

SUMMARY

of

**MASSACHUSETTS WATER RESOURCES AUTHORITY
FISH AND SHELLFISH SCIENCE REVIEW MEETING**

held

May 12, 1994

at

**BATTELLE OCEAN SCIENCES
DUXBURY, MA**

prepared by

**Carlton Hunt
BATTELLE OCEAN SCIENCES**

and

**Margarete Steinhauer
CONSULTANT**

May 31, 1994

INTRODUCTION

The 1994 MWRA fish and shellfish science review meeting was held at Battelle on May 12, 1994. There were 37 attendees, including MWRA personnel, regulators, members of the scientific community, and project scientists (see Appendix A). Carlton Hunt (Battelle) discussed the goals and objectives of the meeting, and the charge to participants (included in agenda handout). Summaries of the sediment chemistry data and benthic features were presented by project scientists. A summary of the meeting presentations and discussion is provided here. Appendix B includes the workshop agenda and copies of the introductory overhead slides. Copies of graphics and written materials provided by the workshop presenters are included in Appendix C and Appendix D presents revised goals of the fish and shellfish study.

The objectives of the workshop were to

- Evaluate questions presented in the outfall monitoring plan (November 1991) to determine, after reviewing the results of the baseline studies, whether the original questions should be modified or if new questions should be asked.
- Evaluate the monitoring parameters being measured, and advance our understanding of meaningful change and appropriate action levels.
- Determine whether the baseline data collected to date are sufficient to detect changes that may occur after the new outfall becomes operational in western Massachusetts Bay.

To solicit discussion around these issues, the following questions were presented to meeting participants:

- Are the objectives of the fish and shellfish studies adequate to meet the goals of the monitoring program?
- Are the baseline data sufficient to understand the ecosystem being monitored?
- Is spatial and temporal coverage provided by the monitoring plan adequate to meet its goals?
- What is the role of the various measurements in the monitoring program? Should any additional measurements be made?
- What key parameters (metrics) could be considered indicators and what are the suggested endpoints?

- Are the meaningful levels of change defined in the draft monitoring plan adequate or should they be changed?
- Can any monitoring questions be phrased as quantitative hypotheses?

Summaries of the formal presentations are included below, followed by a summary of the discussion issues and conclusions.

CAGED MUSSEL STUDIES — Phil Downey (Aquatec)

- As part of the NPDES monitoring requirement, caged mussels have been deployed at three sites (Deer Island, Discovery, and the Large Navigation Buoy offshore). Caged mussels were also deployed off Gloucester in 1991. Composites of the mussels are analyzed for PAHs, pesticides, PCBs, mercury, and lead.
- With some exceptions, the overall trend since 1987 has been decreasing concentrations of most contaminants measured in the mussel tissues. However, 1993 results showed that concentrations of low- and high-molecular-weight PAHs, as well as some chlorinated pesticides were elevated in Deer Island samples.
- In general, higher contaminant concentrations were associated with mussels deployed in the Inner Harbor than with mussels that were deployed at Deer Island.

OVERVIEW OF NATIONAL MUSSEL WATCH PROGRAM Tom O'Connor (NOAA)

- Tissue analyses of mussels and oysters collected nationwide from 140 sites during the last seven years indicate that there were approximately 300 significant decreases and 100 increases (not statistically significant) in contaminant concentrations.
- At the four Boston Harbor sites, trends in contaminant concentrations have been mixed. Some contaminants (e.g., total PAHs) have shown consistent decreases with time at all four sites, while other contaminants (e.g., mercury) have increased at the Hingham Bay site.

HISTOPATHOLOGY OF FLOUNDER — Michael Moore (WHOI)

- Disease and/or abnormalities in winter flounder collected from Deer Island flats has followed a decreasing trend over the last seven years. At the future outfall site, the incidence of hydropic vacuolation in flounder livers has remained at about 30%, unchanged between 1991 and 1993. Incidence of diseased flounder from Cape Cod Bay has been at or near zero between 1991 and 1993.
- The evidence of chemically induced abnormalities in flounder is mostly suggested by strong correlation analyses of the data, particularly with samples from the future outfall site. For example, concentrations of chlorinated pesticides have been highly correlated ($r=0.83$) with incidence of centrotubular vacuolation in flounder livers.
- In the absence of known sources of contamination at the future outfall site, the elevated incidence of vacuolation in flounder livers from that location was examined by $d^{34}S$ stable isotope analysis. However, the $d^{34}S$ signal in scales of fish collected at the future outfall site was not significantly different from the signal in the scales of reference site fish. Because this analysis integrates exposure over the life of the fish, additional isotope analyses of fish scales are not recommended. Examination of isotopes in intestinal or gill epithelium was proposed as a more dynamic sensor.

APOPTOSIS OF FLOUNDER LIVER AND OTHER RESULTS

Bob Hillman (Battelle)

- Histopathology of winter flounder livers included examination for five types of lesions: hydropic vacuolation, macrophage aggregates, biliary proliferation, neoplasia, and “balloon hepatocytes.” For each sample, three tissue sections, prepared and scored in duplicate, were used to generate a lesion index (0-4, least severe to most severe). A site index was developed from the mean lesion index score of all samples examined from the site.
- Flounder liver pathology data from 1993 tissues indicated a sudden and unusual prevalence of “balloon hepatocytes,” a condition of cell death referred to as apoptosis. In 1993 samples, apoptosis was observed in 50-60% of all fish collected from all sites, except Cape Cod Bay, where the condition was observed in about 25% of the samples. If similar conditions are observed in the 1994 samples, possible causes should be examined.

- Between 1992 and 1993, incidence of hydropic vacuolation decreased in fish collected from Broad Sound, but did not change significantly in fish from the other sites. Incidence of all other tissue lesions, except neoplasia, increased sharply in 1993.

DETECTABILITY OF CHANGE : CHEMICALS — Carlton Hunt (Battelle)

- Examination of tissue chemistry data from 1992 ($n=3$) and 1993 ($n=9$ or 10) indicates that contaminant concentrations are consistently and substantially less than the FDA action limits, and that there was little difference between the 1992 and 1993 sample sets. No consistent pattern of change in chemical concentrations was observed across all sampling sites. Many contaminant decreases were observed in samples from Deer Island, some increases were observed in Cape Cod Bay samples, and samples from the future outfall site were highly variable.
- Variability of contaminant concentrations was generally 50%, and was similar in 1992 and 1993, despite an increase in the number of samples ($n=9$ or 10) in 1993.
- Detectable change was in the range of 50-200%. Although the monitoring plan specifies 50-100%, a 100-200% range may be a more reasonable goal.
- Based on the study objectives, the well-defined population variance, and the percent change that can be detected, sample pooling is a reasonable and cost-effective approach for tissue chemistry analyses.

DISCUSSION TOPICS AND RESOLUTIONS

- Discussion concerning the objectives of the fish and shellfish study indicated that the present monitoring objectives were sufficient to address scientific, regulatory, and public concerns raised during the development of the draft monitoring plan. However, based on our current knowledge, and the recent fish and shellfish advisory monitoring guidance, the objectives can be refined. Suggested modifications are found in Appendix D.

- Discussion concerning the adequacy of the baseline data revealed that the baseline data now available, together with our current understanding of the results, suggest that the baseline information is adequate to address the monitoring plan's broad objectives. However, standardization of some procedures and methods is required to ensure data comparability. A recommendation was made to re-evaluate the chemical parameters analyzed in tissues, based on components in the effluent. Compositing of tissue at each of the five stations (versus analysis of individual animals) was strongly endorsed by the meeting participants. Analysis of large individual organisms (lobster and flounder) on an individual basis will continue as specified in the program to ensure that contaminants in these organisms can be detected. Specific compositing schemes will be provided to the OMTF in late May and will be based on the results of the 1993 tissue analyses. A suggestion was made to increase the winter flounder sample size to allow statistical comparison of the data from Deer Island and the future outfall site.
- Concerning the adequacy of the spatial and temporal coverage achieved under the current monitoring plan, it was determined that spatial coverage is adequate and should not be decreased, and that temporal coverage and timing of sample collection are in accordance with EPA guidance that is used to assess potential human health concerns. Adjustments to the specific sampling location within a sampling area and the timing of the sample collection to ensure that animals are collected within the targeted areas were considered appropriate, given the dependence of the target organisms on temperature changes. It was noted that an adequate number of lobster samples collected at varying times is more important in achieving the program's goals than obtaining an insufficient number of samples consistency at the same times each year (i.e., collect lobster any time they are available). Although Cape Cod Bay currently appears to be an adequate reference site for winter flounder tissue analyses, a recommendation was made to also sample winter flounder at a second reference site, well offshore (e.g., Georges Bank) to serve as a backup for the Cape Cod Bay site.
- In the current monitoring program, the parameters being measured in fish and shellfish are for the purposes of (1) human health protection, (2) trend analysis, and (3) fish health as related to the outfall relocation. No changes to these basic goals were recommended by the meeting participants. Various recommendations for additional measurements were made and included the following:

- Analysis of lead in bivalve tissues (caged mussels) but not in edible fish and lobster tissue. The next report on the caged mussel studies will compare lead concentrations in soft-shell clams and in mussel tissues.
 - Continued examination of flounder liver histopathology, with a focus on all major indices related to chemical contamination. Examination of other lesions was discouraged until the reasons for or causes of the observed liver lesions are better understood. Towards this end, continued attempts to relate the histopathology to chemical contaminants in the liver and edible tissues were encouraged. Standardizing the number of histopathology slides was suggested. Some results prior to 1991 were based on single slides, whereas more recent results are based on six slides per animal liver.
 - Continued use of the $d^{34}S$ at the Deer Island and future outfall site was recommended to ensure that the source of the organic matter in the area can be tracked.
 - Addition of histopathology measurements at a distant farfield reference station (e.g., Georges Bank) was recommended.
- Discussions of the meeting participants suggested that the key fish and shellfish indicators might be (1) detection of sustained trends in contaminant concentrations with reference to FDA action levels for edible tissue of lobster and flounder, or (2) detection of trends in the histopathology scores that could suggest that flounder health is deteriorating beyond that documented during the baseline period. The key endpoints should be the FDA action limits in edible tissue. Screening values were briefly considered but were dismissed as less appealing than the FDA action values. Representatives from the regulatory agencies will continue to examine this concept within their purview.
 - Suggestions were made to modify the levels of meaningful change defined in the draft monitoring plan. The plan now specifies detectability of 50-100% change. Additional data on individual organisms suggest that consistently obtaining a 50% detectable change could be cost prohibitive. Because concentrations of contaminants are currently well below the FDA action limits, a 50-200% detectable change (based on specific contaminants) may be a more realistic goal.

- Monitoring questions can now be phrased as quantitative hypotheses are

Ho1: There will be no change in the levels of contaminants in fish and shellfish outside of the levels specified in a matrix showing mean and 95% confidence intervals at a power of 0.8 at each station, tissue type, and contaminant at each of the monitoring stations and as defined in the following matrix.

Ho2: No change in the persistence of histopathology indicators in flounder liver, as identified in the following matrix, will occur.

- More timely fish landing data were requested.

APPENDIX A

WORKSHOP PARTICIPANTS

	NAME	AFFILIATION	PHONE
1	Carlton Hunt	Battelle	(617) 934-0571
2	Jim Robinson	Univ-Boston	(617) 287-7456
3	WINDSOR SUNG	Sung & Associates	(617) 861-9455
4	Jim Bowen	ENSR C&E	(508) 635-9500
5	Robert Buchsbaum	Mass Audubon Society	508 927 1122
6	Damian Shea	NC State Univ	919/515-3391
7	Michael Moore	Woods Hole Oceanog. Inst.	508 457 2000 + 3228 mmore@whoi.edu
8	Mike Connor	MWRA	617-241-6507
9	Tom O'Connor	11211 1st St. Twp. Mass.	911-115 3229 117
10	Bob Hillman	BATTELLE	617 934-0571
11	Linda Hansen	Battelle	617-934-0571
12	Matthew Liebman	EPA Region 1 / Mass Bays Program	617-565-4866
13	Jerry Neff	Battelle	617 934-0571
14	Jack Schwartz	DMF - CAT Cove Lab	508-745-3107
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16	Philip Downey	Induspe Testing Services	802-655-1203
17	Judy Pedron	MCZM	(617) 727-4530
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19	Alan Cooperman	MDEP	(508) 682-5237
20	David Sheperdson	EOEA / MEPA	617-727-5830 x304
21	John Stegeman	WHOI	508 548 1400
22	JACK KELLY	BATTELLE	617-934-0571
23	Margarete Steinhauer	Consultant	617/934-2072
24	Ken Keay	MWRA	617-242-6000
25	Mike Mickelson	"	"
26	Maurv Hall	"	617-242-7310
27.			

APPENDIX B

WORKSHOP AGENDA AND OVERHEAD SLIDES

MASSACHUSETTS WATER RESOURCES AUTHORITY

FISH AND SHELLFISH SCIENCE REVIEW MEETING

MAY 12, 1994

**BATTELLE OCEAN SCIENCES
DUXBURY, MA**

AGENDA
FISH AND SHELLFISH SCIENCE REVIEW MEETING
MAY 12, 1994
BATTELLE OCEAN SCIENCES
DUXBURY, MA

1. WELCOME 0830 - 0845

Mike Connor, MWRA Science Director
Carlton Hunt, Moderator and Battelle Project Manager, Logistics

2. GOALS 0845 - 0915

Carlton Hunt: Workshop Goals, Process, Charge, Product
Predictions
Monitoring Design

3. PRESENTATIONS 0915 - 1115

Phil Downey - Caged mussel studies

→ 10 AM
Michael Moore - Histopathology of flounder

Bob Hillman - Apoptosis of flounder liver and other results

Carlton Hunt - Detectability of change: chemicals
- EPA Fish Advisory Monitoring Guidance

3. Discussion 1115 - 1230

Monitoring objectives
Monitoring Design
Detectable/meaningful change
Monitoring Questions and Hypotheses statements
Design changes

Lunch - Provided 1230 - 1315

4. Discussion (Continued) 1300 - 1400

Wrap up

PERTURBATION:

Question: Has the incidence of disease and/or abnormalities in fish or shellfish changed? (R-11)

FISH AND SHELLFISH CONTAMINATION AND PHYSIOLOGICAL CONDITION

Purpose: Monitor accumulation of toxic contaminants in fish and shellfish consumed by humans to assess human health risk. Determine physiological condition of fish to assess impact on fish health.

Question: Has the level of contaminants in the tissues of fish and shellfish around the outfall changed since discharge began? (R-1, R-11)
Do the levels of contaminants in the edible tissue of fish and shellfish around the outfall represent a risk to human health? (R-1)
Are the contaminant levels in fish and shellfish different between the outfall, Boston Harbor, and a reference site? (R-11)

Measurement: Caged mussels deployed to assess bioaccumulation — PAH, PCB, pesticides
Winter flounder fillet — PCB, pesticides, Hg
Winter flounder liver — PAH, PCB, pesticides, Ag, Cu, Cd, Hg, Pb, Zn
Lobster meat — PCB, pesticides, Hg
Lobster hepatopancreas - PAH, PCB, pesticides, Ag, Cu, Cd, Hg, Pb, Zn
Individual animals will be collected and may be composited according to histological indices to yield six samples of each tissue type for analysis (24 samples total for flounder and lobster at each station) (meant to be six "meat" each for flounder and lobster and six "liver" (flounder) and six hepatopancreas (lobster)). Animal size, mass, and dry/lipid weight will also be recorded. If total Hg exceeds FDA action limit, organic Hg will also be measured. For caged mussels, 2 cages with 30 mussels each will be deployed at middepth or below the pycnocline; mussels from each cage will be pooled for a single sample.

CAGED MUSSELS

Location: Deer Island Flats, near the proposed outfall, and in Cape Cod Bay. Caged mussels will be outside the mixing zone at the proposed outfall only.

Frequency: Caged mussels will be deployed for 2 months during the summer. Flounder and lobster will be sampled every year during spring.

Detectable change: 50%-100% change from present levels for most contaminants, based on power analysis of existing body-burden data in flounder and lobsters.

Data analysis: Perform statistical analysis to determine difference between pre- and post-discharge and between outfall site and other sites. Compare concentrations in edible tissue against either FDA Action Levels or the proposed National Shellfish Sanitation Program Alert Levels

**FISH AND SHELLFISH CONTAMINATION AND PHYSIOLOGICAL CONDITION
(Continued)**

Flounder and Lobster

- Location:** Deer Island Flats, near the proposed outfall, in Cape Cod Bay, near Lynn (flounder only), and east of Stellwagen Bank (flounder only). Lobster sampling will be coordinated with the Massachusetts Division of Marine Fisheries. Flounder collected at 5 locations: Deer Island Flats, Nantasket Beach, Broad Sound, Future Outfall Site, and eastern Cape Cod Bay. Lobster collected at Deer Island Flats, Future Outfall Site, and eastern Cape Cod Bay.
- Frequency:** Once each year, every year during spring.
- Measurement:** Histology in winter flounder, gross abnormalities in lobster. Sample size: $n > 50$ for flounder, $n > 10$ for lobster
- Detectable change:** $< 50\%$ for most flounder and lobster indices (Appendix B), $< 10\%$ for neoplasia in flounder.
- Data analysis:** Perform statistical tests to determine difference between pre- and post-discharge and between outfall site and other sites. Perform regression to test for correlation among physiological and body-burden data. Compare concentrations in edible tissue against either FDA Action Levels or the proposed National Shellfish Sanitation Program Alert Levels.

OBJECTIVES

FISH AND SHELLFISH SCIENCE REVIEW MEETING MAY 12, 1994

- **EVALUATE QUESTIONS POSED IN THE OUTFALL MONITORING PLAN OF NOVEMBER 7, 1991**
 - to determine if the questions should (can) be modified as result of the baseline study
 - to determine if new questions should be asked

- **EVALUATE THE PARAMETERS BEING MEASURED TO DETERMINE**
 - their role in the monitoring program (indicator/endpoint)
 - their ability to detect meaningful change
 - what level of change is acceptable
 - key metrics associated with each variable
(i.e., screening criteria, FDA action limits, increase in contaminant level, etc.)
 - duration/frequency/seasonality associated with meaningful change

- **ADVANCE UNDERSTANDING OF MEANINGFUL CHANGE, KEY MONITORING VARIABLES, AND APPROPRIATE ACTION LEVELS**

- **EVALUATE IF THE BASELINE DATA ON MASSACHUSETTS BAY IS NOW SUFFICIENT TO CAPTURE CHANGES THAT MIGHT OCCUR**
 - to develop testable hypotheses

BASELINE OUTFALL MONITORING SCHEDULE

Jan. Feb. Mar. Apr. May Jun. JUL. Aug. Sep. Oct. Nov. Dec.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	JUL.	Aug.	Sep.	Oct.	Nov.	Dec.
Water Column												
Nearfield	●	●	●	●	●	●	●	●	●	●	●	●
Farfield	●	●	●	●	●	●	●	●	●	●	●	●
Nutrient Flux	●	●	●	●	●	●	●	●	●	●	●	●
Fish & Shellfish												
Benthic Community												
Harbor	●	●	●	●	●	●	●	●	●	●	●	●
Bay												
Sediment Toxics												
Effluent	●	●	●	●	●	●	●	●	●	●	●	●
Hard Bottom Community (proposed)												

Jan. Feb. Mar. Apr. May Jun. JUL. Aug. Sep. Oct. Nov. Dec.

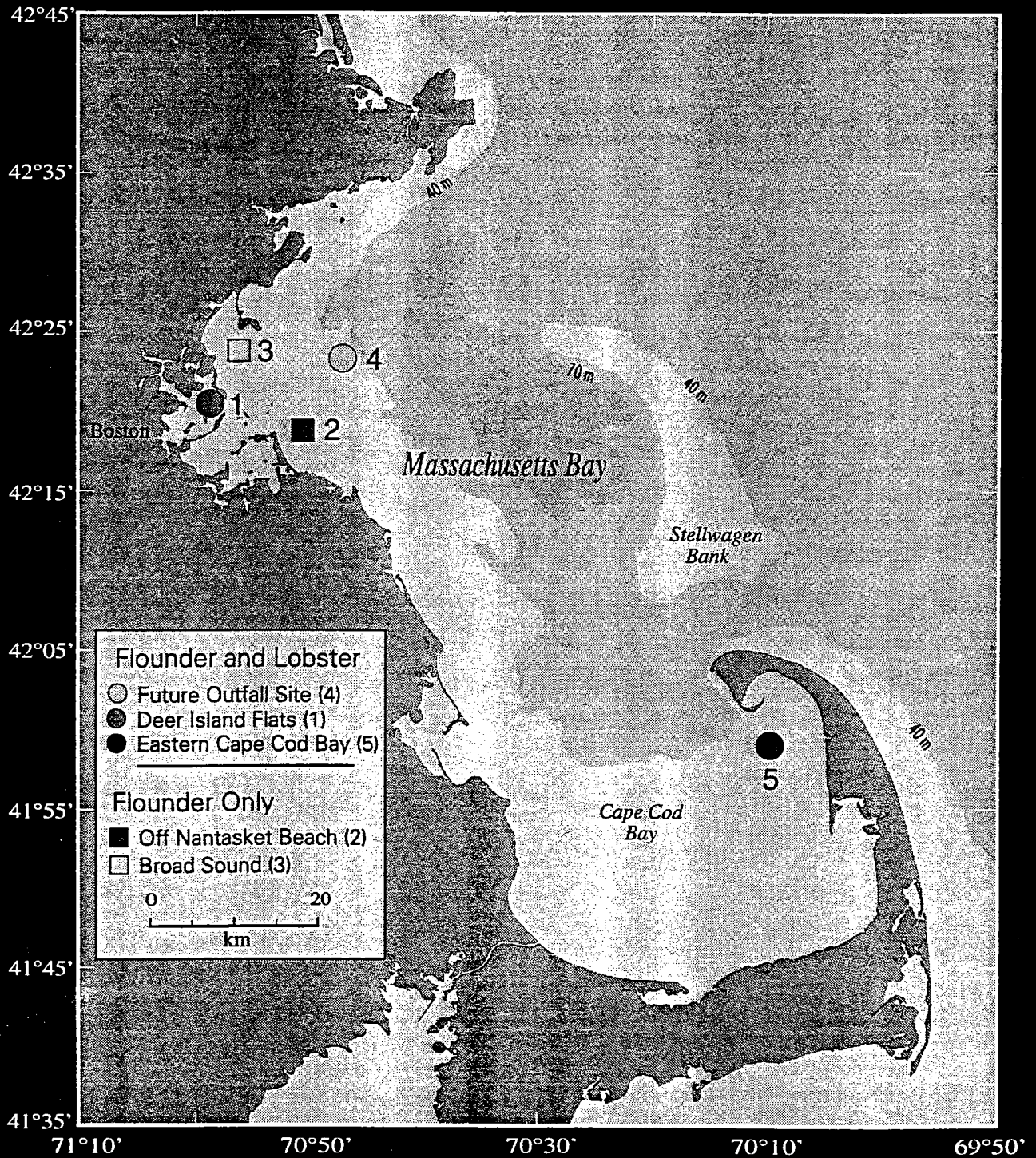
HARBOR MONITORING SCHEDULE

Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Water Column												
CSO												
Nutrients	●	●	●	●	●	●	●	●	●	●	●	●
Nutrient Flux	●				●	●	●	●	●	●	●	●
Fish & Shellfish				●								
Benthic Community												
Traditional							●					
Recon							●	●				
Effluent	●	●	●	●	●	●	●	●	●	●	●	●

Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.

FISH and SHELLFISH STATIONS



Measurements in the MWRA Monitoring Program

Chemical and Related Parameters

Analyte	Effluent	Tissue ^{a,b}	Sediment	Water Column	Benthic Flux
Nutrients	○		○	○	○
Metals	○	○	○		
PCB (congeners)	○	○	○		
Pesticides	○	○	○		
PAHs	○	○	○		
LABs	○		○		
Sterols	○		○		
Coprostanol			○		
<i>C. perfringens</i>	○		○		
Grain Size			○		
TOC			○		
¹⁵ N	○	○			
³⁴ S	○	○			
Lipids		○			

^a Histopathology of flounder liver and flounder age are also determined

^b Lobster tail and hepatopancreas; flounder filet and liver

Table 5. Analytes Included in Tissue Chemistry Analyses.

<p>Major Metals Al Aluminum Fe Iron</p> <p>Trace Metals^a Ag Silver Cd Cadmium Cr Chromium Cu Copper Hg Mercury^b Ni Nickel Pb Lead Zn Zinc</p> <p>Polychlorinated biphenyls (PCBs)^c 2,4-Cl₂(8) 2,2',5-Cl₃(18) 2,4,4'-Cl₃(18) 2,2',3,5'-Cl₄(44) 2,2',5,5'-Cl₄(52) 2,3',4,4'-Cl₄(66) 3,3',4,4'-Cl₄(77) 2,2',4,5,5'-Cl₅(101) 2,3,3',4,4'-Cl₅(105) 2,3',4,4',5-Cl₅(118) 3,3',4,4',5-Cl₅(126) 2,2',3,3,4,4'-Cl₆(128)['] 2,2',3,4,4',5-Cl₆(138) 2,2',4,4',5,5'-Cl₆(153) 2,2',3,3,4,4',5-Cl₇(170) 2,2',3,4,4',5,5'-Cl₇(180) 2,2',3,4,5,5',6-Cl₇(187) 2,2',3,3',4,4',5,6-Cl₈(195) 2,2',3,3',4,4',5,5',6-Cl₉(206) Decachlorobiphenyl-Cl₁₀(209)</p> <p>Polynuclear Aromatic Hydrocarbons (PAHs)^a naphthalene C₁-naphthalenes C₂-naphthalenes C₃-naphthalenes acenaphthylene acenaphthene C₁-fluorenes</p>	<p>NOT ANALYZED FOR</p> <p>Polynuclear Aromatic Hydrocarbons (PAHs) (continued) C₂-fluorenes C₃-fluorenes phenanthrene C₁-Phenanthrenes/anthracene C₂-Phenanthrenes/anthracene C₃-Phenanthrenes/anthracene C₄-Phenanthrenes/anthracene dibenzothiophene C₁-dibenzothiophenes C₂-dibenzothiophenes C₃-dibenzothiophenes fluoranthene pyrene C₁-fluoranthenes/pyrene benzo[a]anthracene chrysene C₁-chrysene C₂-chrysene C₃-chrysene C₄-chrysene benzo[b]fluoranthene benzo[k]fluoranthene benzo[a]pyrene dibenzo[a,h]perylene indeno[1,2,3-c,d]pyrene Perylene Biphenyl Benzo[e]pyrene Dibenzofuran</p> <p>Pesticides^c Hexachlorobenzene Lindane Heptachlor Endrin Aldrin Heptachlorepoxyde alpha-chlordane trans-Nonachlor Dieldrin Mirex 2,4'-DDD 4,4'-DDD 2,4'-DDE 4,4'-DDE 2,4'-DDT 4,4'-DDT</p> <p>Lipids^c</p>
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^a Flounder liver; lobster hepatopancreas

^b Flounder and lobster edible tissue

^c Flounder edible tissue and liver; lobster edible tissue and hepatopancreas

QUESTIONS AND CHARGE FOR THE WORKSHOP

Are the objectives of the fish and shellfish studies sufficient?

Are the baseline data sufficient for understanding the system? Why or why not?

Is coverage in space and time adequate?

What is the role of the measurements in the monitoring program?

Should the levels of meaningful change defined in the draft monitoring plan be modified?

What are the key parameters (metrics) that are considered to be indicators and endpoints?

Are there missing/additional measurements that should be made?

What monitoring questions can be phrased as quantitative hypotheses?

APPENDIX C
PRESENTATIONS

APPENDIX C-1

**P. DOWNEY
AQUATEC**

**Bioaccumulation of
Selected Organic Compounds
and Selected Metals
in Mussels Deployed Near
Deer Island Discharge and in
Massachusetts Bay, 1993**

Prepared for:

**Massachusetts Water Resources Authority
100 First Street
Boston, Massachusetts 02129**

By :

**Philip C. Downey
Inchcape Testing Services
Aquatec Laboratories
55 South Park Drive
Colchester, Vermont 05446**

February 1994

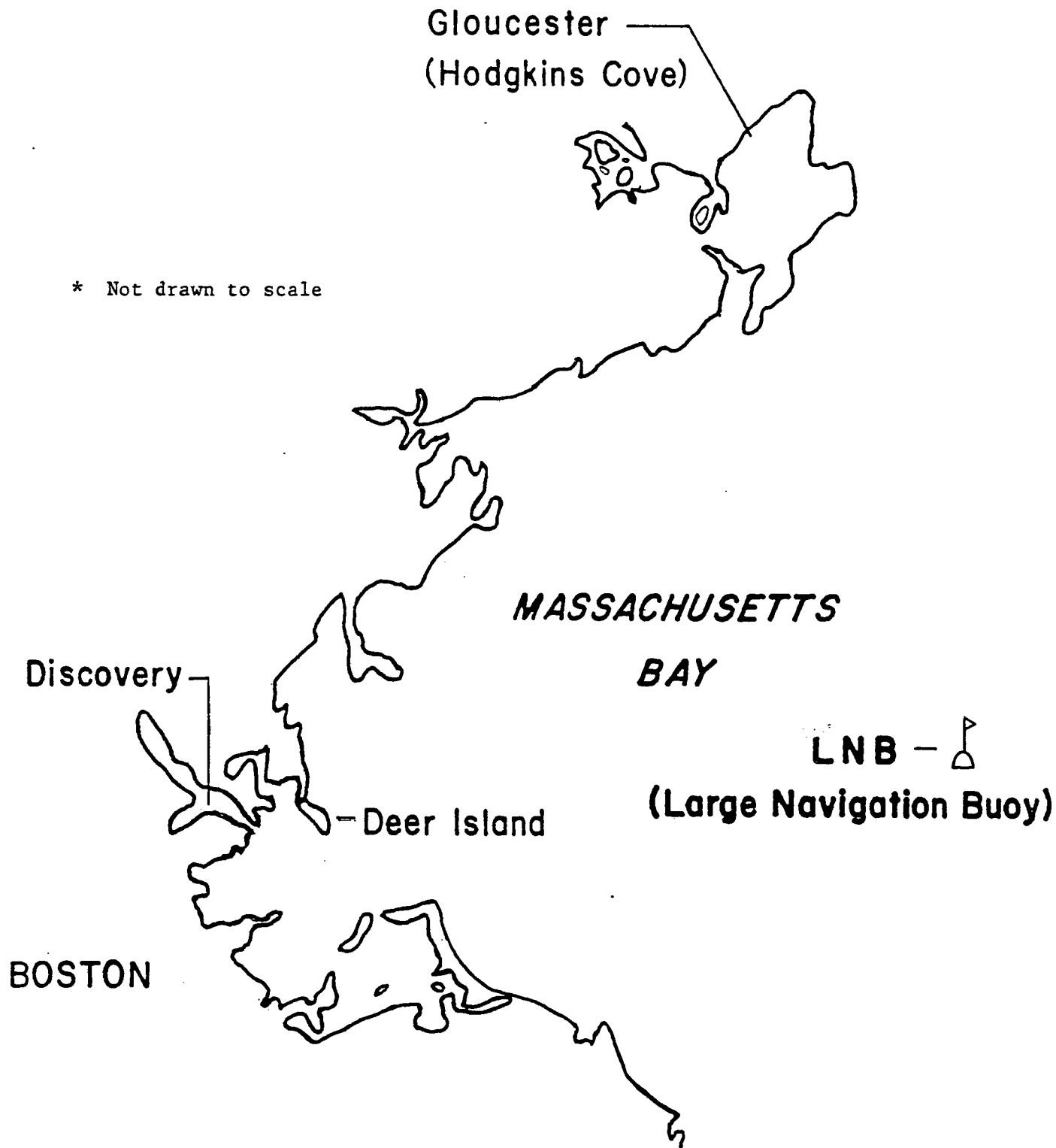


Figure 1. The mussel deployment locations for the 1993 bioaccumulation study. The Gloucester location was the source of all mussels for the study.

