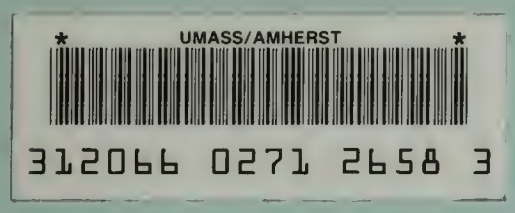

PROGRESS REPORT

PCB CONCENTRATIONS IN MARINE FISH AND SHELLFISH FROM BOSTON AND SALEM HARBORS, AND COASTAL MASSACHUSETTS

Jack P. Schwartz
Division of Marine Fisheries
Cat Cove Marine Laboratory
Environmental Analysis Section
92 Fort Avenue
Salem, MA 01970

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ABSTRACT

Marine Fish and shellfish from Boston Harbor, Salem Harbor, and coastal areas were analyzed to determine tissue concentrations of polychlorinated biphenyls (PCBs). Winter flounder, Pseudopleuronectes americanus, American lobster, Homarus americanus, and several bivalve species were extracted according to U.S. Food and Drug Administration protocol and quantified by gas chromatography as Aroclor 1254. Flounder and lobster collected in Spring, 1986, from Boston Harbor and Salem Harbor were compared to species from coastal sites by analysis of variance.

Results indicate significant increases in PCB body burdens among Boston Harbor flounder compared to coastal flounder. PCBs in flounder from Salem Harbor were not significantly different than PCB concentration in coastal flounder. Lobster from both Boston and Salem Harbor had significantly higher PCB concentrations than coastal lobster. Inclusion of the edible hepatopancreas in lobster samples could be obscuring PCB concentrations in edible muscle tissue. PCB concentrations in coastal lobster were as much as ive times greater than PCBs in coastal flounder. Bivalves from the two harbors do not appear to be bioaccumulating more PCBs than coastal species, however more data is required to conduct further analysis. Factors controlling the uptake and accumulation of PCBs for marine species in each habitat region are discussed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This study could not be possible without the help of many Division of Marine Fisheries personnel. past and present, as well as legislative support for new equipment. Mr. Arnold B. Howe, project leader for the resource assessment program, supervised the collection of coastal samples for the program. Mr. H. Russell Iwanowicz supervised the collection of samples from Boston and Salem Harbors aboard the FC WILBOUR under the command of Captain Shirley Mitchell. Chemical analyses at Cat Cove Marine Laoboratory were performed by Eileen Cox, Selina J. Makofsky, Matthew Pride, and Joan Schlosstein. Laboratory personnel also participated in field sampling at sea. The contaminant monitoring program is directed by W. Leigh Bridges.

INTRODUCTION

Contaminants entering the marine environment have the potential for reducing the survival of marine organisms. When present in sufficient concentrations organic and inorganic compounds can be acutely toxic to marine fishes and invertebrates. At sublethal concentrations chronic effects can decrease survival by reducing fecundity, impaired feeding, higher metabolism, formation of cancerous tumors, and reductions in growth of larval stages. The commercial fishing industry contributes approximately one billion dollars to the Commonwealth's economy each year (Massachusetts Division of Marine Fisheries 1985). It is therefore vital that the Commonwealth of Massachusetts monitor the transfer and uptake of contaminants to manage and protect its renewable marine resources.

In response to growing concern over the impact of contaminants on marine resources, the Division of Marine Fisheries initiated a small-scale contaminant monitoring program. The program is designed to measure tissue concentrations of select organic and inorganic contaminants in commercially important marine fish and shellfish species as well as other chemical and biological parameters. The spatial scope of the program encompasses all territorial waters of the Commonwealth including Boston and Salem Harbors. The goal of the program is twofold:

1. Obtain baseline contaminant information for marine species in polluted harbors and relatively unpolluted coastal areas.

2. Create time series information to monitor changes in contaminant levels within habitats and resources.

This report provides a preliminary assessment for edible tissue concentrations of polychlorinated biphenyls (PCBs) in marine species from harbor and coastal habitats. PCBs are ubiquitous byproducts of several industrial processes, including the manufacture of electrical components. The recent designation of New Bedford Harbor as a Superfund cleanup site by the U.S. Environmental Protection Agency was an outgrowth of PCB contaminantion of the marine environment from discharges associated with the manufacture of electrical components (Weaver 1984). PCBs are suspected carcinogens, readily accumulate in fat tissue as they are transferred from prey to predator along the marine food chain, and can be also toxic to marine fish species (Hansen et al. 1971).

STUDY DESIGN

The winter flounder, Pseudopleuronectes americanus, and American lobster, Homarus americanus, were chosen as the representative

fish and crustacean for predators within their niche, and because of their intimate contact with sediments which can sequester large concentrations of PCBs (Boehm et al. 1984; Brownawell and Farrington 1986). In Boston and Salem Harbors the soft whell clam, Mya arenaria, was chosen as the representative bivalve mollusc shellfish. Offshore bivalve molluscs included the surf clam, Spisula solidissima, quahog, Mercenaria mercenaria, ocean quahog, Arctica islandica, and blue mussel, Mytilus edulis. Three sampling stations were located in Boston Harbor (figure 1) and Salem Harbor (figure 2). Coastal samples were acquired from various random locations that were included with the Division's resource assessment cruise schedule (figures 3, 4, and 5). Harbor sampling employed a variety of gear including a yankee otter trawl, lobster pots, and hand shellfish gear. Coastal sampling was accomplished using a 3/4 size North Atlantic type 2 seam otter trawl. Five legal size flounder, lobster, and a composite of eight bivalves constituted the sample size at each station. Harbor and coastal sampling was performed concurrently in the spring and fall.

SAMPLE PREPARATION AND EXTRACTION

Samples were frozen in polyethylene plastic bags until shipped to Cat Cove Marine Laboratory for PCB analysis. Samples were thawed in a stainless steel trough, and the edible portion from each

