174	7.5840			

R-SQUARE	C.V.	ROOT M.S.E.	PCBL MEAN
0.3649	46.88	0.1678	0.358

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	0.6304	22.38	0.0001
STATION	2	2.5581	45.41	0.0001

Table 14. ANOVA for Pb in liver (PBL) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR

DEPENDENT VARIABLE: PBL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	3	16.5651	5.5217	72.38	0.0001
ERROR	171	13.0451	0.0763		
CORRECTED TOTAL	174	29. 6102			

R-SQUARE	C.V.	ROOT M.S.E.	PBL MEAN
0.5479	34.18	0.2243	0.656

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	10.9146	143.03	0.0001
STATION	2	2.0649	13.53	0.0001

Table 15. ANOVA for Cd in liver (CDL) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
STATION	3	QP WN WR

DEPENDENT VARIABLE: CDL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	3	0.2584	0.0861	5.21	0.0018
ERROR	171	2.8278	0.0165		
CORRECTED TOTAL	174	3.0862			

R-SQUARE	C.V.	ROOT M.S.E.	CDL MEAN
0.0837	61.57	0.1286	0.209

SOURCE	DF	SS	F VALUE	PR>F
STATION	2	0.2210	6.63	0.0017

Table 16. ANOVA for Hg in liver (HGL) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR

DEPENDENT VARIABLE: HGL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	3	1.2979	0.4326	32.13	0.0001
ERROR	171	2.3023	0.0135		
CORRECTED TOTAL	174	3.6002			

R-SQUARE	C.V.	ROOT M.S.E.	HGL MEAN
0.3605	60.78	0.1160	0.191

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	1.1179	83.03	0.0001
STATION	2	0.4958	18.41	0.0001

Table 17. ANOVA for As in liver (ASL) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR

DEPENDENT VARIABLE: ASL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	5	0.0730	0.0146	3.62	0.0306
ERROR	169	0.9737	0.0058		
CORRECTED TOTAL	174	1.0467			

R-SQUARE	C.V.	ROOT M.S.E.	ASL MEAN
0.0697	154.59	0.0759	0.0491

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	0.0010	0.18	0.6759
STATION	2	0.0175	1.52	0.2209
SEASON*STATION	2	0.0517	4.49	0.0126

Table 18. ANOVA for PCBs in muscle (PCBM) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR

DEPENDENT VARIABLE: PCBM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	5	0.4098	0.0820	16.54	0.0001
ERROR	78	0.3864	0.0050		
CORRECTED TOTAL	83	0.7962			

R-SQUARE	C.V.	ROOT M.S.E.	PCBM MEAN
0.515	41.0760	0.0704	0.171

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	0.1631	32.93	0.0001
STATION	2	0.1916	19.34	0.0001
SEASON*STATION	2	0.0550	5.55	0.0056

Table 19. ANOVA for Pb in muscle (PBM) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR

DEPENDENT VARIABLE: PBM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	5	0.2980	0.0596	6.78	0.0001
ERROR	78	0.6860	0.0088		
CORRECTED TOTAL	83	0.9841			

R-SQUARE	C.V.	ROOT M.S.E.	PBM MEAN
0.3028	20.81	0.0938	0.450

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	0.0275	3.13	0.0806
STATION	2	0.1897	10.78	0.0001
SEASON*STATION	2	0.0808	4.59	0.0130

Table 20. ANOVA for Cd in muscle (CDM) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR
LCLASS	4	15 25 35 45

DEPENDENT VARIABLE: CDM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	5	0.1872	0.0234	11.33	0.0001
ERROR	78	0.1549	0.0021		
CORRECTED TOTAL	83	0.3421			

R-SQUARE	C.V.	ROOT M.S.E.	CDM MEAN
0.5471	28.51	0.0455	0.159

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	0.0727	435.21	0.0001
STATION	2	0.0513	12.42	0.0001
LCLASS	3	0.1179	2.90	0.0407
SEASON*STATION	2	0.0365	68.84	0.0004

Table 21. ANOVA for Hg in muscle (HGM) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W

DEPENDENT VARIABLE: HGM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	1	0.0605	0.0605	18.99	0.0001
ERROR	82	0.2611	0.0032		
CORRECTED TOTAL	83	0.3215			

R-SQUARE	C.V.	ROOT M.S.E.	HGM MEAN
0.1880	40.75	0.0564	0.138

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	0.0605	18.99	0.0001

Table 22 ANOVA for As in muscle (ASM) using transformed data

General Linear Models Procedure

CLASS	LEVELS	VALUES
STATION	3	QP WN WR

DEPENDENT VARIABLE: ASM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	2	0.0014	0.0007	11.19	0.0001
ERROR	81	0.0050	0.0001		
CORRECTED TOTAL	83	0.0064			

R-SQUARE	C.V.	ROOT M.S.E.	ASM MEAN
0.2165	41.04	0.0078	0.019

SOURCE	DF	SS	F VALUE	PR>F
STATION	2	0.0014	11.19	0.0001

Table 23. ANOVA for number of MA (macrophage aggregates)(NMA)

General Linear Models Procedure

CLASS	LEVELS	VALUES
STATION	3	QP WN WR
SEX	2	F M

DEPENDENT VARIABLE: NMA

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	5	157.3364	31.4673	44.58	0.0001
ERROR	373	263.2869	0.7586		
CORRECTED TOTAL	378	420.6233			

R-SQUARE	C.V.	ROOT M.S.E.	NMA MEAN
0.3740	106.0568	0.8402	0.7922

SOURCE	DF	SS	F VALUE	PR>F
STATION	2	116.3148	82.39	0.0001
SEX	1	15.8294	22.43	0.0001
SEASON*STATION	2	23.2663	16.48	0.0001

Table 24. ANOVA for percent area of MA (macrophage aggregates)(TOTA1)

General Linear Models Procedure

CLASS	LEVELS	VALUES
STATION	3	QP WN WR
LCLASS	4	15 25 35 45
SEX	2	M F

DEPENDENT VARIABLE: TOTA1

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	8	1996.9569	249.6196	25.07	0.0001
ERROR	370	3684.5568	9.9583		
CORRECTED TOTAL	378	5681.5134			

R-SQUARE	C.V.	ROOT M.S.E.	TOTA1 MEAN
0.3515	97.86	3.1557	3.2246

SOURCE	DF	SS	F VALUE	PR>F
STATION	2	1291.6185	64.85	0.0001
LCLASS	3	210.3026	7.85	0.0001
SEX	1	218.5586	21.95	0.0001
STATION*SEX	2	128.5650	6.46	0.0018

Table 25. ANOVA for mean area of MA (macrophage aggregates)(MEANA1)

General Linear Models Procedure

CLASS	LEVELS	VALUES
STATION	3	QP WN WR
LCLASS	4	15 25 35 45

DEPENDENT VARIABLE: MEANA1

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	10	1148.0115	114.8081	18.48	0.0001
ERROR	403	2503.9123	6.2132		
CORRECTED TOTAL	413	3651.9938			

R-SQUARE	C.V.	ROOT M.S.E.	MEANA1 MEAN
0.3144	106.89	2.4926	2.33

SOURCE	DF	SS	F VALUE	PR>F
STATION	2	136.4733	10.98	0.0001
LCLASS	3	69.7569	3.74	0.0113
STATION*LCLASS	5	94.4769	3.04	0.0104