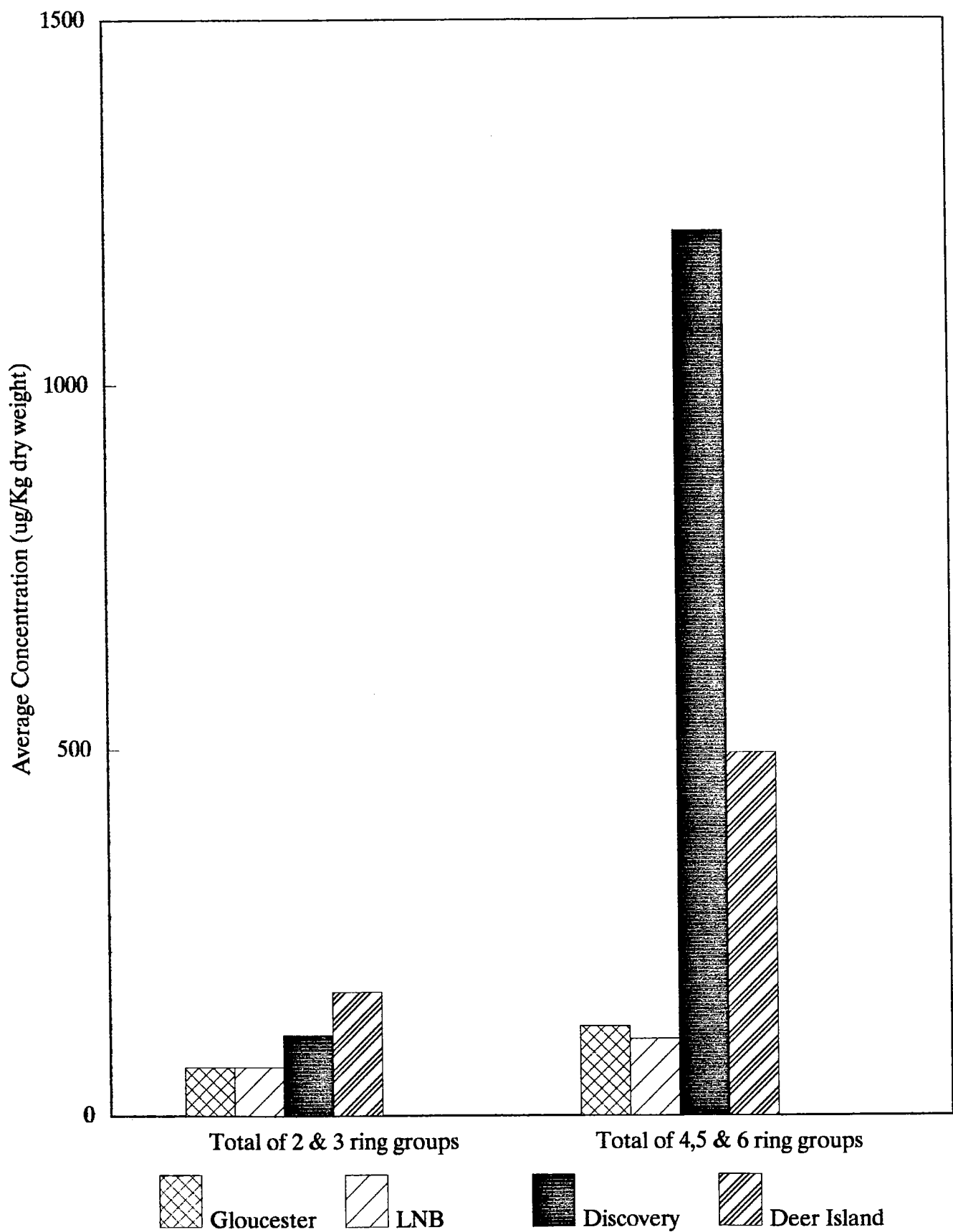


Figure 4. Average concentrations of two groups (2 & 3 ring; 4, 5 & 6 ring) of polynuclear aromatic hydrocarbons in mussel tissue collected from the four stations.

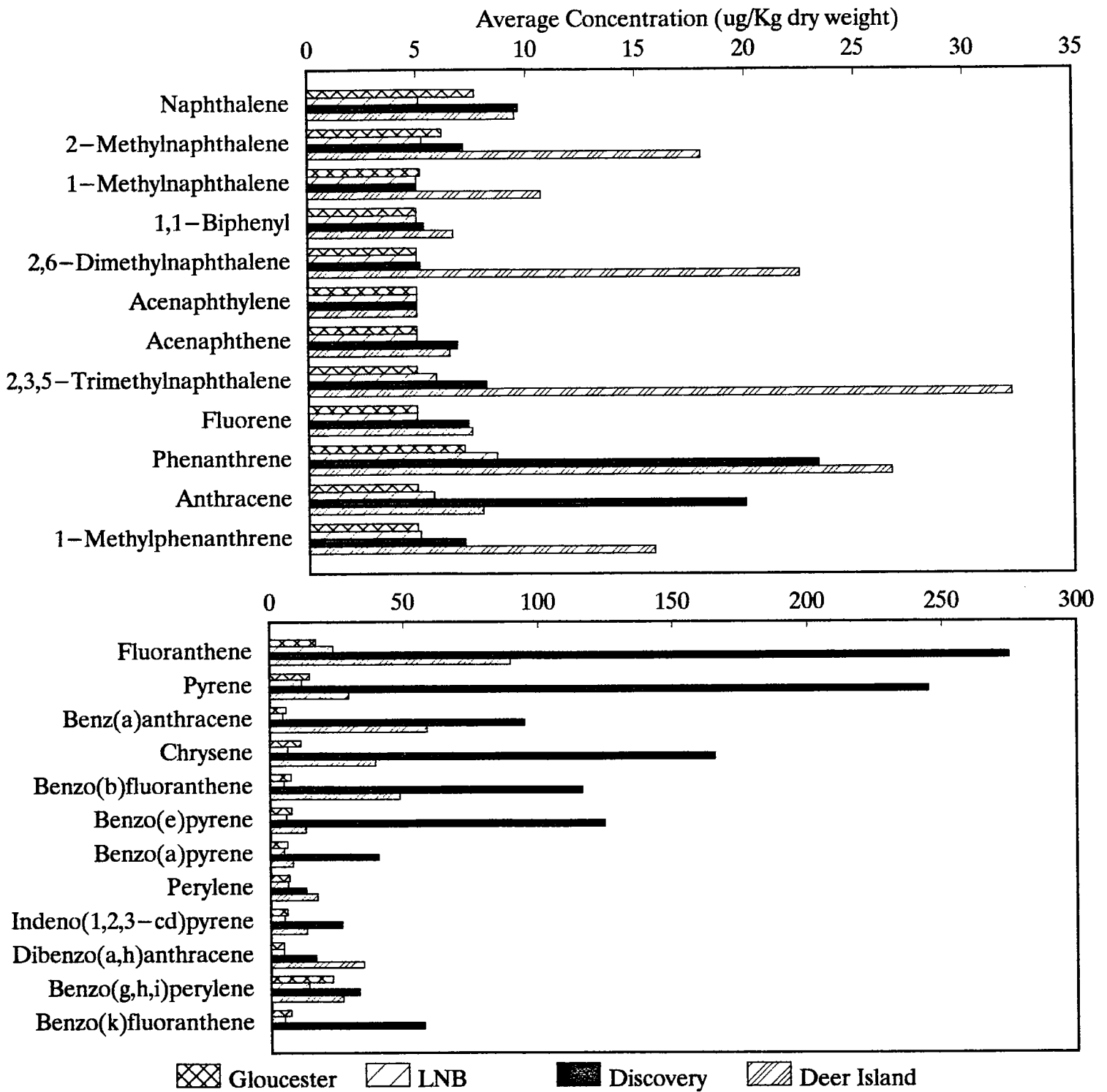


Figure 5. Average concentrations of polynuclear aromatic hydrocarbons in mussel tissue collected from the four stations.

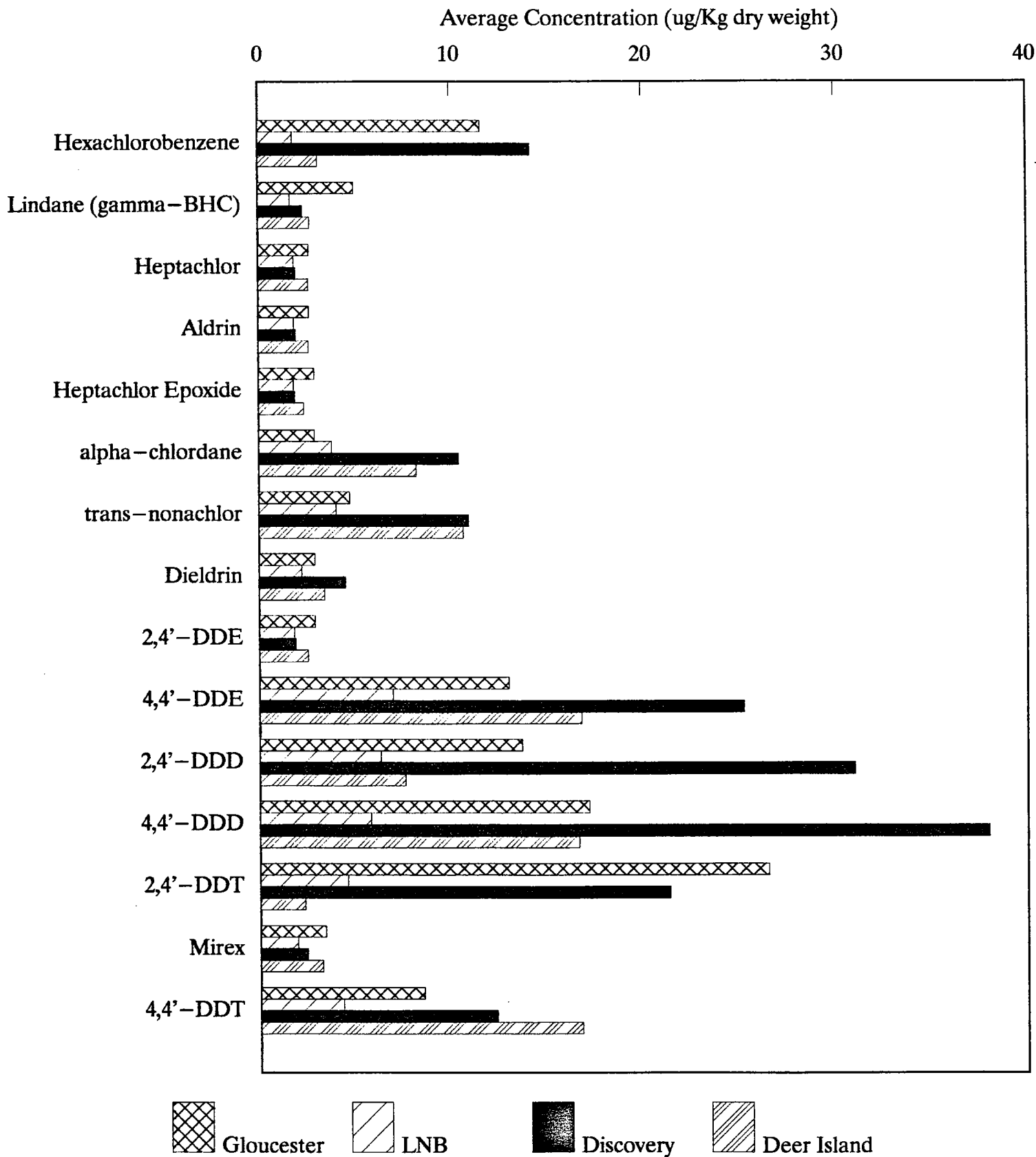


Figure 7. Average concentrations of pesticides in mussel tissue collected from the four stations.

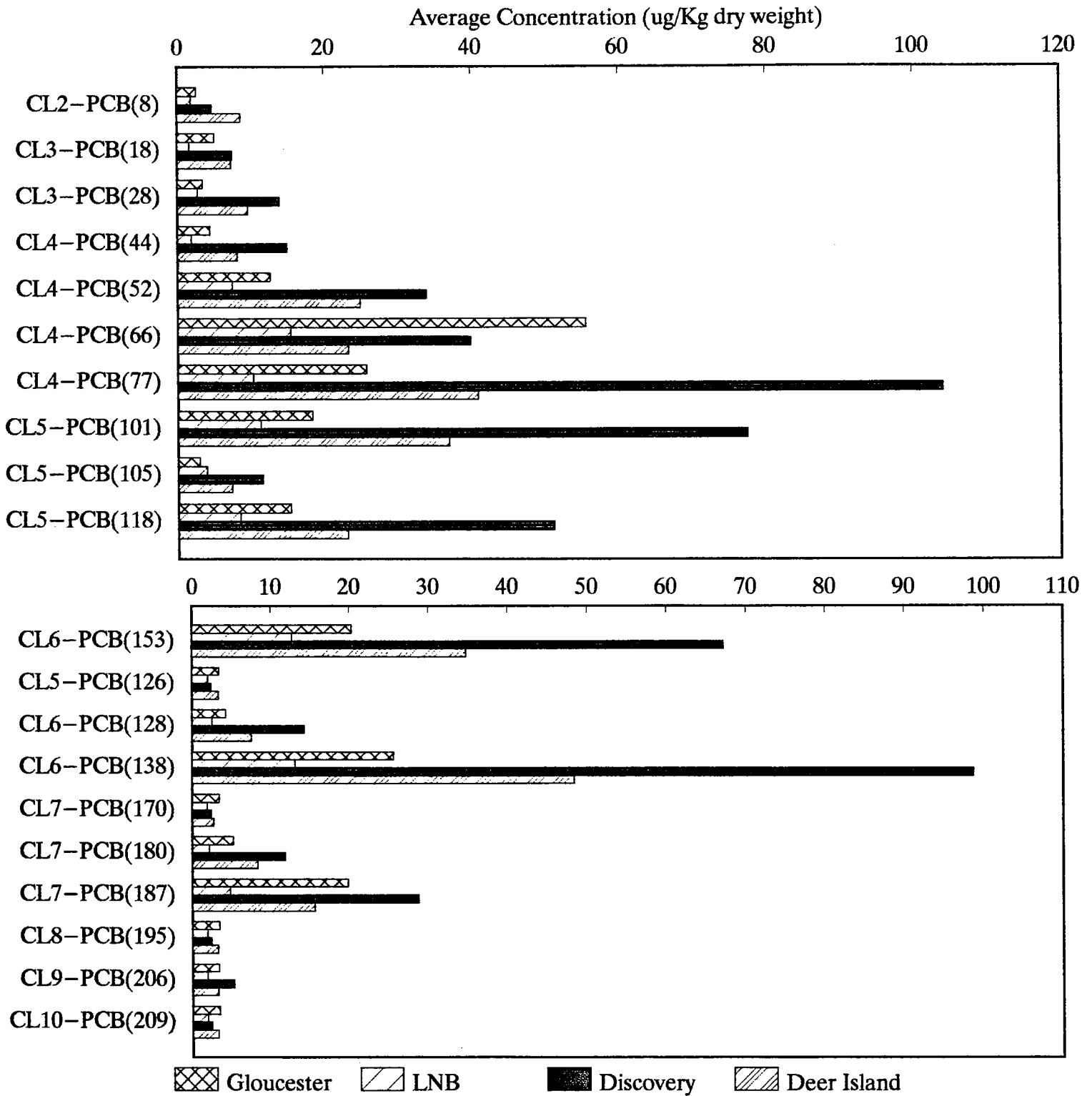


Figure 9. Average concentrations of polychlorinated biphenyls in mussel tissue collected from the four stations.

Table 6. Average concentrations of PAHs (ug/Kg dry weight) from mussels harvested during predeployment at Gloucester and during the 60-day retrieval from the three stations. Averages were calculated using the detection limit value as an estimated concentration when individual analytes were not detected.

Parameter	June Predeployment Gloucester			August 60-Day Retrieval Large Navigation Buoy			August 60-Day Retrieval Discovery			August 60-Day Retrieval Deer Island		
	AVE	SD	Range	AVE	SD	Range	AVE	SD	Range	AVE	SD	Range
Naphthalene	8	5	5- 18	5	0	5- 6	10	1	8- 11	10	3	6- 13
2-Methylnaphthalene	6	2	5- 11	5	0	5- 6	7	2	5- 10	18	7	10- 28
1-Methylnaphthalene	5	0	5- 6	5	0	5- 5	5	0	5- 5	11	5	6- 18
1,1-Biphenyl	5	0	5- 5	5	0	5- 5	5	1	5- 6	7	1	5- 7
2,6-Dimethylnaphthalene	5	0	5- 5	5	0	5- 5	5	0	5- 6	23	7	14- 32
Acenaphthylene	5	0	5- 5	5	0	5- 5	5	0	5- 5	5	0	5- 5
Acenaphthene	5	0	5- 5	5	0	5- 5	7	1	5- 8	7	2	5- 10
2,3,5-Trimethylnaphthalene	5	0	5- 5	6	2	5- 10	8	3	5- 13	32	11	21- 46
Fluorene	5	0	5- 5	5	0	5- 5	7	3	5- 11	8	2	5- 11
Phenanthrene	7	2	5- 10	9	3	6- 13	23	15	10- 51	27	10	17- 43
Anthracene	5	0	5- 5	6	2	5- 10	20	16	11- 52	8	2	6- 11
1-Methylphenanthrene	5	0	5- 5	5	0	5- 6	7	3	5- 13	16	6	9- 25
Total of 2 & 3 ring groups	66	9	60- 83	66	6	61- 75	110	38	75- 181	169	54	112- 249
Fluoranthene	18	7	9- 27	24	5	16- 32	275	35	249- 327	114	38	72- 160
Pyrene	15	5	10- 23	12	2	9- 16	245	35	212- 301	90	30	58- 128
Benz(a)anthracene	6	1	5- 8	5	0	5- 5	95	26	71- 135	30	13	16- 51
Chrysene	12	5	6- 19	7	1	5- 9	166	28	139- 209	59	21	38- 91
Benzo(b)fluoranthene	8	2	5- 11	5	1	5- 7	116	16	99- 145	40	17	18- 61
Benzo(e)pyrene	8	3	5- 12	6	2	5- 12	125	16	106- 150	49	17	25- 68
Benzo(a)pyrene	7	2	5- 9	5	1	5- 7	41	8	33- 55	14	4	6- 19
Perylene	7	3	5- 13	7	5	5- 18	14	2	11- 17	9	5	5- 19
Indeno(1,2,3-cd)pyrene	6	1	5- 8	5	0	5- 6	27	10	15- 41	18	10	7- 31
Dibenzo(a,h)anthracene	5	0	5- 5	5	0	5- 5	17	7	9- 24	14	11	5- 33
Benzo(g,h,i)perylene	23	29	5- 81	14	15	5- 43	33	11	21- 53	35	38	9- 111
Benzo(k)fluoranthene	8	2	5- 11	5	0	5- 6	57	12	45- 75	27	13	12- 43
Total of 4, 5 & 6 ring groups	122	49	70- 194	101	28	75- 163	1210	179	1057- 1510	496	133	289- 680
Total PAH's	188	56	130- 277	166	31	136- 237	1321	183	1139- 1617	665	182	412- 929

Table 8. Average concentrations of pesticides (ug/Kg dry weight) from mussels harvested during predeployment at Gloucester and during the 60-day retrieval from the three stations. Averages were calculated using detection limit value as an estimated concentration when individual analytes were not detected.

Parameter	June Predeployment Gloucester (Sample size - 5)			August 60-Day Retrieval Large Navigation Buoy (Sample size - 8)			August 60-Day Retrieval Discovery (Sample size - 5)			August 60-Day Retrieval Deer Island (Sample size - 5)		
	AVE	SD	Range	AVE	SD	Range	AVE	SD	Range	AVE	SD	Range
Hexachlorobenzene	11.62	8.43	2.40 - 24.20	1.82	0.78	0.49 - 2.70	14.20	11.86	1.80 - 30.00	3.14	1.35	1.60 - 4.80
Lindane (gamma-BHC)	5.00	3.44	2.20 - 11.00	1.69	0.81	0.71 - 2.70	2.33	0.82	1.30 - 3.30	2.70	0.30	2.40 - 3.00
Heptachlor	2.64	1.13	1.50 - 4.20	1.88	0.69	0.93 - 2.70	1.95	1.14	1.00 - 3.50	2.62	1.11	1.30 - 3.90
Aldrin	2.64	1.13	1.50 - 4.20	1.88	0.69	0.93 - 2.70	1.95	1.14	1.00 - 3.50	2.62	1.11	1.30 - 3.90
Heptachlor Epoxide	2.92	1.28	1.50 - 4.50	1.85	0.61	1.00 - 2.60	1.93	0.99	1.00 - 3.20	2.38	0.95	1.30 - 3.90
alpha-chlordane	2.92	1.28	1.50 - 4.50	3.81	0.48	3.30 - 4.60	10.45	1.83	7.80 - 12.00	8.24	1.81	5.20 - 9.70
trans-nonachlor	4.76	1.26	3.70 - 6.90	4.04	0.49	3.30 - 4.60	10.95	2.53	7.80 - 14.00	10.68	2.82	6.80 - 13.00
Dieldrin	2.92	1.28	1.50 - 4.50	2.24	0.18	2.00 - 2.60	4.53	1.65	2.50 - 6.50	3.42	1.57	1.30 - 5.30
2,4'-DDE	2.92	1.28	1.50 - 4.50	1.85	0.61	1.00 - 2.60	1.93	0.99	1.00 - 3.20	2.56	1.02	1.30 - 3.90
4,4'-DDE	13.00	2.83	10.00 - 16.00	6.98	0.79	5.50 - 7.60	25.25	3.10	21.00 - 28.00	16.80	4.32	11.00 - 22.00
2,4'-DDD	13.70	4.09	7.50 - 18.00	6.34	3.05	2.20 - 11.00	31.00	28.91	14.00 - 74.00	7.62	7.17	1.30 - 19.00
4,4'-DDD	17.18	5.58	8.90 - 22.00	5.80	0.93	4.00 - 6.60	38.00	4.69	33.00 - 42.00	16.66	6.64	8.30 - 23.00
2,4'-DDT	26.52	29.99	1.50 - 64.00	4.60	5.68	1.00 - 16.00	21.38	24.90	2.40 - 56.00	2.36	1.15	1.30 - 3.90
Mirex	3.42	2.08	1.40 - 6.00	1.95	0.81	0.90 - 3.10	2.48	1.09	1.00 - 3.30	3.26	1.04	2.00 - 4.40
4,4'DDT	8.56	4.25	4.50 - 14.00	4.34	0.59	3.40 - 5.30	12.35	6.04	8.00 - 21.00	16.80	5.66	9.80 - 23.20
Dieldrin/Aldrin group	5.56	2.40	3.00 - 8.70	4.12	0.60	3.03 - 4.70	6.48	2.38	3.70 - 8.60	6.04	2.29	2.60 - 7.90
Chlordane group	15.60	3.02	10.50 - 18.00	11.39	1.56	9.31 - 14.00	25.65	5.42	17.90 - 30.50	24.00	5.34	15.70 - 28.40
DDD/DDE/DDT	81.88	38.11	38.90 - 137.50	29.90	6.50	23.00 - 40.70	129.90	53.44	83.70 - 188.30	62.80	21.08	35.20 - 86.40

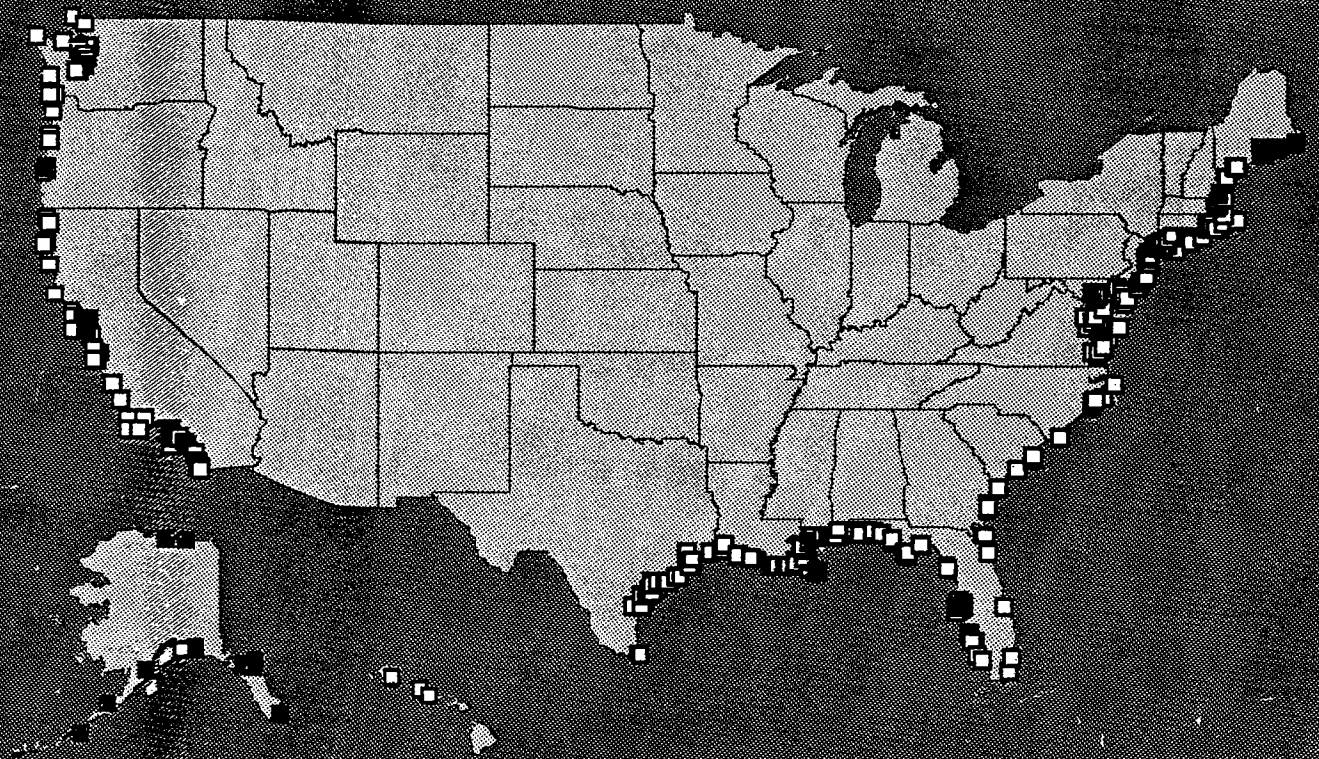
Table 10. Average concentrations of PCBs (ug/Kg dry weight) from mussels harvested during predeployment at Gloucester and during the 60-day retrieval at the three stations. Averages were calculated using the detection limit value as an estimated concentration when individual analytes were not detected.

Parameter	June Predeployment Gloucester (Sample size - 5)			August 60-Day Retrieval Large Navigation Buoy (Sample size - 8)			August 60-Day Retrieval Discovery (Sample size - 4)			August 60-Day Retrieval Deer Island (Sample size - 5)		
	AVE	SD	Range	AVE	SD	Range	AVE	SD	Range	AVE	SD	Range
CL2-PCB(8)	2.64	1.13	1.50 - 4.20	1.88	0.69	0.93 - 2.70	4.75	4.21	2.00 - 11.00	8.64	10.67	1.30 - 27.00
CL3-PCB(18)	5.04	3.52	1.50 - 9.20	1.66	0.42	1.30 - 2.50	7.48	1.18	6.50 - 9.10	7.36	2.86	3.30 - 9.70
CL3-PCB(28)	3.44	1.60	1.50 - 5.50	2.75	0.87	1.90 - 4.60	14.00	1.83	12.00 - 16.00	9.68	3.45	5.80 - 13.00
CL4-PCB(44)	4.42	1.27	2.60 - 5.90	1.88	0.22	1.60 - 2.20	15.00	1.63	13.00 - 17.00	8.16	2.19	5.30 - 10.00
CL4-PCB(52)	12.70	3.15	9.50 - 16.00	7.48	2.25	5.20 - 12.00	34.00	4.69	27.00 - 37.00	25.00	11.58	14.00 - 43.00
CL4-PCB(66)	55.58	70.48	6.90 - 179.00	15.50	3.70	12.00 - 24.00	40.00	6.22	33.00 - 47.00	23.40	5.55	16.00 - 28.00
CL4-PCB(77)	25.80	6.42	19.00 - 34.00	10.36	3.45	2.00 - 12.00	104.00	12.11	96.00 - 122.00	41.00	12.19	23.00 - 52.00
CL5-PCB(101)	18.40	4.16	14.00 - 24.00	11.33	1.51	8.60 - 13.00	77.50	12.71	63.00 - 94.00	37.00	9.46	23.00 - 45.00
CL5-PCB(105)	2.92	1.28	1.50 - 4.50	3.94	0.47	3.20 - 4.50	11.58	12.29	1.00 - 25.00	7.34	5.47	1.30 - 13.00
CL5-PCB(118)	15.40	3.29	12.00 - 19.00	8.46	0.39	7.90 - 9.00	51.25	6.75	44.00 - 57.00	23.20	7.29	13.00 - 29.00
CL6-PCB(153)	20.30	9.98	4.50 - 31.00	12.75	1.49	10.00 - 14.00	67.25	10.05	57.00 - 80.00	34.80	10.23	20.00 - 47.00
CL5-PCB(126)	3.42	2.08	1.40 - 6.00	2.05	0.68	1.10 - 3.10	2.48	1.09	1.00 - 3.30	3.36	0.96	2.00 - 4.40
CL6-PCB(128)	4.30	2.21	1.40 - 7.20	2.55	0.40	1.80 - 2.90	14.33	6.77	9.30 - 24.00	7.58	2.79	4.70 - 11.30
CL6-PCB(138)	25.60	6.73	18.00 - 33.00	13.13	1.73	10.00 - 16.00	98.75	56.08	59.00 - 180.00	48.40	17.83	31.00 - 74.00
CL7-PCB(170)	3.42	2.08	1.40 - 6.00	1.95	0.81	0.90 - 3.10	2.45	1.17	1.00 - 3.50	2.74	1.09	1.40 - 4.40
CL7-PCB(180)	5.20	1.97	3.00 - 7.30	2.18	0.37	1.80 - 2.90	11.88	3.71	8.50 - 17.00	8.34	3.40	4.90 - 13.20
CL7-PCB(187)	19.80	7.36	12.00 - 30.00	4.81	0.37	4.40 - 5.50	28.75	14.66	19.00 - 50.00	15.60	5.57	9.60 - 23.40
CL8-PCB(195)	3.42	2.08	1.40 - 6.00	1.95	0.81	0.90 - 3.10	2.48	1.09	1.00 - 3.30	3.26	1.04	2.00 - 4.40
CL9-PCB(206)	3.40	2.10	1.30 - 6.00	1.95	0.81	0.90 - 3.10	5.30	4.69	2.30 - 12.30	3.26	1.04	2.00 - 4.40
CL10-PCB(209)	3.42	2.08	1.40 - 6.00	1.95	0.81	0.90 - 3.10	2.48	1.09	1.00 - 3.30	3.26	1.04	2.00 - 4.40
Total PCB's	238.62	91.70	160.20 - 394.20	110.49	8.87	94.80 - 123.60	595.67	104.06	482.60 - 701.10	321.38	96.39	191.50 - 407.20