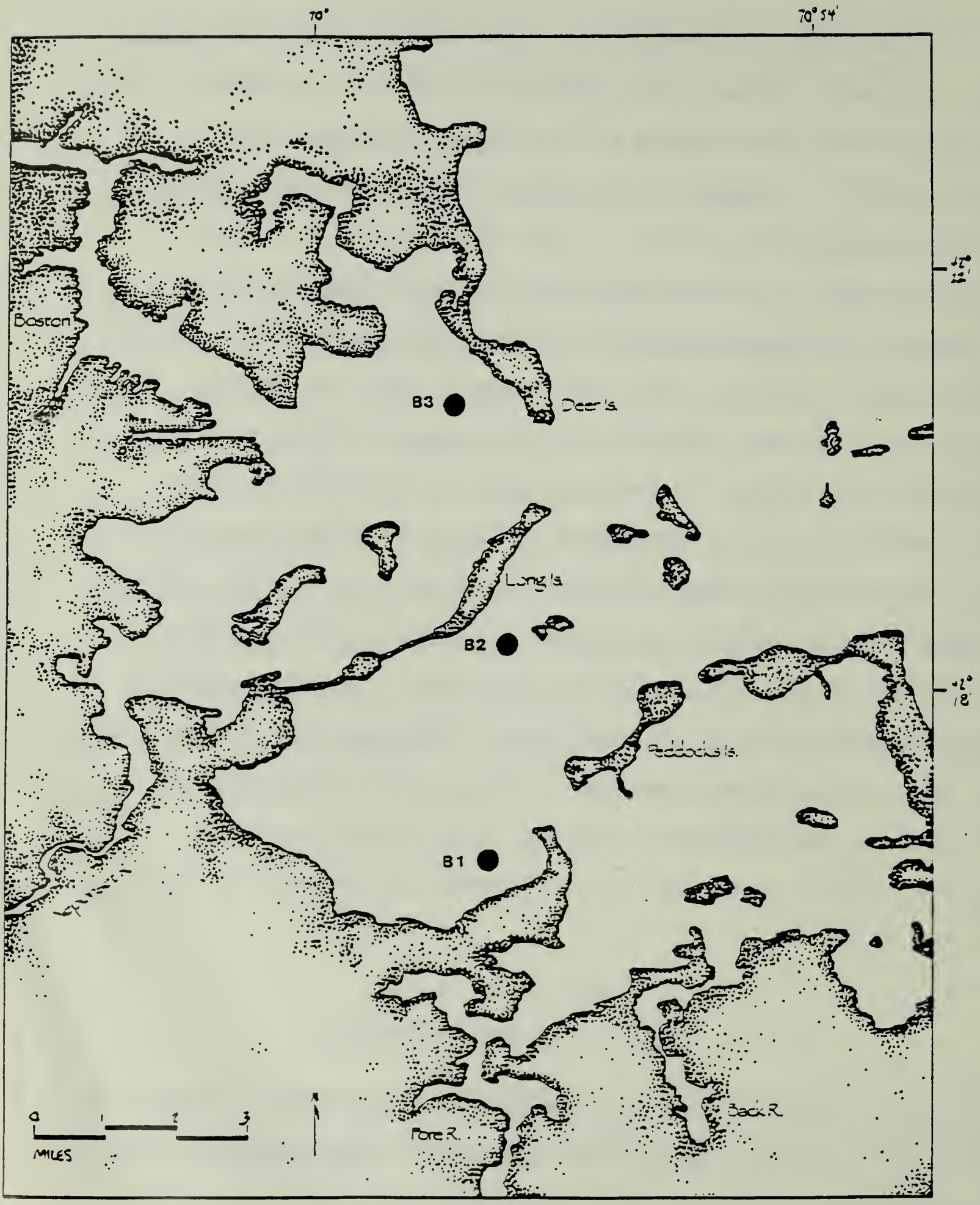

Figure 1. Division of Marine Fisheries Boston Harbor sample sites.

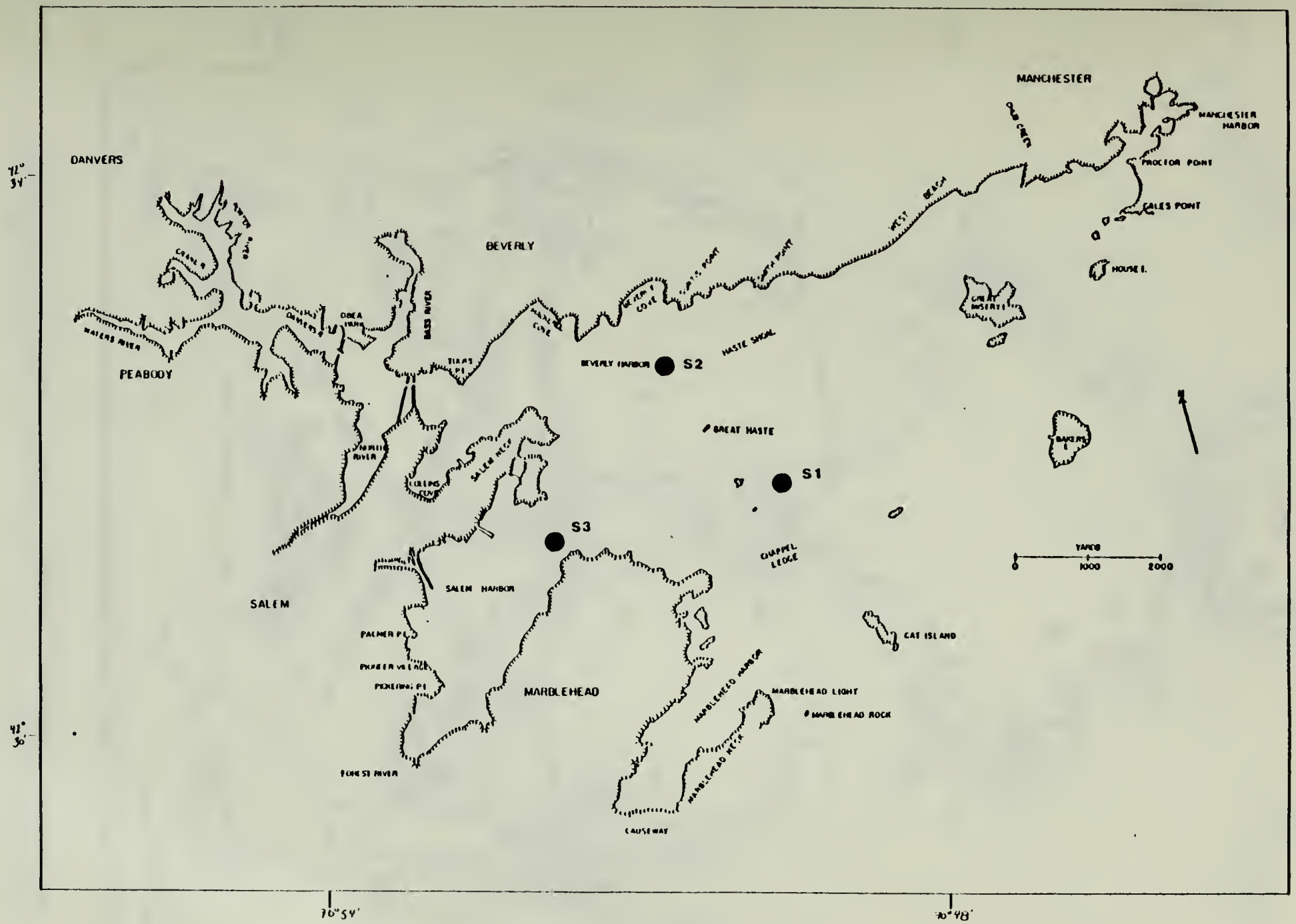

Figure 2. Division of Marine Fisheries Salem Harbor Sites.