Table 26. ANOVA for vacuolated cells (RAM)

General Linear Models Procedure

CLASS	LEVELS	VALUES
SEASON	2	S W
STATION	3	QP WN WR
SEX	2	M F

DEPENDENT VARIABLE: RAM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR>F
MODEL	11	41.8369	3.8033	12.36	0.0001
ERROR	367	112.9176	0.3077		
CORRECTED TOTAL	378	154.7545			

R-SQUARE	C.V.	ROOT M.S.E.	RAM MEAN
0.2703	217.446	0.5547	0.255

SOURCE	DF	SS	F VALUE	PR>F
SEASON	1	5.2342	17.01	0.0001
STATION	2	16.5615	26.91	0.0001
SEX	2	0.4593	1.49	0.2226
SEASON*STATION	2	6.5209	10.60	0.0001
SEASON*STATION*SEX	5	5.9310	3.86	0.0020

Table 27. Comparisons of hepatic macrophage aggregate mean parameters and vacuolated cells (RAM) by site, all fish

Site	n	x cm	Hepatic Aggregates			VC/4HPF
			% area	#/mm^2	size (mm^2 x 10^{-4})	
QP[1]	97	35.5	0.21^a	2.79^a	2.32^a	0.07^a
WR[2]	179	24.3	0.02^a	0.52^b	0.60^a	0.26^b
WN[3]	138	29.5	0.31^b	6.46^c	3.65^b	1.89^c

[1]QP = Quonochontaug Pond (minimal contamination)
[2]WR = Whale Rock (moderate contamination)
[3]WN = Warwick Neck (severe contamination)

Means with the same superscript are not significantly different from each other at the 5 percent level of probability.

Table 28. Comparison of hepatic macrophage aggregate mean parameters by site, by season, all fish

Site	n		x cm		% area		#/mm^2		size (mm^2 x 10^{-4})	
	W	S	W	S	W^a	S^a	W^a	S^a	W^a	S^a
QP[1]	28	69	34.5	35.9	0.11^a	0.26^a	1.25^a	3.42^a	1.4^a	2.7^a
WR[2]	85	94	26.7	82.2	0.03^a	0.02^a	0.72^a	0.34^a	0.5^a	0.6^a
WN[3]	75	63	29.4	29.6	0.37^a	0.24^a	4.20^a	5.60^a	3.6^a	3.7^a

[1]QP = Quonochontaug Pond
[2]WR = Whale Rock
[3]WN = Warwick Neck

Means with the same superscript are not significantly different from each other at the 5 percent level of probability for site subsets.

Table 29. Comparison of male and female winter flounder by site, all fish

Parameter	QP[1] m	QP[1] f	WR[2] m	WR[2] f	WN[3] m	WN[3] f
n	28	65	49	102	56	79
xcm	32.6	37.7	26.1	25.2	28.3	30.4
% area	0.13[a]	0.27[a]	0.04[a]	0.02[a]	0.56[a]	0.15[b]
#/mm^2	2.10[a]	3.20[a]	0.98[a]	0.44[a]	11.3[a]	3.2[b]
size (mm^2 x 10^{-4})	2.29[a]	2.48[a]	0.90[a]	0.63[a]	4.5[a]	3.1[b]

[1]QP = Quonochontaug Pond
[2]WR = Whale Rock
[3]WN = Warwick Neck

Means with the same superscript are not significantly different from each other at the 5 percent level of probability.

Table 30. Comparison of winter flounder hepatic macrophage mean parameters at three sites of varying environmental degradation by season[1], by length class

Site	n		L. class	% area		#/mm^2		size (mm^2 x 10^{-4})	
	W	S		W	S	W	S	W	S
QP[2]	3	0	10.0 - 20.0 cm	---	---	---	---	---	---
WR[3]	4	29		0.01	0.005	0.25	0.03	1.1	0.5
WN[4]	2	0		0.46	---	14.0	---	3.6	---
QP	1	8	21.0 - 30.0 cm	0.00	0.05	0.00	1.63	0.00	0.03
WR	61	61		0.02	0.01	0.49	0.33	0.5	0.5
WN	38	30		0.31	0.18	6.84	5.63	3.2	3.1
QP	18	40	31.0 - 40.0 cm	0.16	0.23	1.66	2.78	1.8	2.1
WR	20	4		0.05	0.20	1.50	2.75	0.6	4.4
WN	34	32		0.44	0.31	7.44	5.65	4.2	4.4
QP	6	21	41.0 -α	0.36	0.39	0.83	5.33	1.3	4.6
WR	0	0		---	---	---	---	---	---
WN	1	1		0.00	0.00	0.00	0.00	0.00	0.00

[1]Seasons were winter and spring 1987
[2]QP = Quonochontaug Pond
[3]WR = Whale Rock
[4]WN = Warwick Neck

Table 31. Comparison of winter flounder hepatic pollutant levels (ppm) by site and by season

Site	n			cm	all fish					winter					spring				
	A	W	S		PCB	Cd	As	Pb	Hg	PCB	Cd	As	Pb	Hg	PCB	Cd	As	Pb	Hg
QP[1]	0	0	0	10-20.0	ND					ND					ND				
WR[2]	0	0	0		ND					ND					ND				
WN[3]	0	0	0		ND					ND					ND				
QP[1]	1	1	0	21.0-30.00	.04	.14	.04	1.7	.02	.04	.14	.04	1.7	.02	ND				
WR[2]	10	8	2		.27	.21	.05	3.5	.16	.20	.22	.03	4.1	.09	.55	.18	.11	1.2	.44
WN[3]	2	2	0		2.0	.55	.12	5.9	.17	2.0	.55	.12	5.9	.17	ND				
QP[1]	45	13	32	31.0-40.00	.35	.23	.04	.87	.16	.27	.36	.03	1.9	.05	.38	.17	.04	.45	.21
WR[2]	11	9	2		.31	.22	.05	1.9	.13	.27	.18	.04	2.0	.12	.50	.41	.07	1.2	.20
WN[3]	11	11	0		.71	.21	.19	1.9	.21	.71	.21	.19	1.9	.21	ND				
QP[1]	24	5	19	41.α	.35	.19	.03	.54	.20	.20	.23	.03	1.3	.05	.39	.18	.93	.36	.24
WR[2]	0	0	0		ND					ND					ND				
WN[3]	1	1	0		.55	.30	.49	.97	.11	.55	.30	.40	.97	.11	ND				

QP[1] = Quonochontaug Pond
WR[2] = Whale Rock
WN[3] = Warwick Neck

ND = not determined

Table 32. Number of winter flounder hepatic lesions, NBP, winter 1987

Site	Toxic (RAM	Pre-neoplastic	Neoplastic	Total
WN (75)	36[a]	3	1	40
percent	64	4	1.3	69.1
WR (85)	12[b]	2	0	14
percent	14	2.3	0	16.3
QP	1[c]	2	0	3
percent	3.5	7	0	10.5

Means with the same superscript are not significantly different from each other at the 5 percent level of probability.