APPENDIX C-2

**T. O'CONNOR
NOAA**

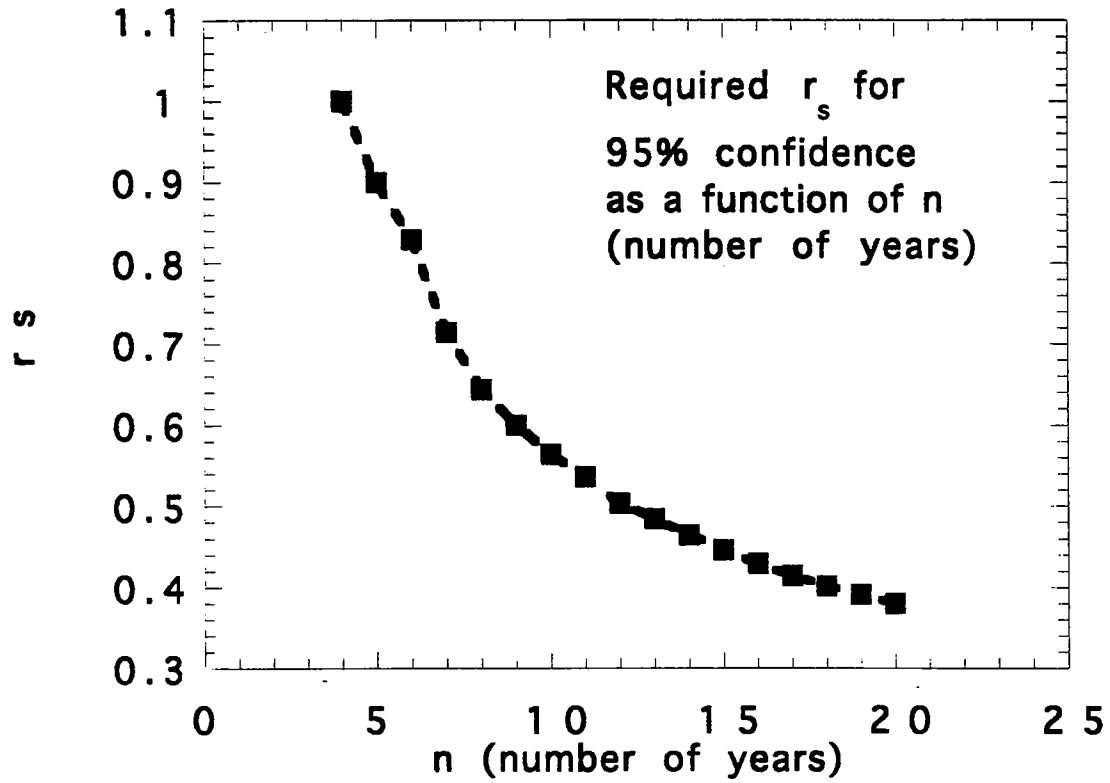
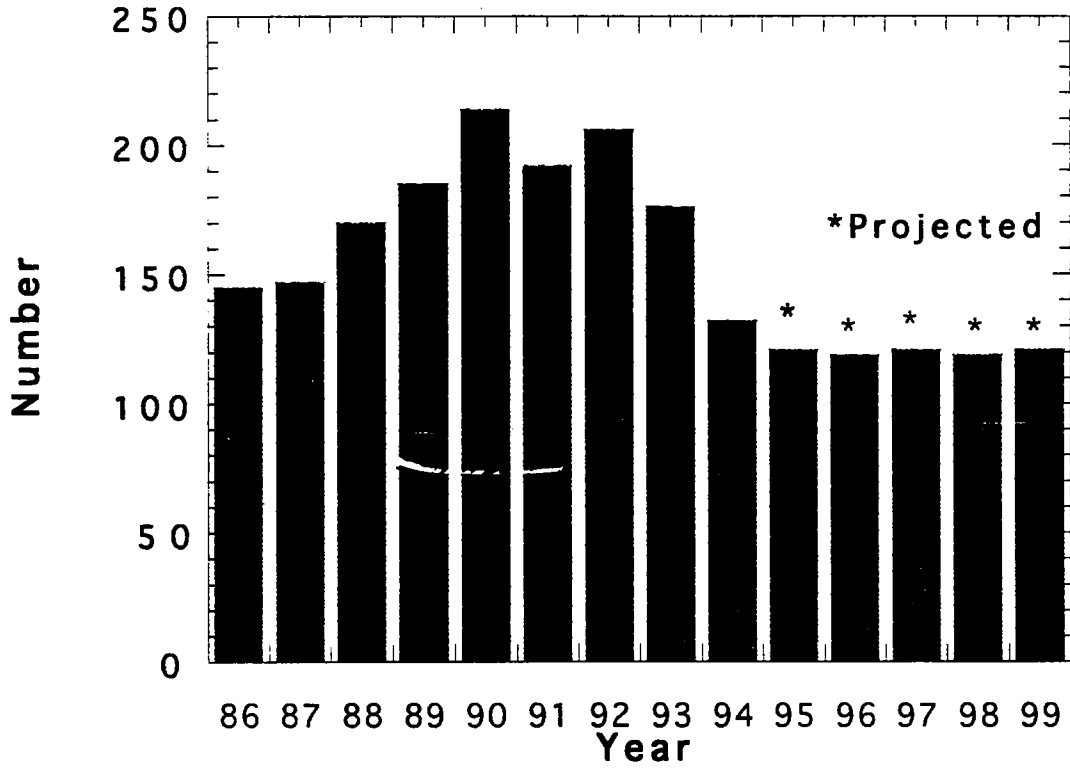
The National Status and Trends Program



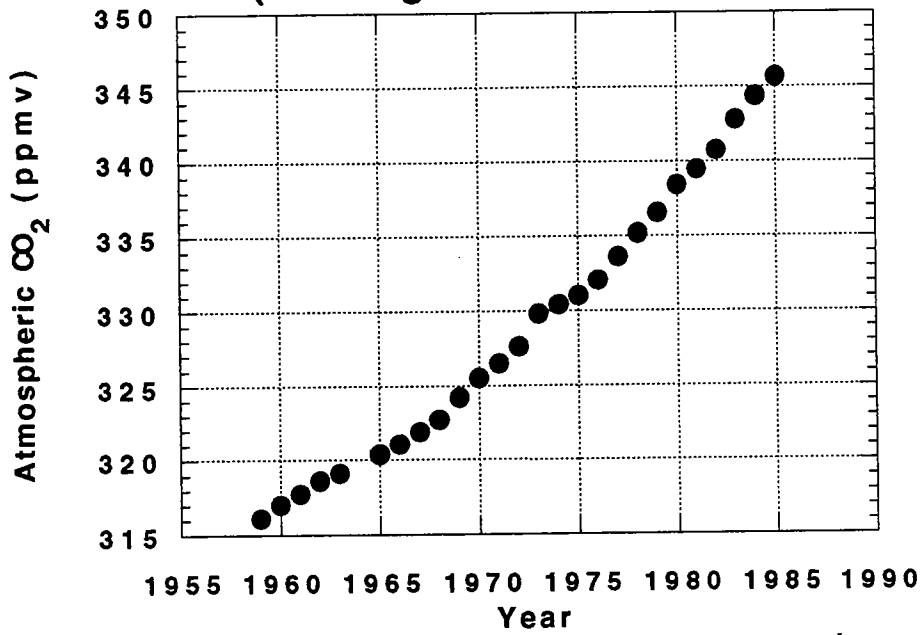
■ Benthic Surveillance Sites

□ Mussel Watch Sites

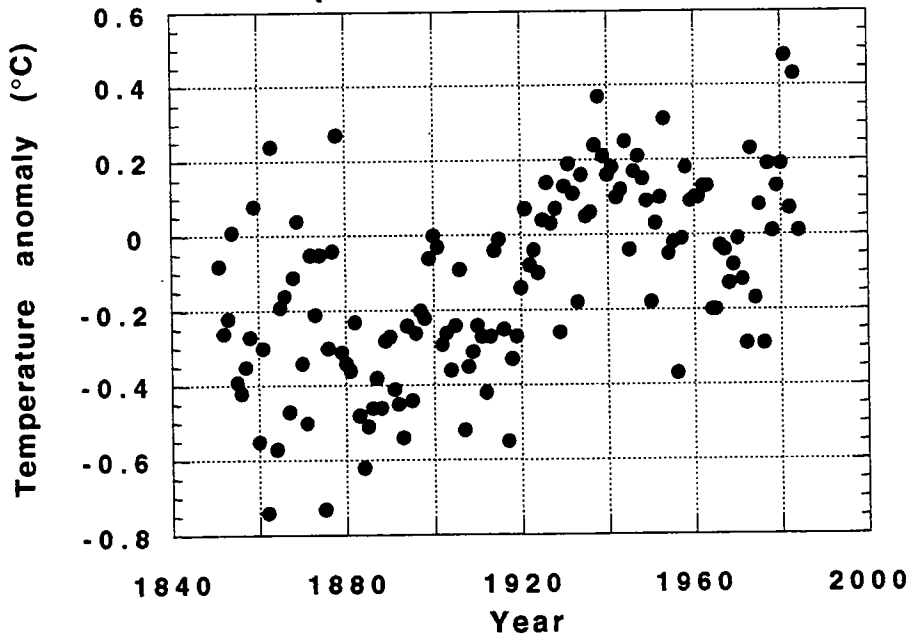
Number of Sites Sampled for Mollusks



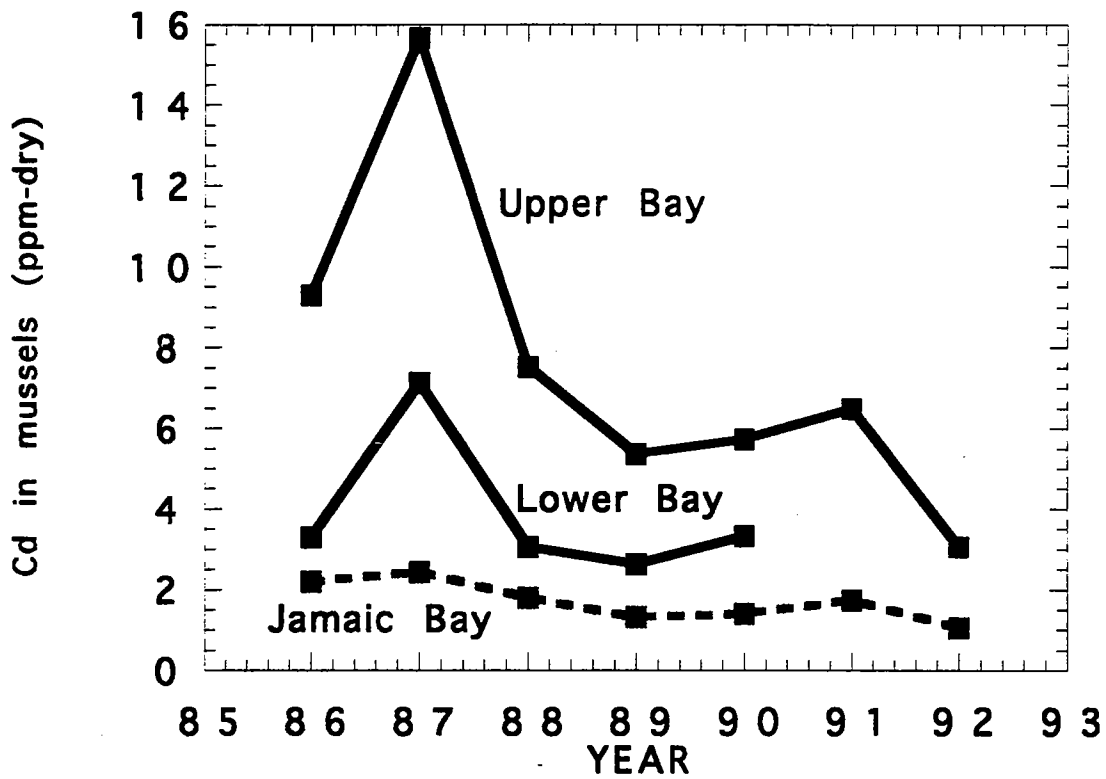
**Monotonic trend data
(Keeling and Boden, 1986)**



**Noisy trend data
(Jones et al. 1986)**



Cd in mussels at three sites in Hudson estuary

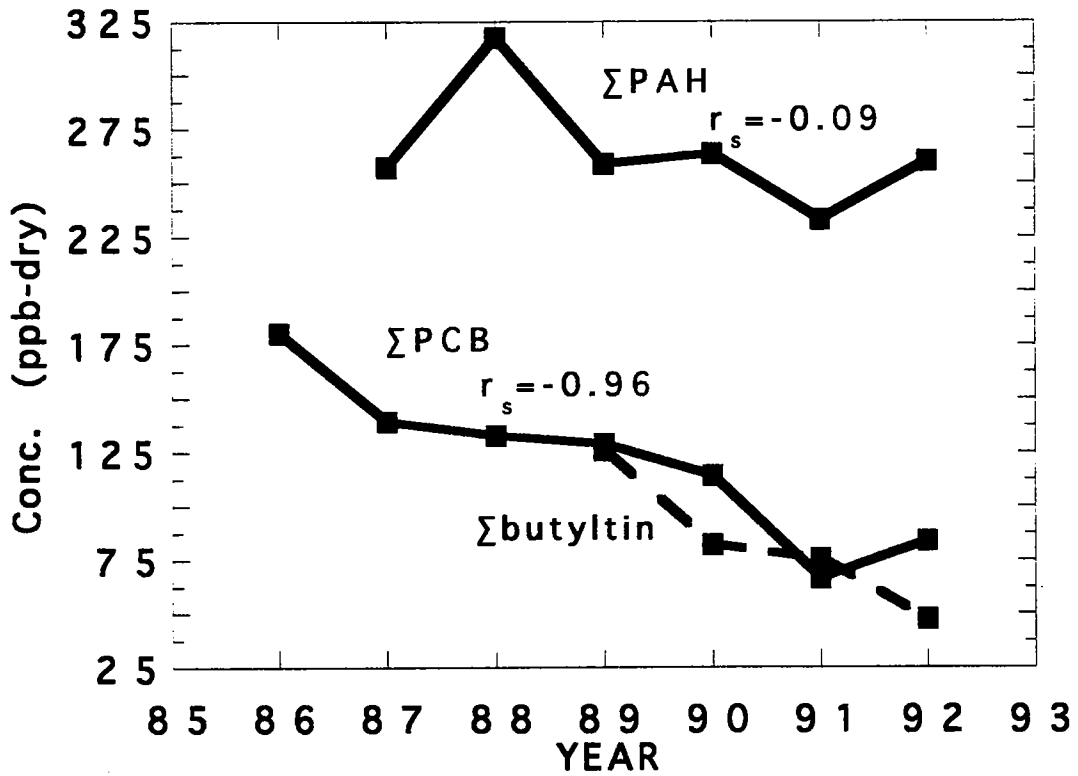


**291 significant decreases and
110 increases over 7 years at 136 sites***

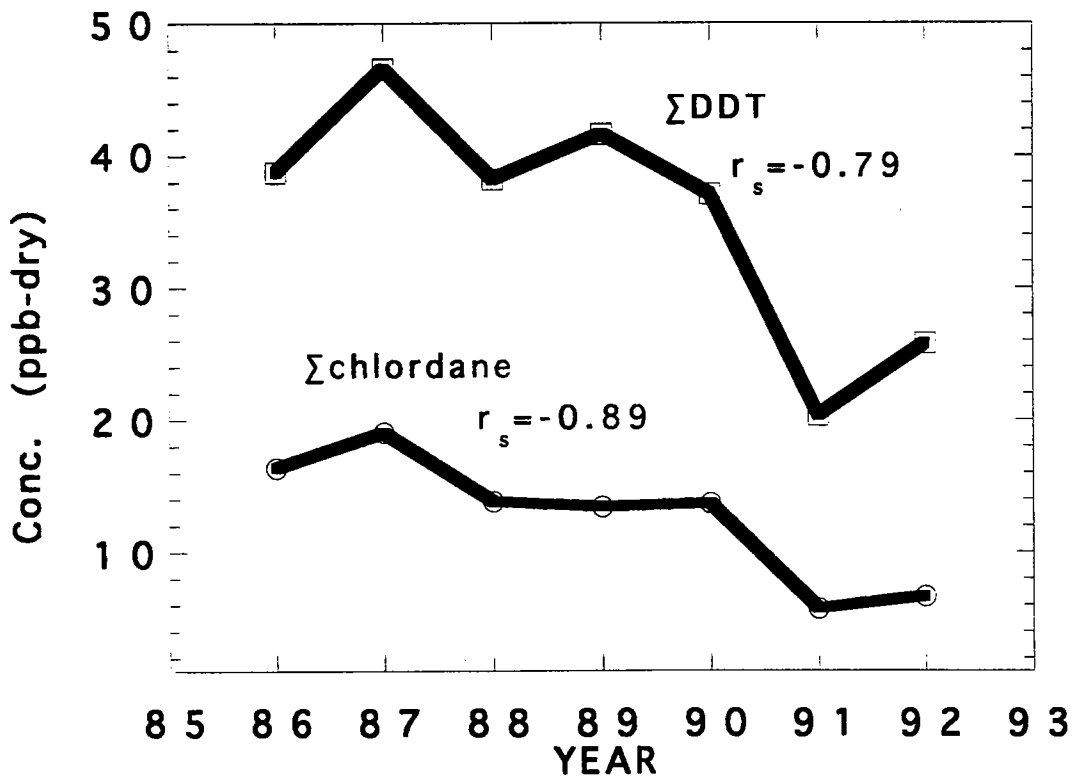
	Increases	Decreases
• Ag	9	13
• As	13	18
• Cd	9	36
• Cu	11	17
• Hg	11	9
• Ni	11	9
• Pb	9	16
• Se	7	20
• Zn	10	11
• tPCB	2	42
• tDDT	1	32
• tCdane	0	58
• tPAH	17	10
• <u>TOTALS</u>	<u>110</u>	<u>291</u>

• *7 random inc or dec per chemical, 88 per total

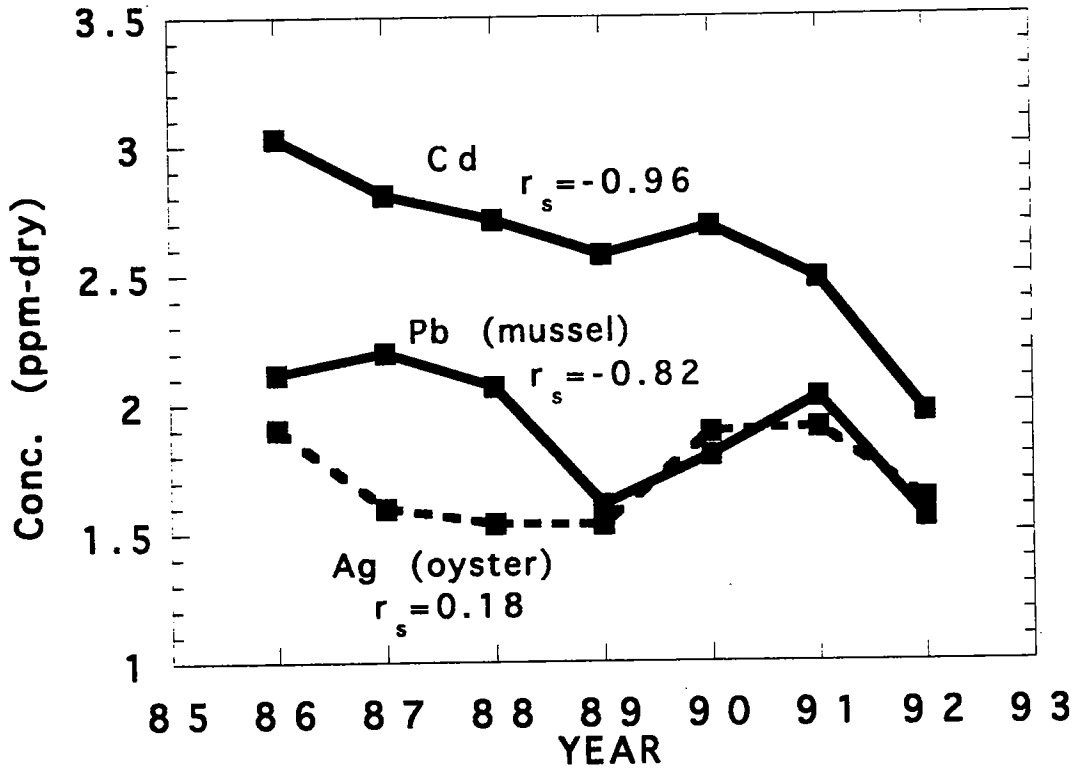
National Geometric Mean Concentrations



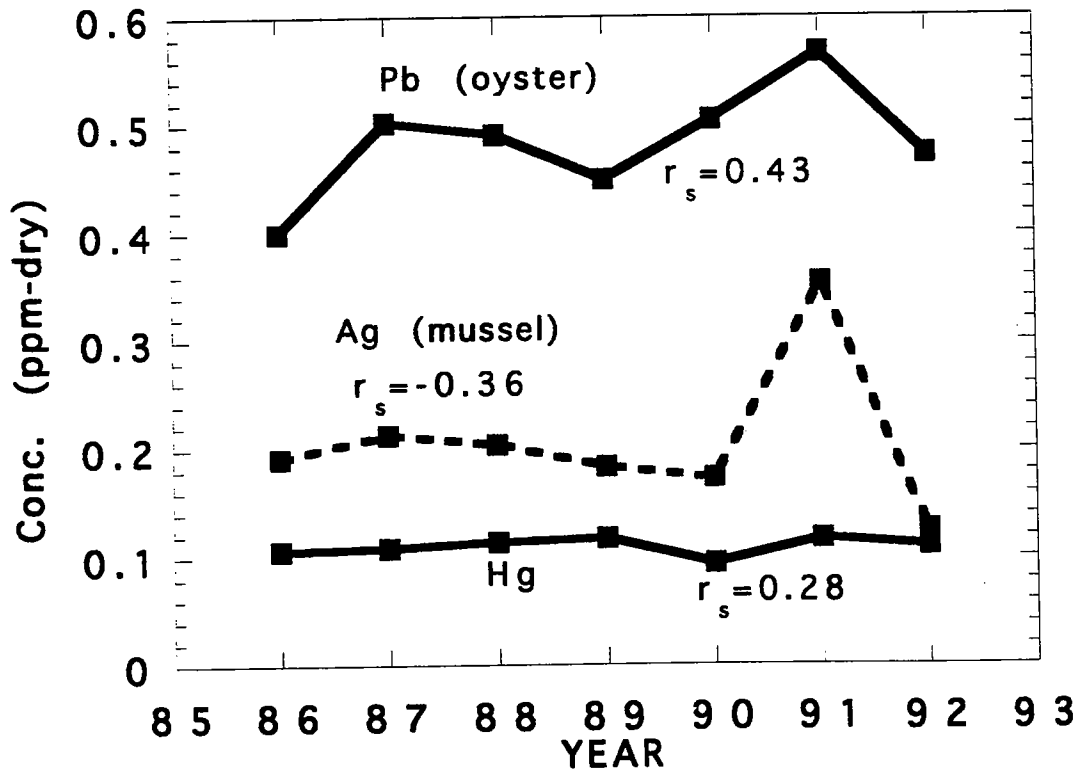
National Geometric Mean Concentrations



National Geometric Mean Concentrations



National Geometric Mean Concentrations



71.10°W

71.03°W

70.95°W

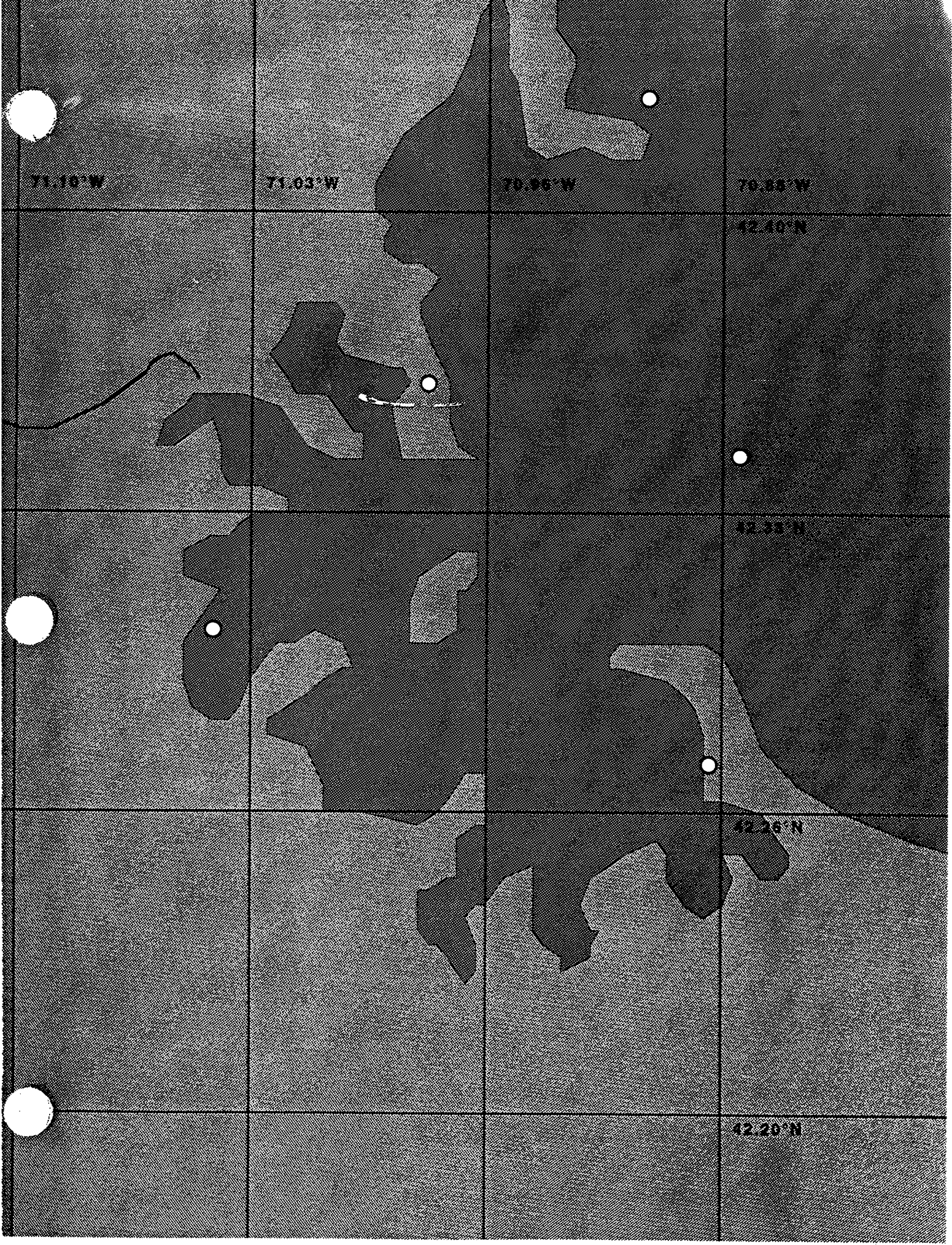
70.88°W

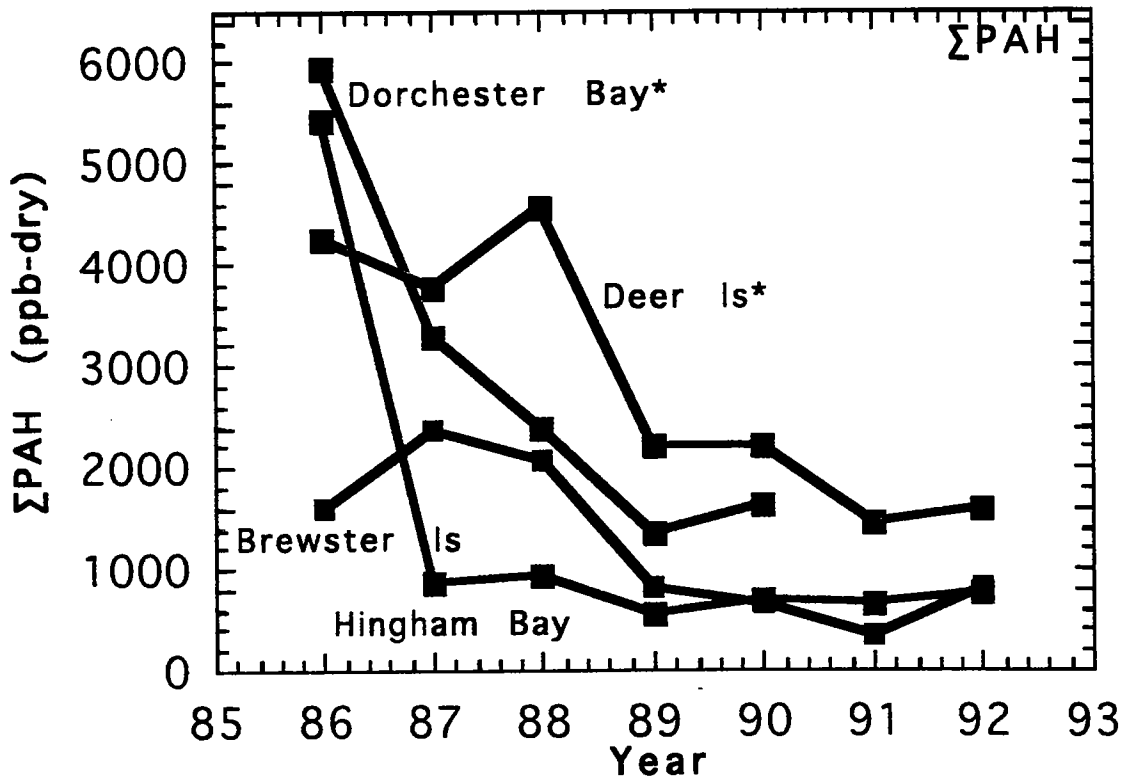
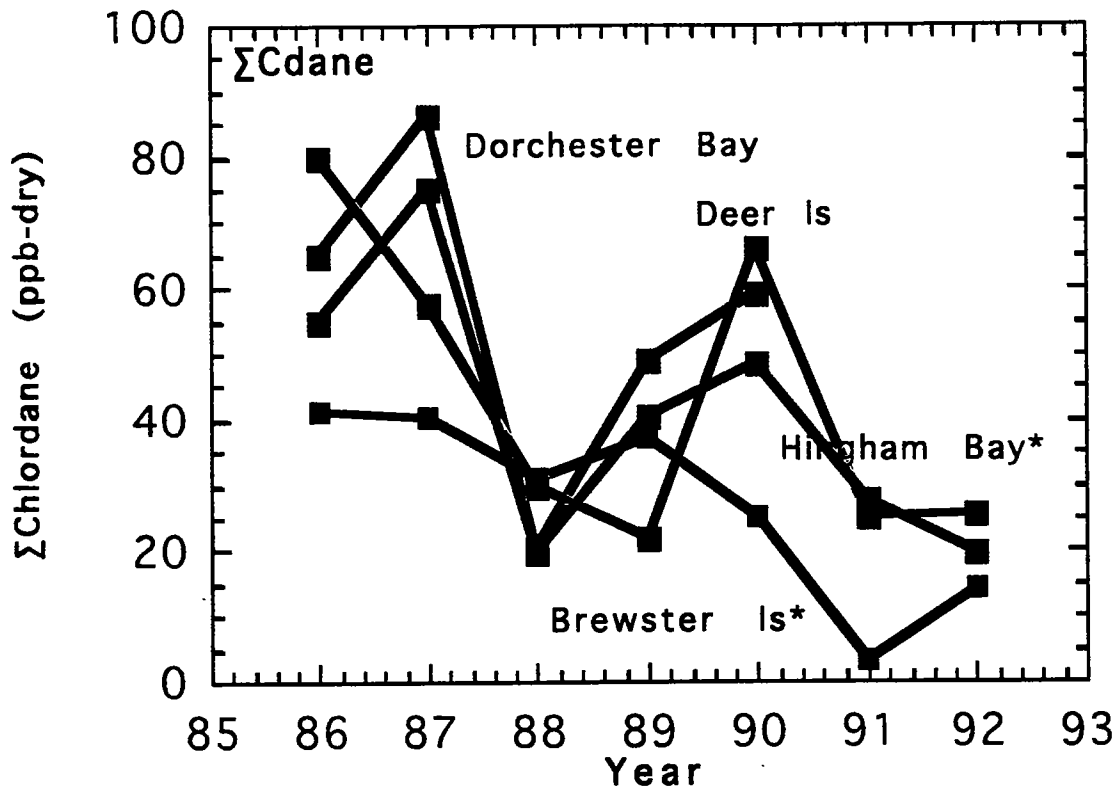
42.40°N

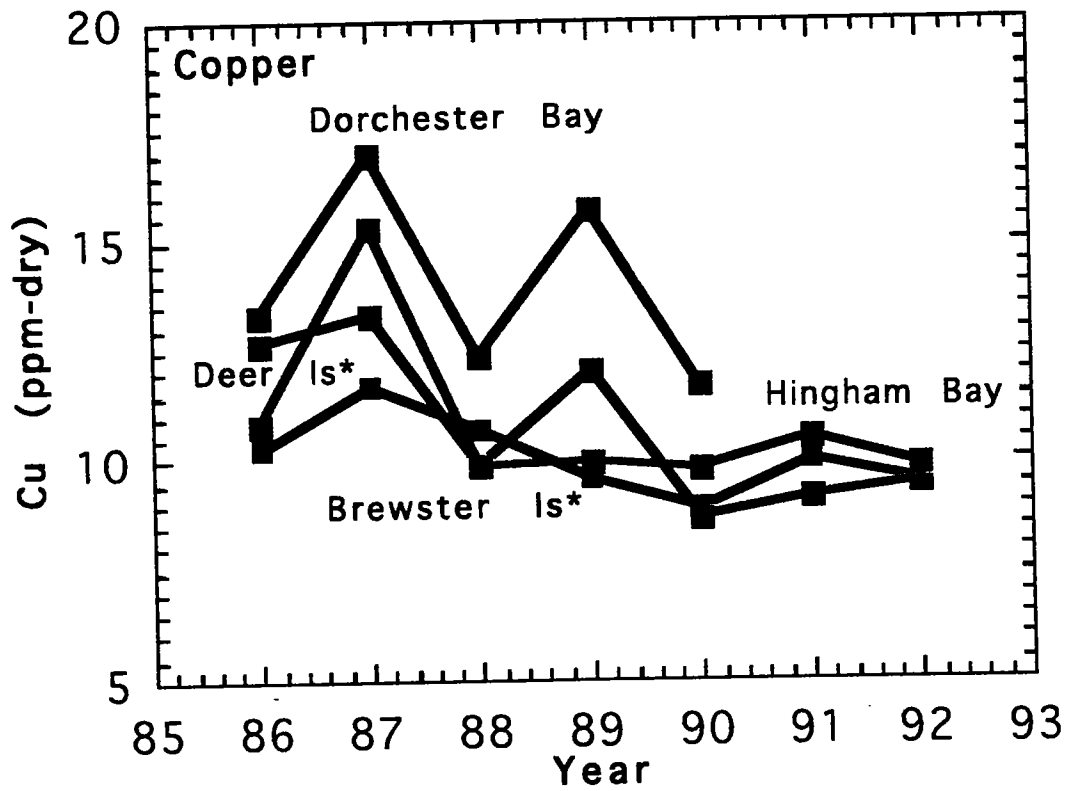
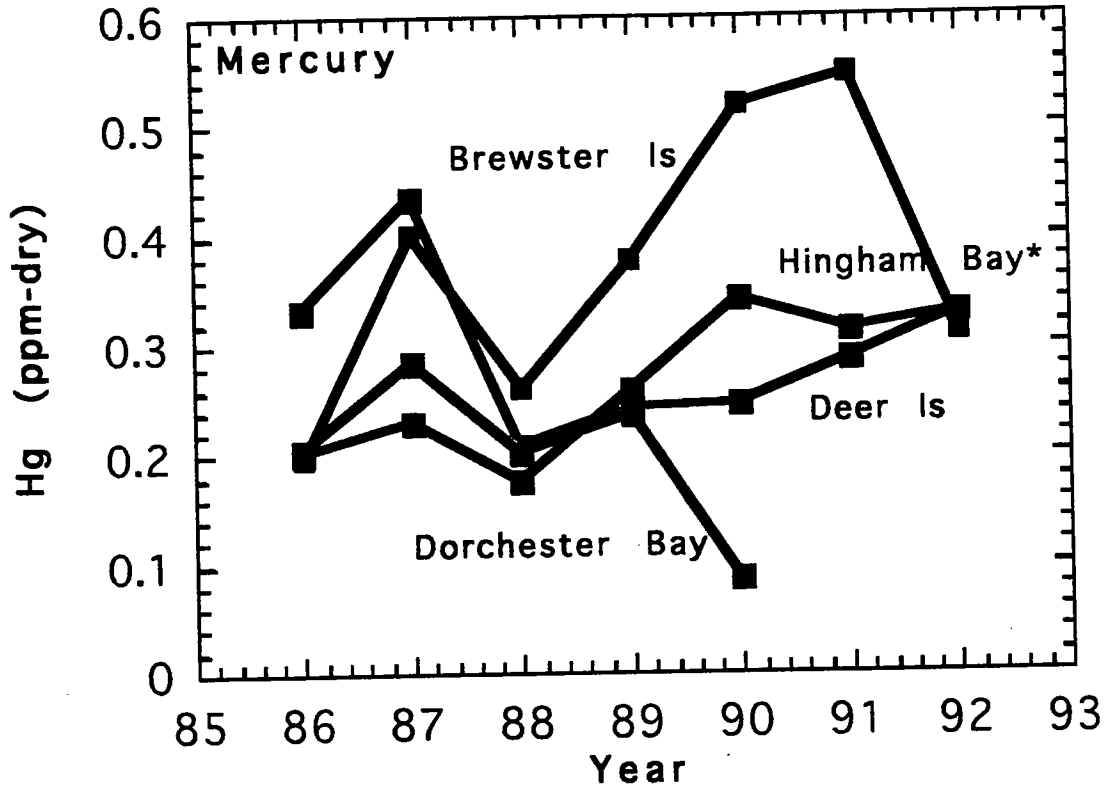
42.35°N