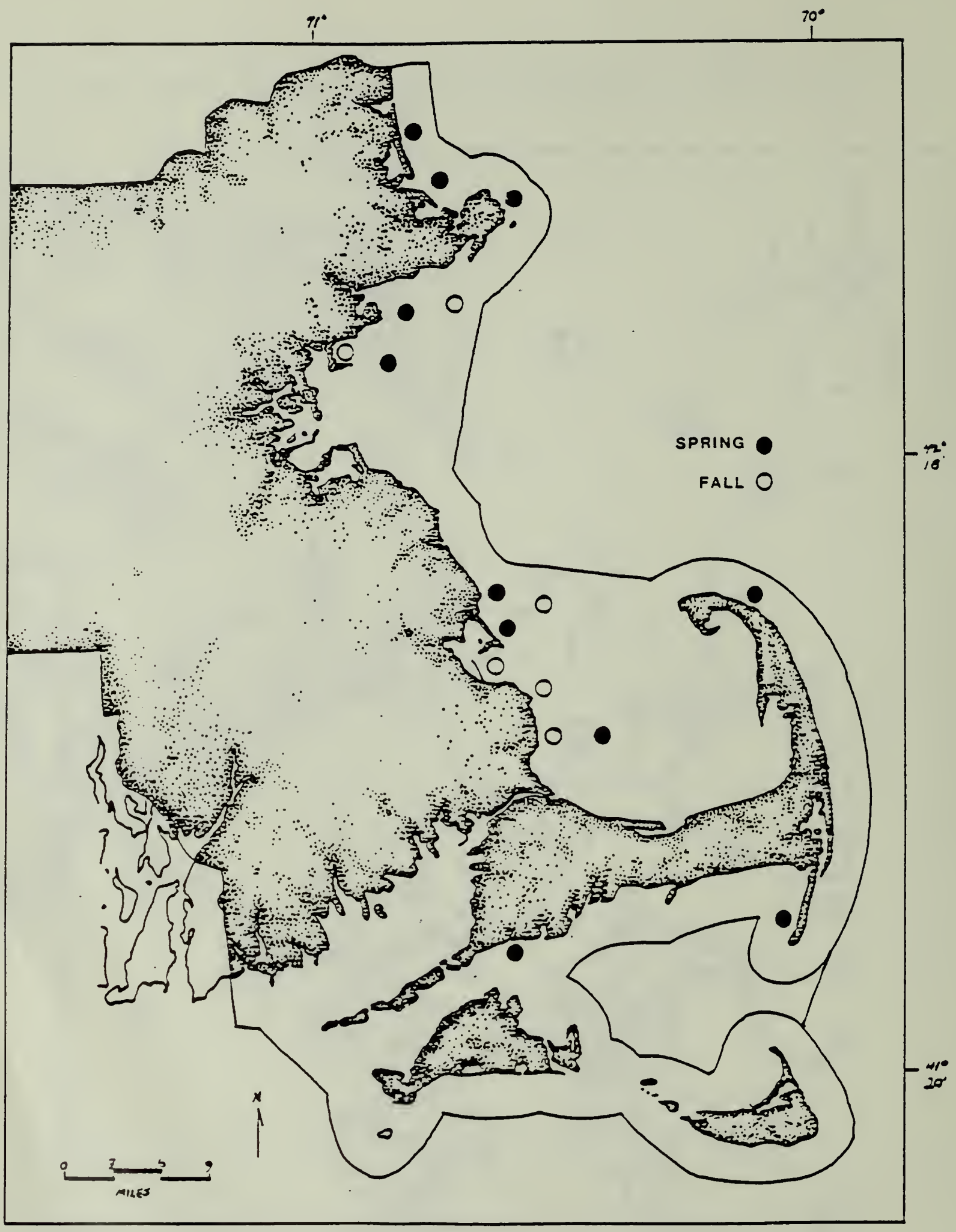

Figure 3. Division of Marine Fisheries coastal sample sites, spring and fall, 1984.

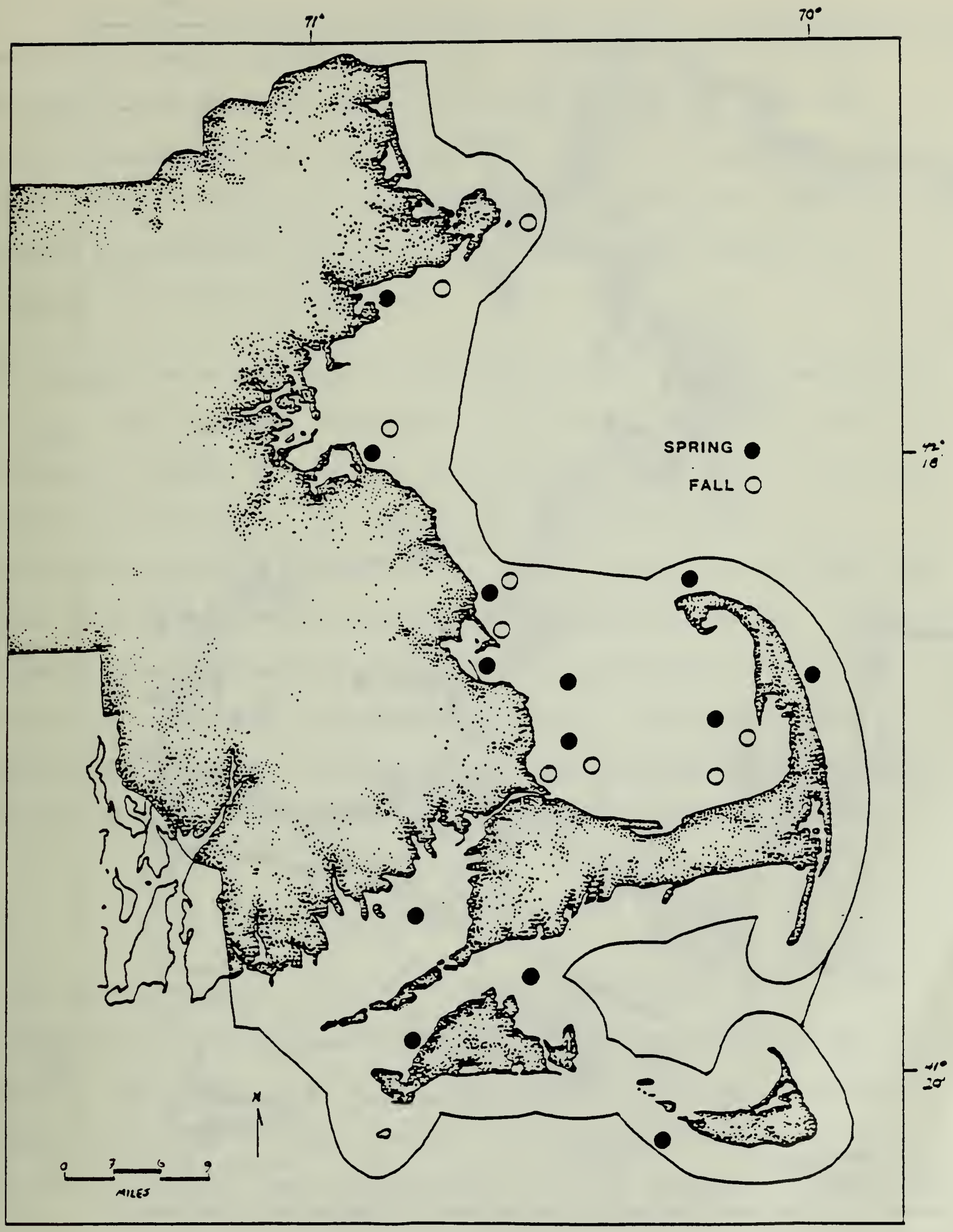

Figure 4. Division of Marine Fisheries coastal sample sites, spring and fall, 1985.

