Chemical Impacts in Fish and Shellfish
from Cape Cod and Massachusetts Bays

Michael J. Moore[1], Roxanna M. Smolowitz[2], Dale F. Leavitt [1] and John J. Stegeman[1]
[1] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543
[2] Laboratory for Aquatic Animal Medicine and Pathology, University of Pennsylvania,
Marine Biological Laboratory, Woods Hole, MA 02543, USA.

Abstract

Mummichogs, soft shell clams, and blue mussels from some or all of 10 sites in Boston Harbor and Massachusetts and Cape Cod Bays were examined histologically: a suite of pathological changes previously known to be associated with chemical contamination were found in animals from the more contaminated sites. In particular, liver tumors were evident in 14% of the adult mummichogs from the Island End River, a tributary of the Mystic River in Boston Harbor. Additionally, a number of pathologies previously shown to be associated with chemical exposure were seen in the two bivalve species at a number of contaminated sites. Induction of cytochrome P450 1A (CYP1A) was also seen in mummichogs from the more contaminated sites: CYP1A induction is a biochemical change associated with exposure to dioxin and other planar halogenated and aromatic hydrocarbons. These findings suggest that there are measurable biochemical and pathological changes in intertidal fish and shellfish from the more contaminated parts of the Massachusetts Bays system. These types of changes were less evident in the two reference sites in Cape Cod Bay.

Introduction

The objective of this study was to evaluate biological impacts associated with chemical contaminants in salt marsh systems of the Massachusetts Bays, by examining multiple species, stations and endpoints, using sensitive biochemical and less sensitive pathological measures of exposure and effect. The studies were carried out over two years in 1992 and 1993.

Biological changes that can be explicitly linked to chemical contaminants, are now commonly referred to as biomarkers (Huggett 1992). These may be used to determine spatial and temporal differences in the identity and source of pollutants and their effects. In using the biomarker approach in this study we have determined 1) whether there is contamination by selected toxicants, 2) whether the contaminants are present at biologically significant concentrations, 3) the severity and geographic extent of these effects, and thus 4) which species/populations are at risk.

Mixed function oxidase (MFO) activity is the generic name given to the type of reaction carried out by a family of enzymes known as cytochrome P450. It has been known for over 20 years that the rate of some MFO reactions could be induced (increased) by exposure of animals or animal cells to selected kinds of chemicals. Thus, aromatic hydrocarbons, and chlorinated aromatic hydrocarbons (PCBs, dioxins) induce aryl hydrocarbon hydroxylase (AHH) activities by causing synthesis of an increased amount of a specific enzyme. Induction of MFO activities in liver of fish was suggested in the mid-1970s as an index or marker of exposure to petroleum. Numerous studies have confirmed that use of induction of MFO. In the mid-1980s we provided the first evidence that antibodies to the particular MFO enzyme (the specific P450 induced by hydrocarbons, called cytochrome P450 1A or CYP1A) could be used to measure the degree of induction in fish liver, related to exposure in the environment. The induction of CYP1A is one

of the few molecular responses to pollutants for which we have a good understanding of the mechanism (transcriptional activation), chemical specificity (planar toxic aromatic compounds), biological specificity (vertebrates), sensitivity (dose-response relationships) and influence of environmental variables (Stegeman et al. 1992).

Several recent studies, with different fish species and in different parts of the world, have revealed close correlations between the levels of induced cytochrome CYP1A and levels of PCBs or PAH either in the organisms or in their immediate environment. Studies in the flounder *Platichthys flesus* from Langsundsfjord, Norway (Stegeman et al. 1988), in starry flounder (*Platichthys stellatus*) from San Francisco Bay (Stegeman *et al.*, unpublished), and in rattail (*Coryphaenoides armatus*) from the deep ocean (Stegeman et al. 1986) have all shown close correlation between the levels of induction of CYP1A in hepatic microsomes and the levels of total PCB residues.

Over protracted periods such chemical exposure and molecular alteration can result in histopathological change. Such change is a definitive marker of biological effect, which can range from moderate to severe parasitic infestations or cellular abnormalities culminating in frank neoplasms. Such changes rarely can be linked to specific chemicals but usually can indicate the combined effects of the suites of chemicals found in a particular environment, which can include immunosuppressive agents, cell toxins, tumor initiators and tumor promoters.

The role of environmental chemicals as causative agent in the appearance of numerous diseases in fish is clear, for there are abundant data linking the prevalence of hepatic neoplasms and associated (putative) preneoplastic conditions to organic chemical pollutants (Harshbarger and Clark 1990). Less clear is the significance of increased or decreased prevalences of parasitism and other infectious diseases in fish from polluted sites.

Hepatic neoplasms detected in mummichogs (*F. heteroclitus*) have been described as hepatocellular carcinoma and hemangiosarcoma (Vogelbein et al. 1990). These have been closely associated with a high concentration of PAH, suggesting similar etiology to that suggested for hepatic tumors in winter flounder from Boston Harbor (Murchelano and Wolke 1985; Johnson et al. 1993; Moore et al. 1996).

Environmental contaminants as a cause of pathology in molluscs is "...becoming increasingly well established." (Sindermann 1990). Pathology associated with molluscs from polluted waters are inflammation of the gills, and focal epithelial proliferation of the gills (Gardner and Pruell 1988), regression and atrophy of digestive tubules, increased occurrence of ceroid, depression of gametogenic activity, and mantle recession (Sindermann 1990). Impaired immunocompetence with increased prevalence of infectious etiologies (Anderson 1988) such as rickettsia (Gardner and Pruell 1988) are also important findings.