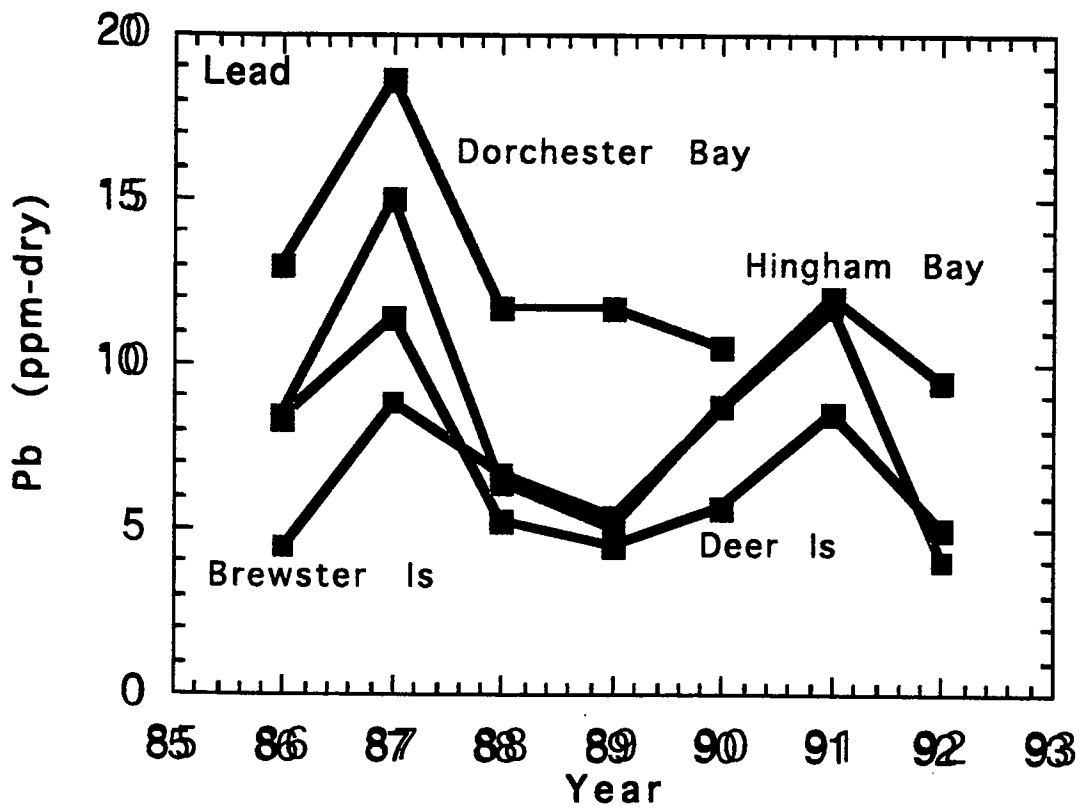
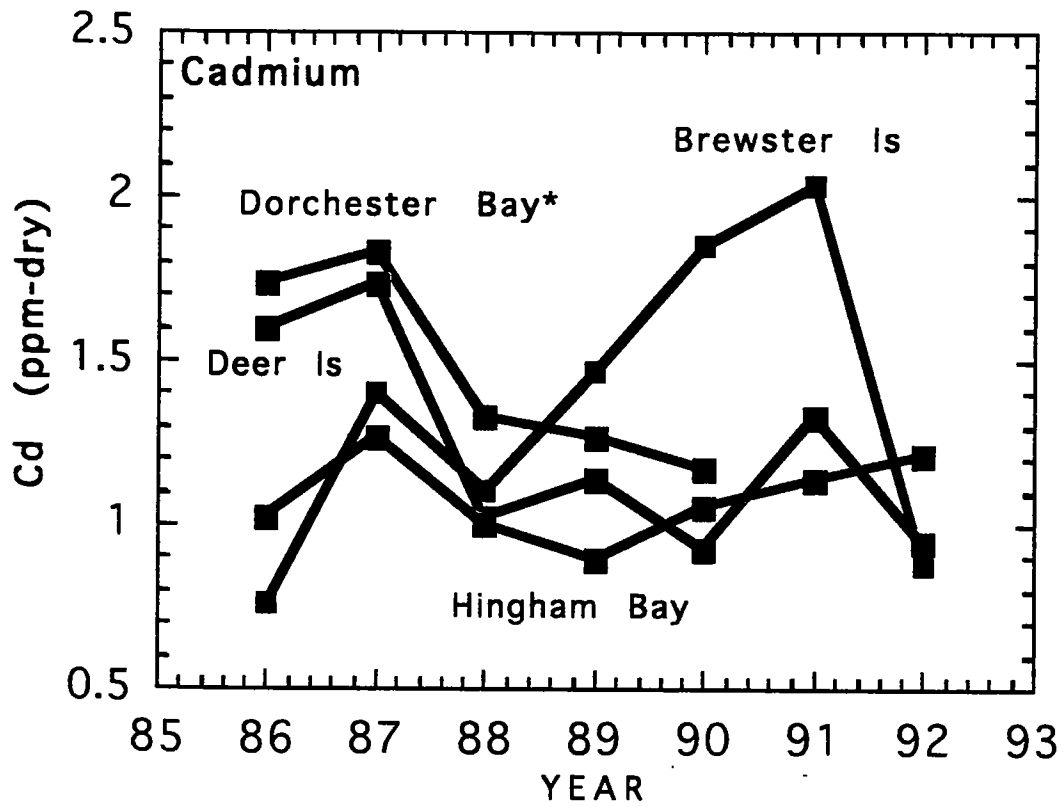
42.28°N

42.20°N









APPENDIX C-3

**M. MOORE
WHOI**

What is the role of the various measurements in the monitoring program?

The 7 year data series on winter flounder pathology from Deer Island Flats has allowed and will continue to give an appreciation of the biological consequences of changes in the management of Greater Boston's sewerage.

The 3 year data series at the future outfall site and 3 other reference sites will, by 1995, allow an appropriate baseline to assess perturbations induced by the activation of the new diffusers, assuming monitoring is continued annually till at least the year 2000.

Documenting the presence or absence of chemical-associated biological change in appropriate biomarker species such as the winter flounder is an essential ingredient in the assessment of risk to humans of evolving sewage management practices in the Boston area.

Is coverage in time and space adequate?

Time: These biomarkers integrate chronic exposure. Liver tumors only develop in fish at least five years old. Hydropic vacuolation is first seen in three year old fish. Therefore an annual sampling effort is adequate. That effort should always be conducted at the same time of year. i.e. April to allow reference to the data in hand. The time of year was chosen to ensure availability of fish at all chosen sites at the same time. Given the chronic nature of the exposure the precise month chosen is less important.

Space: The stations used range from the most contaminated to the least contaminated areas of the Mass. Bays. To maintain a baseline for any future effluent intrusion into Eastern Cape Cod Bay, a further reference station should be established east of Cape Cod, or on Stelwagen Bank. Georges Bank is the obvious choice. Samples could be obtained via NOAA research cruises.

Has the incidence of disease and/or abnormalities in fish or shellfish changed?

Deer Island Flats

For the period 1987 through 1983 the prevalence (sic) of liver neoplasia in winter flounder has reduced from greater than 5 to 11% prior to 1989 to 2 to 5% in 1989 to 1991 and zero in 1992 and 1993.

Centrotubular vacuolation has fallen from 60-70% to 30-50% over the same period.

Future Outfall Site

Hydropic vacuolation prevalence has not changed from 1991 through 1993. It remains at around 30%.

These data provide a baseline to compare with the years following the activation of the offshore diffuser.

Fish have presumably been collected in April 1994 and should be collected every April thereafter till 2000.

Eastern Cape Cod Bay

Disease prevalence has been essentially zero at this site 1991 through 1993. It is therefore an excellent reference site for both Boston Harbor and the Future Outfall Site.

What are meaningful levels of change in the system?

Detectable change - as per 1991 Monitoring plan:

"< 50% for most flounder and lobster indices"

"< 10% for neoplasia in flounder"

In this point the plan is ambiguous, as the above criteria do not include the element of change. Assume it means > 50% change from a predetermined baseline would be a meaningful change. Thus for the future outfall site, the prevalence of hydropic vacuolation should not exceed 45% (it is now c.30%) after the diffuser is activated. Liver tumors are currently absent. 10% of zero is zero, thus liver tumors should not be seen after the diffuser is activated. This is reasonable.

Are baseline data sufficient to address the objectives stated in the monitoring plan?

R-11 Will finfish and shellfish that live near or migrate by the diffuser be exposed to elevated levels of some contaminants, potentially contributing to adverse health in some populations?

There is already evidence of chemically-induced biological damage at the Future Outfall Site. This level appears to be relatively constant. It is possible that this change is a result in part of transport of toxics released from the current outfalls, although the stable isotope data makes this seem less likely.

This damage is best regarded as a harbinger of neoplastic change. Neoplastic change would occur given longer and/or greater chemical exposure.

For fish health it is reasonable to conclude that baseline data are adequate. By 1995 there will be 9 consecutive years of data for Deer Island Flats, and 5 consecutive years for the other 4 stations. Both the reference site at Eastern Cape Cod Bay and the Future Outfall Site have proven to be highly reproducible with the current level of sampling effort, which should be maintained.

Sources of contaminants to Boston Harbor

30 to 70% of the reduction in loadings of heavy metals are attributed to source reduction and to improved contaminant estimates. (MWRA 94-1 page iii)

Once the treatment plant upgrade and the new diffuser are complete, ongoing source reduction will be the primary tool for further improvement of the toxic chemical problem. We need to understand where the best gains have been and will be achieved

An important issue to establish is the relative contribution to source reduction of:

1. Active source reduction of ongoing industrial activity through altered process resulting from corporate, Federal, State and MWRA programs.
2. Passive source reduction resulting from cessation of industrial activity resulting from economic failure.

Stable isotopes

Aim:

To study the elevated level of hydropic vacuolation at the Future Outfall Site, given the absence of obvious nearby sources.

Result:

Sewage sludge appeared to induce a detectable shift in isotope ratios in Deer Island Fish. The Future Outfall Site fish were not detectably different from reference fish. Therefore the chemical exposure of these fish was not sludge-borne to a significant degree. The exposure could have come from one or more of the following sources:

1. **Boston Sewage effluent.** Effluent particles that were lighter and traveled further than the sludge particles could have significantly contaminated area(s) where the fish caught at the Future Outfall Site spend a significant amount of time. Given that the Future Outfall Site fish did not have a sludge signal it would seem that they at least did not visit Deer Island Flats significantly

2. **Historic dumping of chemical waste.** The intensity of current data coverage is inadequate to rule out the presence of hotspots in the western Mass. Bay area that resulted from historic short-dumping of material barged from Boston destined for the Mass. Bay Disposal Site (MBDS) and other dumping areas to the east, or that the Future Outfall Site fish may visit the MBDS.

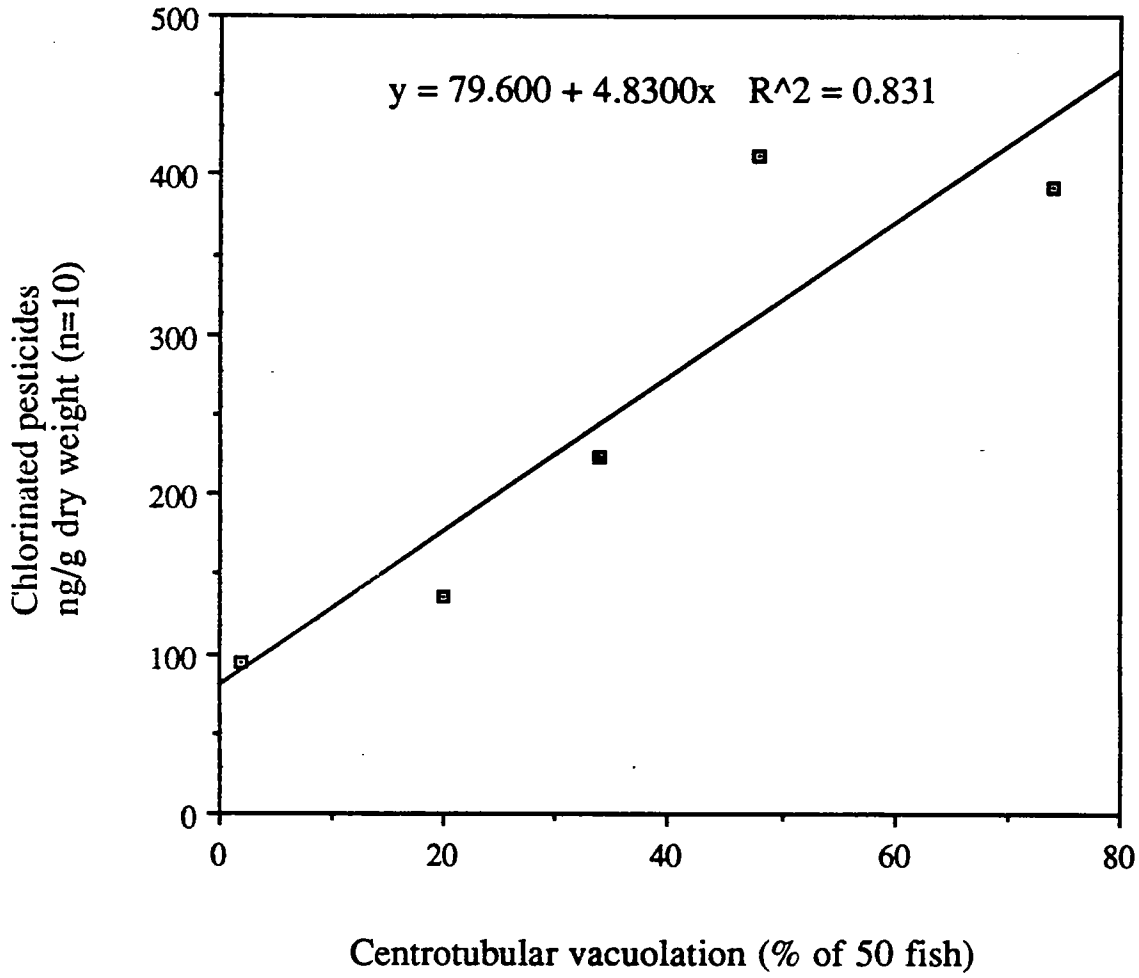
Further stable isotope analysis?

The sludge related signal will presumably continue to wane in the Deer Island Fish. Once the effect of sludge has fully disappeared, if there is a residual shift between sewage effluent impacted fauna in the absence of sludge, as compared to reference conditions, then it would be worth continuing to monitor this parameter. Using fish scales for this parameter integrates the exposure over the life of the fish. A more dynamic sensor would be intestinal or gill epithelium.

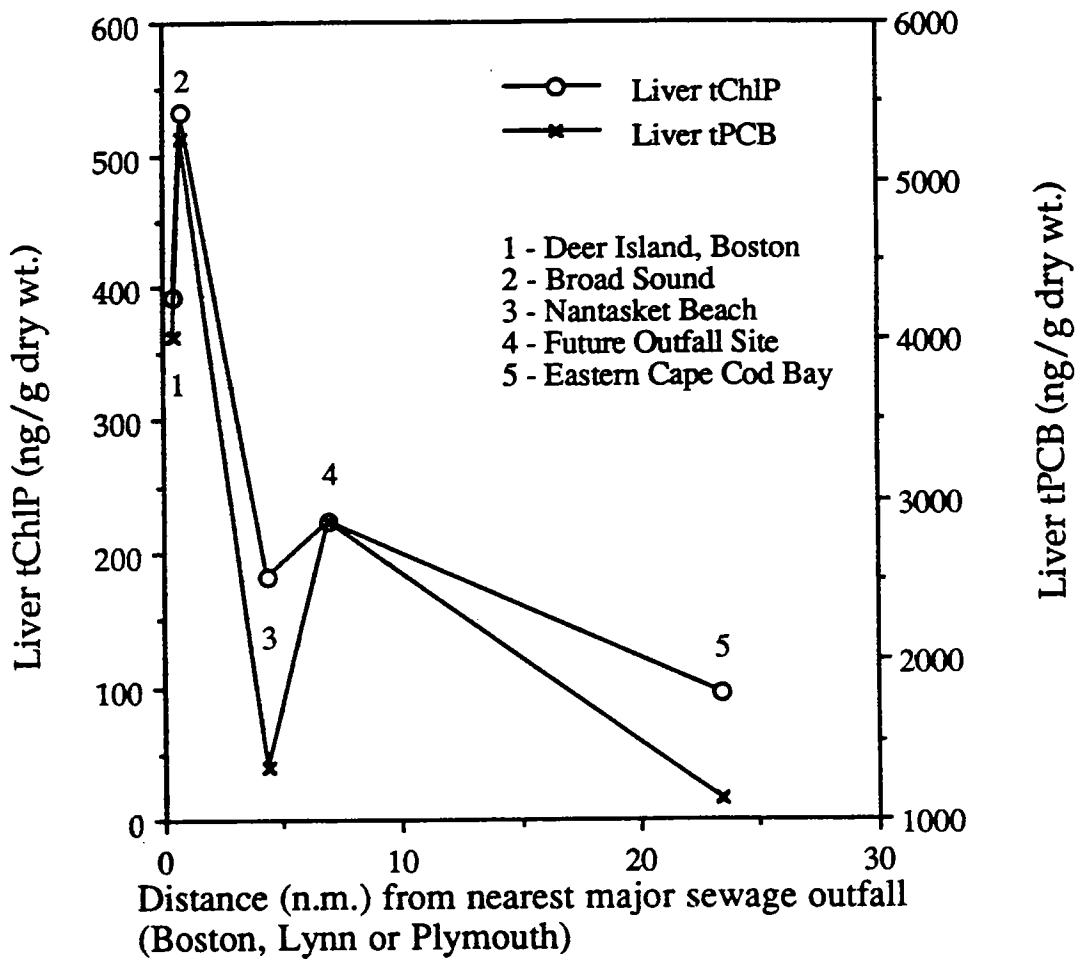
d34S in WINTER FLOUNDER SCALES

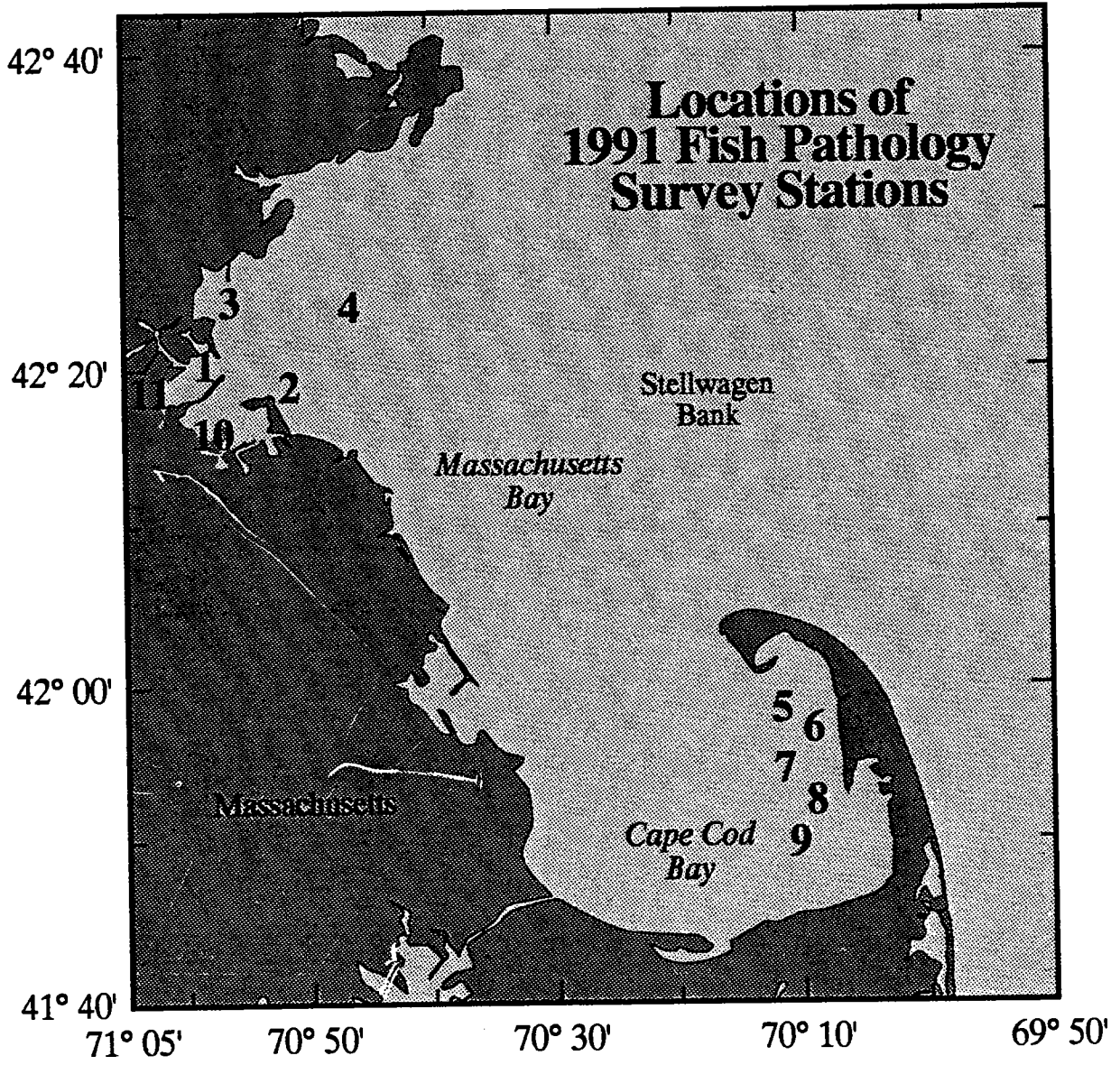
	'93	'92	'91	'90	'89
Lynn Outfall	12.5		11.6		
Deer Island	10.5	9.8	9.8	9.2	9.9
Nantasket Beach	13.0		12.9		
Future Outfall	12.2	12.7	13.3		
Eastern Cape Cod Bay	11.6	12.2	13.0		

Winter flounder livers - 1992



Winter flounder liver contaminants, compared with distance from site of capture to nearest major sewage outfall -1992





PREVALENCE OF LIVER NEOPLASIA IN WINTER FLOUNDER (%)

	'93	'92	'91	'90	'89	'88	'87
Lynn Outfall	2	0	7				
Deer Island	0	0	3	5	3	12	6
Nantasket Beach	0	0	0				
Future Outfall	0	0	0				
Eastern Cape Cod Bay	0	0	0				

**PREVALENCE OF CENTROTUBULAR HYDROPIIC VACUOLATION
IN WINTER FLOUNDER LIVER (%)**

	1993	1992	1991
Lynn Outfall	38	74	61
Deer Island	38	48	45
Nantasket Beach	30	20	36
Future Outfall Site	28	34	28
Eastern Cape Cod Bay	6	2	3

APPENDIX C-4

**B. HILLMAN
BATTELLE**

KEY LESIONS STUDIED

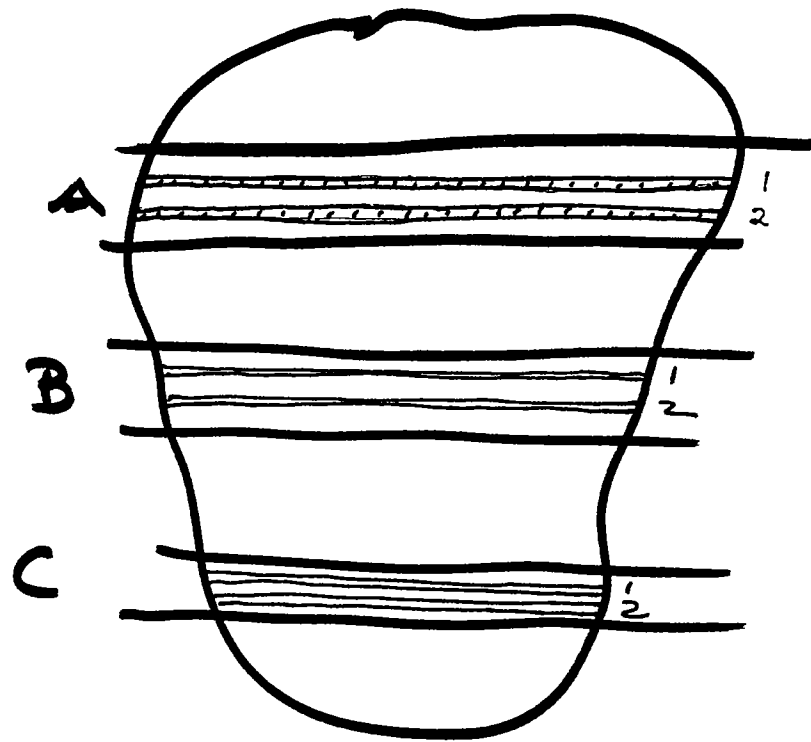
Hydropic Vacuolation
Centrotubular
Tubular
Focal

Macrophage Aggregates

Biliary Proliferation

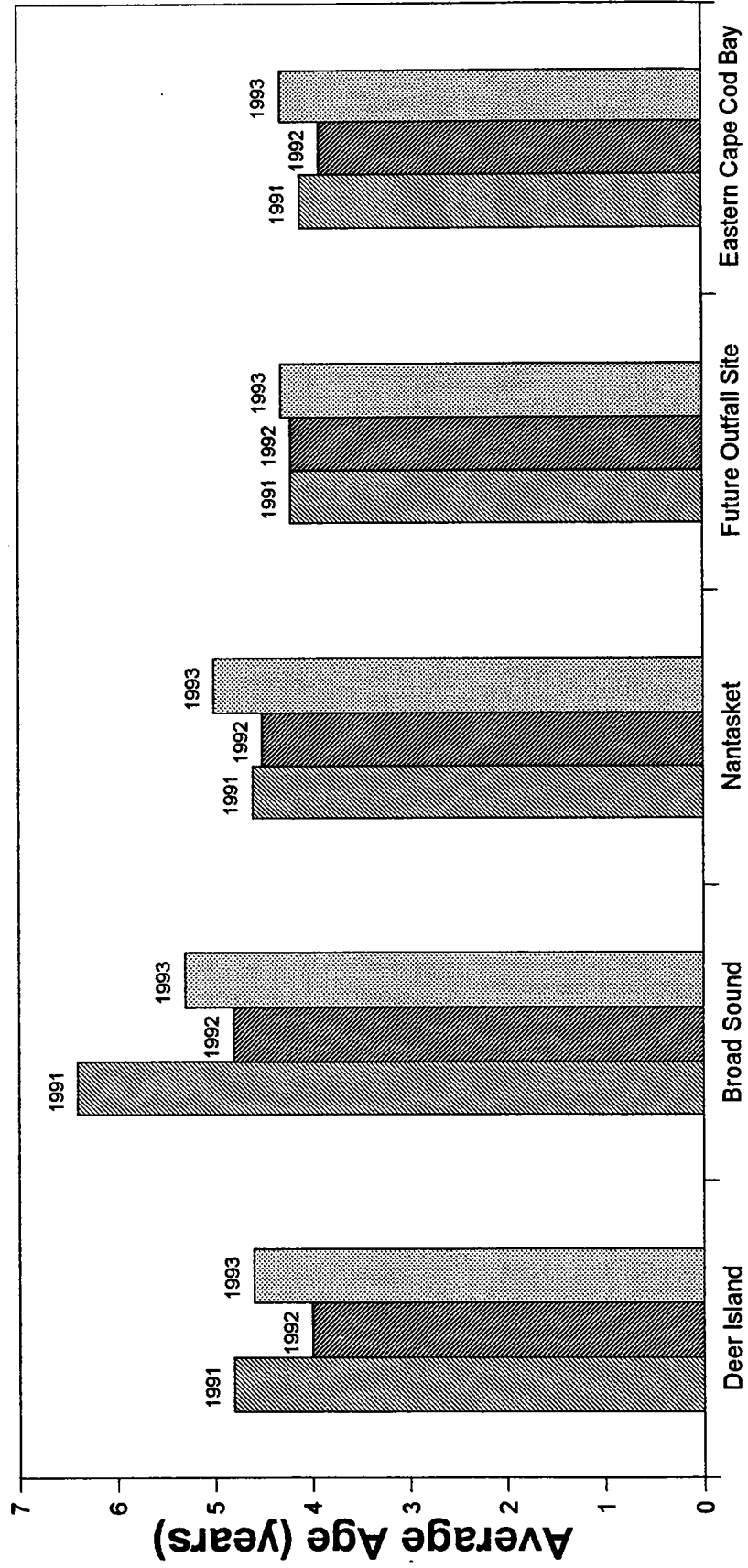
Neoplasia

“Balloon Hepatocytes”