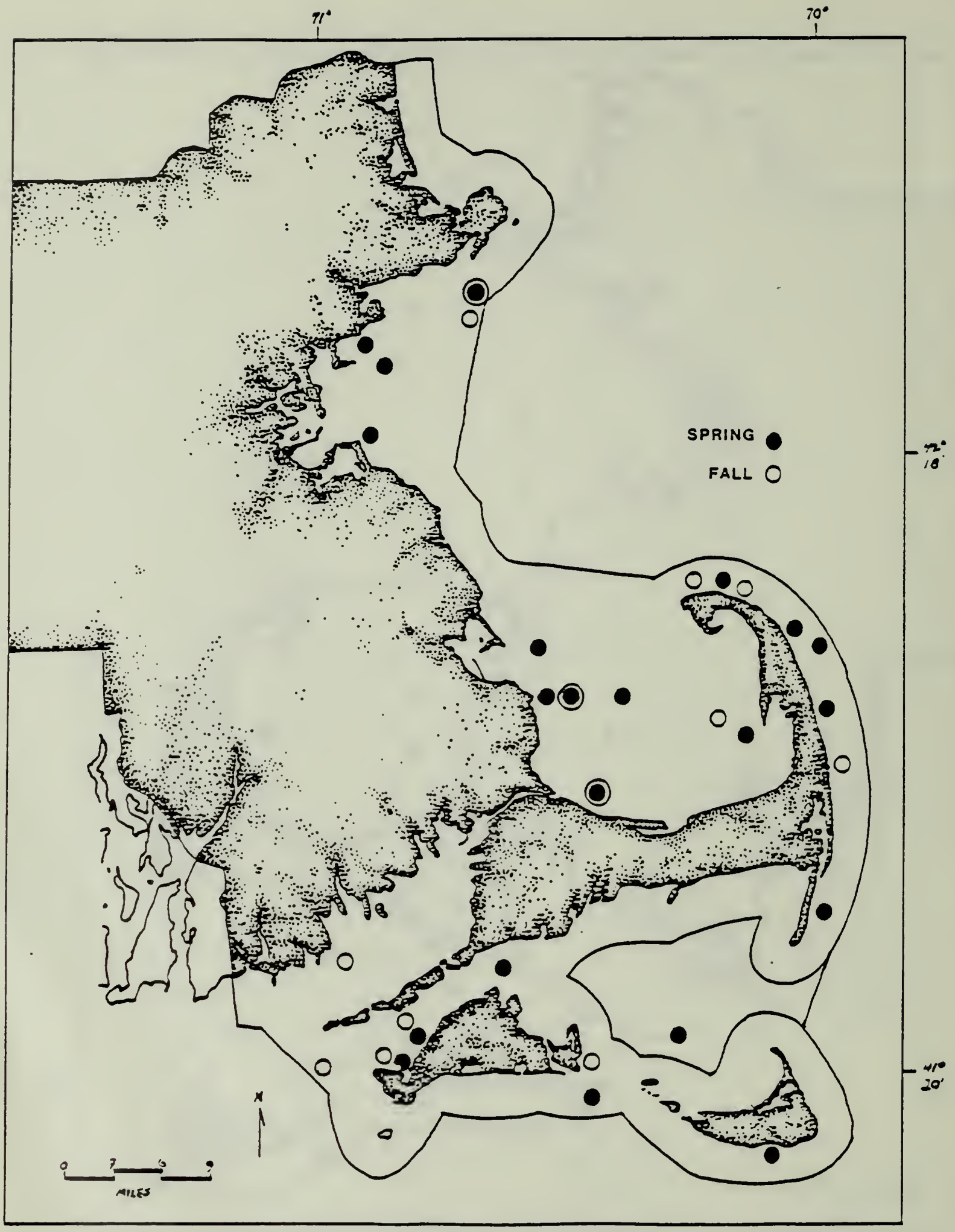

Figure 5. Division of Marine Fisheries coastal sample sites, spring and fall, 1986.

sample was removed for PCB analysis. The edible portion was defined as the skinless filet of flounder, combined meat and edible hepatopancreas, i.e. tomale, of lobster, and shucked meat of bivalves. Samples were homogenized in a stainless steel blender and refrozen in either a glass beaker or wrapped in aluminum foil until extracted.

All samples were extracted following the high moisture method of the U.S. Food and Drug Administration (method 212, Pesticide Analytical Manual, Volume 1). Essentially, this method involved initial extraction of the sample with acetonitrile in an explosion-proof high speed blender. The extract was filtered and added to a separatory funnel containing petroleum ether. The organic phase was partitioned into petroleum ether and treated with sodium chloride to minimize emulsions. The subsequent extract was isolated, dried with anhydrous sodium sulfate, passed through florisil, and concentrated to 10 ml (see appendix A).

PCB QUANTIFICATION

PCBs were quantified using a Hewlett-Packard 5880A gas chromatograph equipped with an electron capture detector and a 2 m packed column of 1.5% SP2250/1.95% SP2401 on 100/120 Supelcoport. Injection volume = 1 ul, flowrate (95% Argon/5% Methane) = 40 ml/min, isothermal oven temperature = 205^{o}C, injector temperature

= 225°C, and detector temperature = 300°C. Total PCBs were identified by comparison to the chromatographic pattern of Aroclor 1254, which has typically been found in marine fish and shellfish in the Northwest Atlantic Ocean (Boehm and Hirtzer 1982). The average area of four peaks from the Aroclor moiety were used to quantify total PCBs. These four peaks were never obscured by co-eluting compounds and were universally present in all samples (figure 6).

QUALITY CONTROL

Routine quality control included tracking all samples as they proceeded through each step of the analysis. Logbooks were maintained for all samples received, stored in freezers, extracted on the bench, and released as analytical reports. Final data is also stored on computer files. A logbook of calculations was maintained for samples used as quality control during the extraction and quantification process. Quality control during extraction consisted of a blank, a sample spiked with a known concentration of Aroclor 1254, and duplicates of the spiked sample. Every five samples (20%) were accompanied by a set of quality control extractions.