Table 33. Number of winter flounder hepatic lesions, NBP, spring 1987

WN (63)	12	3	1	16
percent	19	4.7	1.5	25.2
WR (94)	8	2	0	10
percent	8.5	2	0	10.5
QP	0	1	0	1
percent	0	1.4	0	1.4

Table 34. Number of winter flounder hepatic lesions, NBP, 1987

WN (138)	48	6	2	56
percent	34.7	4.3	1.4	40.4
WR (179)	20	4	0	24
percent	11	2.2	0	13.2
QP (97)	1	3	0	4
percent	1	3.1	0	4.1

Total fish = 414
Total lesions = 84
Percent lesions = 20.2

WR = Warwick Neck
WR = Whale Rock
QP = Quonochontaug Pond

Table 35. Pollutant residues (µg/g, wet weight) in the liver of winter flounder collected during winter season (November 1986-February 1987).

	WN	WR	QP
	n=26	n=27	n=20
PCBs	0.630 ± 0.283	0.259 ± 0.237	0.196 ± 0.182
	n=24	n=23	n=19
Pb	1.465 ± 0.546	1.748 ± 0.896	1.211 ± 0.631
	n=24	n=23	n=19
Cd	0.274 ± 0.350	0.194 ± 0.112	0.182 ± 0.086
	n=24	n=27	n=21
Hg	0.142 ± 0.105	0.131 ± 0.090	0.051 ± 0.038
	n=24	n=27	n=21
As	0.051 ± 0.034	0.043 ± 0.033	0.033 ± 0.014

Table 36. Pollutant residues (µg/g, wet weight) in the liver of winter flounder collected during spring season (May-June 1987).

	WN	WR	QP
	n=19	n=23	n=57
PCBs	0.823 ± 0.326	0.501 ± 0.255	0.381 ± 0.136
	n=19	n=23	n=57
Pb	0.525 ± 0.134	0.869 ± 0.216	0.408 ± 0.241
	n=19	n=23	n=57
Cd	0.289 ± 0.081	0.234 ± 0.090	0.174 ± 0.057
	n=19	n=21	n=57
Hg	0.441 ± 0.158	0.252 ± 0.105	0.228 ± 0.099
	n=19	n=21	n=57
As	0.027 ± 0.010	0.057 ± 0.037	0.039 ± 0.028

Table 37. Pollutant residues (µg/g, wet weight) in the muscle of winter flounder collected during winter season (November 1986-February 1987).

	WN	WR	QP
	n=14	n=14	n=14
PCBs	0.397 ± 0.157	0.163 ± 0.070	0.202 ± 0.119
	n=14	n=14	n=14
Pb	0.574 ± 0.5152	0.513 ± 0.147	0.557 ± 0.145
	n=14	n=14	n=14
Cd	0.146 ± 0.075	0.127± 0.019	0.134 ± 0.040
	n=14	n=14	n=14
Hg	0.197 ± 0.084	0.161 ± 0.044	0.189 ± 0.101
	n=14	n=14	n=14
As	0.027 ± 0.009	0.012± 0.005	0.022 ± 0.010

Table 38. Pollutant residues (µg/g, wet weight) in the muscle of winter flounder collected during spring season (May-June 1987).

	WN	WR	QP
	n=14	n=14	n=14
PCBs	0.170 ± 0.058	0.102 ± 0.043	0.139 ± 0.027
	n=14	n=14	n=14
Pb	0.750 ± 0.127	0.453 ± 0.187	0.624 ± 0.116
	n=14	n=14	n=14
Cd	0.268± 0.024	0.147 ± 0.088	0.229 ± 0.054
	n=14	n=14	n=14
Hg	0.135 ± 0.057	0.123 ± 0.064	0.115 ± 0.046
	n=14	n=14	n=14
As	0.020 ± 0.006	0.015 ± 0.007	0.021 ± 0.009

Table 39. ANOVA for pollutant residues in the liver using the contrasts

	WN vs WR & QP	WR vs WN & QP	QP vs WN & WR
PCBL	0.0001* 105**	0.0005 105	0.0009 105
PbL	NS	0.0035 105	0.0001 105
CdL	NS	NS	NS
HgL	NS	NS	NS
AsL	0.002 105	NS	0.015 105

* Probability > F value
** Number of observations

Table 40. ANOVA for pollutant residues in the muscle using the contrasts

	WN vs WR & QP	WR vs WN & QP	QP vs WN & WR
PCBM	0.0001* 84**	0.001 84	NS
PbL	0.008 84	0.0002 84	NS
CdM	0.005 84	0.0009 84	NS
HgM	NS	NS	NS
AsM	0.002 84	0.0001 84	

* Probability > F value
** Number of observations

Narragansett Bay Winter Flounder Macrophage Aggregate Number Corrected For Age

An Addendum

R.E. Wolke
C.W. Recksiek

INTRODUCTION

This study is an addendum to the 1986-87 Narragansett Bay Project entitled "Winter Flounder Contaminant and Pathology Study in Narragansett Bay". One of the purposes of the original study was to test the hypothesis that certain histological structures known as macrophage aggregates (MAs) found in Psuedopleuronectes americanus would vary across a gradient of clean to contaminated environments and could therefore serve as a monitor of both fish health and the degree of environmental degradation. The hypothesis had been tested previously in other geographic locations and had been found both valid and useful. An additional dimension was added to the original study in that individual fish were also examined for burdens of PCB, Pb, Cd, Hg and As in an attempt to determine if a relationship existed between particular contaminants and the presence of MAs.

The original study, however, failed to take into consideration an important variable controlling the numbers of MAs, that of fish age. This study reports results obtained when the number of MAs and their correlation with PCB, Pb, Cd, Hg and As are statistically evaluated within known age groups of fish from three sites.

MATERIALS AND METHODS

Winter flounder were collected during the Winter and Spring of 1986-87 by demersal trawl from three sites in Rhode Island: Warwick Neck (Narragansett Bay, 41° 39' 50" N, 71° 22'35" W), Whale Rock (West Passage of Narragansett Bay, 41° 39'45"N, 71° 24'45"W) and Quonochontaug Pond. The sites had been classified as contaminated, moderately contaminated and non-contaminated repectively in the initial study. Two-hundred ninety four of four-hundred fourteen fish were aged using an otolith sectioning technique similar to that presently used by the NMFS and modified by Recksiek and Haas (see NBP Tech. Rept., Ageing of Winter Flounder Otoliths from Rhode Island, 1989).

Initially, scatter plots of MA number vs age were produced to visually evaluate relationships and number/age clusters. Then age groups (2-4yrs; 4+-6yrs) were chosen for statistical evaluation. The non-parametric, independent variable, rank sum test was used to compare the mean number of MAs between sites and simple correlations were conducted comparing MA number and contaminants for each site.

In addition, apoptotic, uniquely vacuolated hepatocytes were counted at each site and correlations with MAs and contaminant levels were investigated.

RESULTS AND DISCUSSION

The ages of fish vary widely among sites and, due to the constraints of sampling, year classes were unevenly distributed (Figs. 1,2,3). For statistical purposes the best grouping of ages appeared to be two to four years (Group 1) and four-plus to six years Group 2).