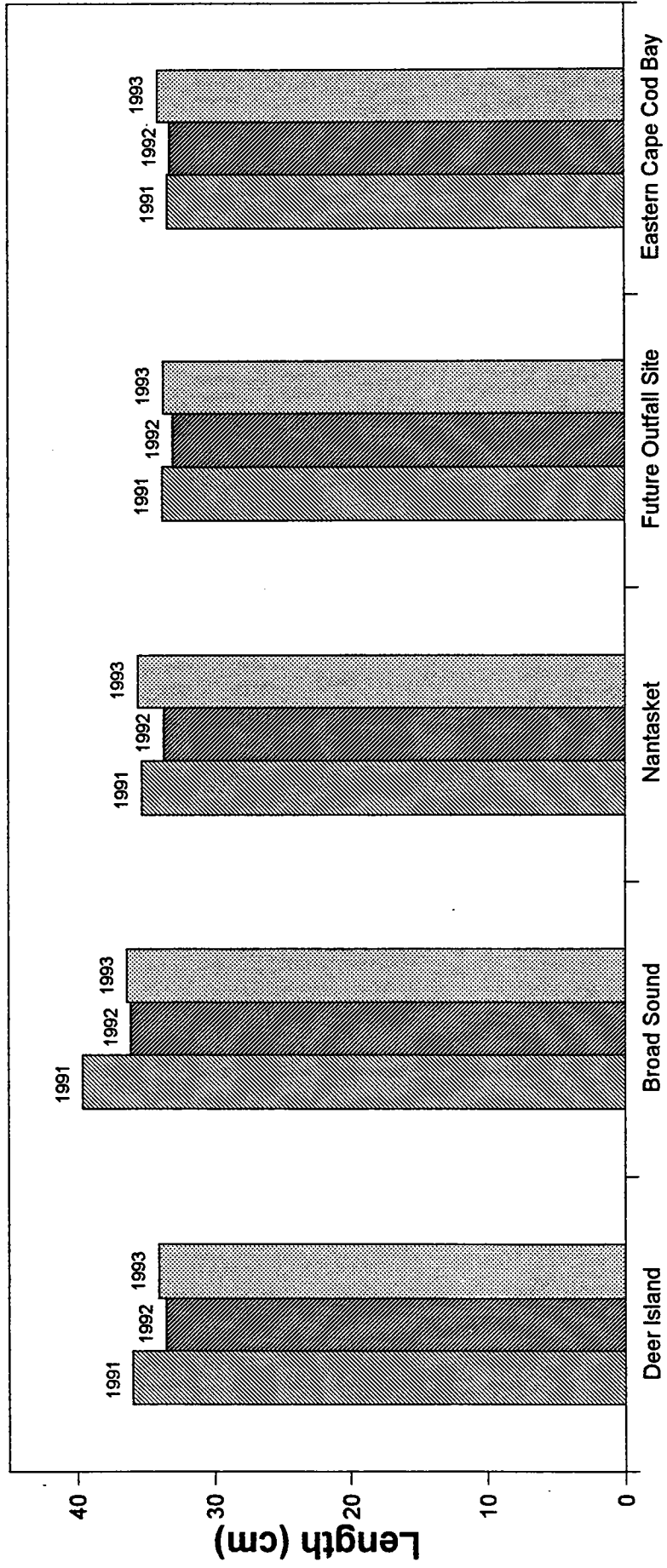


ST	ID	SEX	YR	TL	SL	WT	FIN	GR	GS	LC	CENT A	CENT B	CENT C	CENT	CENT	CENT	TUBU
-----	-----	-----	1	1	1	1	1	1	1	1	2	2	1	C	C	A	1
F12	584	F	4	30	24.5	375	0	1-	1-	YB	0	0	0	0	0	0	0
F13	425	F	5	34.5		850	0	0	0	YB	1	0	0	0	0	0	0
F13	426	M	6	38.5		700	0	0	0	B	3	2	2	3	3	0	0
F13	427	F	5	31		450	0	1	0	B	0	0	0	0	0	0	0
F13	428	M	4	30.5		425	0	0	0	B	2	2	2	3	3	2	2
F13	429	F	8	44		875	0	0	0	B	0	0	0	0	0	0	0
F13	430	F	5	38.5		650	0	0	0	YB	1	0	0	0	0	0	0
F13	431	F	7	42		850	0	0	0	B	3	3	2	3	2	3	1
F13	432	F	4	31		425	0	0	0	B	0	0	0	0	0	0	0
F13	433	F	6	42.5		1200	0	0	0	YB	0	0	0	0	0	0	0
F13	434	F	5	34.5		525	0	0	0	B	0	0	0	0	0	0	0
F13	435	F	6	41.5		825	0	0	0	B	3	3	2	3	2	3	1
F13	436	M	5	37.5		750	0	0	0	B	0	0	0	0	0	0	0

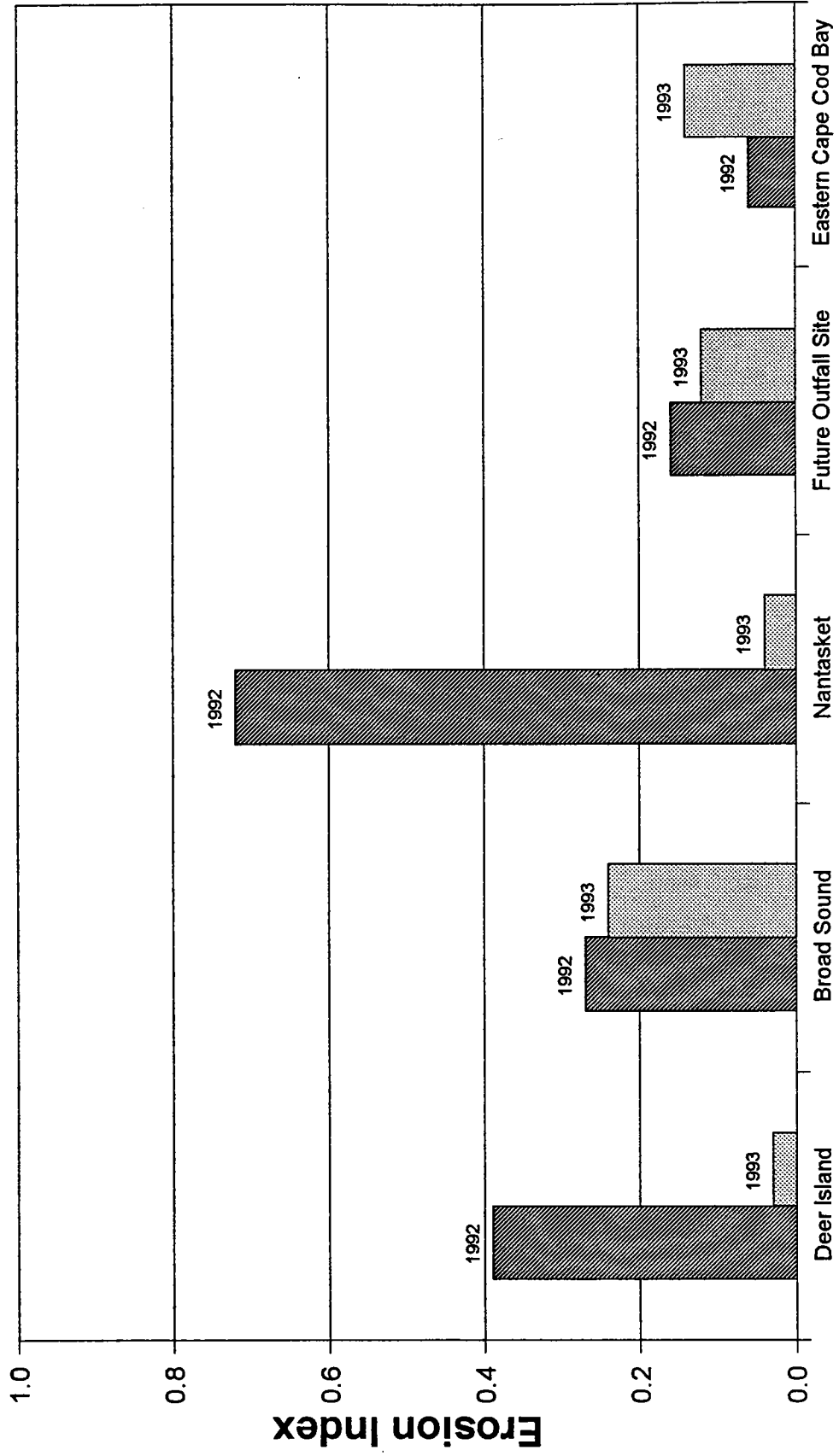
Average Age of Winter Flounder By Site in 1991, 1992, 1993



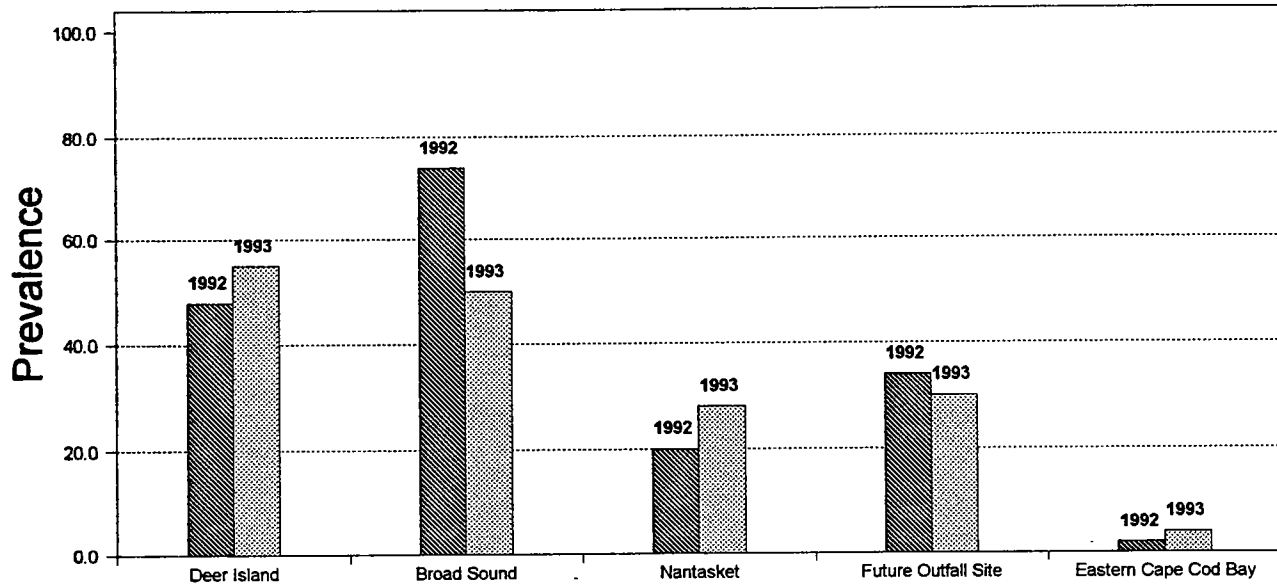
Average Length of Winter Flounder By Site in 1991, 1992, 1993



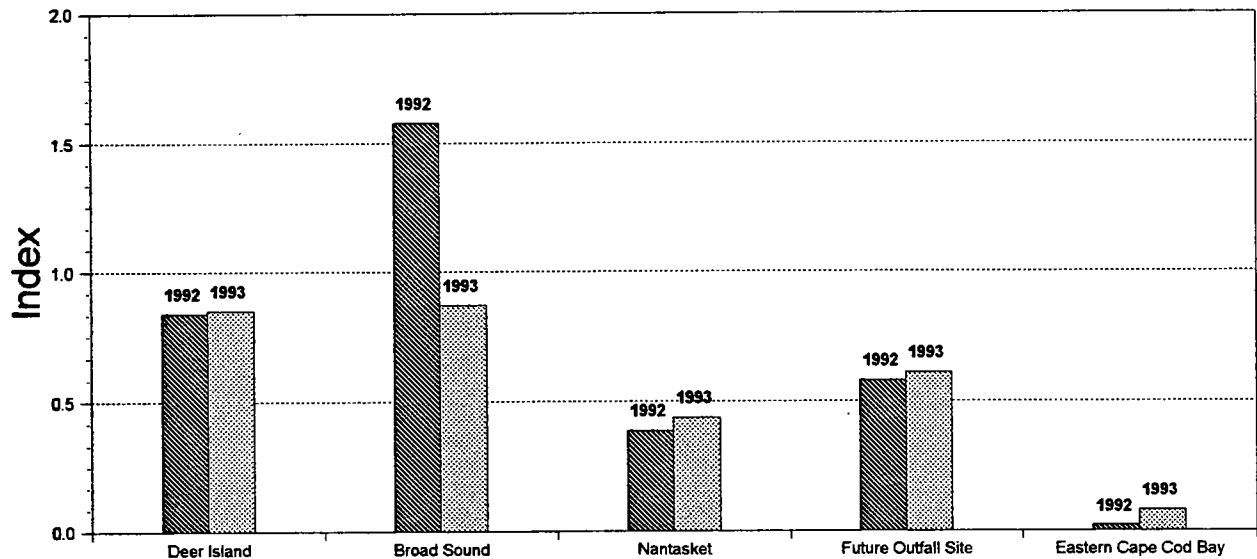
Fin Erosion Index By Site in 1992 and 1993



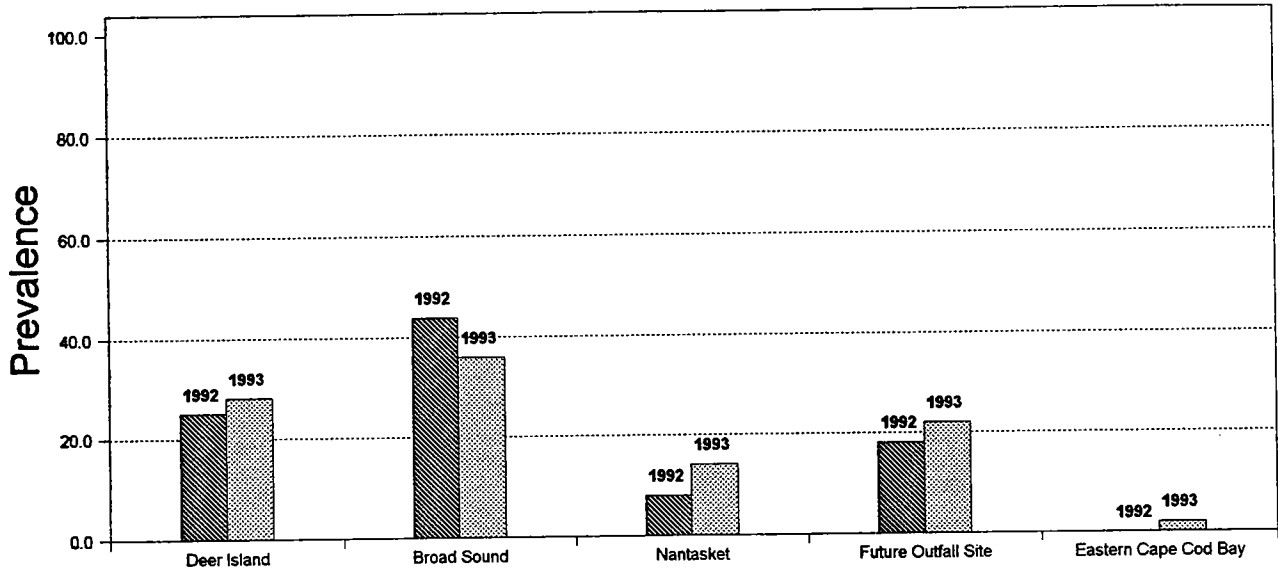
Centrotubular Hydropic Prevalence By Site in 1992 and 1993



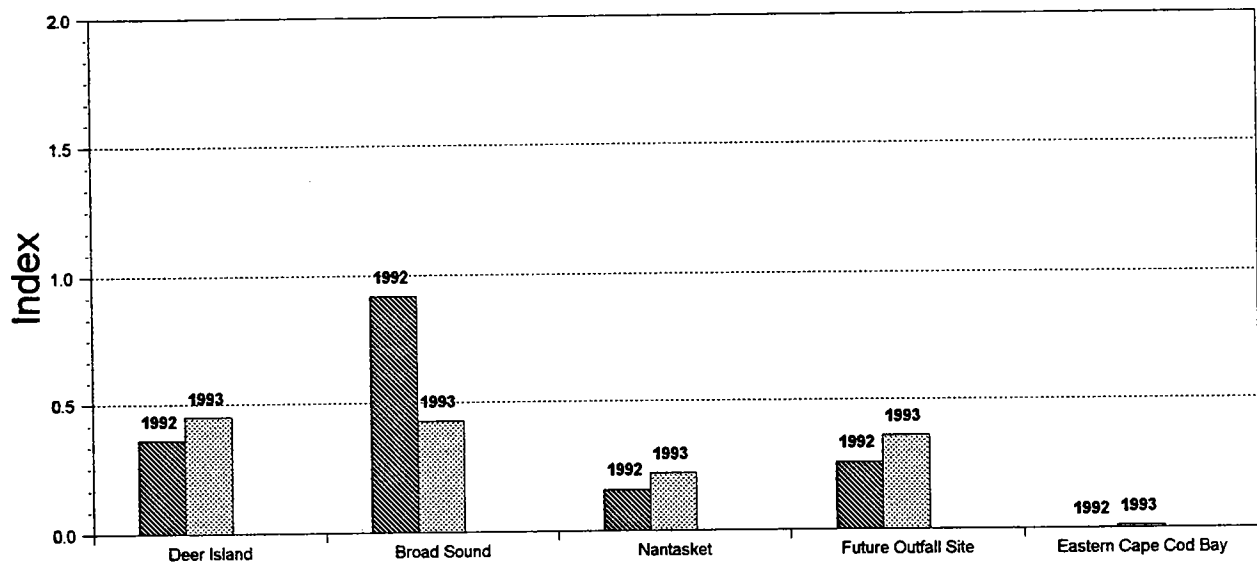
Centrotubular Hydropic Index By Site in 1992 and 1993



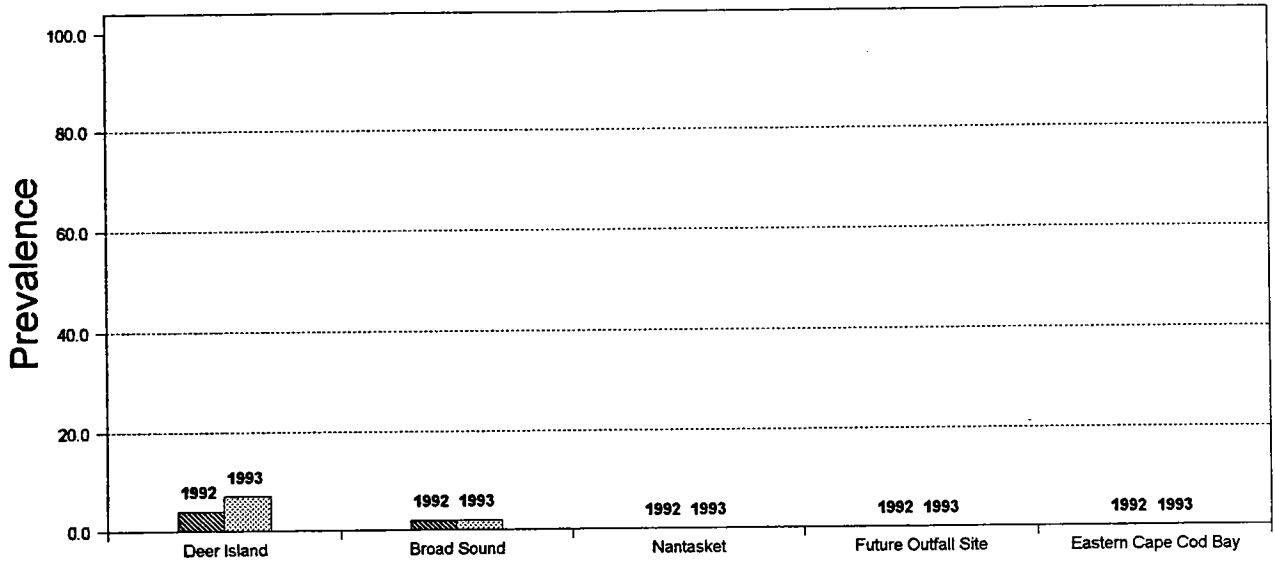
Tubular Hydropic Prevalence By Site in 1992 and 1993



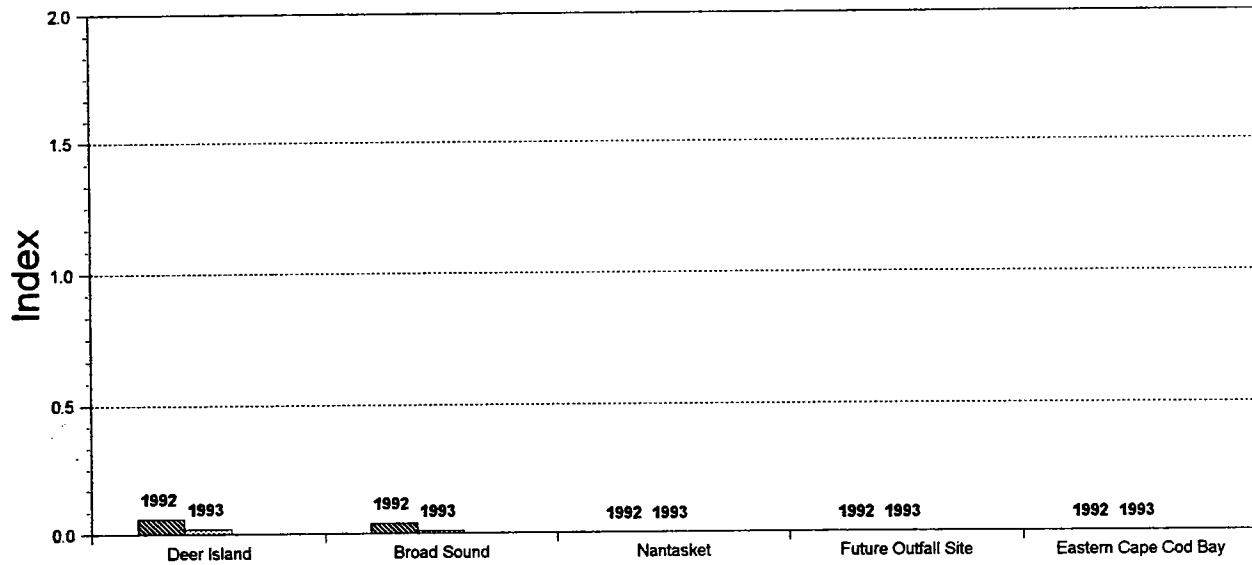
Tubular Hydropic Index By Site in 1992 and 1993



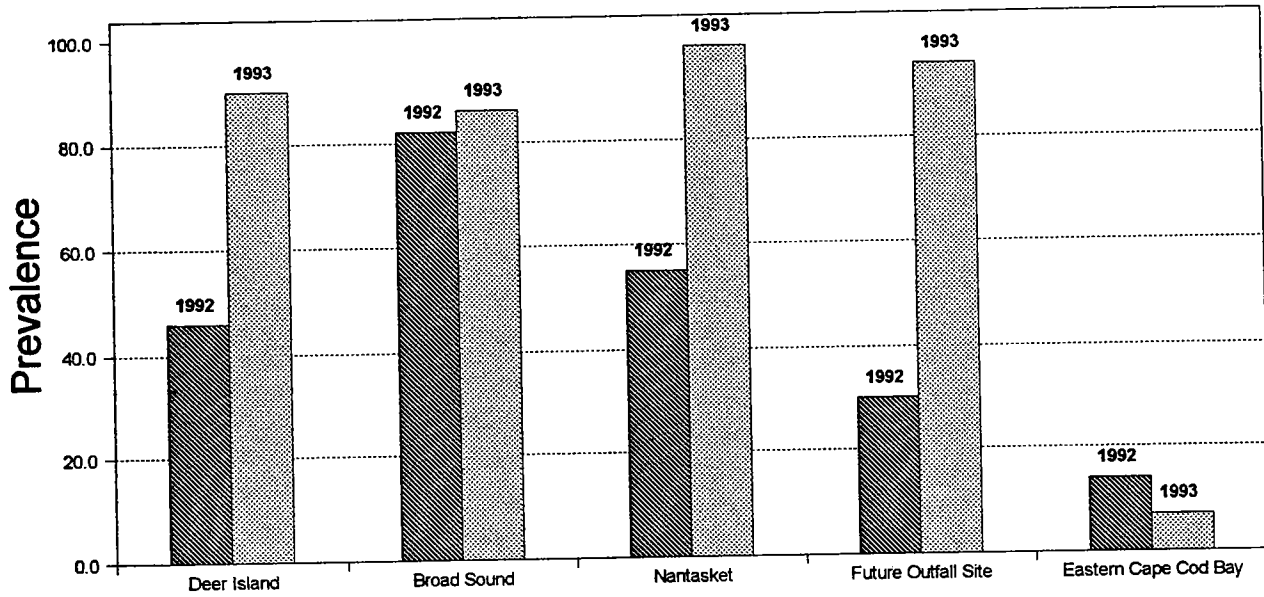
Focal Hydropic Prevalence By Site in 1992 and 1993



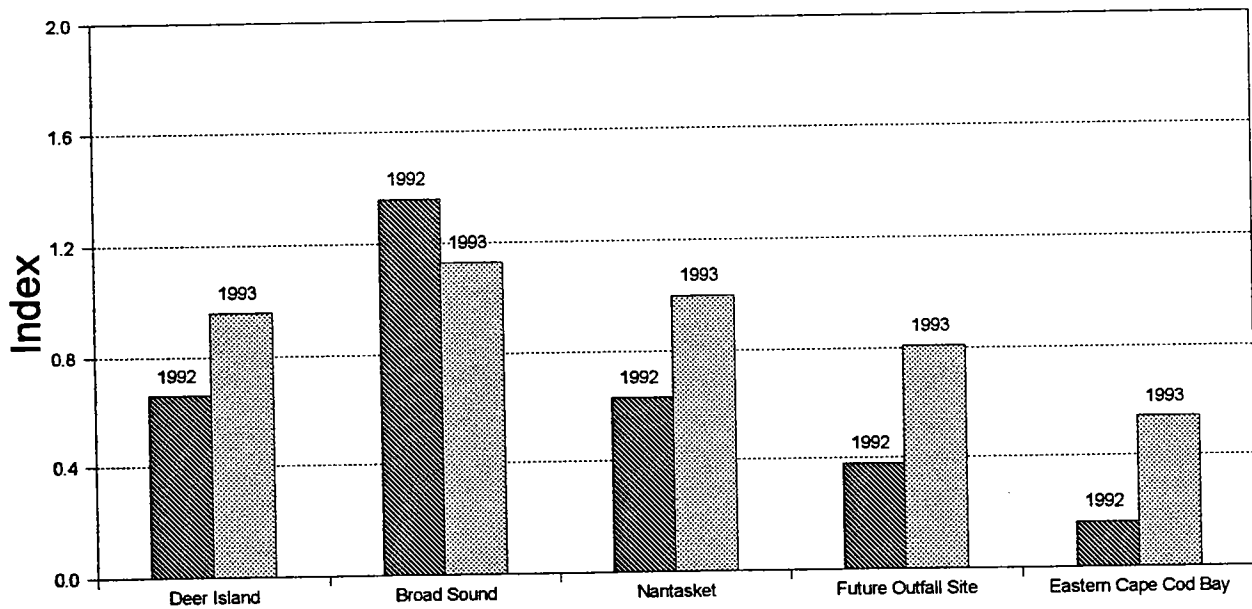
Focal Hydropic Index By Site in 1992 and 1993



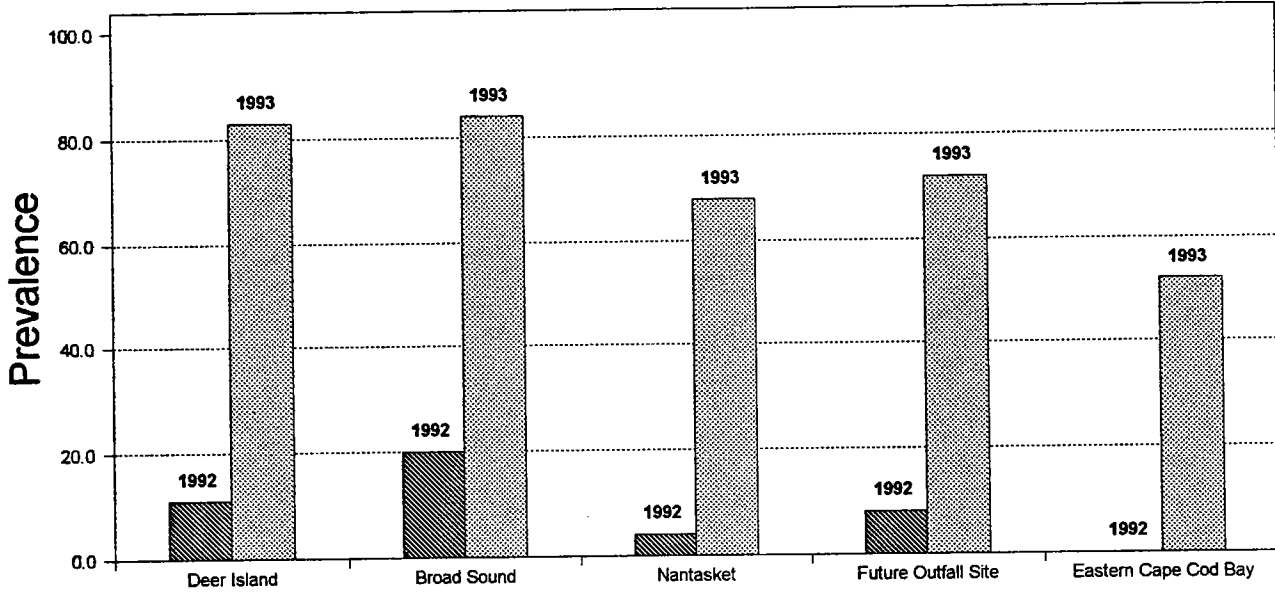
Macrophage Prevalence By Site in 1992 and 1993



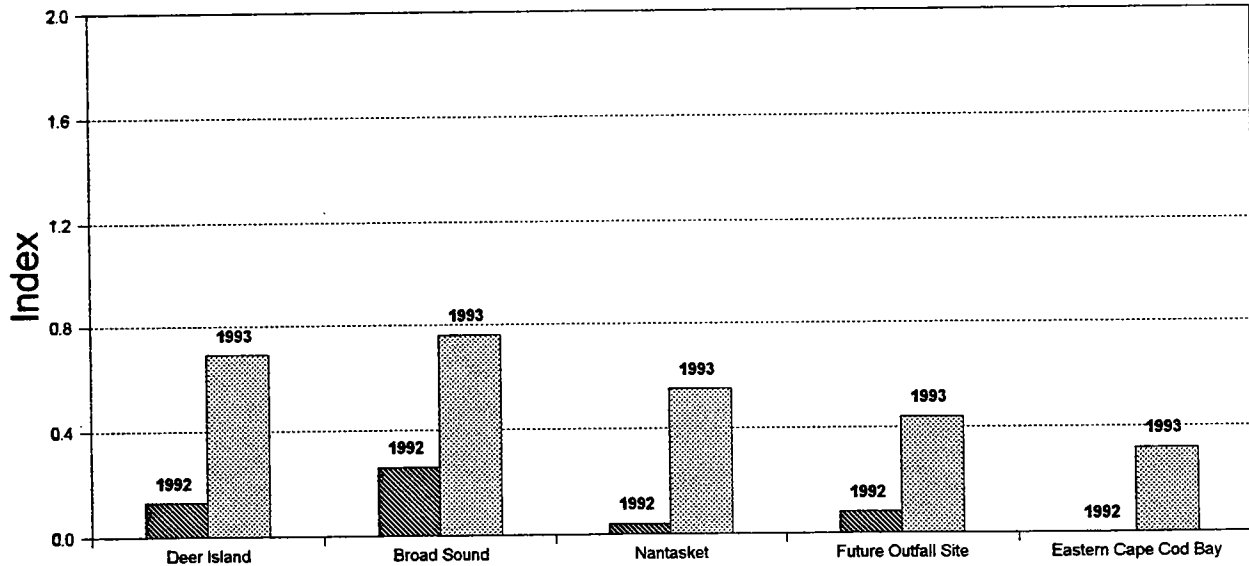
Macrophage Aggregate Index By Site in 1992 and 1993



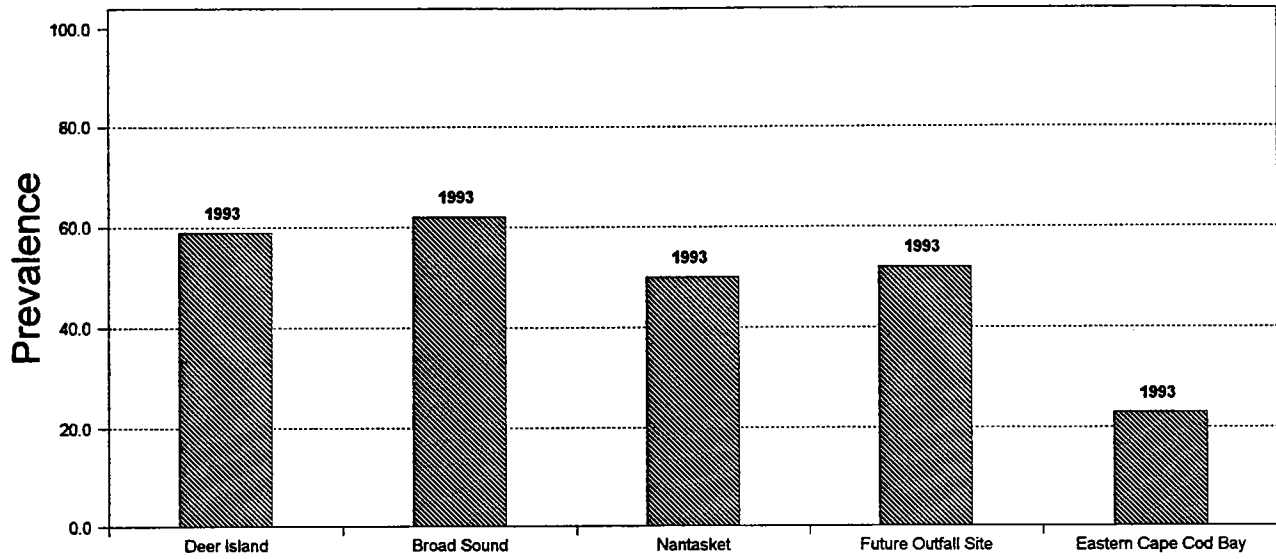
Biliary Proliferation Prevalence By Site in 1992 and 1993