In this study we show both biochemical and pathological changes in fish and shellfish to be predominantly present in the more contaminated areas around Boston Harbor.

Methods

In 1992 and 1993 we sampled fish and shellfish when available and in 1992 we sampled sediment from a total of nine intertidal sites and one pier around the Mass Bays as illustrated in Figure 1 and listed in Table 1. Sediment samples were analyzed for polynuclear aromatic hydrocarbons (PAH), moisture, inorganic carbon and silt-clay content using standard EPA methods. Mummichogs (*F. heteroclitus*) were sampled by beach seine, examined for gross pathology and dissected. Multiple organs were examined using histology, and for cytochrome CYP1A expression, using immunohistochemical (Smolowitz et al. 1991) and biochemical techniques (Kloepper-Sams et al. 1987). Softshell clams (*Mya arenaria*) and blue mussels (*Mytilus edulis*) were examined histopathologically. Clams were also evaluated for the presence of hematopoietic neoplasia (Smolowitz and Reinisch 1986).

Table 1. Station Locations for Mass. Bays Program Field Samples

Stn. #	Location	Lat.	Long.	Comment
1	Town River Bay, Weymouth	42 14.93	70 57.86	In bay just west of Germantown Point.
2	Neponset River	42 17.42	71 01.89	By double culvert under road which runs south of old Jordan Marsh warehouse. *Fundulus* to south of road in creek (with hydrocarbon sheens) running west at foot of road embankment, clams on north shore of creek to north of road. Both samples within 50 yds of drain mouths.
3	Saugus River	42 27.55	70 59.29	On south bank just downstream of East Saugus road bridge.
4	Fort Point Channel	42 20.65	71 03.64	50 yds downstream of drains leaving Boston Police parking lot at head of channel. Also took 3 mid-channel sediment samples from lower, middle and upper parts of the channel.
5	Scorton Creek	41 44.57	70 25.59	At old RR Bridge in creek running east from across main creek from parking area.
6	Herring River, Wellfleet	41 55.66	70 03.29	Chequesset Neck. At mouth of Herring R.
7	Island End River, Mystic River	42 23.61	71 03.01	On flats at northeast east tip of creek just north of travelift dock. No live clams.
8	Chelsea River	42 23.95	71 00.91	Intertidal immediately north of green can # 7.
9	Earhart Dam	42 23.30	71 04.10	Intertidal to northeast of railroad tracks.*
10	Pier 2 NavyYard	42 22.36	71 03.21	Pilings at inner end of Pier 2.*

Positions were determined with a Magellan 5000 global positioning system reciever.
*No sediment chemistry analysis.

Figure 1. Sites at which mummichugs (*Fundulus heteroclitus*) and soft shell clams (*Mya arenaria)* were sought. Clams were only present as dead shells at the Island End river. Numbers in parenthese are the concentraions of polynuclear aromatic hydrocarbons in sediment expressed as µg/ g dry weight.
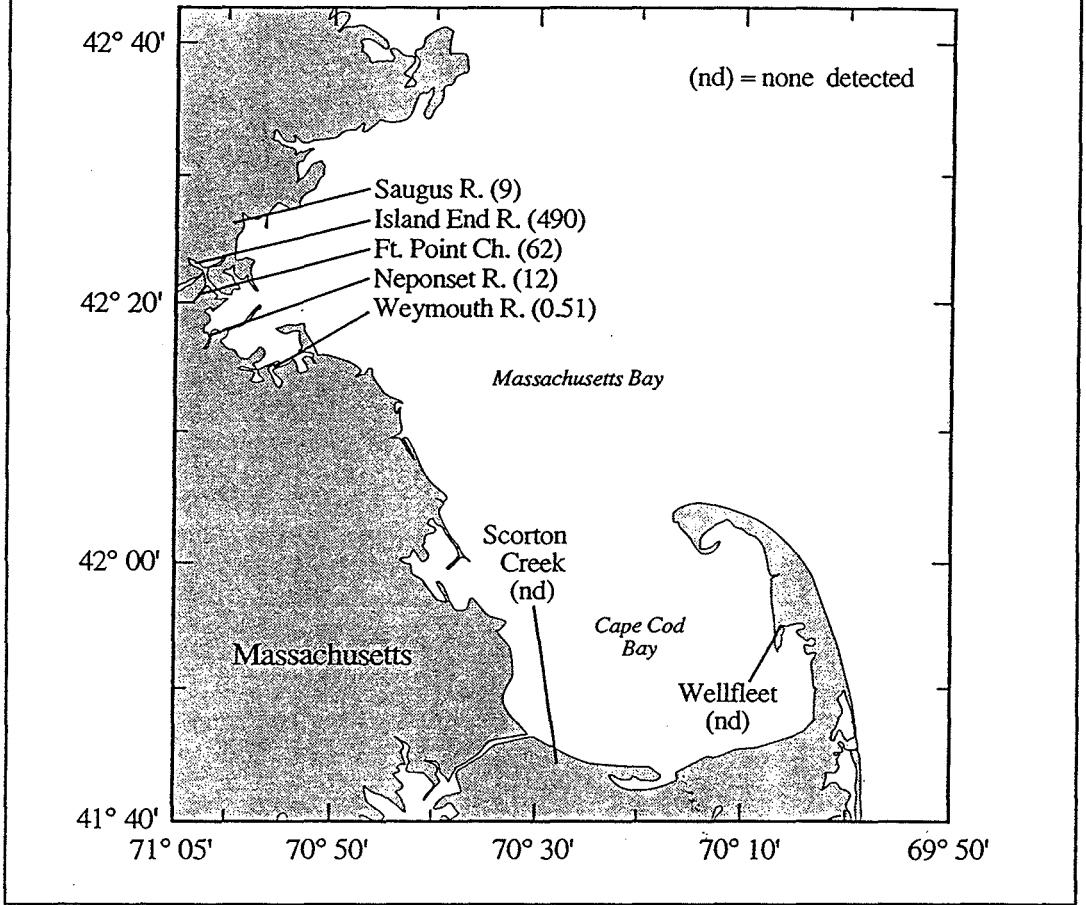
The histopathological data were analyzed using multivariate statistical techniques (Tabachnick and Fidell 1983) and subjected to discriminant function analysis. CYP1A and EROD data were analyzed for between-station differences by ANOVA and with levels of sediment contamination by simple logistic regression.