The gas chromatograph was calibrated daily prior to the start of a batch run. Multilevel calibration using Aroclor 1254 was

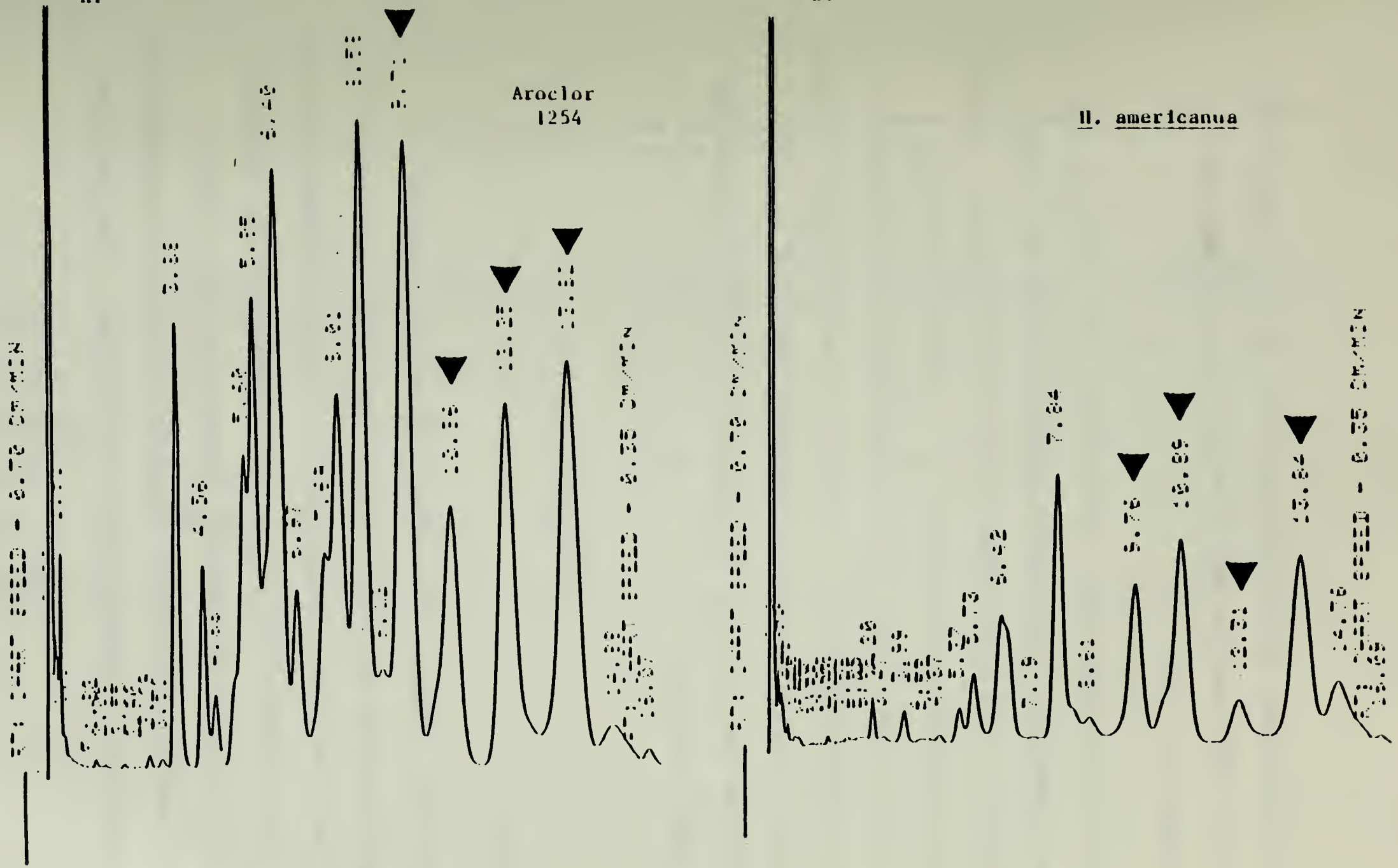

Figure 6. Gas chromatogram of A) Aroclor 1254 standard, and B) a coastal lobster, H. americanus. Triangles denote the four peaks used to identify and quantify total PCBs in all samples.

performed from 0.01 ppm (parts per million) to 20.0 ppm at five concentrations. Each sample was injected twice to ensure precision within +/- 10%.

Cat Cove Marine Laboratory also participates semi-annually in a routine interlaboratory calibration study for PCBs and trace metals sponsored by the Environmental Protection Agency. In 1985 and 1987 the laboratory participated in an interlaboratory calibration study with other states conducting PCB analysis in striped bass, Morone saxatilis. Analytical results from Cat Cove Marine Laboratory in both studies were within 90% of the true values established by Hazelton Laboratories, Inc., which served as the principal reference.

RESULTS

Coastal Species

Table one lists the average PCB values for marine species collected during spring and fall of 1984 and 1985, and spring of 1986. Fixed gear conflicts, size selectivity of the sampling gear for sublegal size groups, and patchy distribution of all species prevented the collection of complete samples at each station. Therefore, all coastal PCB tissue concentrations were combined by species and season for baseline levels (controls). Samples from

Table 1. Average PCB concentrations (ppm) in coastal fish and shellfish species collected from 1984 to 1986 including the standard error of the mean and sample size (parentheses).

	1984		1985		1986
	Spring	Fall	Spring	Fall	Spring
P. americanus	0.10 ±0.03 (6)	0.16 ±0.09 (5)	0.17 ±0.11 (6)	0.05 ±0.02 (4)	0.12 ±0.03 (20)
H. americanus	0.59 ±0.16 (4)	0.73 ±0.10 (4)	0.88 ±0.25 (5)	0.44 ±0.07 (9)	0.24 ±0.04 (12)
S. solidissima	----	0.02 (1)	0.00 (2)	0.00 (3)	----
A. islandica	0.50 ±0.47 (2)	0.00 (1)	0.00 (3)	0.01 ±0.01 (2)	----
M. edulis	0.48 ±0.32 (2)	0.00 (1)	----	0.12 (1)	----
M. mercenaria	----	----	----	0.13 ±0.01 (2)	----

fall 1986, are being analyzed and will be included in a later report.

All coastal flounder samples had PCB tissue concentrations below 0.5 ppm. PCB concentrations in flounder averaged 0.12 ppm (± 0.02 ppm) over the three year period and did not significantly change by season or year. The largest seasonal difference in PCBs in flounder was 1985 when spring flounder samples had PCB body burdens that were three times the concentrations found in flounder in the fall. PCB concentrations in lobster were significantly different between spring and fall of 1985 [Student's $t(12) = 10.5$; $p<0.001$]. This difference could be an artifact of the relatively small sample size and large standard error for spring, 1985, lobster. The average PCB concentration in lobster from all five cruises was 0.58 ppm (± 0.11 ppm) which was five times greater than the average PCB concentration in flounder over the same three-year period. Lobster collected in spring, 1986, had the lowest PCB concentrations of any seasonal group. With the exception of two ocean quahogs and two mussels collected in spring, 1984, all bivalves accumulated very small amounts of PCBs.