Using this criterion, the numbers for each site are: Group 1, Quonochontaug Pond (Q) N=24, Whale Rock, (WH) N=75 and Warwick Neck (WN) N=54. For Group 2 they are: Q N=16, WH N=7 and WN N=41, for a total of 207 fish.

Pages six and eight list descriptive statistics for Group 1 MA number and contaminant levels. The mean number of MAs for sites Q and WH (1.08 and 0.6) are less than those of the contaminated site, WN (2.96). While there is no significant difference between the Q and WH sites (P=0.49), there is a highly significant difference between WN and Q (P=0.005) and WN and WH (P=0.0001). Mean contaminant liver levels of fish from the three sites show a gradient for PCB, lead, cadmium and mercury but not for arsenic which is slightly higher at WH. There are no correlations between number of MAs and contaminant levels at any site.

Page 11 lists the descriptive statistics for Group 2 MA number and contaminant levels. The mean number of MAs for sites Q and WH (1.125 and 3.143) are less than those of the contaminated site, WN (9.732). These figures reflect a site contamination gradient. While there is no significant difference between the Q and WH sites (P=0.135), there is a highly significant difference between WN and WH (P=0.008) and WN and Q (P=0.0000). Mean contaminant liver levels for the three sites are difficult to evaluate since there are so few samples at site WH. In general, however, levels are highest in livers from WN. There are no correlations between number of MAs and contaminant levels at any site.

An additional parameter to measure degraded environments in Winter Flounder is the presence of uniquely vacuolated hepatocytes. These cells have not been reported in other species of fish and were first reported in Boston Harbor flounder with hepatocellular carcinoma. For that reason, their numbers were calculated in this study. It is

interesting to note that these cells are far more prevalent at WN (1.33) than at Q (0.00), however, there is no correlation to MA number nor to particular contaminants.

CONCLUSIONS

The following conclusions may be reached:

1. Hepatic MA number may be used in the Winter Flounder to monitor fish health (stress) and the condition of the environment in which they live. This confirms the work we have been conducting for over two years with the State of Massachusetts and Winter flounder from Boston Harbor, Buzzards Bay, Cape Cod and Georges Bank.

2. There is no apparent relationship between MA number and the specific contaminant burdens measured in this study. This is a disappointment but may be an important finding as regards causation of these structures.

3. Winter flounder vacuolated hepatocytes (apoptotic cells) are more prevalent at the contaminated site and their number follows the gradient of defined environmental degradation.

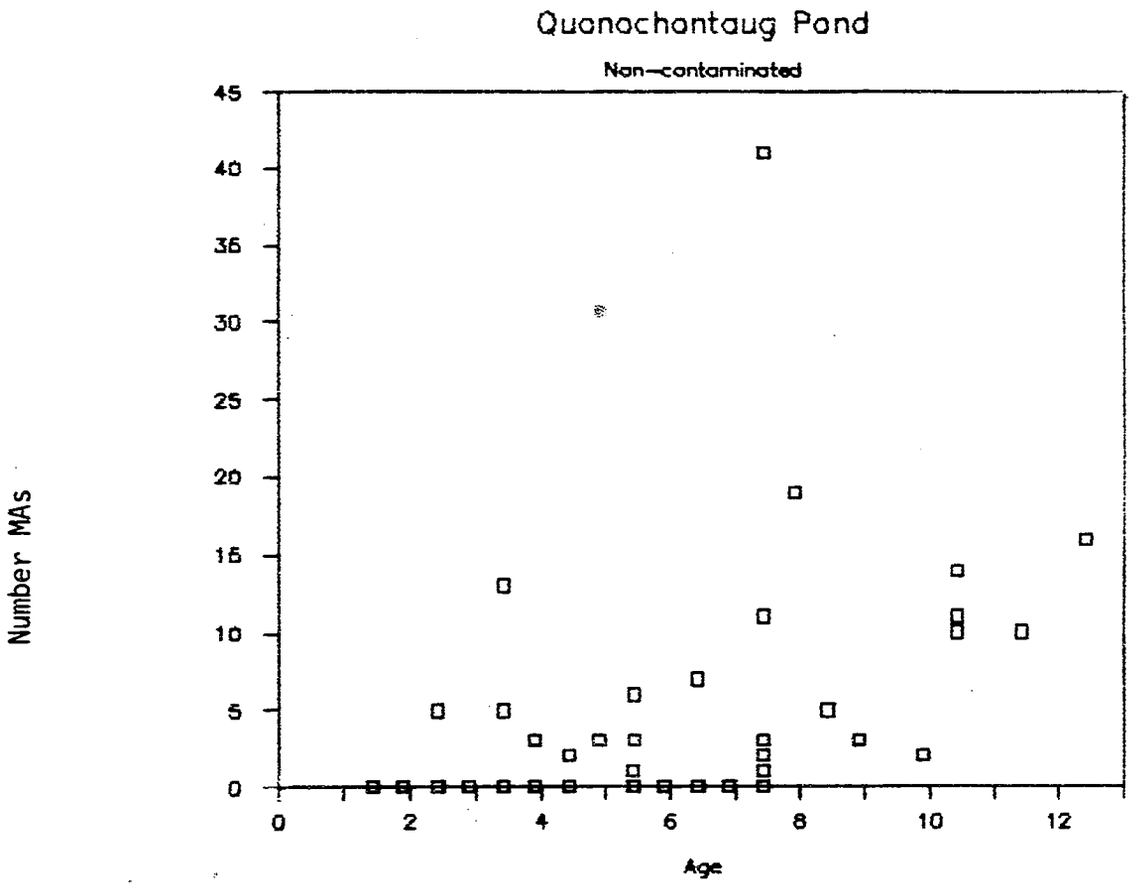


Fig. 1. Scatter plot, all fish, Quonochontaug Pond, number of macrophage aggregates vs age. *

* Plot shows duplicate data as a single point, especially on age axis.