Results

Silt, clay, moisture and organic carbon content varied substantially but without any marked parallel with PAH content. In order of increasing PAH burden per gm. sediment the stations fell into three major groups (Figure 1): 1) Low: Wellfleet, Scorton, 2) Medium: Weymouth Fore River, Neponset River, Chelsea River, Saugus River, and 3) High: Fort Point Channel, Island End River.

Mummichogs were often hard to find in the Boston Harbor system. The Island End River site appeared to be on the border of being azootic. There appeared to be a more viable icthyofauna in 1993 than in 1992. Likewise, mummichogs were hard to find in the Neponset River: Attempts to find fish failed at Fox Point, the gas tanks, and further up the river, before we

Table 2. Frequency of Histopathological Lesions in *Fundulus heteroclitus* from 4 sites in the Massachusetts Bays - 1993

|		Wellfleet	Island End	Chelsea R.	Earhart Dam, Mystic R.
Hepatic MA		0	26	0	0
	N	14	48	2	14
Hepatic coccidia		7	0	0	0
	N	14	48	2	14
Hepatic necrosis		0	7	0	0
	N	14	48	2	14
Hepatic neoplasm		0	7	0	0
	N	14	48	2	14
Altered hepatocyte foci		0	19	0	0
	N	14	48	2	15
Hepatic clear cell patches		0	5	0	1
	N	14	48	2	14
Hepatic Megalocytosis		3	2	1	0
	N	14	48	2	14
Cardiac nematodes		1	0	0	1
	N	15	27	2	12
Gill Trematodes		1	2	0	2
	N	15	28	2	14
Gill hyperplasia		14	20	1	9
	N	16	28	2	14
Spleen MA		0	11	0	0
	N	8	20	1	9

The numbers of fish showing each lesion, and the number (N) of fish examined for each cell type for each station is given. MA = Macrophage aggregations.

Table 3 Frequency of Histopathological Lesions in *Fundulus heteroclitus* from 8 Sites in the Massachusetts Bays – 1992

		Weymouth	Neponset	Saugus	Fort Pt. Ch.	Scorton	Wellfleet	Island End	Chelsea R.
Hepatic MA		0	0	0	0	0	0	0	0
	N	19	20	19	19	20	19	20	20
Hepatic coccidia		1	0	0	0	0	15	0	0
	N	19	20	19	19	20	19	20	20
Hepatic necrosis		1	0	0	0	0	3	0	0
	N	19	20	19	19	20	19	20	20
Hepatic clear cells		0	0	0	3	0	0	0	0
	N	20	20	20	19	20	19	20	20
Hepatic Megalocytosis		0	0	0	0	2	0	0	0
	N	20	20	20	20	20	19	20	20
Cardiac nematodes		0	1	0	0	0	0	0	0
	N	3	3	5	1	8	9	5	4
Gill Trematodes		0	10	1	0	0	4	0	0
	N	18	16	16	3	20	14	13	8
Gill hyperplasia		10	13	13	1	8	4	0	0
	N	18	16	16	3	20	14	14	7
Spleen		2	0	0	0	0	0	0	0
	N	14	15	17	7	12	0	16	12
Spleen MA		3	5	9	0	5	0	0	0
	N	13	15	17	7	12	0	16	12

The numbers of fish showing each lesion, and the number (N) of fish examined for each cell type for each station is given. Liver neoplasms and altered foci of hepatocytes were not seen in any fish, nor were abnormalities seen in cardiac muscle, renal tubules, or intestine. Island End fish were subadult in 1992. MA = Macrophage aggregation. Lesions are defined in the text.