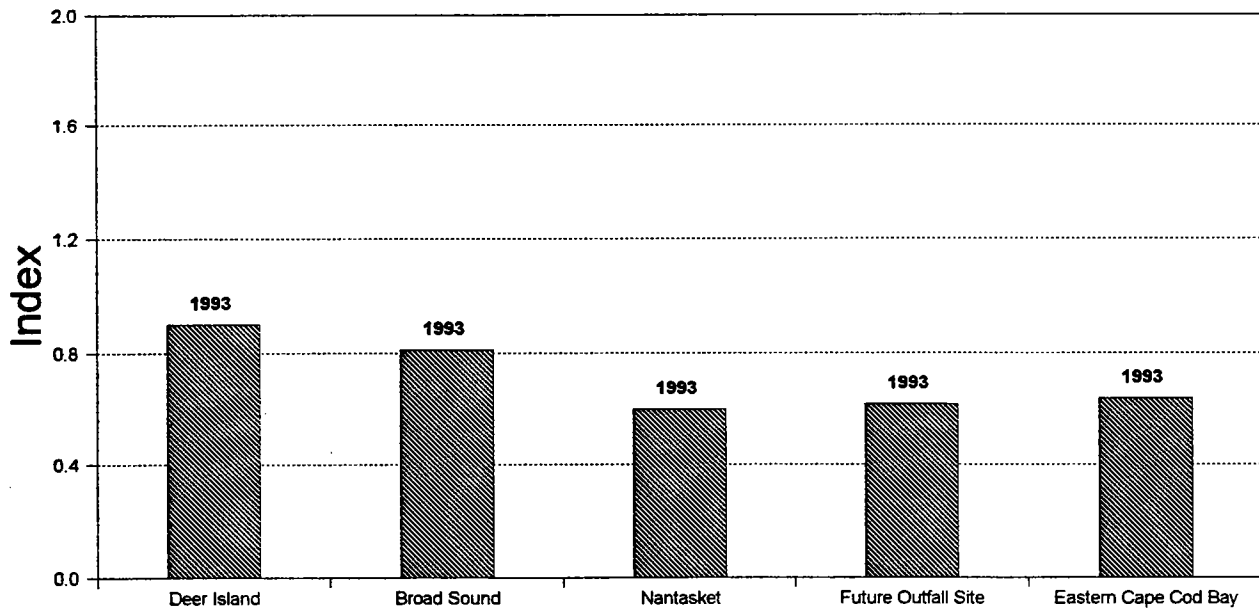
Biliary Proliferation Index By Site in 1992 and 1993



Balloon Hepatocytes Prevalence By Site in 1993



Balloon Hepatocytes Index By Site in 1993



CONCLUSIONS

1. **Fin erosion decreased in 1993 at all sites except Eastern Cape Cod Bay, especially at Deer Island and Nantasket Beach. Probably the result of cessation of sludge release.**
2. **Hydropic vacuolation decreased at Broad Sound; not much different at other sites. Other lesions, except neoplasia, increased sharply at all sites. However, data have to be interpreted with caution because of difference in examination technique between '93 and previous years.**
3. **Apparent apoptosis is significant lesion that should be given further scrutiny. If '94 sampling shows lesion at intensity equal to or greater than in '93, we should look more closely for the possible causes.**

APPENDIX C-5

**C. HUNT
BATTELLE**

OBJECTIVES

Estimate level of change that can be observed based on 1992 and 1993 sampling

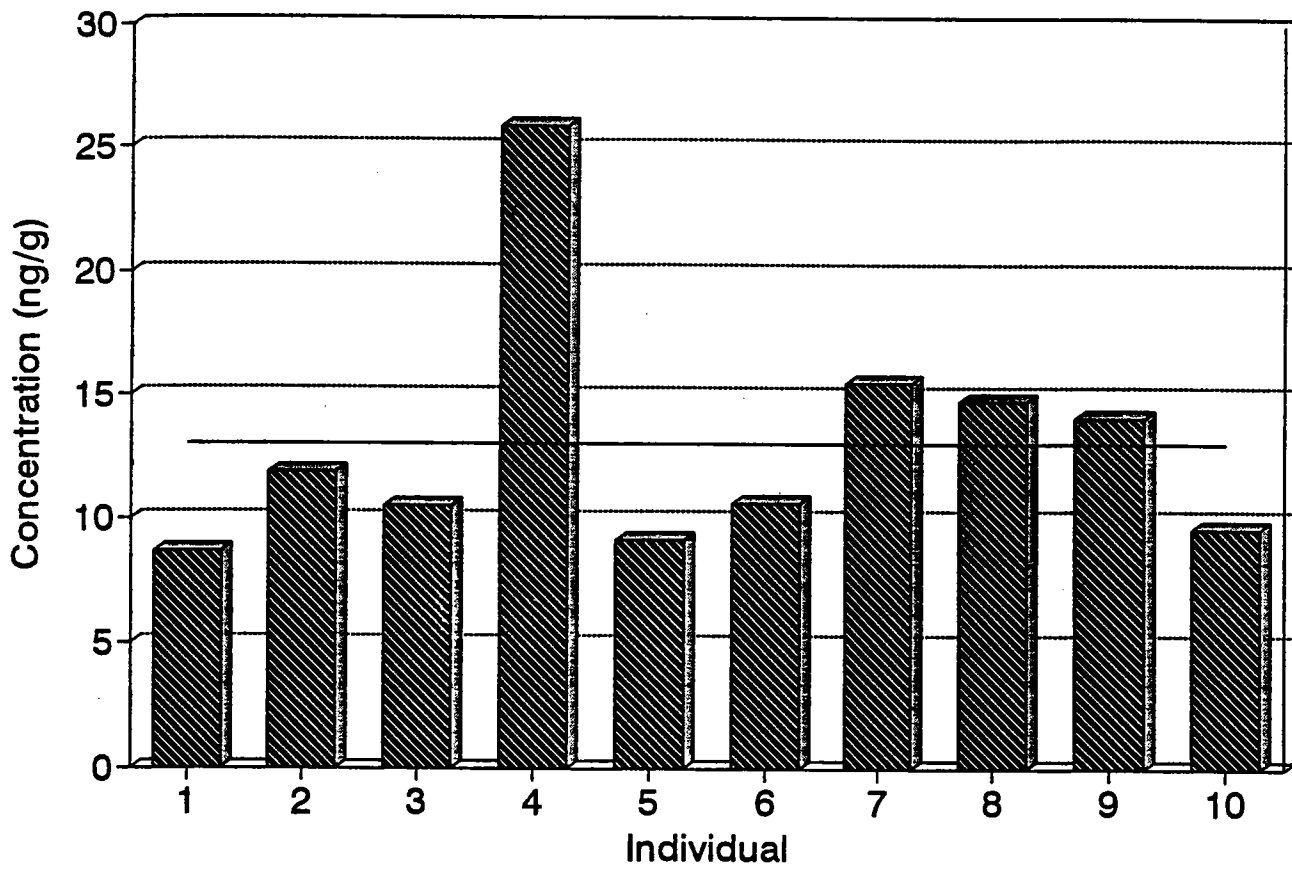
Examine variability in individual organisms

Compare 1992 and 1993 tissue contaminant concentrations

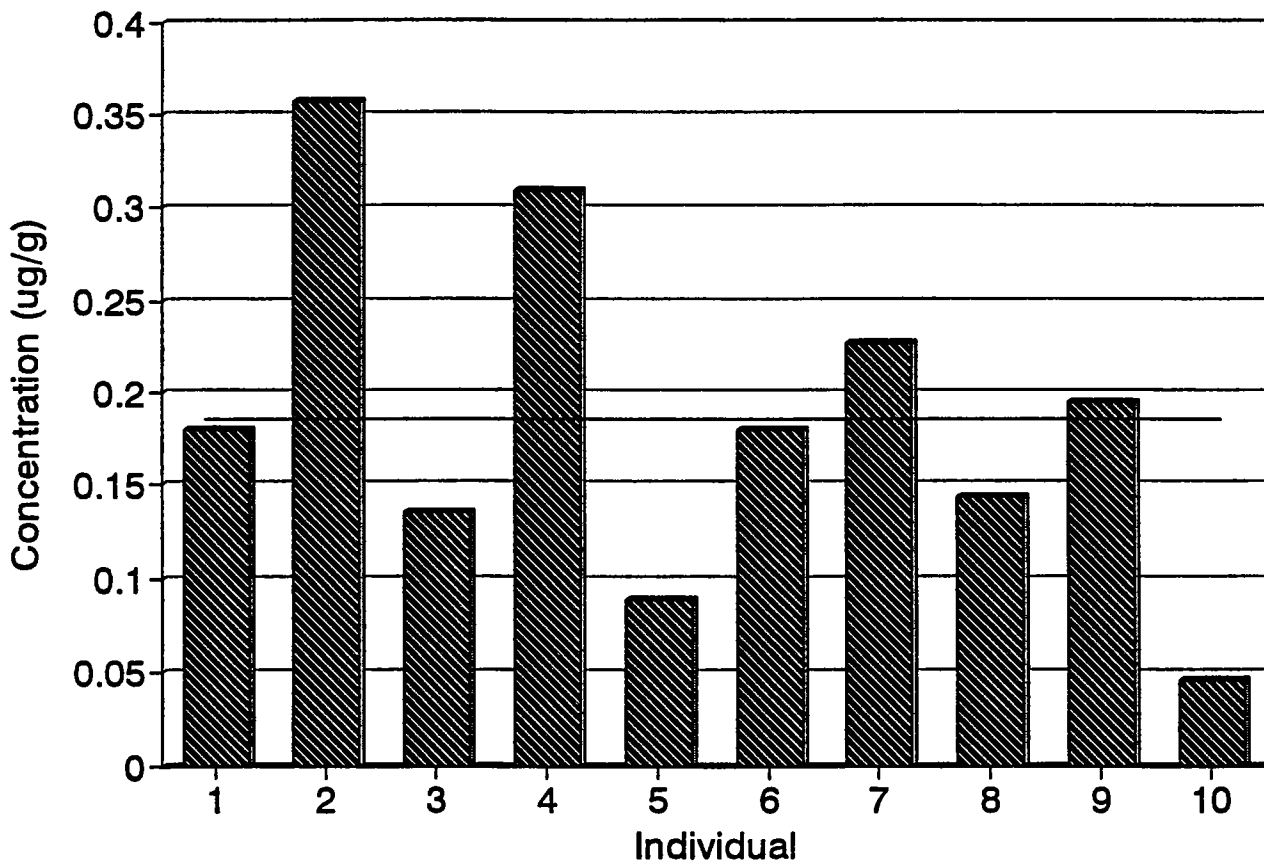
SUMMARY OF TISSUE SAMPLES

TISSUE TYPE	<u>NUMBER OF INDIVIDUALS ANALYZED PER SITE</u>	
	1992	1993
Flounder Tissue	3	9 or 10
Flounder Liver	3 (one composite of 7)	1 composite of 10
Lobster Tail	3	2, 3, 10
Lobster Hepatopancreas	3	2, 3, 10

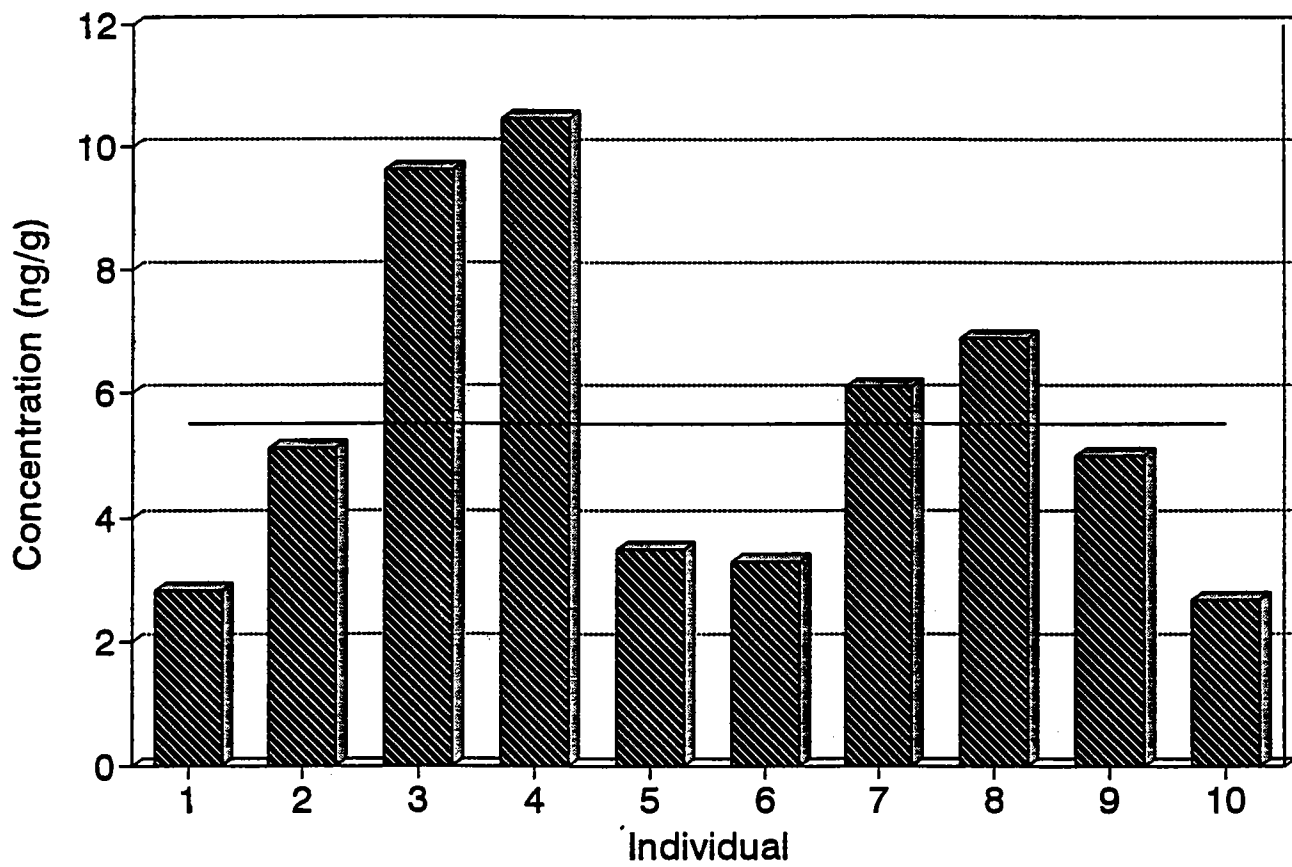
1993 Flounder Tissue Total DDT Levels (dry weight) for Cape Cod Bay



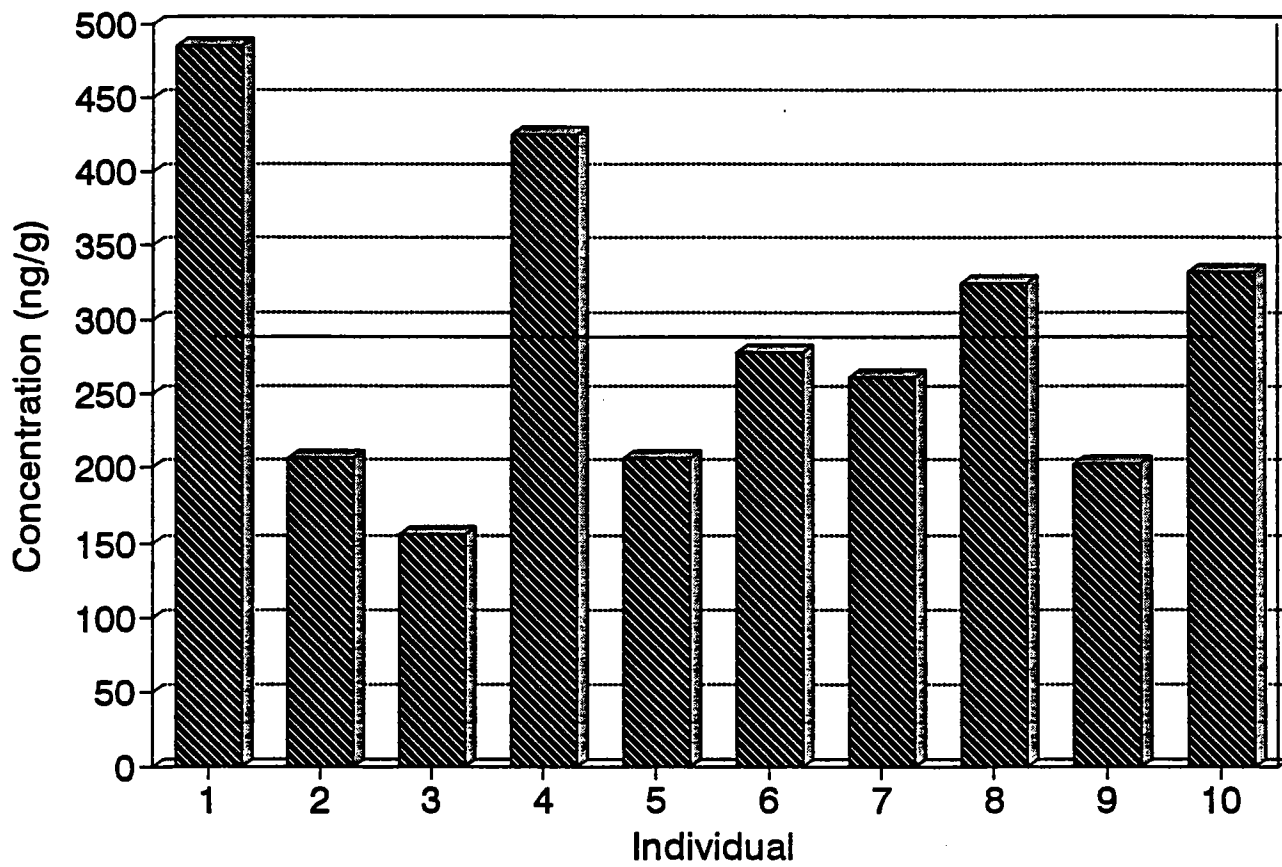
1993 Flounder Tissue Mercury Levels (dry weight) for Cape Cod Bay



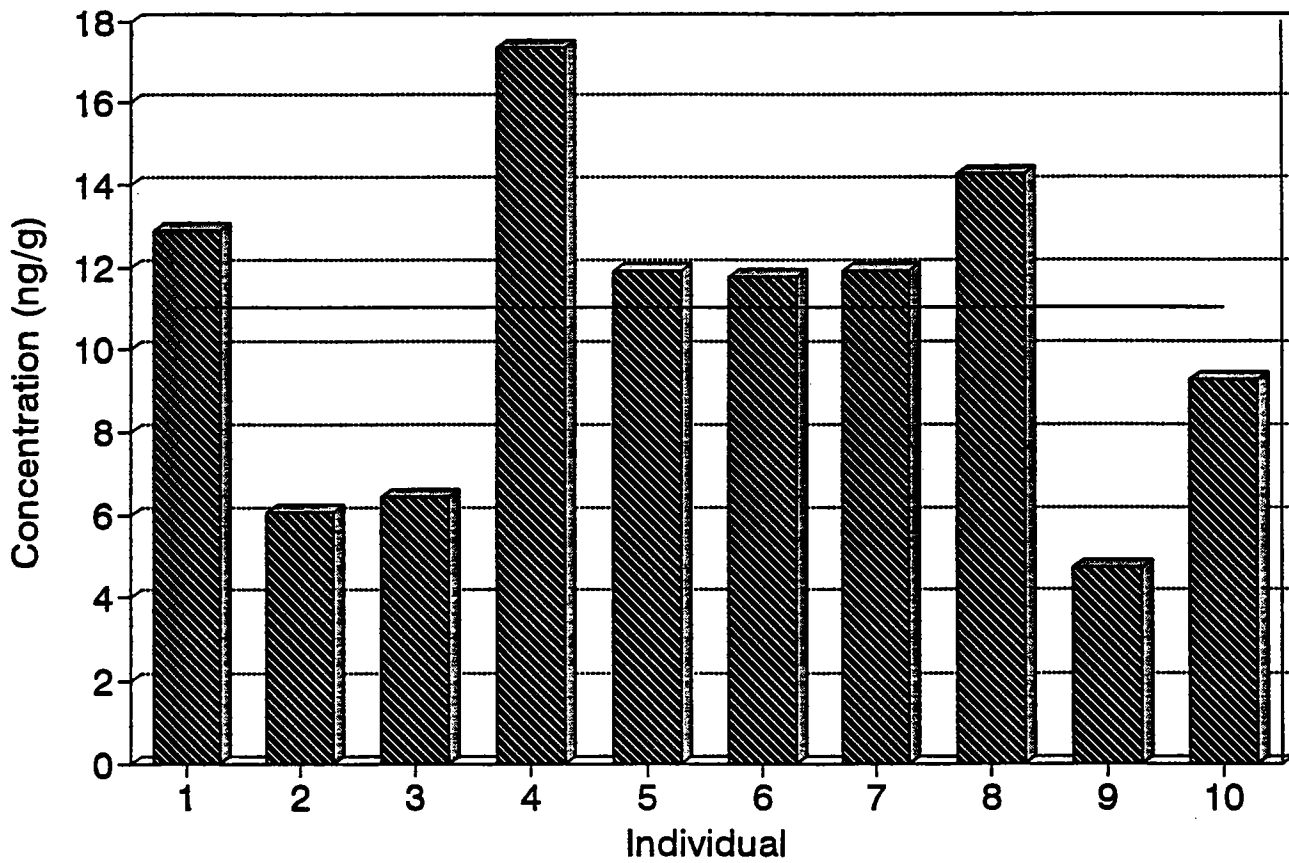
1993 Flounder Tissue Total Chlordane Levels (dry weight) for Cape Cod Bay



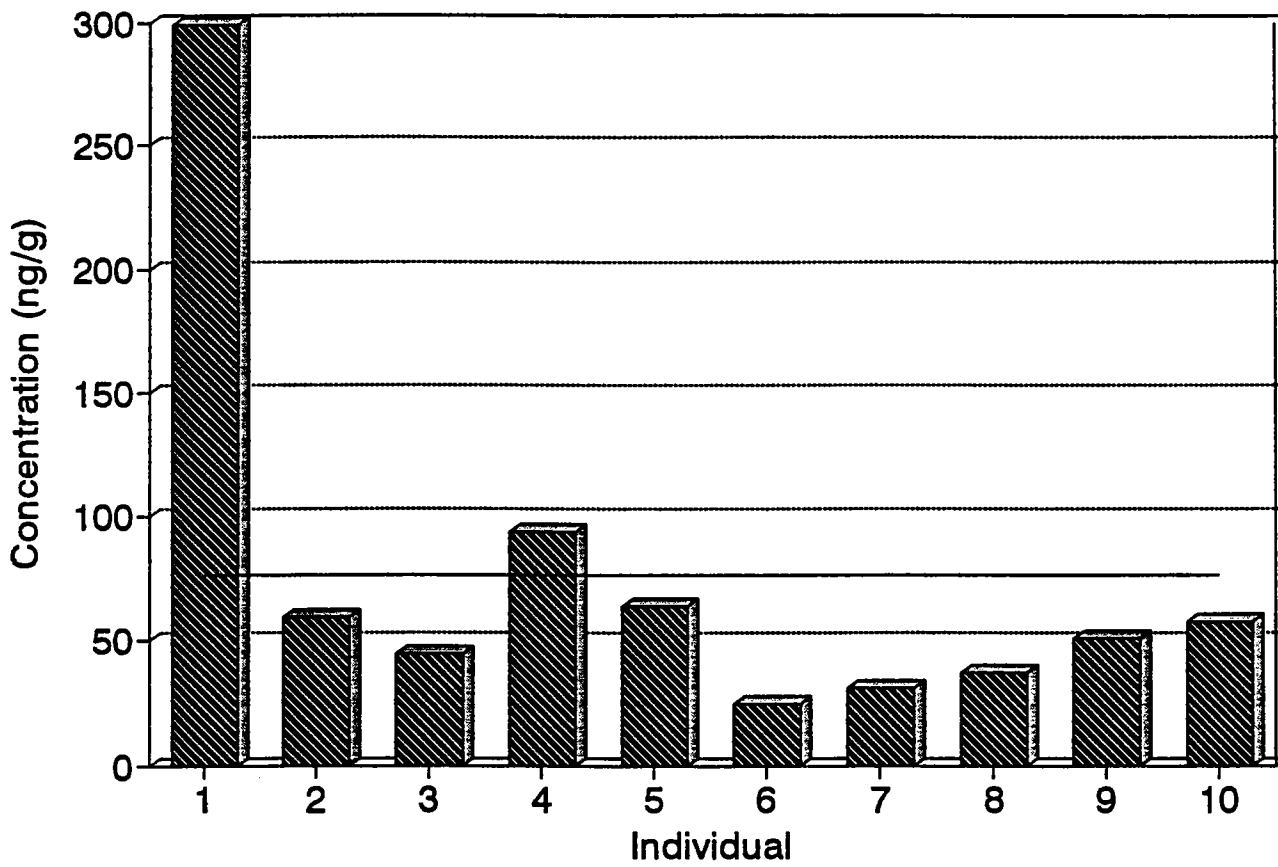
1993 Lobster Hepatopancreas DDT Levels (dry weight) for Cape Cod Bay



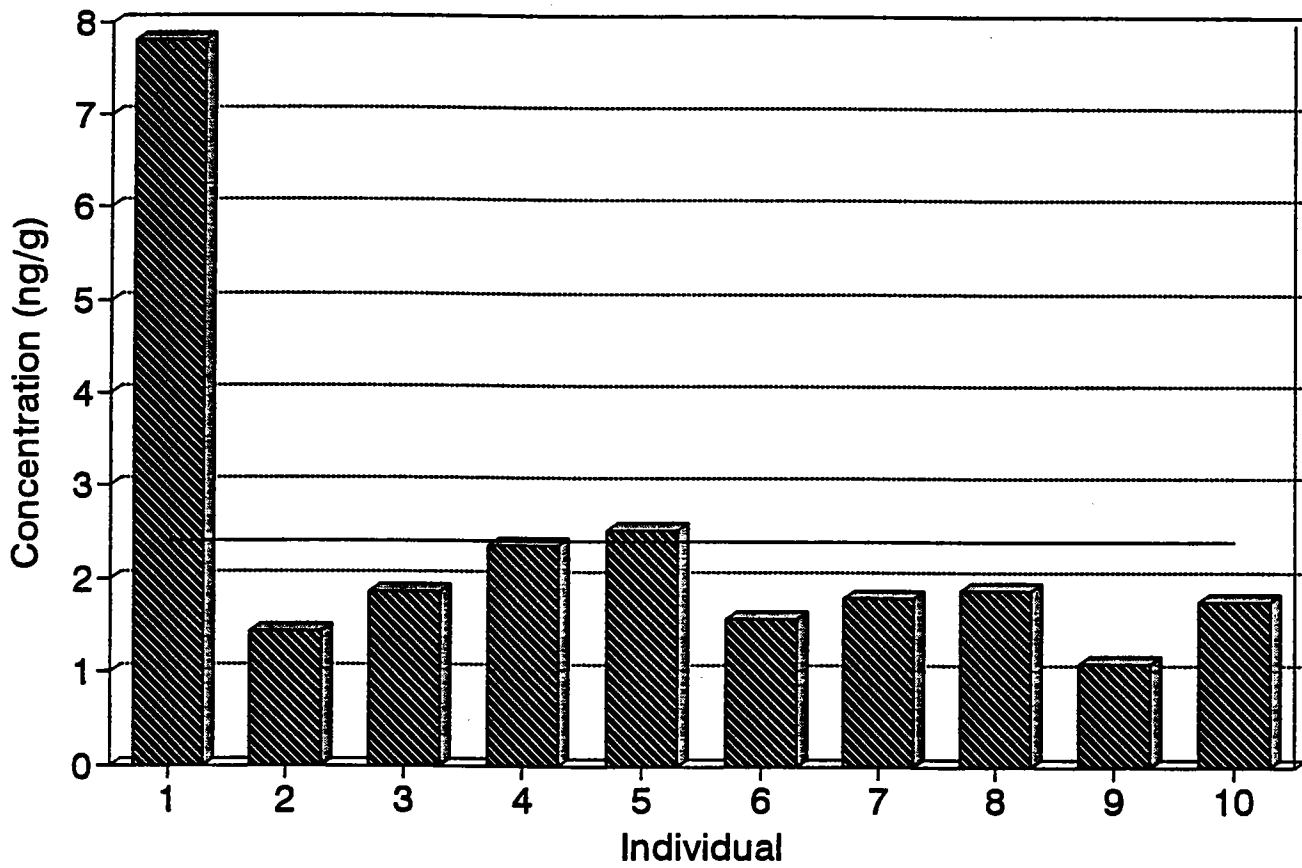
1993 Lobster Tissue Total DDT Levels (dry weight) for Cape Cod Bay



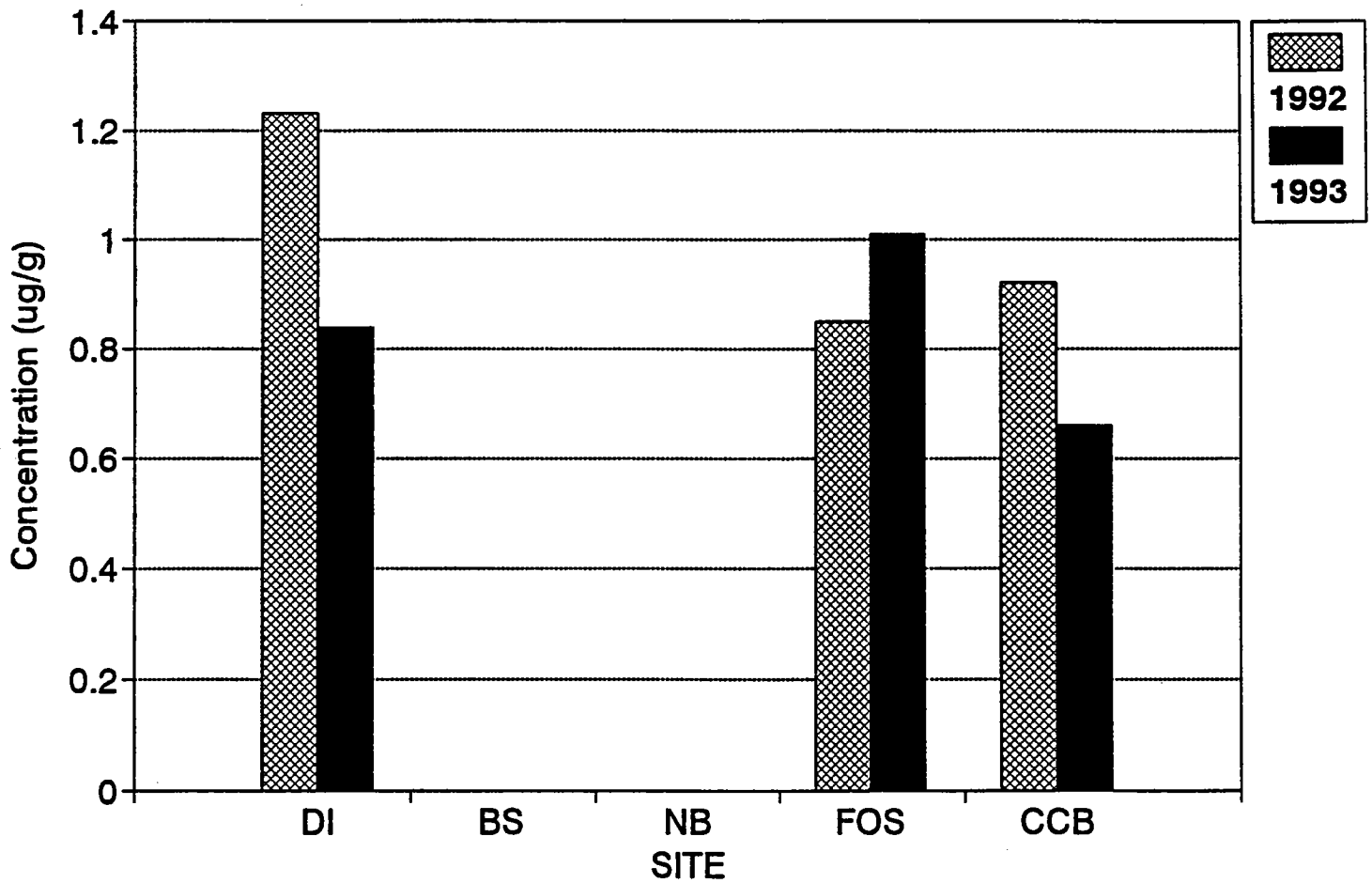
1993 Lobster Hepatopancreas Chlordane Levels (dry weight) for Cape Cod Bay