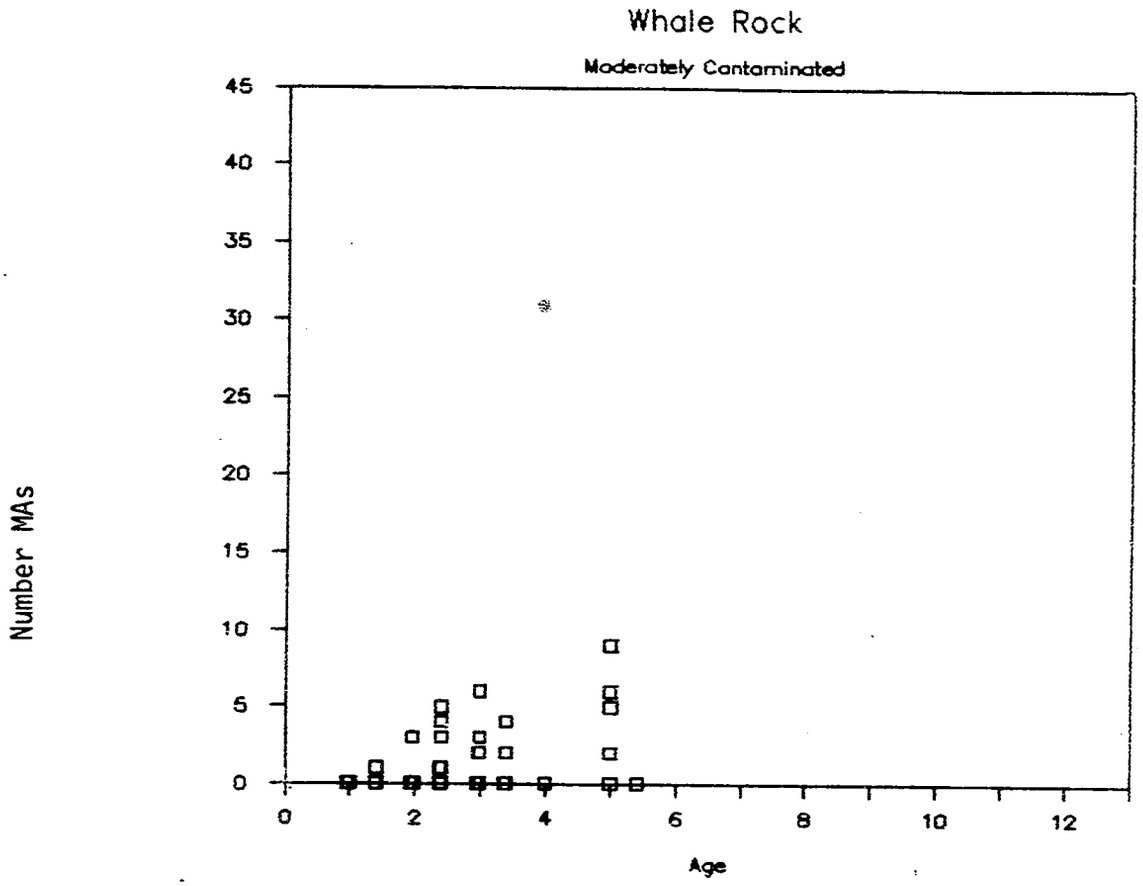


Fig. 2. Scatter plot, all fish, Whale Rock, number of macrophage aggregates vs age. *

* Plot shows duplicate data as a single point, especially on age axis.

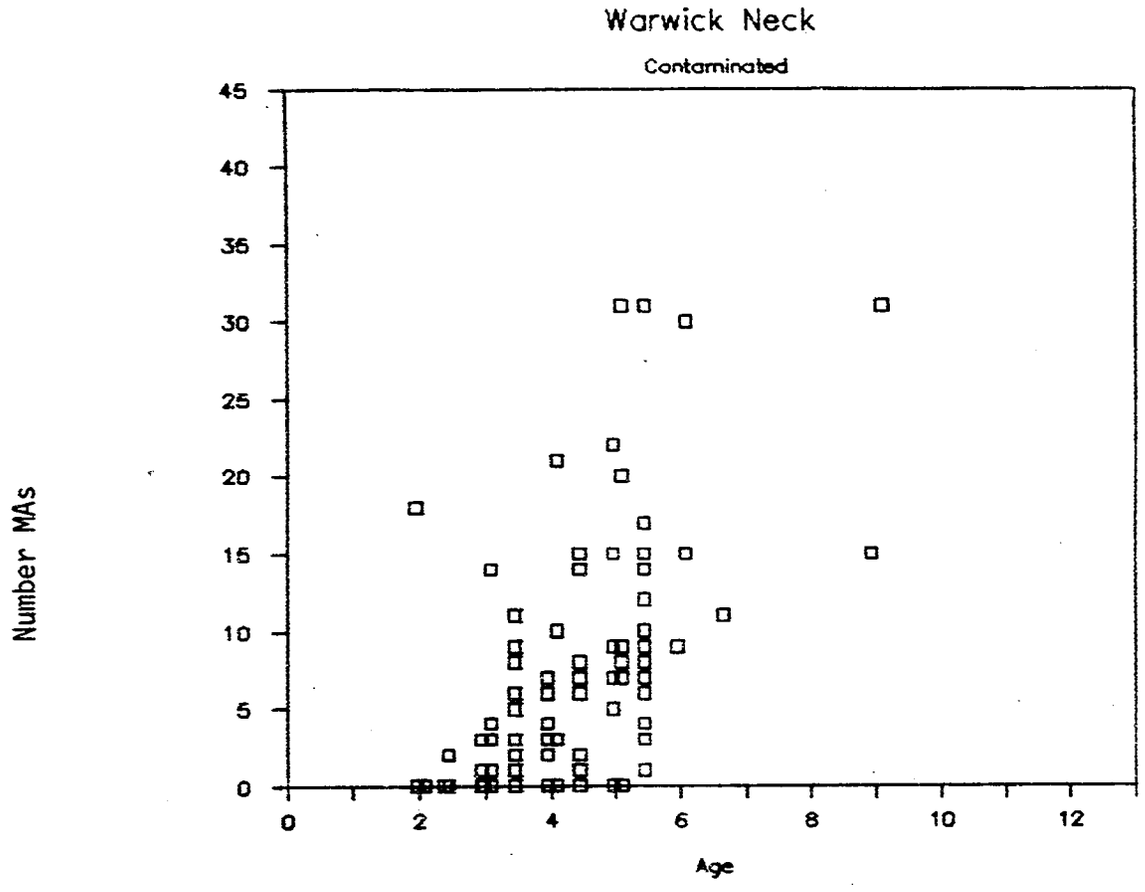


Fig. 3. Scatter plot, all fish, Warwick Neck, number of macrophage aggregates vs age. Note number of MA above 5 in 2 to 4 and 4 to 6 age groups. *

* Plot shows duplicate data as a single point, especially on age axis.

DESCRIPTIVE STATISTICS

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
NMAQ2	1.083	2.948	24	0.000	0.000	13.00
NMAWH2	6.000E-01	1.443	75	0.000	0.000	6.000
NMAWN2	2.963	4.269	54	1.000	0.000	21.00
HGQ2	1.547E-01	1.267E-01	24	1.175E-01	1.100E-02	4.580E-01
HGWH2	2.377E-01	2.980E-01	73	1.250E-01	3.000E-02	1.568
HGWN2	3.220E-01	2.447E-01	52	3.480E-01	4.100E-02	1.568
ASQ2	3.712E-02	1.983E-02	24	3.050E-02	1.200E-02	7.800E-02
ASWH2	5.330E-02	8.040E-02	73	2.600E-02	9.000E-03	5.660E-01
ASWN2	4.942E-02	7.640E-02	52	3.600E-02	1.400E-02	5.660E-01

RANK SUM TWO SAMPLE TEST FOR NMAQ2 VS NMAWH2

VARIABLE	RANK SUM	SAMPLE SIZE	U STAT	AVERAGE RANK
NMAQ2	1.198E+03	24	898.0	49.9
NMAWH2	3.752E+03	75	902.0	50.0
TOTAL	4.950E+03	99		

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION 0.012
TWO TAILED P VALUE FOR NORMAL APPROXIMATION 0.9902

TOTAL NUMBER OF VALUES WHICH WERE TIED 98
MAX. DIFF. ALLOWED BETWEEN TIES 1.00E-05

CASES INCLUDED 99 MISSING CASES 51

RANK SUM TWO SAMPLE TEST FOR NMAQ2 VS NMAWN2

VARIABLE	RANK SUM	SAMPLE SIZE	U STAT	AVERAGE RANK
NMAQ2	718.0	24	418.0	29.9
NMAWN2	2.363E+03	54	878.0	43.8
TOTAL	3.081E+03	78		

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION 2.485
TWO TAILED P VALUE FOR NORMAL APPROXIMATION 0.0130

TOTAL NUMBER OF VALUES WHICH WERE TIED 71
MAX. DIFF. ALLOWED BETWEEN TIES 1.00E-05

CASES INCLUDED 78 MISSING CASES 72

Continued from page 6.