Figure 2. Anti-CYP1A immunoblots of *Fundulus heteroclitus* liver microsomes. The density of the band is proportional to the concentration of the protein. Greater induction was seen in fish from around Boston. In the spring spawning season induction was depressed in females from the heavily contaminated Island End River.
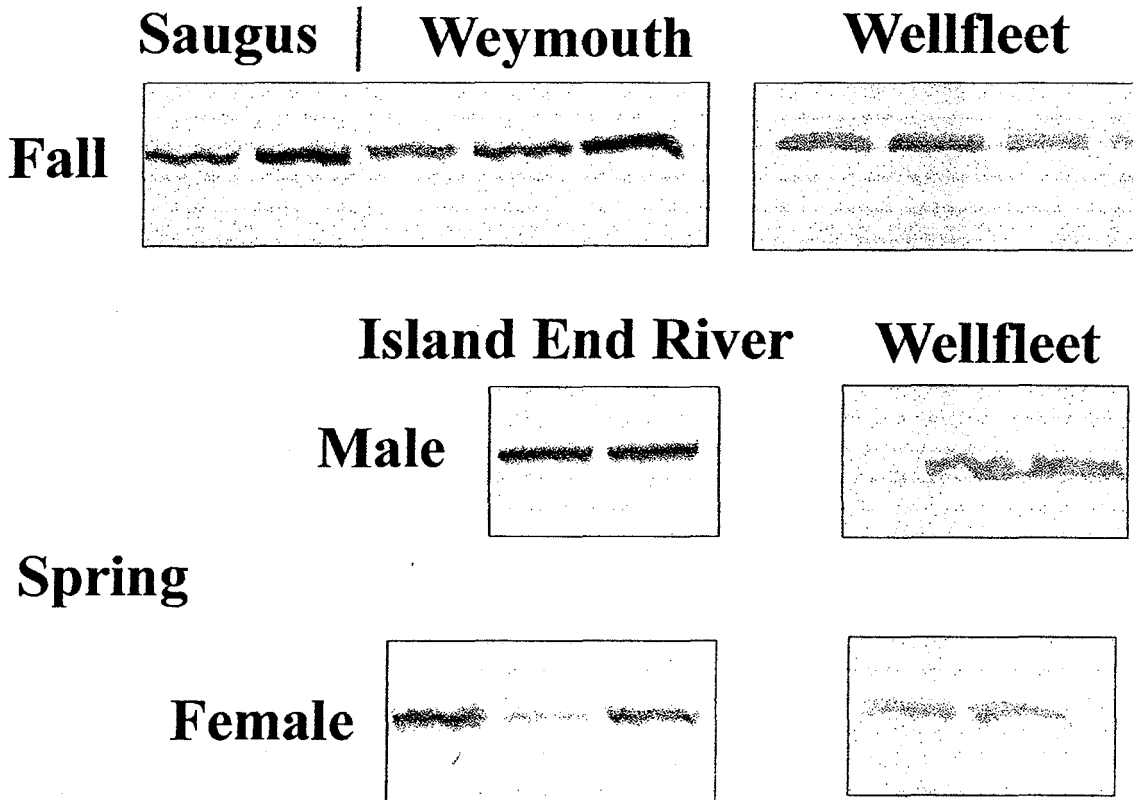
found fish at the site described. The Fort Point Channel was hard to sample, given the extensive bulkheading, and the available fish were small. The Saugus River failed to yield fish at 4 different sites downstream of the described site. In contrast, mummichogs were abundant at both of the reference sites on Cape Cod.

The most dramatic histological change evident in the mummichogs was in adults from the Island End River, the most contaminated site, in 1993 (Table 2). At that site 14% of the fish had liver neoplasms, and 40% had associated foci of tinctorially altered hepatocytes. Neoplasms and altered hepatocyte foci were absent from fish from all the other less contaminated sites. Other lesions were also observed in a variety of organs from a variety of sites, as shown in Tables 2 and 3.

Biochemical data on *F. heteroclitus* are listed in Tables 4 and 5 and illustrated in Figure 2. Data were segregated by site and season of collection and by gender, for the spring collection. It is important to account for inherent physiological differences in addition to chemically-induced changes. Specifically, we know that spawning female *F. heteroclitus* have a markedly reduced

Table 4. Comparison of Sediment PAH and Liver P4501A and EROD in *Fundulus heteroclitus* Caught in the Fall of 1992

	Stn #	Sediment tPAH (µg/g dry wt.)	Sediment tPAH (µg/g C)	Liver P4501A /mg Mean	SD	N	Liver EROD pmol/min/mg Mean	SD	N
Island End River	7*	490.49	8567.42	No data			753.2		1
Fort Point Channel	4*	62.06	822.39	67.8 [5,6,8]	41.6	2	482.5	320.6	2
Neponset River	2	11.51	261.66	28.2 [3,4]	9.1	3	324.7	74.1	4
Saugus River	3	9.32	502.35	60.4 [5,6]	16.1	5	638.4	217.9	5
Chelsea River	8	3.83	153.15	35.0	5.4	4	320.6	57.7	4
Weymouth Fore River	1	0.51	56.38	44.2 [4,5,6]	12.5	4	387.1	151.3	5
Scorton Creek	5	nd	nd	24.4	7.2	4	302.5	109.7	4
Wellfleet	6*	nd	nd	14.8 [8]	8.1	11	471.8	284.9	9

* Pooled samples of 2-4 fish per pool
Superscripts indicate significant difference(s) with station(s) of that number (ANOVA $p<0.05$)
P4501A data are relative values /mg microsomal protein. Absolute data cannot be given in the absence of purified *Fundulus* P4501A protein as a standard.
nd= below detection limit

Table 5. Comparison of sediment PAH and liver P4501A and EROD in *Fundulus heteroclitus* caught in the spring of 1993