Harbor species

The harbor sampling component of the program began in late 1985. The most complete data set was collected in spring, 1986. Tables

two and three list average PCB concentrations by station in Boston and Salem Harbors, respectively. A one-way analysis of variance (Sokal and Rohlf 1969) was applied to the station data to determine if PCB concentrations were significantly different between stations in each harbor. The one-way analysis of variance (ANOVA) for flounder samples between stations in Boston Harbor, spring, 1986, indicated no significant difference between each station. Similarly, a one-way ANOVA for lobster and flounder samples between stations in Salem Harbor, spring, 1986, indicated no significant difference between stations for each species. Therefore, the 1986 Boston Harbor flounder were combined as were the Salem Harbor lobster and flounder. Harbor species were compared to respective coastal species collected in spring, 1986.

A one-way ANOVA for PCB concentrations in flounder samples from Boston Harbor, Salem Harbor, and coastal areas indicated a highly significant difference between groups (table 4). The highest tissue concentrations of PCBs in flounder from all three regions were in fish from Boston Harbor which were five times higher than tissue concentrations in coastal flounder (figure 7). There was a significant difference in PCB concentrations between these two groups by paired ANOVA [$F(1,35) = 37.75$; $p<0.001$]. PCB concentrations in Salem Harbor flounder were higher than coastal flounder but not significantly different by paired ANOVA.

Table 2. Average PCB concentrations (ppm) in fish and shellfish species from Boston Harbor, including the standard error of the mean and sample size (parentheses).

	B1	B2	B3	
	Spring '86	Spring '86	Fall '85	Spring '86
P. americanus	0.67 ±0.18 (6)	0.59 ±0.11 (6)	0.24 ±0.07 (5)	0.49 ±0.07 (6)
H. americanus	----	1.17 ±0.44 (5)	----	
M. arenaria	0.14 (1)	0.14 (1)	----	

Table 3. Average PCB concentrations (ppm) in fish and shellfish species from Salem Harbor, including the standard error of the mean and sample size (parentheses).

	S1		S2	S3
	Fall '85	Spring '86	Spring '86	Spring '86
P. americanus	0.03 ±0.03 (5)	0.20 ±0.05 (5)	0.19 ±0.02 (6)	0.17 ±0.02 (6)
H. americanus	0.38 ±0.12 (2)	----	1.48 ±0.83 (5)	0.83 ±0.17 (5)
M. arenaria	----	----	0.02 (1)	0.04 ±0.01 (2)

Table 4. One-way ANOVA table comparing PCBs in flounder, P. americanus, between Boston Harbor, Salem Harbor, and coastal samples, Spring 1986. A = between station variance, B = within station variance.

Source of variation	S.S.	d.f.	M.S.	F-ratio
A	2.3167	2	1.1584	29.90***
B	2.0144	52	0.0387	
error	4.3311	54	0.0802	

*** = P < 0.001

Table 5. One-way ANOVA table comparing PCBs in lobster, H. americanus, between Boston Harbor, Salem Harbor, and coastal samples, Spring 1986. A = between station variance, B = within station variance.

Source variation	S.S.	d.f.	M.S.	F-ratio
A	5.6317	2	2.8159	3.45*
B	19.5898	24	0.8162	
error	25.2216	26	0.9701	

* = P < 0.05

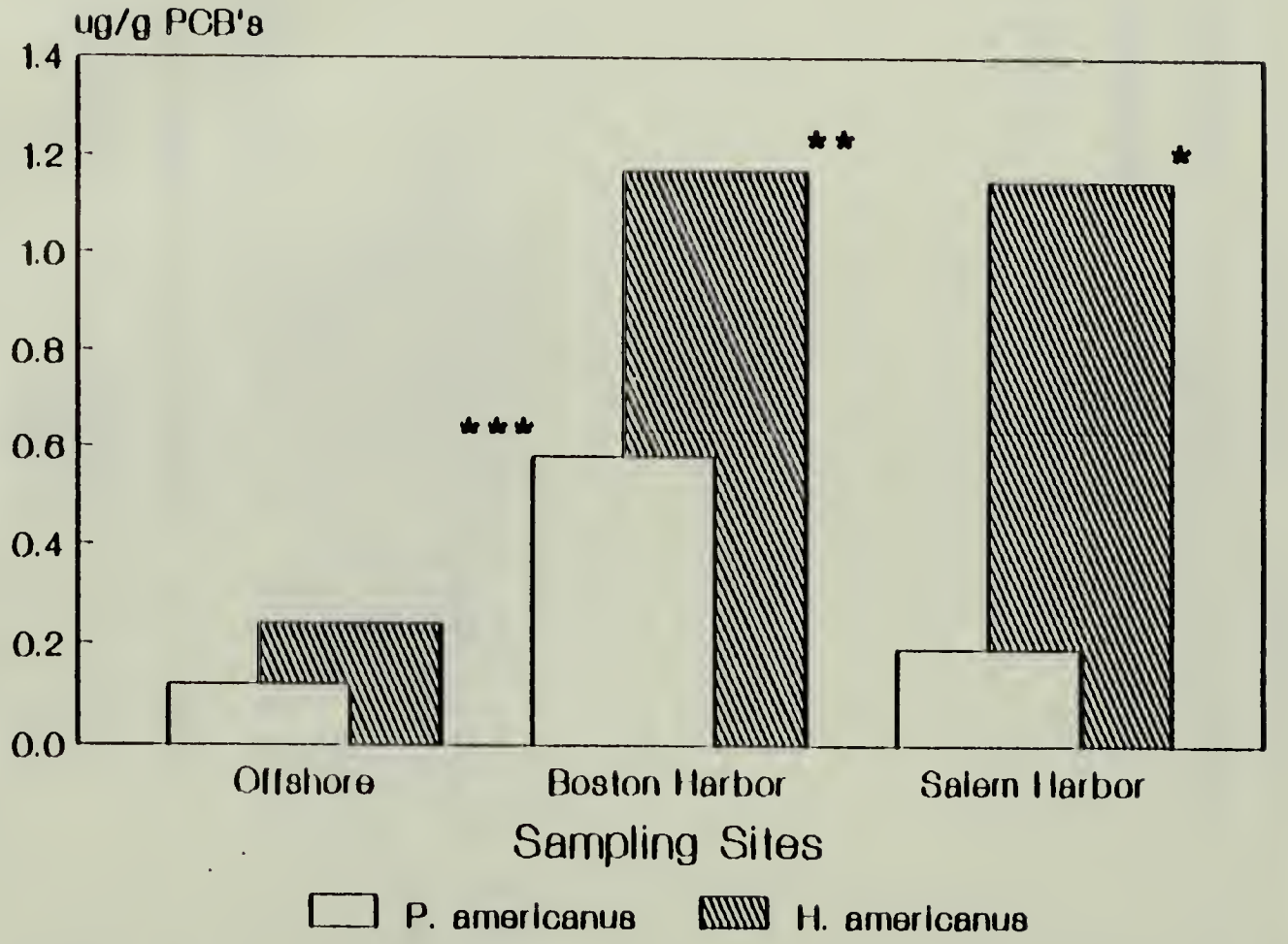

Figure 7. Average PCB concentrations (ppm) for flounder, P. americanus, and lobster, H. americanus, from all coastal, Boston Harbor, and Salem Harbor locations, Spring 1986. Levels of significant difference between coastal and harbor species are indicated by one, two or three asterisks ($P < 0.05$, 0.01, and 0.001, respectively.