RANK SUM TWO SAMPLE TEST FOR NMAWH2 VS NMAWN2

VARIABLE	RANK SUM	SAMPLE SIZE	U STAT	AVERAGE RANK
NMAWH2	4.103E+03	75	1.253E+03	54.7
NMAWN2	4.282E+03	54	2.797E+03	79.3
TOTAL	8.385E+03	129		

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION 3.683
TWO TAILED P VALUE FOR NORMAL APPROXIMATION 0.0002

TOTAL NUMBER OF VALUES WHICH WERE TIED 123
MAX. DIFF. ALLOWED BETWEEN TIES 1.00E-05

CASES INCLUDED 129 MISSING CASES 21

SIMPLE CORRELATIONS

	NMAQ2	HGQ2
NMAQ2	1.0000	
HGQ2	0.3324	1.0000

CASES INCLUDED 24 MISSING CASES 51

SIMPLE CORRELATIONS

	NMAWH2	HGWH2
NMAWH2	1.0000	
HGWH2	-0.1005	1.0000

CASES INCLUDED 73 MISSING CASES 2

SIMPLE CORRELATIONS

	NMAWN2	HGWN2
NMAWN2	1.0000	
HGWN2	0.0052	1.0000

CASES INCLUDED 52 MISSING CASES 23

SIMPLE CORRELATIONS

	NMAQ2	ASQ2
NMAQ2	1.0000	
ASQ2	-0.1251	1.0000

CASES INCLUDED 24 MISSING CASES 51

Continued from page 7.

SIMPLE CORRELATIONS

	NMAWH2	ASWH2
NMAWH2	1.0000	
ASWH2	-0.1597	1.0000

CASES INCLUDED 73 MISSING CASES 2

SIMPLE CORRELATIONS

	NMAWN2	ASWN2
NMAWN2	1.0000	
ASWN2	-0.1052	1.0000

CASES INCLUDED 52 MISSING CASES 23

DESCRIPTIVE STATISTICS

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
PCBQ2	3.080E-01	1.754E-01	24	3.020E-01	3.600E-02	7.060E-01
PCBWH2	4.245E-01	2.556E-01	73	3.790E-01	7.000E-03	1.232
PCBWN2	7.357E-01	2.951E-01	52	7.740E-01	3.700E-02	1.737
PBQ2	8.307E-01	6.588E-01	24	6.190E-01	2.640E-01	3.119
PBWH2	1.167	8.027E-01	73	9.080E-01	4.490E-01	5.239
PBWN2	1.153	7.653E-01	52	8.940E-01	3.230E-01	3.293
CDQ2	1.605E-01	5.537E-02	24	1.425E-01	4.400E-02	2.930E-01
CDWH2	2.274E-01	9.100E-02	73	2.250E-01	7.500E-02	5.230E-01
CDWN2	3.530E-01	3.316E-01	51	2.840E-01	6.100E-02	1.864

SIMPLE CORRELATIONS

	NMAWN2	PCBWN2
NMAWN2	1.0000	
PCBWN2	0.0733	1.0000

CASES INCLUDED 52 MISSING CASES 23

SIMPLE CORRELATIONS

	NMAWN2	PBWN2
NMAWN2	1.0000	
PBWN2	-0.1477	1.0000

CASES INCLUDED 52 MISSING CASES 23

SIMPLE CORRELATIONS

	NMAWN2	CDWN2
NMAWN2	1.0000	

Continued from page 8.

SIMPLE CORRELATIONS

	NMAWN2	CDWN2
NMAWN2	1.0000	
CDWN2	0.1484	1.0000

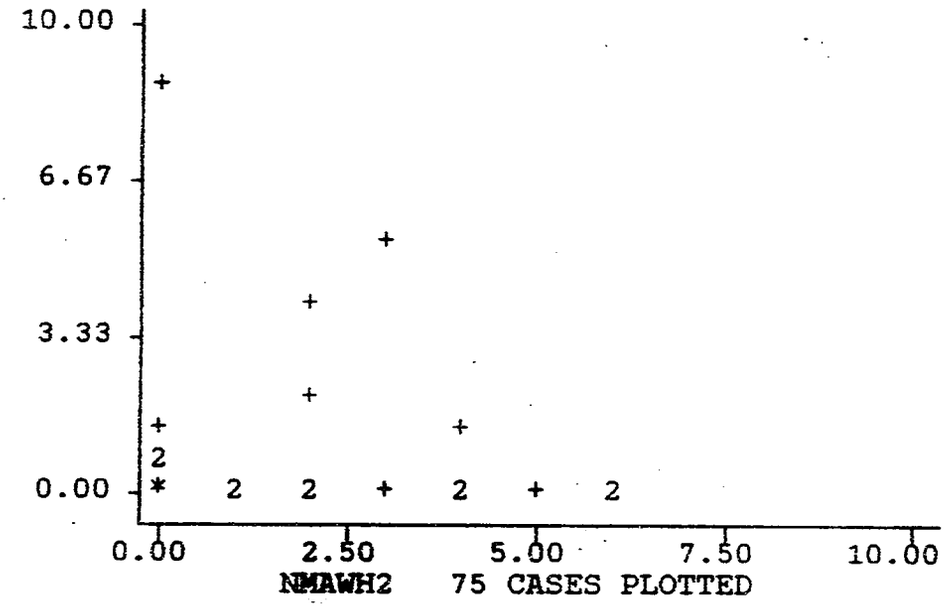
CASES INCLUDED 51 MISSING CASES 24

Descriptive statistics for vacuolated (apoptotic) hepatocytes (RAM) for Group 2 fish.