		Wellfleet (Stn 6)			Island End River (Stn 7)		
Sediment tPAH (μg/g dry weight)		Below detection limits			490.5		
Sediment tPAH (μg/gC)		Below detection limits			8567.4		
		Mean	SD	N	Mean	SD	N
P4501A	Males	20.6 [7]	1.2	2	65.9	2.4	3
	Females	2.5	2.3	5	14.0	20.4	3
EROD	Males	150.2 [7]	74.9	2	374.7	64.4	3
	Females	115.4	81.9	4	186.5	158.6	3

Superscripts indicate significant difference(s) with station(s) of that number (ANOVA $p<0.05$)

Figure 3. Simple regression of sediment polynuclear aromatic hydrocarbon content per gram carbon vs.Cytochrome CYP1A content in Fundulus heteroclitus caught from 8 stations in the Massachusetts Bays in the Fall of 1992.

$$y = 0.065x + 23.362$$

$$r^2 = 0.545 \quad p < 0.001$$
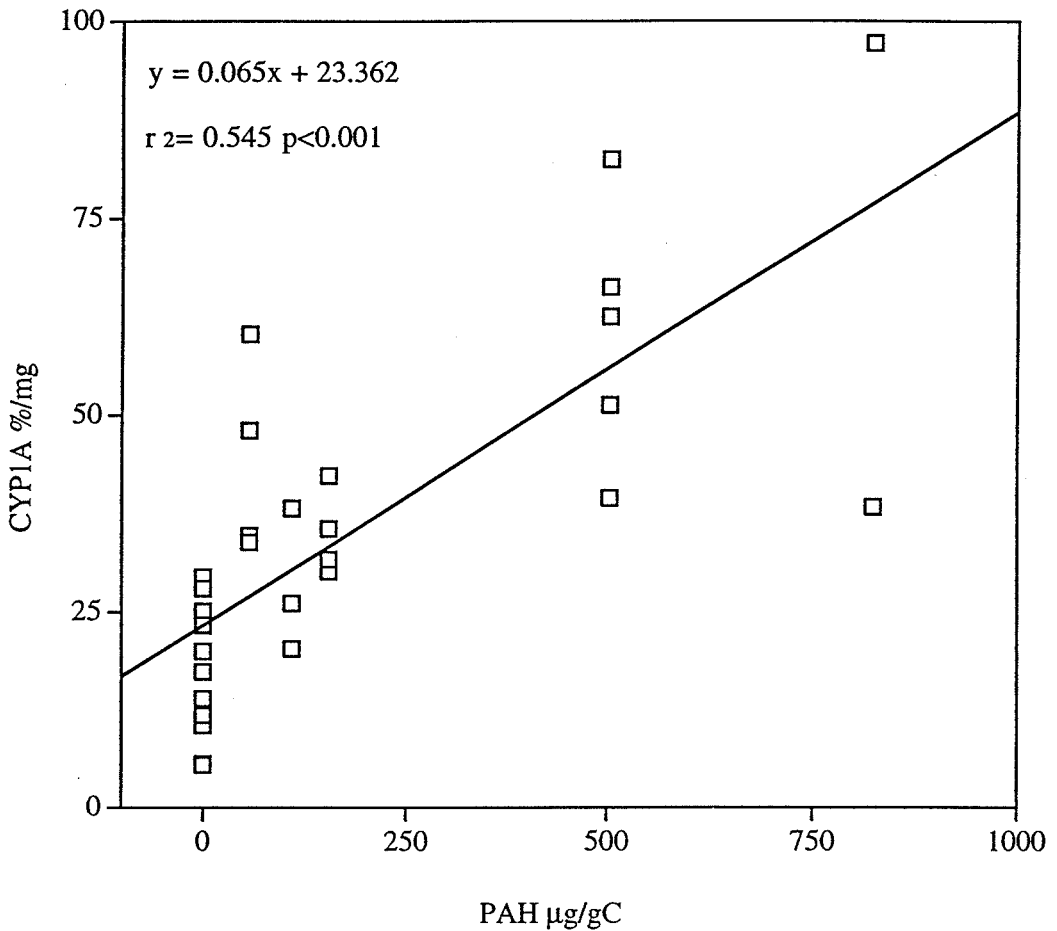
CYP1A expression even in the face of chemical exposure (Elskus et al. 1991). This reduction results from the liver of spawning females being primarily occupied with production of an egg constituent, vitellogenin. This effect is not seen in *F. heteroclitus* caught in the Fall. Thus genders were not separated for Fall collected samples. Tables 4 and 5 respectively show Fall and Spring values of CYP1A protein levels, and CYP1A enzymatic activity, as shown by ethoxyresorufin-o-deethylase (EROD) activity. In Table 4 there is a general trend of increasing CYP1A protein content and EROD activity with increasing chemical exposure (Figure 3). In Table 5 the Spring 1993 data shows a marked induction of CYP1A protein and EROD activity in the males in fish from the Island End River, as compared to Wellfleet. In contrast females from this Spring sample set showed lower CYP1A protein, and EROD activity from both sites, although there was still a relative increase in EROD of females from the Island End River as compared to Wellfleet.