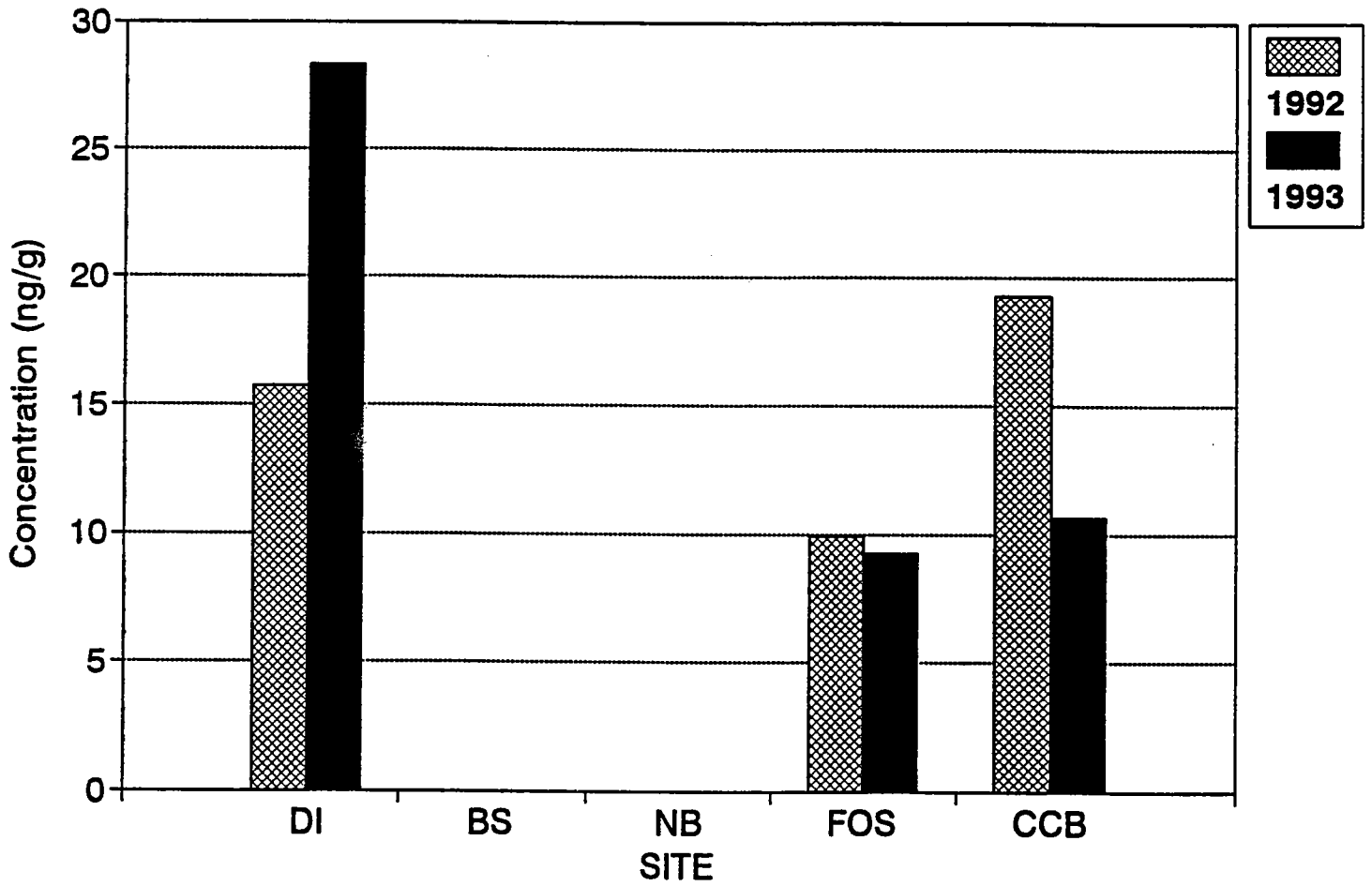
1993 Lobster Tissue Total Chlordane Levels (dry weight) for Cape Cod Bay



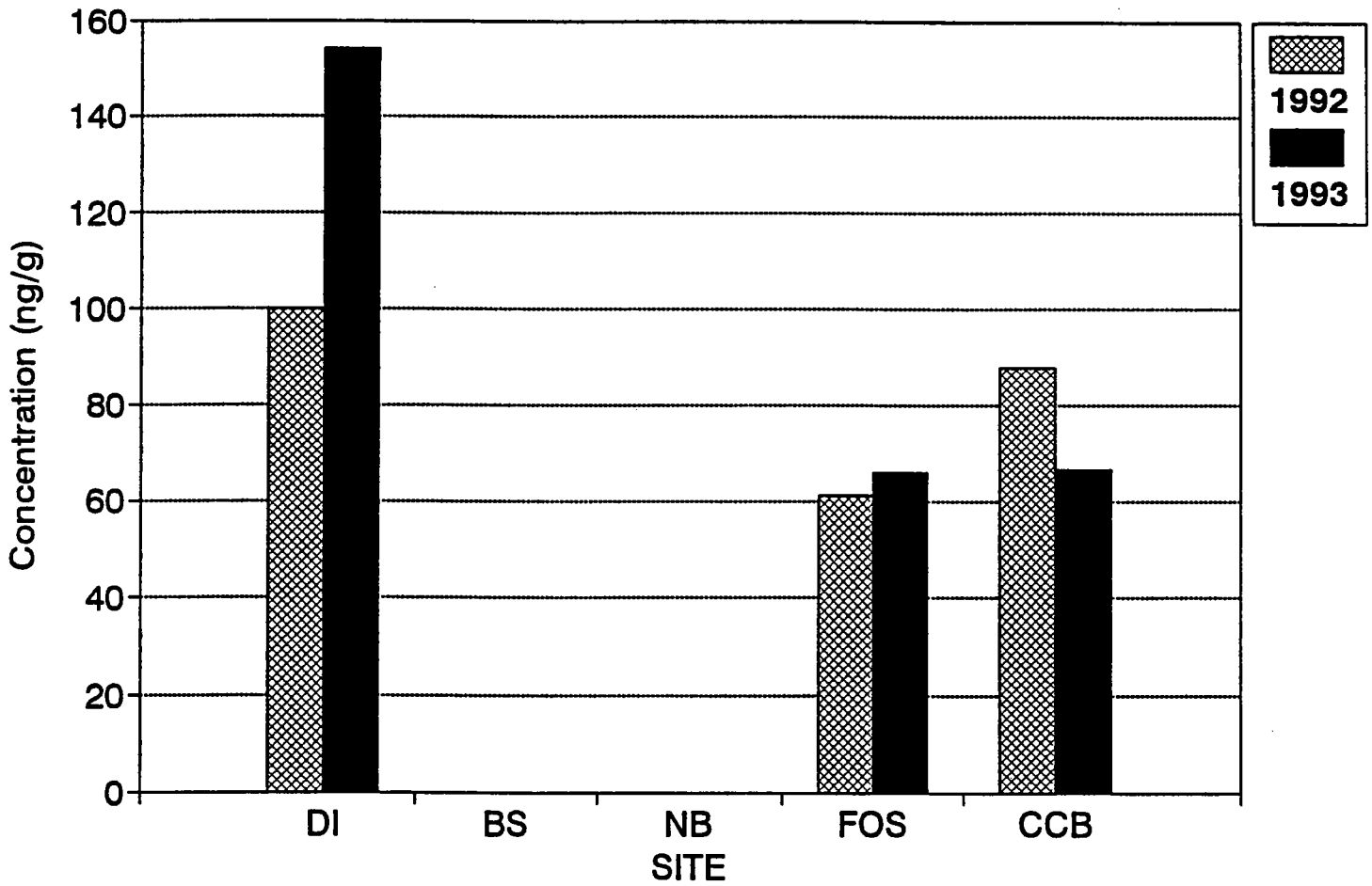
Comparison of 1992 and 1993 Mercury Levels in Lobster Tissue (dry wt)



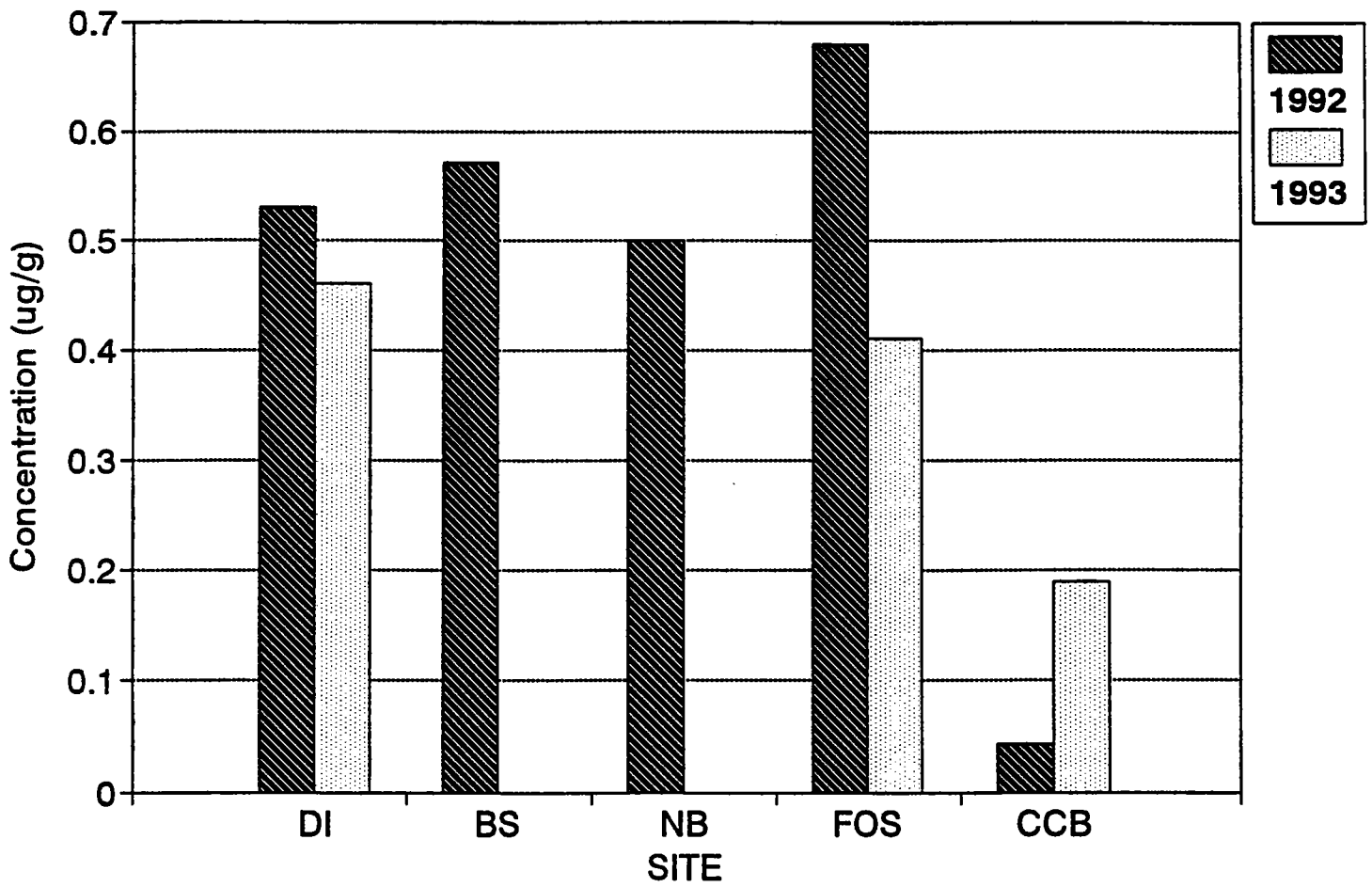
Comparison of 1992 and 1993 DDT Levels in Lobster Tissue (dry wt)



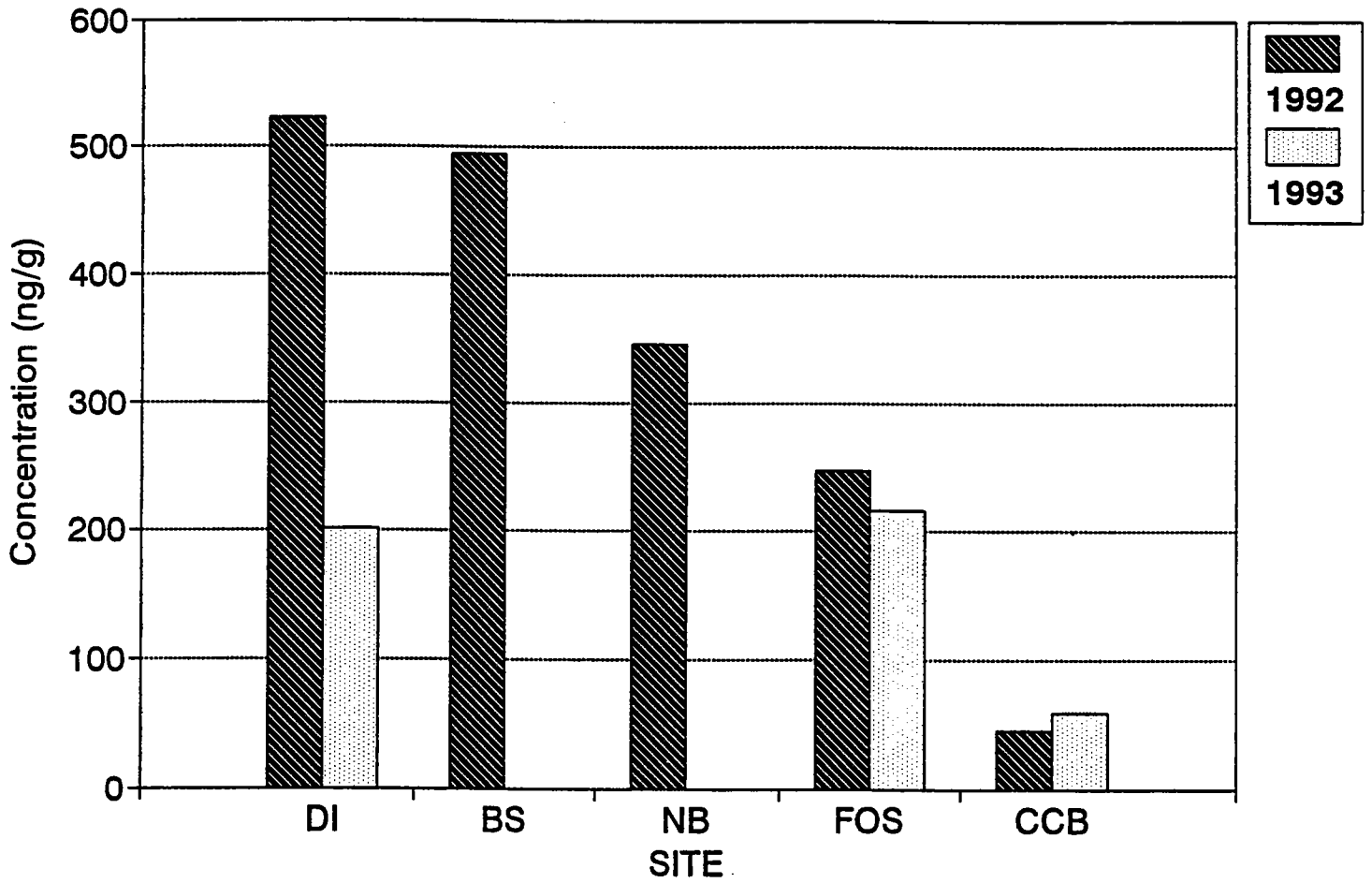
Comparison of 1992 and 1993 PCB Levels in Lobster Tissue (dry wt)



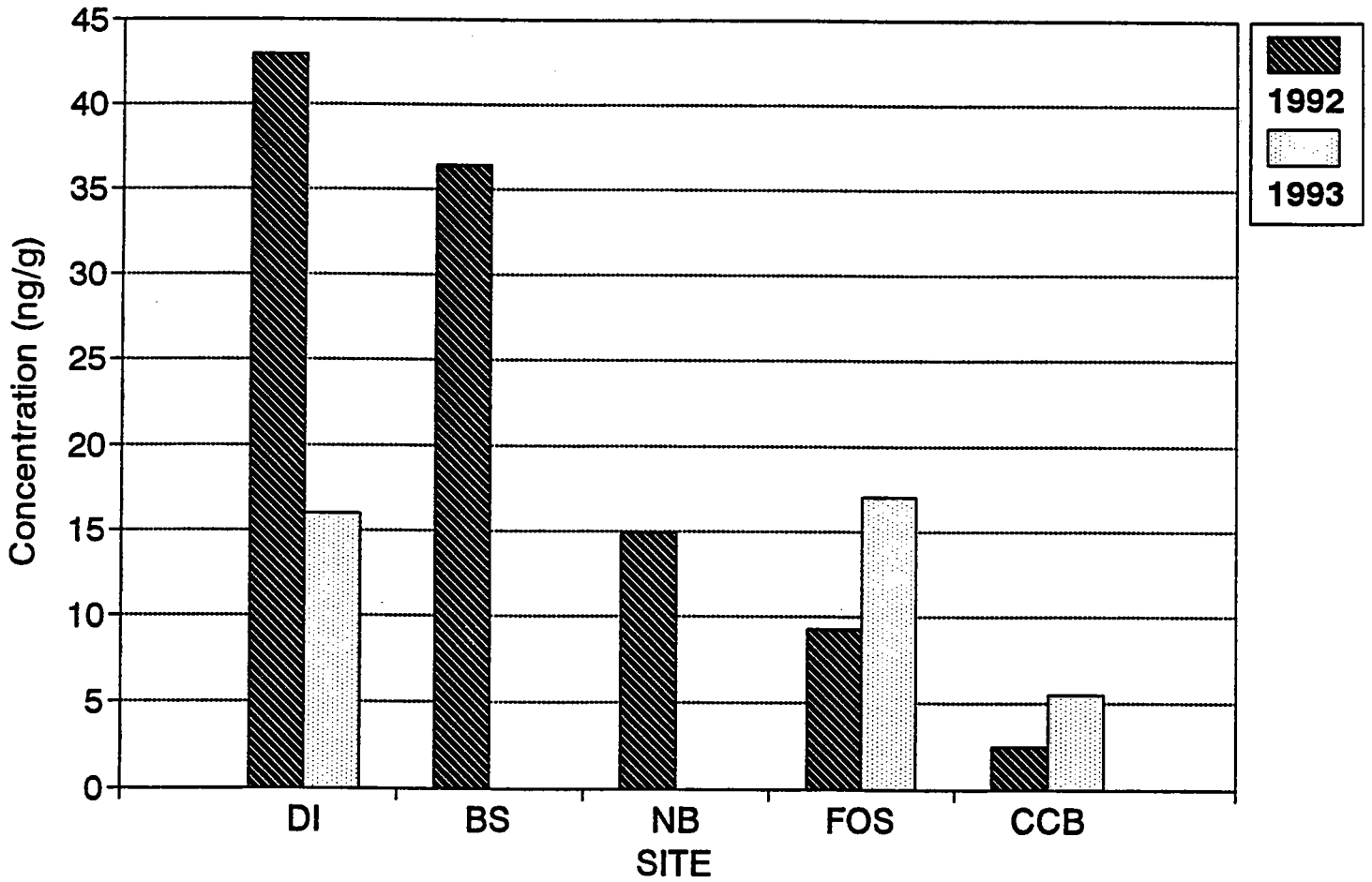
Comparison of 1992 and 1993 Mercury Levels in Flounder Tissue (dry wt)



Comparison of 1992 and 1993 PCB Levels in Flounder Tissue (dry wt)



Comparison of 1992 and 1993 Chlordane Levels in Flounder Tissue (dry wt)



DETECTABLE CHANGES IN TISSUES - 1993
PRELIMINARY DATA

Station	Fraction	Compound	N	Average	STD	P80 Detectable Change	
				ng/g dry	ng/g dry	ng/g	%
DI	FISH	Chlordane	10	16.04	8.17	10.82	67.47
FOS	FISH	Chlordane	9	17.01	11.74	16.52	97.13
CCB	FISH	Chlordane	10	5.54	2.76	3.66	66.03
DI	FISH	Dieldrin	10	3.30	1.07	1.42	42.89
FOS	FISH	Dieldrin	9	2.96	1.92	2.70	91.31
CCB	FISH	Dieldrin	10	2.02	0.53	0.70	34.51
DI	FISH	Hg (ug/g)	10	0.46	0.33	0.44	95.43
FOS	FISH	Hg (ug/g)	9	0.41	0.22	0.32	76.52
CCB	FISH	Hg (ug/g)	10	0.19	0.09	0.12	66.92
DI	FISH	PCB	10	200.39	94.65	125.43	62.59
FOS	FISH	PCB	9	215.05	116.44	163.82	76.18
CCB	FISH	PCB	10	59.24	25.93	34.37	58.01
DI	FISH	DDT	10	32.17	18.26	24.20	75.23
FOS	FISH	DDT	9	27.64	12.30	17.30	62.58
CCB	FISH	DDT	10	13.05	5.12	6.79	52.05
DI	LOBSTER	Chlordane	3	6.73	1.96	5.96	88.48
FOS	LOBSTER	Chlordane	2	2.11	0.10	0.53	25.16
CCB	LOBSTER	Chlordane	10	2.41	1.94	2.57	106.63
DI	LOBSTER	Dieldrin	3	9.02	1.87	5.66	62.80
FOS	LOBSTER	Dieldrin	2	4.66	0.62	3.34	71.62
CCB	LOBSTER	Dieldrin	10	3.52	0.66	0.87	24.86
DI	LOBSTER	Hg (ug/g)	3	0.84	0.02	0.06	6.81
FOS	LOBSTER	Hg (ug/g)	2	1.01	0.43	2.33	230.36
CCB	LOBSTER	Hg (ug/g)	10	0.66	0.18	0.24	36.52
DI	LOBSTER	PCB	3	154.21	101.54	308.20	199.86
FOS	LOBSTER	PCB	2	65.79	3.73	19.99	30.38
CCB	LOBSTER	PCB	10	66.46	49.39	65.44	98.47
DI	LOBSTER	DDT	3	28.36	18.63	56.56	199.46
FOS	LOBSTER	DDT	2	9.24	2.11	11.30	122.31
CCB	LOBSTER	DDT	10	10.65	3.99	5.28	49.62

DI	HEPATOPANCREAS	Chlordane	3	194.42	8.92	27.06	13.92
FOS	HEPATOPANCREAS	Chlordane	2	48.56	6.97	37.39	77.00
CCB	HEPATOPANCREAS	Chlordane	10	76.12	80.75	107.00	140.56
DI	HEPATOPANCREAS	Dieldrin	3	124.70	43.89	133.23	106.84
FOS	HEPATOPANCREAS	Dieldrin	2	56.60	14.98	80.32	141.91
CCB	HEPATOPANCREAS	Dieldrin	10	39.79	17.11	22.67	56.98
DI	HEPATOPANCREAS	Pb (ug/g)	3	0.33	0.17	0.50	151.51
FOS	HEPATOPANCREAS	Pb (ug/g)	2	0.38	0.17	0.94	246.83
CCB	HEPATOPANCREAS	Pb (ug/g)	10	0.10	0.08	0.11	107.26
DI	HEPATOPANCREAS	Hg (ug/g)	3	0.30	0.10	0.29	99.29
FOS	HEPATOPANCREAS	Hg (ug/g)	2	0.24	0.06	0.33	141.41
CCB	HEPATOPANCREAS	Hg (ug/g)	10	0.21	0.11	0.14	67.28
DI	HEPATOPANCREAS	Ni (ug/g)	3	0.65	0.33	1.00	152.78
FOS	HEPATOPANCREAS	Ni (ug/g)	2	0.47	0.05	2.47	528.42
CCB	HEPATOPANCREAS	Ni (ug/g)	10	1.31	0.67	0.89	67.80
DI	HEPATOPANCREAS	Ag (ug/g)	3	6.53	0.81	2.45	37.58
FOS	HEPATOPANCREAS	Ag (ug/g)	2	2.43	1.06	5.69	234.10
CCB	HEPATOPANCREAS	Ag (ug/g)	10	6.35	6.34	8.41	132.49
DI	HEPATOPANCREAS	Cd (ug/g)	3	3.33	1.18	3.59	107.55
FOS	HEPATOPANCREAS	Cd (ug/g)	2	13.26	6.00	32.16	242.53
CCB	HEPATOPANCREAS	Cd (ug/g)	10	10.92	5.12	6.78	62.13
DI	HEPATOPANCREAS	Cr (ug/g)	3	1.46	0.09	0.28	19.36
FOS	HEPATOPANCREAS	Cr (ug/g)	2	1.27	0.08	0.46	35.83
CCB	HEPATOPANCREAS	Cr (ug/g)	10	1.09	0.35	0.46	42.67
DI	HEPATOPANCREAS	Cu (ug/g)	3	642.00	281.02	852.97	132.86
FOS	HEPATOPANCREAS	Cu (ug/g)	2	309.00	251.73	1350.11	436.93
CCB	HEPATOPANCREAS	Cu (ug/g)	10	463.51	400.18	530.27	114.40
DI	HEPATOPANCREAS	Zn (ug/g)	3	74.80	59.82	181.58	242.75
FOS	HEPATOPANCREAS	Zn (ug/g)	2	83.55	47.31	253.71	303.67
CCB	HEPATOPANCREAS	Zn (ug/g)	10	49.73	21.83	28.93	58.18
DI	HEPATOPANCREAS	PCB	3	2857.83	489.46	1485.62	51.98
FOS	HEPATOPANCREAS	PCB	2	2262.60	1028.06	5513.81	243.69
CCB	HEPATOPANCREAS	PCB	10	2151.32	2162.26	2865.20	133.18
DI	HEPATOPANCREAS	PAH	3	11727.20	9642.59	29267.70	249.57

FOS	HEPATOPANCREAS	PAH	2	5862.35	3102.69	16640.70	283.86
CCB	HEPATOPANCREAS	PAH	10	3248.79	3126.43	4142.80	127.52
DI	HEPATOPANCREAS	DDT	3	642.21	47.81	145.13	22.60
FOS	HEPATOPANCREAS	DDT	2	290.29	100.21	537.46	185.15
CCB	HEPATOPANCREAS	DDT	10	287.68	105.40	139.66	48.55

PARAMETERS REQUIRED FOR POWER ANALYSIS

n = number of individuals

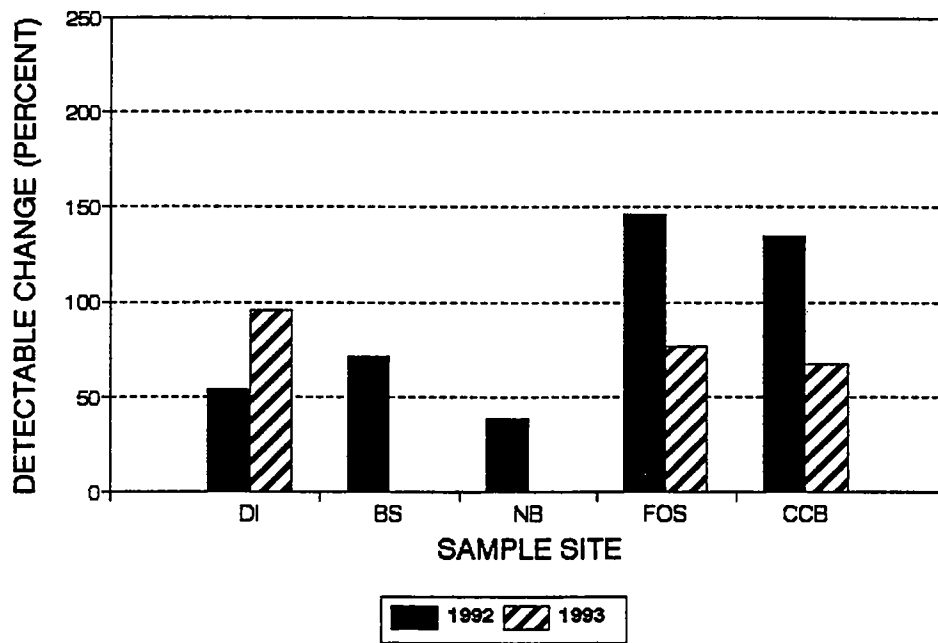
α = Probability of committing a Type I error **[0.05]**