PCBs in spring, 1986, lobster from Boston and Salem Harbors were approximately five times the concentration found in coastal lobster (figure 7), and there was a significant difference between the three groups by ANOVA (table 5). Both Boston and Salem Harbor lobster had significantly higher PCB concentrations than coastal lobster by paired ANOVA [$F(1,15) = 10.94$; $p<0.01$, Boston Harbor vs. coastal; $F(1,20) = 6.10$; $p<0.05$, Salem Harbor vs. coastal]. Very little data is available on PCB concentrations in bivalves for Boston and Salem Harbor. From the data that is available it appears that PCB concentrations are similar to PCBs in coastal bivalves.

DISCUSSION

Factors controlling the rate of PCB accumulation in marine species have not been determined in this survey although PCB concentrations in coastal lobster and flounder indicate differences in total body burdens between species. Lobster, for example, contained five times the average PCB concentration in flounder. Differences in PCB values for these two species in the Northwest Atlantic Ocean have previously been reported by Boehm and Hirtzer (1982). They detected PCBs in lobster (as Aroclor 1254) between 0.10 - 0.15 ppm, and in winter flounder between 0.002 - 0.031 ppm. This order of magnitude difference between species was obtained from analyzing the edible muscle tissue from

each organism. In contrast, the present study included the edible hepatopancreas with lobster PCB determinations. Detoxifying organs such as the hepatopancreas are known to sequester substantially higher concentrations of PCBs than muscle tissue (Farrington et al. 1986). Higher PCB concentrations in the hepatopancreas could contribute to the overall increase in lobster PCB body burdens and obscure lower PCB concentrations in the muscle tissues of lobster. Other factors contributing to differences in PCB concentrations in lobster could be seasonal changes in body composition as suggested by previous studies in New Bedford Harbor (Division of Marine Fisheries, unpbl. data). However, Roberts, et al. (1982) reported no correlation between sex and PCB concentrations in lobster from the New York Bight and Long Island Sound, and Reid et al. (1982) found no correlation between body fat and PCBs in lobster from these same two areas.

Recent investigations by Boehm et al. (1984) and the U.S. Dept. of Commerce (1987) found that Boston Harbor sediments contain high concentrations of PCBs which are available to flounder either through direct contact on the sediment surface or through ingestion of contaminated prey species. Fowler et al. (1978) demonstrated that the polychaete worm, Nereis diversicolor, is capable of concentrating PCBs from water or sediments, and concluded that contaminated sediments contribute the bulk of these compounds in the natural environment. The worms were capable of attaining equilibrium concentrations three to four times greater

than the sediments depending on the initial PCB concentrations in sediments. Rubinstein et al. (1984) compared PCB uptake in spot, Leiostomus xanthurus, exposed to PCBs in sediments and fed contaminated worms, Nereis virens. They found that fish exposed to PCB-contaminated sediments and fed polychaetes from the same sediments accumulated more than twice the PCBs than fish exposed to similar conditions but fed uncontaminated worms. PCBs in the diet added to the body burdens the fish acquired from the contaminated sediments.

PCBs in flounder form Salem Harbor were not significantly idfferent than coastal flounder. PCB concentrations in Salem Harbor sediments have been reported at 0.534 ppm, whereas PCBs in Boston Harbor sediments are 17.105 ppm (U.S. Dept. of Commerce 1987) Municipal discharge in Salem Harbor is also an order of magnitude below the discharge rate in Boston Harbor (Estrella 1984). Despite the relatively low concentrations of PCBs in Salem Harbor sediment and flounder, PCB concentrations in Salem Harbor lobster were similar to concentrations in Boston Harbor lobster, and significantly higher than coastal lobsters. The reason for this increase is unclear since PCB concentrations in Salem Harbor sediments is over thirty times less than Boston Harbor sediment. The inclusion of the edible hepatopancreas could increase the overall tissue concentration as previously described for coastal lobster, or this increase may simply reflect a greater degree of PCB exposure for lobsters due to differences in

diet, habitat, or residence time in the Salem Harbor region between lobster and flounder.

Of all species analyzed the bivalve molluscs had some of the lowest detectable PCB concentrations in all three study sites. Bivalve PCB concentrations in the present study were similar to tissue concentrations reported for bivalves in the Northwest Atlantic Ocean and the New York Bight (Reid et al. 1982; Steimle et al. 1986). However, spatial comparisons between the two harbor regions and coastal areas will require more data than is presently available. Apparently soft shell clams living in PCB-contaminated sediments of Boston and Salem Harbors can isolate themselves from PCBs in surrounding sediments or discharge accumulated PCBs while filtering seawater to feed on phytoplankton. Because of the low trophic position of phytoplankton and lipophilic nature of PCBs to accumulate in animal fat, the clams would be exposed to much lower PCB concentrations than flounder or lobster.

SUMMARY

This information provides a first order approximation of the magnitudes of difference that could be expected when comparing marine organisms from other contaminated nearshore areas with

similar organisms from deeper waters. Determining temporal changes in PCB body burdens, however, will require more extensive sampling in each habitat region. Despite the small size of the existing data set, differences in PCB body burdens by spatial area have been detected, and some general statements concerning these differences can be made:

1. Flounder, lobster, and various bivalve mollusc species were analyzed for total PCB concentrations in edible portions. Samples were collected from Boston Harbor, Salem Harbor, and random coastal sampling sites.

2. The highest tissue concentrations of PCBs among all species tested were found in lobster from Boston and Salem Harbors.

3. Lobster from Boston and Salem Harbors have significantly greater body burdens of PCBs than coastal lobsters.

4. Flounder from Boston Harbor have a significantly greater body burden of PCBs than coastal flounder. Evidence suggests that exposure to PCBs in sediments and prey organisms are probably pathways for accumulation. PCB concentrations in Salem Harbor flounder are not significantly different than coastal flounder.

5. Tissue concentrations of PCBs in coastal lobsters were higher

than coastal flounder. Factors controlling differences in PCB accumulation between species are not known at this time. Determining these factors is complicated by the inclusion of the edible lobster hepatopancreas with muscle tissue which could be increasing lobster PCB concentrations, while PCBs in flounder are based exclusively on edible muscle tissue. Separate analysis of lobster hepatopancreas and muscle tissue is necessary to determine accurate PCB concentrations in all edible tissue between species.