Clams were collected in both years. One of the more dramatic observations we made was the extensive presence of empty softshell clam valves in the mudflats of the lower half of the Island

End River, with a total absence of live animals, even after prolonged digging. Extensive histopathologic evaluations of soft shell clams were done in 1992 in order to determine what pathologies might be correlated with polluted stations. In 1993, (based on the results from 1992) soft shell clams and blue mussels were examined for an abbreviated list of pathologies that were identified as potentially resulting from pollution. The frequencies of pathologies in clams are shown in Tables 6 and 7 for 1992 and 1993 respectively. Published pathologies as well as previously undescribed diseases/ conditions were identified in *M. arenaria* in 1992. Leukemia, gill hyperplasia (papilloma), and parasitic metacercaria were present in connective tissues of various portions of the animals' bodies. Also seen were siphon, gill and mantle inflammation and brown cell accumulation in the digestive gland and kidney. Brown cells are probably lipofuscin-filled inactive hemocytes also called ceroid bodies (Lauckner 1983; Sparks and Morado 1988; Sindermann 1990). Chlamydial infection of the digestive gland, pericardial/brown exudate in the kidney, gill parasites and green gland (pericardial gland) changes were also seen. All of the above have previously been reported in *Mya*. New pathologies/conditions identified in this study are 3 types of parasitic infections of the kidney, kidney hyperplasia, gonadal inflammation, and probable gill carcinomas. This last lesion was only found in animals from one location.

Immunohistochemical evaluation of hemocyte samples revealed the presence of varying levels of hemopoietic neoplasia in clams from 4 sites (Table 8). The most severely impacted site was the Fort Point Channel, with 25% of the sample affected. Low prevalences were also seen at Neponset, Chelsea and Wellfleet, but not Scorton, Saugus, or Weymouth. No apparent correlation with chemical exposure was detected.

The blue mussel *Mytilus edulis* was collected at selected sites during 1993 and subjected to a similar protocol of pathological evaluation as for the soft shell clam. Pathologies observed (Table 9) included trematode infections of the gonads with associated inflammation, possible coccidial infections of the gonad, gill parasites, green gland (pericardial gland) abnormalities, leukemia (sarcoma) and brown cells in the digestive gland. All of the above have been previously identified in *M. edulis* or related bivalves Trematode infections were severe at the Chelsea River and Pier 2 sites.

Discussion

F. heteroclitus

From our field observations, the abundance of fish from the areas sampled appeared to be inversely related to the degree of chemical contamination. This suggests that the chemical-associated pathological and biochemical changes evident in this study may well have influenced population level recruitment to some degree. The methods utilized in this study were not designed to make quantitative estimates of population abundance, but our qualitative observations suggest this supposition. Another factor in the low availability of this species in the Boston stations may be substantial habitat degradation. In particular the extensive bulkheading of the intertidal zone, with loss of marsh grass and other salt marsh organisms presumably restricts the alongshore colonization, and recolonization following die-offs, of suitable habitat by mummichogs. It should be noted that only subadult fish were found in 1992 at the Island End River site, whereas at one time point in 1993 a good collection of adults was made. This must imply significant alongshore movements of this species with time.

The most important observations on fish histology in this study are those of chemical-associated liver neoplasms and altered cellular foci in the adult fish from the Island End River. These two lesions have been closely associated with chemical exposure in other wild populations of fish (Harshbarger and Clark 1990; Moore and Myers 1994), including *F. heteroclitus*

Table 6 - Frequency of Occurence of Specific Histopathological Conditions in *Mya arenaria* -1992

Tissue		Lesion	Station number						
			1	2	3	4	5	6	8
blood cells		leukemia	0	1	0	4	0	1	2
gonad		inflammation	6	6	12	10	1	0	3
		parasites	0	2	0	0	6	1	3
		neoplasia	0	0	3	0	0	0	0
gill		parasites	5	13	16	6	10	4	3
		inflammation	7	13	19	12	5	9	10
		mucous cell (avg)	2.15	2.05	2.10	2.00	2.00	2.00	2.00
		hyperplasia	5	14	13	1	2	9	6
kidney		brown cell # (avg)	2.35	2.70	2.10	2.65	2.00	2.11	2.65
		parasites	3	9	7	0	10	9	4
		gr/br debris	7	3	13	17	1	5	12
		hyperplasia (avg)	2.75	3.15	2.95	3.00	2.10	2.26	3.00
heart		sac hyperplasia	0	0	0	0	0	0	0
		inflammation	0	0	0	0	1	0	0
colon			0	0	0	0	0	0	0
mantle		inflammation	3	4	4	4	1	2	2
		other abnorm	0	2	3	1	0	2	1
neural tissue			0	0	2	1	0		0
foot		inflammation	0	0	0	0	1	1	1
		other	0	1	1	0	1	3	0
style sac			1	3	0	0	2	0	0
intestine epithelia		parasite	0	2	0	0	3	1	1
		inflammation	0	0	0	0	0	0	0
		necrosis	0	1	0	0	0	0	0
		hyperplasia	0	1	0	0	0	1	0
		brown cell # (avg)	2.16	1.84	2.05	2.30	1.30	2.00	2.10
	lumen	parasite	0	1	0	0	0	0	1
		other abnorm	0	0	0	0	0	0	0
digestive gland		parasites	8	7	0	3	4	5	0
		brown cells in ct (avg)	1.8	2.3	2.3	1.75	0.85	1.79	1.95
	absorptive cells	vacuolation (avg)	4.15	4.7	4.45	4.65	4.5	4.79	3.95
		height (avg)	4.1	4.7	4.45	4.7	4.5	4.79	3.9
	reserve cells	occurrence (avg)	4.1	4.4	4.25	4.45	3.75	4.89	3.95
mantle edge		inflammation	0	1	4	2	1	1	1
		mucous cell # (avg)	0	0	0.22	0	0	0	0
green gland		abnorm (avg)	2.69	2.87	3.38	2.94	1.91	2.54	2.80
adductor muscle		abnormality	0	0	1	1	0	0	0
siphon		inflammation	2	1	3	1	0	6	0
		hyperplasia	1	1	0	0	0	0	0
		parasite	0	1	0	0	1	1	0
		other	0	0	0	0	0	0	0