$1-\beta$ = Power (β = Probability of committing a Type II error) **[0.8]**

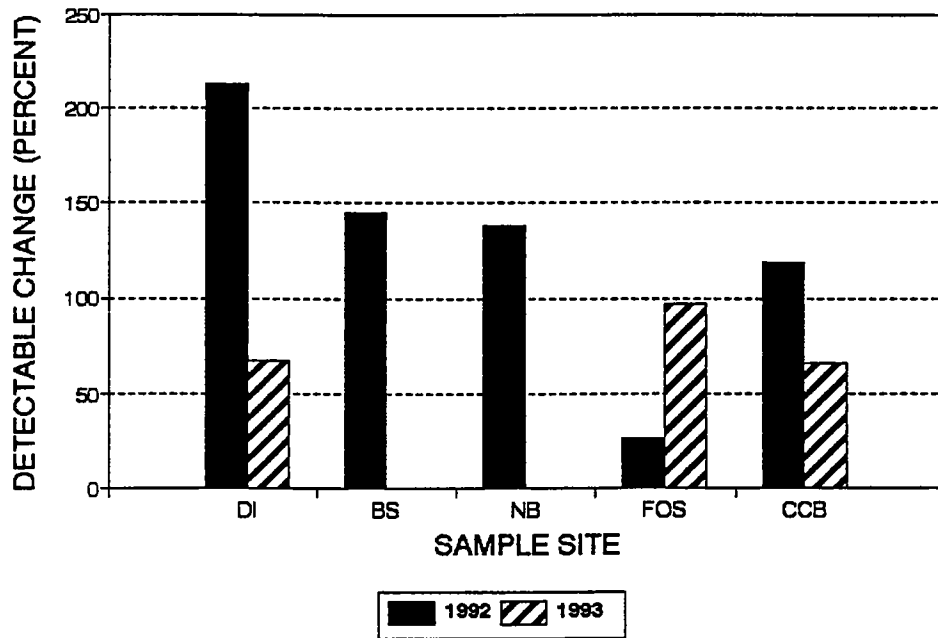
σ = Standard error of the estimate **[Determined from real data]**

es = Effect size **[Detectable change defined by analysis or set]**

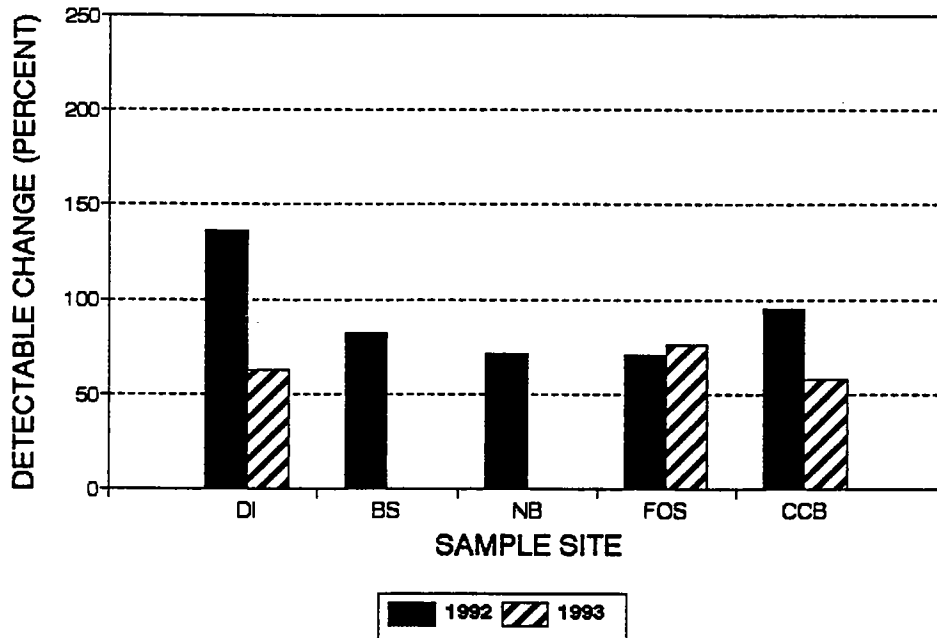
FLOUNDER TISSUE MERCURY



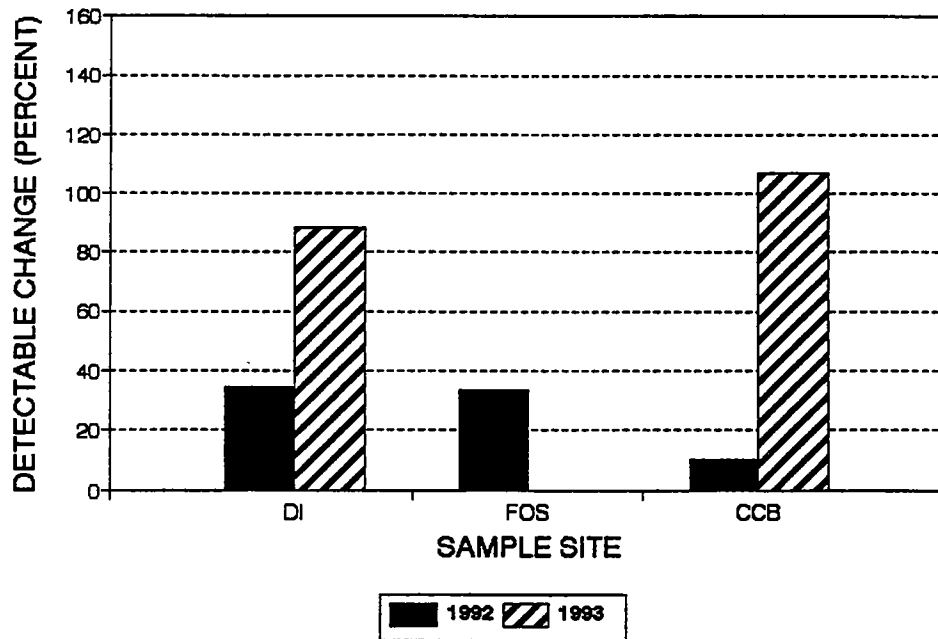
FLOUNDER TISSUE CHLORDANE



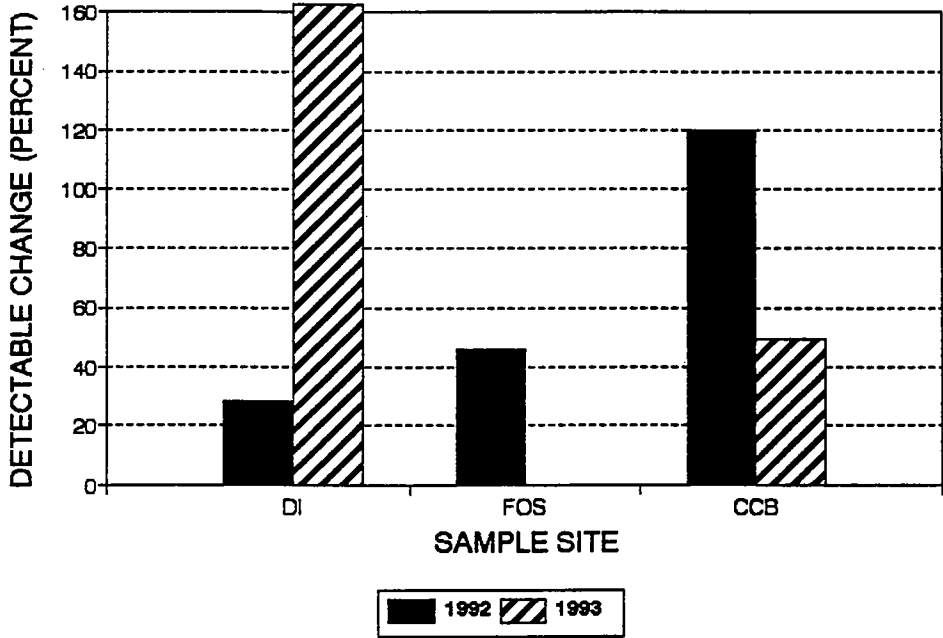
FLOUNDER TISSUE TOTAL PCB



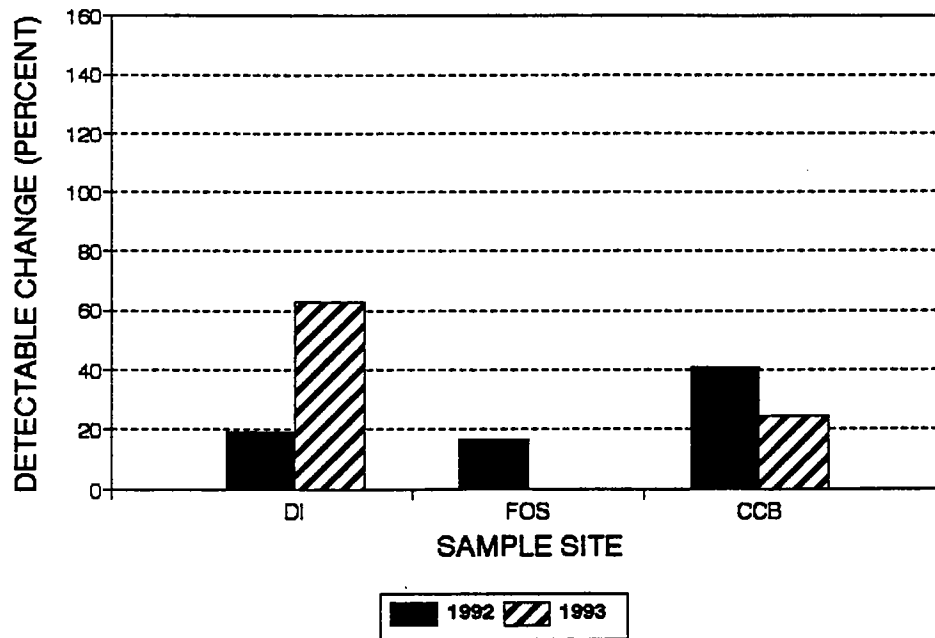
LOBSTER TISSUE Chlordane



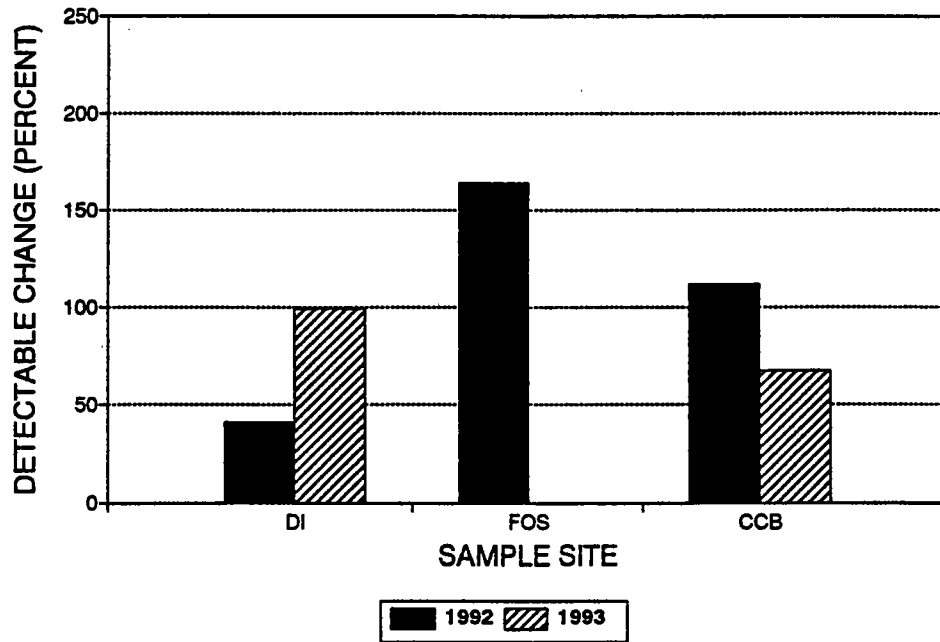
LOBSTER TISSUE TOTAL DDT



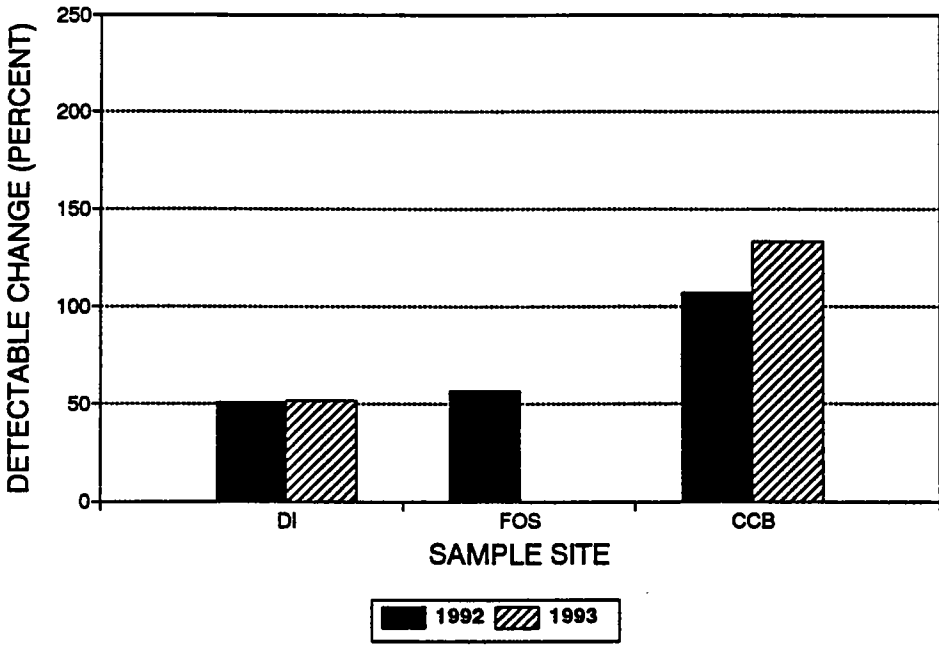
LOBSTER TISSUE DIELDRIN



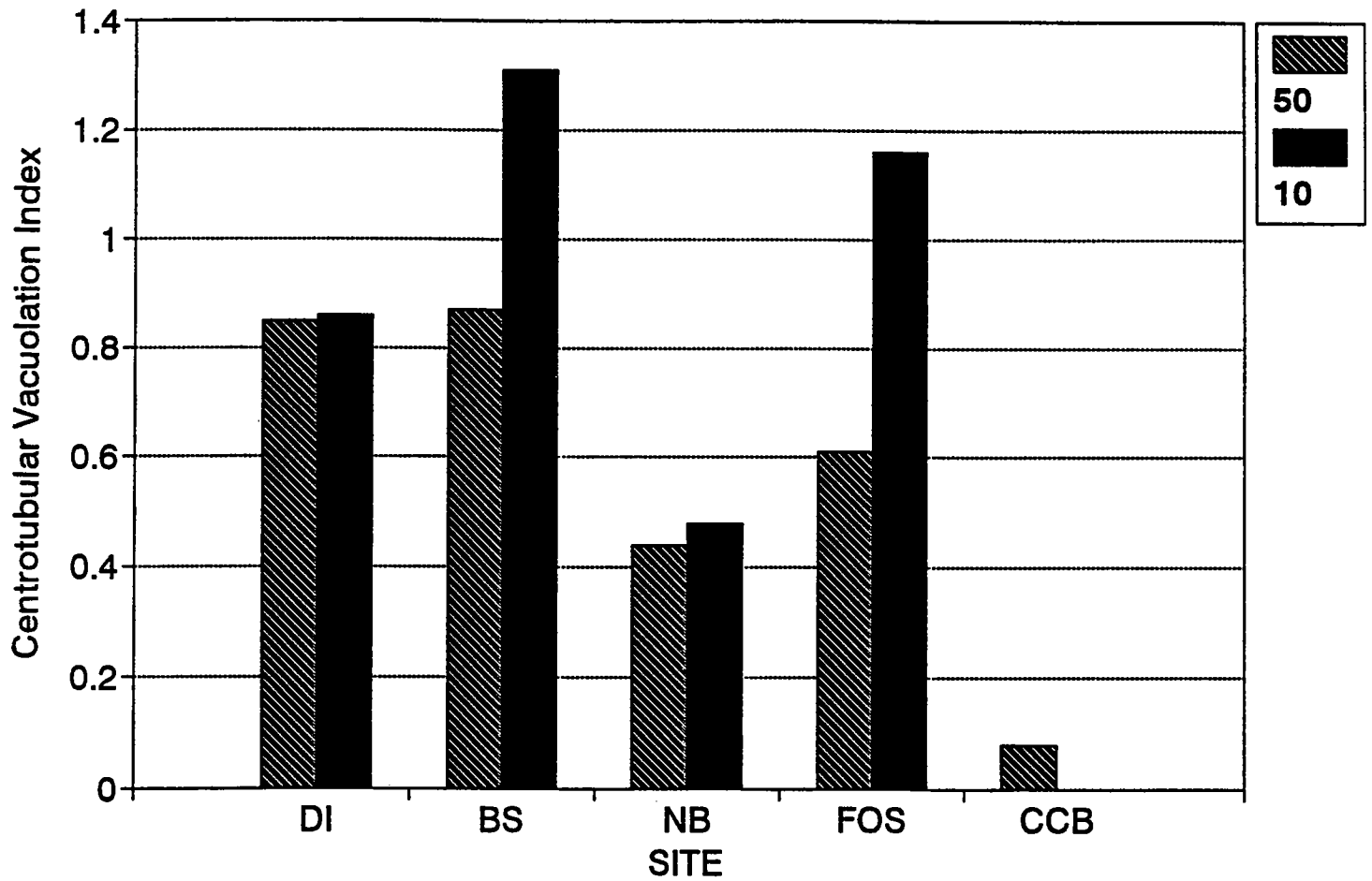
LOBSTER HEPATOPANCREAS MERCURY



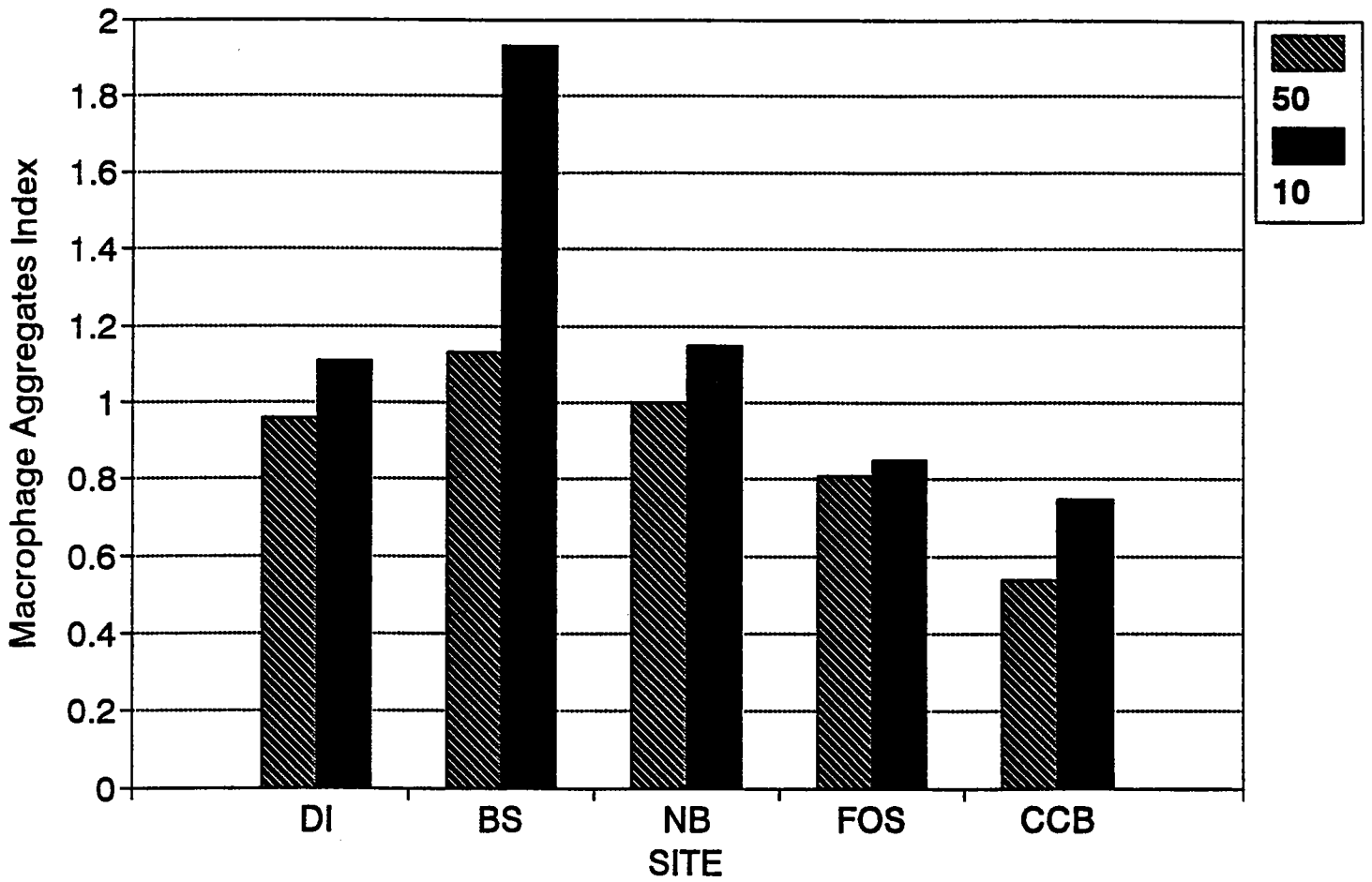
LOBSTER HEPATOPANCREAS TOTAL PCB



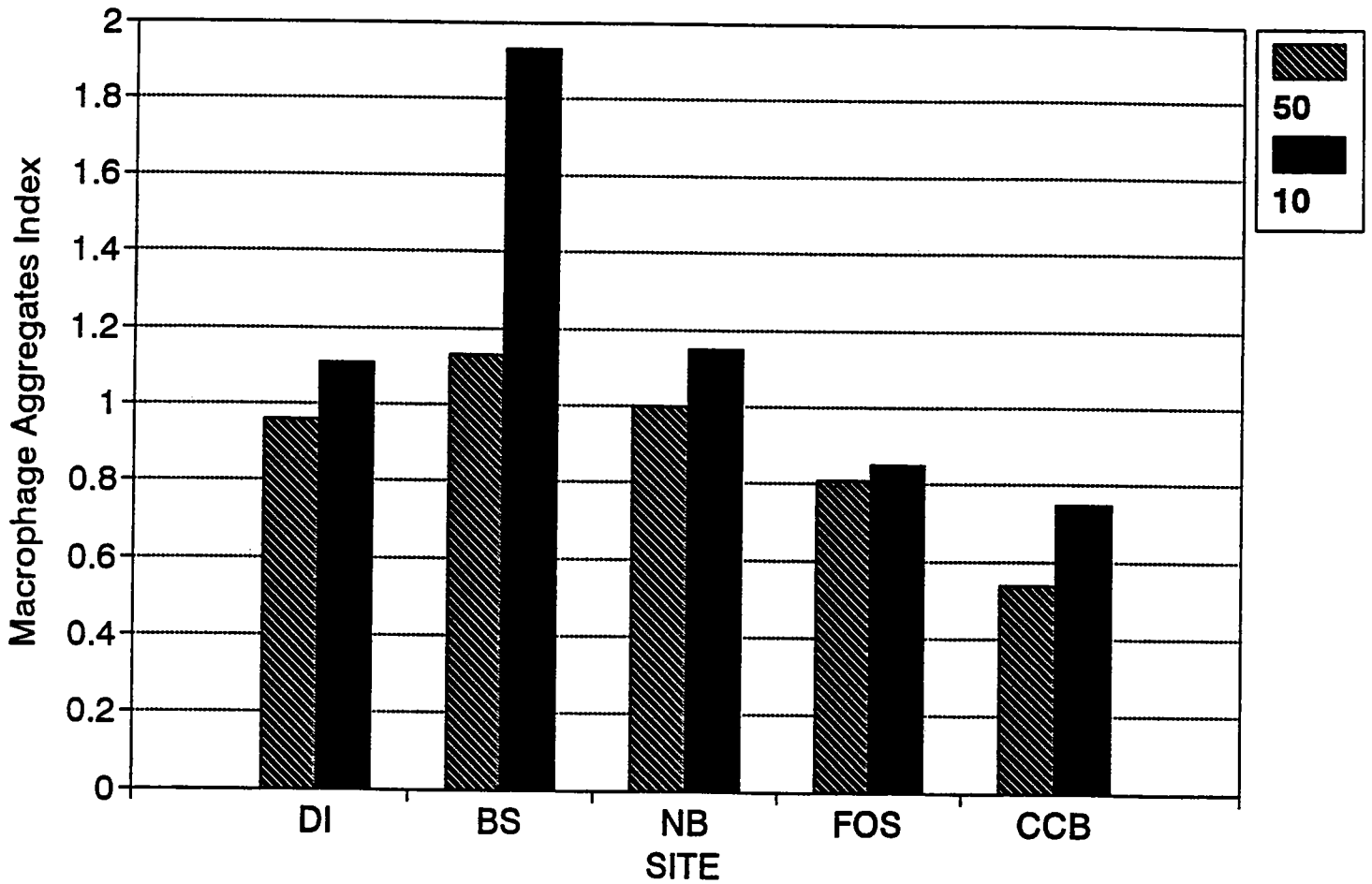
Comparison of Histo/Pathology in Flounder Livers (50 vs 10)



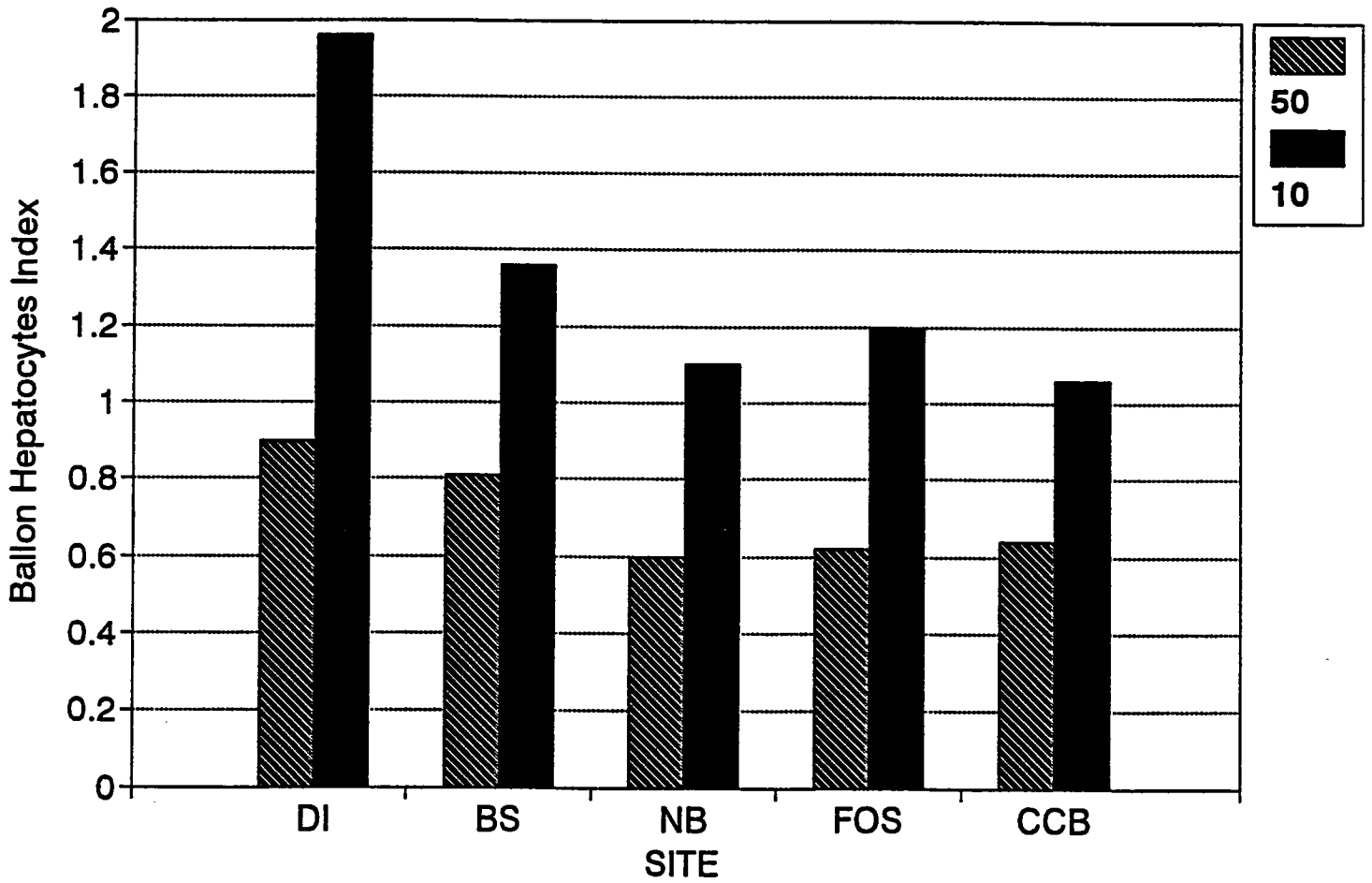
Comparison of Histo/Pathology in Flounder Livers (50 vs 10)



Comparison of Histo/Pathology in Flounder Livers (50 vs 10)



Comparison of Histo/Pathology in Flounder Livers (50 vs 10)



FINANCIAL IMPACT OF INDIVIDUALS VERSUS COMPOSITES

ASSUMPTIONS

- 1) Field sampling effort not substantially different
- 2) Processing time tie is not included
- 3) 10 individuals per site
- 4) 3 composites of 5 individuals each
- 5) Cost per sample \$1400 for metals, PAH, PCB, pesticides

Cost of individual analysis = \$14,000

Cost of pooled samples = \$ 4,200

Cost savings with composites 70%.

More individuals included in the analytical pool (10 verse 15)

Power of analysis increases

Ability to detect change is greater

SUMMARY (PRELIMINARY)

No consistent pattern in change in concentrations among chemicals across sites

- Possible decrease in contaminants at Deer Island
- Possible increases in CCB
- FOS variable

Variability is similar in 1992 and 1993 even with added individuals

Detectable change

- Is slightly better using ten individuals
- A consistent improvement in detectable change is not apparent

Detectable change remains in the 50 to 200 percent range

Concentrations are substantially less than FDA action limits

Cost of analyzing individual animals relative to gain in information on variability and the occasional organism with high contaminant concentration is high

Settling on a consistent analytical scheme is imperative to ensure sufficient consistent baseline data is available

**EPA GUIDANCE FOR ASSESSING CHEMICAL CONTAMINANT DATA
FOR USE IN FISH ADVISORIES**

Purpose: To provide methods for sampling and analysis of contaminants in fish and shellfish in relation to state fish consumption advisories.

Process: Tiered Approach

Tier 1: Screening approach to assess magnitude of contamination for commonly consumed target species

Tier 2, Phase I: Assessment of contaminant magnitude assessment

Tier 2, Phase II: Assessment of geographic extent assessment

Conduct Tier 2 if exceed screening value in screening study.

TIER 1 SCREENING STUDIES

Species and size:	Commonly consumed animals (1 shellfish and one fish OR two fish with one a bottom feeder)
Sampling:	Areas where various types of fishing is practiced Clean and dirty sites for comparison During legal harvest season but not spawning period
Sample type:	Composite filet samples of finfish and edible portions of target shellfish; samples of similar length (individuals should not exceed 10% of average size for composite; larger organisms should be targeted (page 6-11))
Replicates:	One composite for each species (or tissue type); replicates are optional and encouraged
Sample Analysis:	Methods capable of accurately quantifying contaminants at or below screening values (Use clean methods); Analysis of all analytes of concern
Data Reporting and analysis:	Concentrations on a wet weight basis and compared to screening values Screening results determine if Tier 2 studies are needed

- NOTES:**
- Analysis of oldest (larger) individuals to represent highest likely exposure can be conducted
 - Analysis of individual fish is allowed; it is recommended that a portion of the individual fish homogenate be retained to allow analysis of individual organisms.
 - Analysis of individual organisms allow estimates of underlying population variance which facilitates sample size determinations.

TIER 2 INTENSIVE STUDIES

- Species:** Commonly consumed animals (one shellfish and one fish OR two fish with one a bottom feeder); Use three size classes for Phase 2, however, for estuarine systems size based sampling is recommended only if it is likely to serve the potential risk management outcome (Page 6-7)
- Sampling:** Areas where various types of fishing is practiced
Clean and dirty sites for comparison
During legal harvest season but not spawning period
Optional to collect replicate composites of three size classes in Phase 1
- Sample type:** Composite filet samples of finfish and edible portions of target shellfish; samples of similar length (individuals should not exceed 10% of average size for composite)
Composite samples for three size classes for Phase 2
- Replicates:** One composite for each species (or tissue type); replicates are optional but encouraged
Phase 1, Replicate composites for each target species
Phase 2, Replicate composites at each Phase 2 site
- Sample Analysis:** Methods capable of accurately quantifying contaminants at or below screening values (Use clean methods); Only those analytes exceeding screening values
- Data Reporting and analysis:** Concentrations on a wet weight basis and compared to screening values

COMPARISON OF GUIDANCE TO CURRENT MWRA PROGRAM

PROGRAM ELEMENT	EPA GUIDANCE	1992-1994 MWRA PROGRAM
Data use	Fish advisories from human health perspective	Human health Trend analysis Fish health
Species	1 shellfish 1 fish	2 shellfish 1 bottom fish
Sampling	Several areas; various types of fishing activity Clean and dirty sites for comparison During legal harvest season Not spawning	Five areas flounder (clean to dirty) Three areas lobster (clean to dirty) April/May time frame Non-gravid (lobster)
Animal Size	Single size (with 10% of average) Optional three size classes Uniform within 10% of average	Single size class Legal size lobster 4/5 year old flounder
Sample type	Single composite (Number of individuals depends on variance and desired power) 5 individuals per composite rule of thumb	Composites planned (# per replicate and # replicates TBD)
Replicates	One composite for species Individual fish for population variance	Three composites planned (or more?) Individuals analyzed in 1992 (7 flounder) and 1993 (10 flounder) Composite of 3 individual flounder in 1992
Analytes	Bioaccumulating metals and organics	Major analytes of concern in Mass Bay
Reporting units	Wet weight concentrations	Dry weight (Wet is optional)
Indicators	Comparison to screening value (arithmetic mean)	Comparison to FDA action limits, individuals or composite Relation of chemicals to indications of flounder health Histopathology; gross lesions

REVISED OBJECTIVES OF MWRA FISH AND SHELLFISH MONITORING

1. To ensure, from a human health perspective, the safety of fish and shellfish taken from the vicinity of the Massachusetts bay outfall.

Baseline

- Modified screening approach at five sites for flounder, three for lobster
- Triplicate composites with 5 individuals per composite (power of 80)
- Optional analysis of large individual (old) organisms
- Caged mussels, flounder meat and liver; lobster meat and hepatopancreas
- Single size (4/5 year class) of for flounder
- Screening value calculation and comparison; FDA action limit

Post discharge

- Same design
- Option to collect screening replicates from subareas in nearfield region
- Comparison to risk criteria and FDA action limits
- Optional analysis of individual large (old) organisms
- Reduced set of parameters keyed to risk assessment
- Wet weight reporting units
- Screening value calculation and comparison; FDA action limit

2. To detect trends in contaminant concentrations in important commercial/recreational fish in the vicinity of the MWRA outfall in Massachusetts Bay.

- Triplicate composite samples of 5 organisms composite
- Use small (younger) animals as early indication of unacceptable uptake
- Dry weight reporting units

3. To evaluate any effect on fish and lobster health and relate changes to contaminant burdens in the organisms

- Histopathology
- Gross lesions
- Relationships between chemistry and histopathology

APPENDIX D

**SUGGESTED REVISED OBJECTIVES OF THE MWRA
FISH AND SHELLFISH MONITORING STUDY**

**CARLTON HUNT
BATTELLE**

**SUGGESTED REVISED OBJECTIVES OF THE MWRA
FISH AND SHELLFISH MONITORING STUDY**

**CARLTON HUNT
BATTELLE**

1. To ensure, from a human health perspective, the safety of fish and shellfish collected in the vicinity of the future outfall.

Baseline

- Retain five (or possibly three) sampling sites for flounder tissue chemistry, five sites for flounder histopathology, and three sampling sites for lobster tissue analyses
- Retain caged mussel study; flounder meat and liver analyses; lobster meat and hepatopancreas analyses
- Include replicate composites at each site (the number of replicates and individual fish to be determined based on 1993 analysis of individual tissues)
- Set power of detection ($1-\beta$) at 80% with $\alpha = 0.05$
- Retain optional analysis of large individual (old) organisms
- Collect one size class (4/5 year) of flounder
- Compare chemical concentrations in tissues to FDA action limit
- Include wet weight reporting units

Post-Discharge

- Follow same sampling design
- Allow an option to collect more replicates in subareas in nearfield region
- Compare chemical concentrations in tissues to FDA action limits
- Analyze individual large (old) organisms (lobster)
- Analyze for a reduced set of parameters that are keyed to risk assessment
- Include wet weight reporting units

2. To detect trends in contaminant concentrations in important commercial/recreational fish in the vicinity of the MWRA outfall in Massachusetts Bay.

- Analyze triplicate composite samples of five organisms/composite
- Use dry weight reporting units

3. To evaluate any effect of outfall discharge on fish and lobster health and relate changes to contaminant burdens in the organisms.

- Examine gross lesions
- Conduct histopathology of selected lesions that may be indicative of stress
- Examine relationships between chemistry and histopathology