DESCRIPTIVE STATISTICS

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
RAMQ2	0.000	0.000	24	0.000	0.000	0.000
RAMWH2	3.133E-01	1.238	75	0.000	0.000	8.500
RAMWN2	1.637	4.100	54	0.000	0.000	26.00

RAMWH2 VS NMAWH2



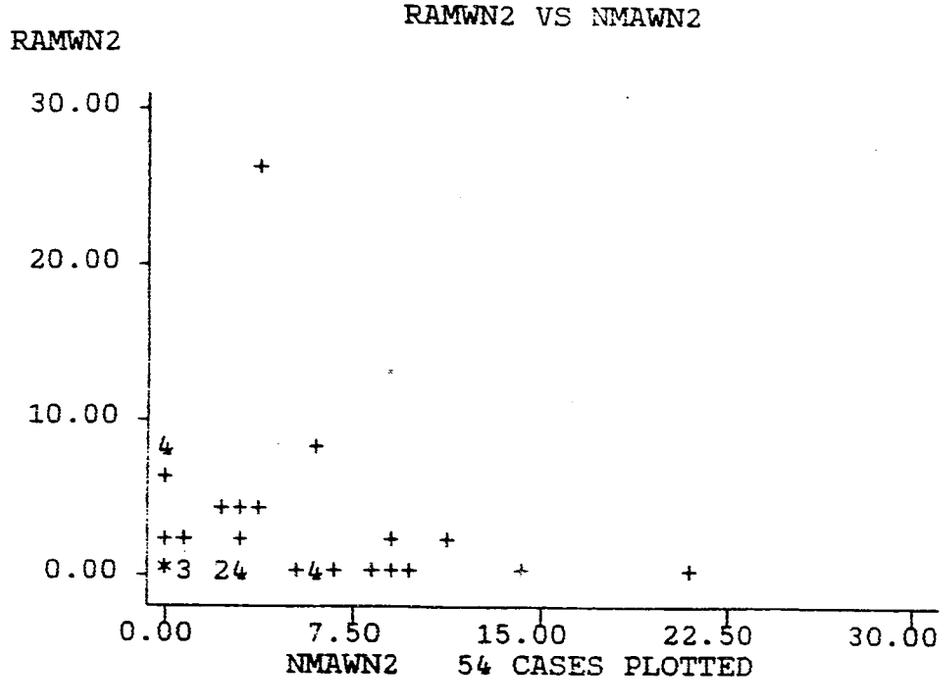
SIMPLE CORRELATIONS

	NMAWH2	RAMWH2
NMAWH2	1.0000	
RAMWH2	0.1339	1.0000

CASES INCLUDED 75 MISSING CASES 0

Continued from page 9.

RAMWN2 VS NMAWN2



SIMPLE CORRELATIONS

	NMAWN2	RAMWN2
NMAWN2	1.0000	
RAMWN2	-0.0450	1.0000

CASES INCLUDED 54 MISSING CASES 21

RANK SUM TWO SAMPLE TEST FOR RAMWH2 VS RAMWN2

VARIABLE	RANK SUM	SAMPLE SIZE	U STAT	AVERAGE RANK
RAMWH2	4.527E+03	75	1.677E+03	60.4
RAMWN2	3.858E+03	54	2.373E+03	71.4
TOTAL	8.385E+03	129		

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION 1.659
TWO TAILED P VALUE FOR NORMAL APPROXIMATION 0.0971

TOTAL NUMBER OF VALUES WHICH WERE TIED 121
MAX. DIFF. ALLOWED BETWEEN TIES 1.00E-05

CASES INCLUDED 129 MISSING CASES 21

DESCRIPTIVE STATISTICS

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
NMAQ4	1.125	1.784	16	0.000	0.000	6.000
NMAWH4	3.143	3.579	7	2.000	0.000	9.000
NMAWN4	9.732	8.127	41	8.000	0.000	31.00
PCBQ4	3.381E-01	1.926E-01	16	3.135E-01	6.100E-02	6.890E-01
PCBWH4	3.271E-01	2.231E-01	7	3.070E-01	6.700E-02	6.540E-01
PCBWN4	6.900E-01	2.513E-01	41	6.800E-01	1.470E-01	1.304
PBQ4	6.850E-01	4.999E-01	16	5.305E-01	2.170E-01	2.222
PBWH4	1.705	1.379	7	1.172	7.460E-01	4.730
PBWN4	0.963	7.778E-01	41	6.960E-01	3.230E-01	4.762
CDQ4	2.626E-01	3.431E-01	16	1.630E-01	8.200E-02	1.518
CDWH4	2.869E-01	1.525E-01	7	2.340E-01	1.570E-01	5.230E-01
CDWN4	3.867E-01	2.924E-01	41	3.220E-01	1.170E-01	1.864

RANK SUM TWO SAMPLE TEST FOR NMAQ4 VS NMAWH4

VARIABLE	RANK SUM	SAMPLE SIZE	U STAT	AVERAGE RANK
NMAQ4	175.0	16	39.00	10.9
NMAWH4	101.0	7	73.00	14.4
TOTAL	276.0	23		

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION 1.102
TWO TAILED P VALUE FOR NORMAL APPROXIMATION 0.2703

TOTAL NUMBER OF VALUES WHICH WERE TIED 20
MAX. DIFF. ALLOWED BETWEEN TIES 1.00E-05

CASES INCLUDED 23 MISSING CASES 59

RANK SUM TWO SAMPLE TEST FOR NMAQ4 VS NMAWN4

VARIABLE	RANK SUM	SAMPLE SIZE	U STAT	AVERAGE RANK
NMAQ4	207.5	16	71.50	13.0
NMAWN4	1.445E+03	41	584.5	35.3
TOTAL	1.653E+03	57		

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION 4.546
TWO TAILED P VALUE FOR NORMAL APPROXIMATION 0.0000

TOTAL NUMBER OF VALUES WHICH WERE TIED 49
MAX. DIFF. ALLOWED BETWEEN TIES 1.00E-05

CASES INCLUDED 57 MISSING CASES 25

RANK SUM TWO SAMPLE TEST FOR NMAWH4 VS NMAWN4

Continued from page 11.

RANK SUM TWO SAMPLE TEST FOR NMAWH4 VS NMAWN4

VARIABLE	RANK SUM	SAMPLE SIZE	U STAT	AVERAGE RANK
NMAWH4	89.50	7	61.50	12.8
NMAWN4	1.086E+03	41	225.5	26.5
TOTAL	1.176E+03	48		