20 individuals examined per station
Integers represent number of affected individuals per station.
Fractional numbers are averages of extent of condition at each station

(Vogelbein et al. 1990). Similar lesions have been induced experimentally in a variety of aquarium fish species following exposure to a number of hepatocarcinogens, including polynuclear aromatic hydrocarbons (Schultz and Schultz 1982; Hendricks et al. 1985; Hawkins et al. 1988; Hawkins et al. 1989) and to extracts of contaminated sediments (Metcalfe et al. 1990). The absence of chemical-associated lesions in mummichogs from the Island End River in 1992 is unlikely to reflect major chemical changes at that site between the two years. It is almost certainly a result of the much smaller size of the fish sampled in 1992 (32 mm mean length), as compared to 1993 (76 mm total length). It is known that altered liver cell foci and liver tumors apparently take a substantially longer time to develop in wild species than in aquarium-bred species such as medaka and trout. The time frame is not known for mummichogs, but in winter flounder from Boston Harbor we know that the youngest tumor-bearing animals were 5 years old (Moore and Stegeman 1994), i.e. about 50% of their life expectancy. Therefore, we would expect the tumor-bearing mummichogs to be at least 2 years old. The fish sampled in 1992 were probably spawned in 1991 and hence below the likely tumor-bearing age. Further sampling and age analysis of this population is warranted.

Macrophage aggregates were only evident in fish from the Island End River in 1993. Age, along with chemical exposure, parasitism, and nutrition are known factors to affect macrophage aggregation (Blazer et al. 1987). The absence of macrophage aggregations from other contaminated sites in the Boston area may reflect the short lifespan of this species in contrast to other species in Boston that have shown severe macrophage aggregation at similar levels of contamination (Murchelano and Wolke 1985). The other lesions described in these fish are of uncertain relevance to chemical exposure.

Cytochrome CYP1A protein levels have been shown to increase with exposure to dioxin and other aromatic compounds that mimic dioxin in their ability to bind the aromatic hydrocarbon receptor and induce cytochrome CYP1A. These compounds include halogenated biphenyls, dibenzofurans and polynuclear aromatic hydrocarbons. Increasing levels of these compounds increase the amount of CYP1A protein usually with concomitant increases in enzymatic activity, measured here by metabolism of a substrate ethoxyresorufin-o-deethylase (EROD). The data presented in Tables 4 and 5 clearly show statistically significant increased CYP1A content at the contaminated sites in comparison with the two reference sites. Specifically fish from the Fort Point Channel, Saugus River, and Weymouth Fore River showed statistically elevated CYP1A protein content as compared with the Wellfleet and Scorton Creek sites. As described in the results section there are physiological factors that affect CYP1A expression involving oogenesis in the spring caught females. The reduced expression in females caught in the spring (Table 5) is evidence of this effect. The residual variability in CYP1A expression and EROD activity is presumably a result of numerous other unmeasured variables, including other CYP1A inducers, such as coplanar PCB's, dioxins and dibenzofurans, and possibly natural plant products.

Bivalves

Soft shell clams were present at all of the intertidal sites sampled, except the Island End River. The methods we employed to collect these animals were not designed to be quantitative, but clams were surprisingly abundant at the Fort Point Channel, Chelsea River and Neponset River sites, indeed their abundance throughout the Boston Harbor system, wherever a shelving intertidal soft bottom was present, was as remarkable as the relative absence of mummichogs.

Previous studies have identified many types of lesions in various species of mollusks that exclusively occur in, or appear to be more commonly found in, animals from polluted waters or in animals exposed in the laboratory to polluted sediments. Bivalve lesions previously associated with pollution and found in the soft shell clams and blue mussels examined in this study (Tables 6, 7, 8 and 9) are: gill hyperplasia (papilloma) (Barry et al. 1971; Gardner et al. 1991),

Table 7 - Frequency of Occurence of Specific Histopathological Conditions in *Mya Arenaria* - 1993

Tissue	Lesion	Station # 8	Station # 9
Blood Cells	leukemia	1/20	1/20
Gonad	inflammation	7/20	5/20
	parasites	0/20	1/20
	neoplasia	5/20	0/20
Gill	parasites	5/20	11/20
	inflammation	20/20	20/20
	hyperplasia/ papilloma	8/20	2/20
Kidney	brown cell	19/19	14/14
	parasites	0/19	0/14
	gr/br debris	19/19	7/14
	hyperplasia	19/19	
Mantle	inflammation	17/19	15/20
Digestive Gland	brown cells in ct	19/19	20/20
Green Gland	abnorm	20/20	20/20

20 Individuals Examined Per Station
Integers Represent Number Of Affected Individuals Per Station.
Fractional Numbers Are Averages Of Extent Of Condition At Each Station

leukemia(Peters 1988), kidney hyperplasia (Barry et al. 1971; Gardner et al. 1991), increased brown cells (ceroid bodies) (Sindermann 1990), and gonadal inflammation (unassociated with parasites) (Sindermann 1990). Of the other lesions seen in these animals, the association of kidney parasites and green gland (pericardial gland) hyperplasia with pollution has not been described to date.