6. Bivalve molluscs had the lowest PCB concentrations of any phylogenetic group in the survey. There is unsufficient data to compare PCBs in bivalves between habitats.

7. Considerable more data is necessary to successfully monitor the spatial and temporal distribution of PCBs and other contaminants in marine species simultaneously from different habitats.

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APPENDIX A

EXTRACTION PROCEDURE

FOR PCBs (according to P.A.M. 212.13a)

Reagents:

Acetonitrile
Petroleum Ether
Saturated Salt Solution
Distilled Water
Florisil: 60-100 mesh; activated @ 650C stored at 130°C
Sodium Sulfate (anhydrous)

1. Add homogenized biota sample to blender jar. Approximate wet weight for different organisms is as follows:

lobster	25g
flounder	50-70g
striped bass	25-30g
bivalves	50-70g

2. Measure 200 ml acetonitrile in graduated cylinder and add to blender. Graduated cylinder remains with sample through extraction procedure.

   Note: When preparing spiked sample, add known standard to sample prior to blending using hexane rinsed pipette. Blanks receive identical treatment at all times.

3. Start blending at low speed for a few seconds, then set blender at high speed for one minute. Stop after one minute and scrape caked sample material on the sides of blender jar into the acetonitrile using a stainless steel spatula. Stir any caked material on the bottom of the blender with the stainless steel spatula. Resume blending for 1 minute, first at low speed for a few seconds, then at high speed.

   Note: Use individual stainless steel spatulas for each sample.

4. Pour entire contents of blender cup through a buchner funnel (containing no. 614 VWR filter paper, placed in buchner using clean tweezers) into a 500ml filter flask attached to an operating suction pump. Collect as much extract and sample material as possible from the blender jar using the stainless steel spatula. Allow sample to drip through the filter paper until dripping stops. Do not allow the sample to sit more than one minute.

   Note: Both the filter flask and the graduated cylinder remain with the sample throughout the extraction procedure.

5. Pour the extract into the graduated cylinder and record the volume. This volume is "F" in the adjusted wet weight calculations.

6. Pour extract into a 1L separatory funnel. Measure 100ml petroleum ether in a separate graduated cylinder. Pour the 100ml petroleum ether into the graduated cyclinder assigned to the sample to rinse any remaining sample residue. Add the 100ml petroleum ether to the 1L separatory funnel.

7. Vigorously shake the 1L separatory funnel for 90 seconds.

   Caution: Vent the separatory funnel once or twice after 1 or 2 initial shakes to relieve vapor pressure and repeat periodically while shaking.

8. Add 10ml of saturated salt solution to the 1L separatory funnel, followed by approximately 600ml distilled water. Distilled water is added by measuring approximately 350ml in the filter flask and 250ml in the graduated cylinder to rinse both pieces of glassware.

9. Hold separatory funnal horizontally and shake for 35 seconds. Remember to vent funnel periodically.

10. Allow phases to separate. Drain lower phase and watch for an emulsion. If an emulsion (foam) is present between phases, attempt breaking up the emulsion with a glass rod (use a separate glass rod for each sample). If emulsion still persists, proceed as follows:

    a. Add another 10ml saturated salt solution, after draining as much of the lower phase as possible without losing any emulsified material. Allow some time for the emulsion to disperse.
    b. Drain any water that has been released.
    c. Wash 4X with 100ml distilled water, draining lower phase each time.

11. Add 100ml distilled water to separatory funnel. Invert the separatory funnel, open stop cock (carefully!) and gently swirl for 5 seconds. Drain lower phase then repeat procedure with a second 100ml distilled water.

12. Drain lower phase from separatory funnel. Collect upper phase in a glass stoppered graduated cylinder. Record this volume, which is called "P" in the adjusted wet weight calculation.

    Note: Samples can be stored in a refrigerator at this step.

Column Chromatography Cleanup

1. Wearing dry disposable gloves, construct column by placing a glass wool plug, (handling the glass wool with tweezers and a glass rod) at the bottom of the column. Add 20g Florisil (cool to the touch) and approximately 1/2" of sodium sulfate. Rinse column with petroleum ether. Drain off excess petroleum ether but always leave 1/2" of petroleum ether covering the top of the column. (Do not let column go dry)

2. Connect a 10ml concentrator tube containing 4 glassbeads to a 500ml Kuderna-Danish (K-D) flask, and place under the column.

3. Before putting extract on the column, add sodium sulfate up to the 3-5ml mark of the glass-stoppered graduated cylinder. Invert the cylinder (keep your thumb on the stopper) and watch to see if all the sodium sulfate forms clumps. If so, add more sodium sulfate up to the 5-7ml mark and invert the cylinder. If all the sodium sulfate forms clumps, add more but do not exceed the 10ml mark on the graduated cylinder. Let the extract and sodium sulfate stand no less than 10 minutes but no more than 30 minutes before putting the extract on the column.

4. Adjust the flow rate on the column to approximately 5ml/min (or 1ml/12 seconds). As petroleum ether drains from top of column, carefully pour extract onto column. Do not let the column go dry. Adjust flow rate to approximately 5ml/min. (1ml/12 seconds).

5. Add 10ml of petroleum ether to the empty glass-stoppered graduated cylinder. When the last of the extract disappears from the top of the column, add the 10ml petroleum ether rinse

6. Repeat the 10ml graduated cylinder rinse 2X adding the rinse each time to the column.

7. After the last 10ml petroleum ether rinse disappears from the top of the column, rinse the column with petroleum ether using the teflon squeeze bottle, and add 170ml petroleum ether to the column. Collect all petroleum ether from the column in the K-D flask.

Sample Concentration

1. Remove the K-D flask concentrator tube from the column. Place a three ball snyder column on top of the K-D flask. Rinse the snyder column with a small amount of petroleum ether. Place the entire K-D flask assembly on a steambath. Steam-bath should be set at #7 or 80-85C. Strap down the K-D assembly with rubber bands. As the petroleum ether begins to boil, watch to see if the vapors are passing freely around the balls while the petroleum ether heats up in the snyder column. If not, tap the side of the snyder column to loosen the balls, otherwise vapor pressure will build up in the K-D flask and it could violently rupture (bump).

2. Concentrate the sample to 5-6ml. Remove the K-D assembly from the steam bath and rinse the snyder column with 1-2ml petroleum ether.

3. Disconnect the concentrator tube from the K-D flask when it is cool to the touch. Absorb moisture around the outer joint with a kimwipe. Allow the concentrator tubes to air dry.

   Note: Leaving the concentrator tube joined to the K-D flask for prolonged periods can result in frozen ground glass joints.

4. Bring total volume of sample in concentrator tube to 10ml with hexane. Cap the concentrator tube with a ground glass stopper and mix contents.

5. Transfer 1-2ml aliquots to autosampler vials using disposable pipettes that have been hexane rinsed and are dry. Make up 2 sample vials for each sample. Crimp and seal the vials. Store one in refrigerator in back up file. Store second vial in small beaker in refrigerator until G.C. analysis.