NORMAL APPROXIMATION WITH CONTINUITY CORRECTION 2.381
TWO TAILED P VALUE FOR NORMAL APPROXIMATION 0.0173

TOTAL NUMBER OF VALUES WHICH WERE TIED 40
MAX. DIFF. ALLOWED BETWEEN TIES 1.00E 05

CASES INCLUDED 48 MISSING CASES 34

SIMPLE CORRELATIONS

	NMAWN4	PCBWN4
NMAWN4	1.0000	
PCBWN4	0.1465	1.0000

CASES INCLUDED 41 MISSING CASES 0

SIMPLE CORRELATIONS

	NMAWN4	PBWN4
NMAWN4	1.0000	
PBWN4	0.1431	1.0000

CASES INCLUDED 41 MISSING CASES 0

SIMPLE CORRELATIONS

	NMAWN4	CDWN4
NMAWN4	1.0000	
CDWN4	0.0127	1.0000

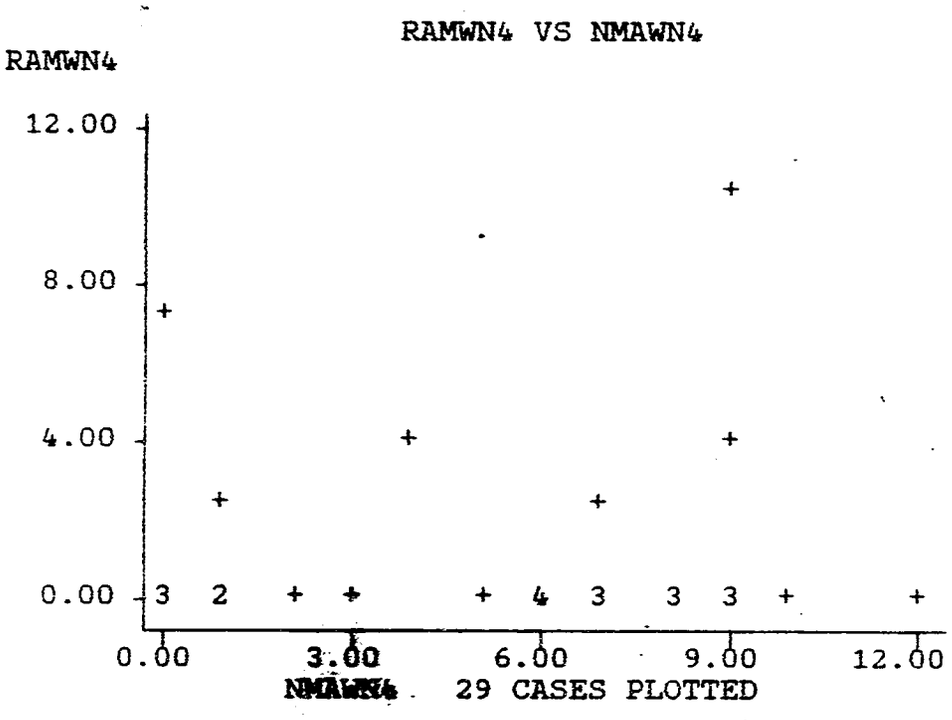
CASES INCLUDED 41 MISSING CASES 0

Continued from page 12.

DESCRIPTIVE STATISTICS

VARIABLE	MEAN	S.D.	N	MEDIAN	MINIMUM	MAXIMUM
HGQ4	1.745E-01	1.193E-01	16	1.775E-01	2.300E-02	4.450E-01
HGWH4	9.657E-02	1.375E-02	7	9.500E-02	7.900E-02	1.150E-01
HGWN4	3.150E-01	1.724E-01	41	3.420E-01	9.000E-03	6.340E-01
ASQ4	3.744E-02	1.925E-02	16	3.900E-02	1.400E-02	7.700E-02
ASWH4	1.814E-02	6.568E-03	7	1.800E-02	9.000E-03	2.700E-02
ASWN4	3.412E-02	2.033E-02	41	3.000E-02	6.000E-03	1.000E-01
RAMQ4	0.000	0.000	16	0.000	0.000	0.000
RAMWH4	1.357	2.809	7	0.000	0.000	7.500
RAMWN4	1.520	3.075	41	0.000	0.000	11.00

Descriptive statistics for vacuolated (apoptotic) hepatocytes and MA numbers; and contaminant levels and MA numbers for Warwick Neck.



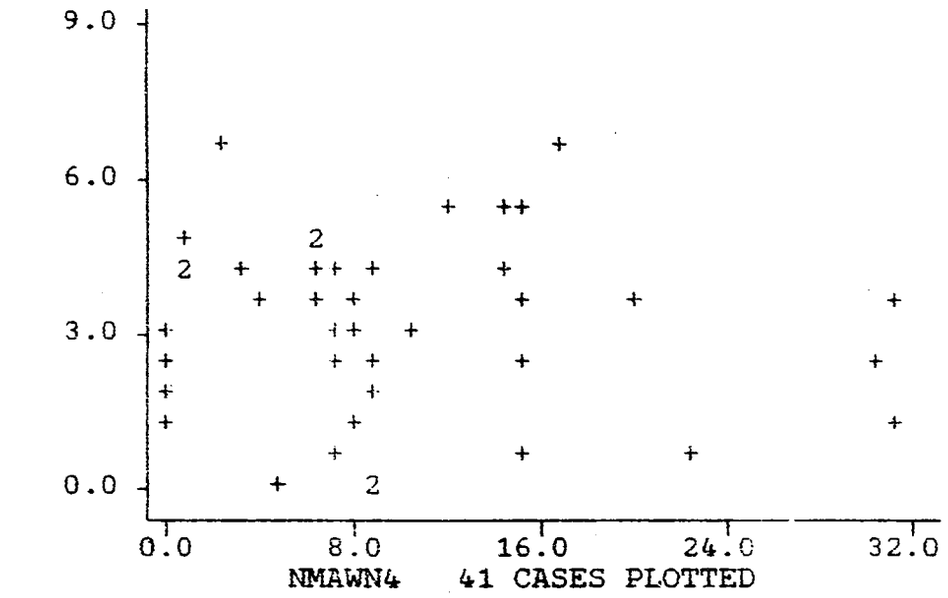
SIMPLE CORRELATIONS

	NMAWN4	RAMWN4
NMAWN4	1.0000	
RAMWN4	0.1320	1.0000

CASES INCLUDED 41 MISSING CASES 0

Continued from page 13.

HGWN4 VS NMAWN4



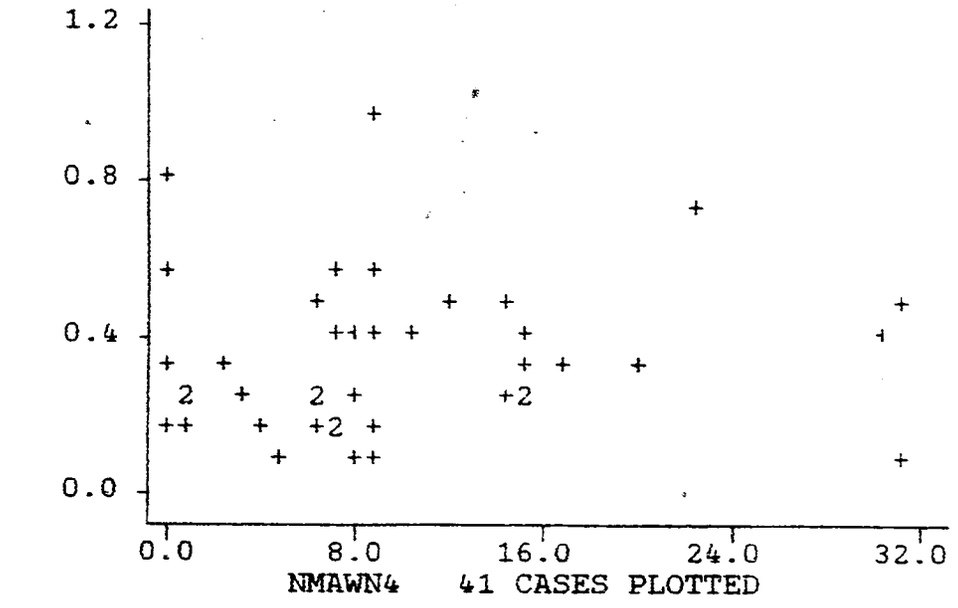
SIMPLE CORRELATIONS

	NMAWN4	HGWN4
NMAWN4	1.0000	
HGWN4	-0.0528	1.0000

CASES INCLUDED 41 MISSING CASES 0

Continued from page 14.

ASWN4 VS NMAWN4



SIMPLE CORRELATIONS

	NMAWN4	ASWN4
NMAWN4	1.0000	
ASWN4	0.0963	1.0000

CASES INCLUDED 41 MISSING CASES 0