While gill papillomas have been induced with polluted sediments in the laboratory (Gardner et al. 1991) and have been associated with polluted natural environments (Barry et al. 1971), the occurrence of carcinomas of possible gill origin has not been identified before. Extensive metastasis of tumor to various other tissues of the clams was identified in all affected animals. However, only in one animal was a possible gonadal tubule identified as containing neoplastic cells. While gonadal tumors with metastasis to the gill have been identified in *M. arenaria* (Yevich and Barszcz 1977), because of the large size of the gill tumors, appearance of continuity with the gill epithelium, extensive metastatic foci of tumors within other tissues of these animals, and lack of involvement of gonadal tubules with tumor, carcinoma of primary gill origin was favored over carcinoma of gonadal origin. However, transmission electron microscopic examination of the tumor cells should confirm the cell of origin. Carcinomas of possible gill origin were identified in several soft shell clams in this study, but from only one location (Saugus). It is not known if this lesion is directly due to the effects of a specific pollutant or combination of pollutants, or to some other reason such as virus infection , or even a combination of the above afflictions. However, the occurrence of the tumor in animals from one area indicates

Table 8 - Intensity of Hemopoietic Neoplasia in *Mya arenaria*

	Stn. #	O	I	II	III
		Number of clams in each group			
Weymouth	1	20	0	0	0
Neponset	2	19	1	0	0
Saugus	3	20	0	0	0
Fort Point Channel	4	16	2	1	1
Chelsea River	8	18	2	0	0
Scorton Creek	5	20	0	0	0
Wellfleet	6	20	2	0	0

Groups of clams defined as:
O: No evidence of any neoplastic hemocytes
I: Between 1 to 15 % of hemocytes were neoplastic
II: Between 16 to 70 % of hemocytes were neoplastic
III: Greater than 70 % of hemocytes were neoplastic

that it is a site specific lesion. Thus it will probably not be a useful lesion for monitoring pollution. Further studies of the cause of this lesion are needed. *M. edulis* showed decreased numbers of pathologies associated with polluted waters as compared with *M. arenaria* However, the possible causative relationship of pollution with parasitic gonadal infections in *Mytilus* needs to be further investigated.

An increased occurrence of leukemia (sarcoma) in *M. arenaria* and *M. edulis* collected from polluted waters was not identified in this study. The finding of 4/20 clams from the Fort Point Channel with leukemia was not statistically significant. However, it may well be biologically significant. To evaluate this, a larger sample from that site should be obtained and analyzed. It is possible that the lack of positive correlation between leukemia in bivalves and pollution resulted either from the type of pollution in the sites studied, or the lack of some other promoting or initiating factor necessary for the development of leukemia in the clams and mussels.

In this study individual lesion types (such as gonadal inflammation or kidney hyperplasia) show an increase in animals from more severely polluted sites. The more important finding however, is the cumulative increase in various lesion types and severities in animals from polluted waters. Such an increase in the number and severity of lesions in polluted animals may reflect a decrease in condition and cellular and humoral defense abilities (Sindermann 1990) as well as a possible direct effect on the target cells by the pollutants (Gardner et al. 1991). The use of bivalves as biological monitors of pollution may be very appropriate and could provide information both in assessing the severity of pollution at a point in time as well as the effectiveness of remedial efforts.

Conclusion

We suggest that extreme chemical stress at the Island End River appears to have lead to a high prevalence of liver neoplasia in mummichogs from this site. Soft shell clams were assumed to once being abundant but only shells were found at this site in 1992 and 1993. This and other sites in and around Boston Harbor showed elevated levels of cytochrome CYP1A protein and

Table 9 - Frequency of Occurence of Specific Histopathological Conditions in *Mytilus edulis* - 1993

Tissue	Lesion	Station # 7	Station # 8	Station # 10
blood cells	leukemia	0/20	1/20	0/20
gonad	inflammation	4/20	7/20	15/20
	parasites	2/20	1/20	4/20
	neoplasia	0/20	0/20	0/20
gill	parasites	0/20	1/20	2/20
	inflammation	0/20	1/20	0/20
	hyperplasia/ papilloma	0/20	0/20	0/20
kidney	granularity of cells	19/19	17/17	20/20
	parasite	0/20	17/17	1/20
	hyperplasia	19/19	17/17	20/20
	cells in lumen	13/19	13/17	15/20
	brown cells in epith.	18/19	8/17	10/20
digestive gland	brown cells in ct	0/20	5/20	3/20
	parasite	No data	1/20	3/20
green gland	abnorm	8/8	16/16	17/17

20 individuals examined per station
Integer represent numbers of affected individuals per station

activity in fish indicative of exposure to significant levels of hydrocarbons known to induce that protein. Soft shell clams and mummichogs exhibited a range of pathological change, some associated with chemical exposure, that allow prediction of levels of chemical contamination at each sample site. In contrast the Cape Cod Bay sites, Wellfleet, and Scorton Creek both exhibited low levels of contamination, and little biological change associated with chemical exposure. Attention should continue to be paid to the sources of chemical contamination in and around Boston Harbor. This area, especially its inner reaches, such as the Island End River were substantially more impacted than cleaner sites in Cape Cod Bay. In particular, attempts to minimize the ongoing toxic chemical inputs into the Mystic River and the Island End River could allow gradual reduction of the severe biological problems evident in that area.

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