

Polycyclic Aromatic Compound Profiles From Extracts of Dreissenid Mussels and Gammarid Amphipods Coexisting in Hamilton Harbour

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ABSTRACT

Aggregates of dreissenid mussels were collected in Hamilton Harbour (western Lake Ontario) from a south shore site (Randle Reef) in an area characterized by coal tar-contaminated sediments, and from a site on the north shore exposed to particulates circulating in the harbour water column. Samples were separated into three components: dreissenid mussels, gammarid amphipods (*Gammarus fasciatus*), and particulate material. The samples were freeze-dried, and extracted using ultrasonication in dichloromethane. The organic solvent extracts were subjected to an open-column alumina and Sephadex LH-20 gel column clean-up procedure, and characterized by gas chromatography-mass spectrometry (GC-MS). The chromatographic profiles of all sample extracts were dominated by polycyclic aromatic hydrocarbons (PAH). The concentrations of the individual compounds were normalized for contaminant profile comparison of the extracts of dreissenids, amphipods, and particulates associated with aggregates of dreissenid mussels. These profiles were also compared with extracts of coal tar-contaminated sediment from the Randle Reef area, and extracts of suspended particulates obtained from sediment traps. The similarities in the PAH profiles provide evidence of exposure to a common source of contaminants. These data also show that PAH associated with suspended particulates in Hamilton Harbour are being accumulated by dreissenid mussels and gammarid amphipods.

INTRODUCTION

Hamilton Harbour (Figure 1) is a 40 km² embayment of Lake Ontario exposed to discharges of industrial effluents, treated municipal sewage, atmospheric deposition, and roadway runoff from an urban watershed of approximately 900 km². The harbour is designated as an Area of Concern by the International Joint Commission. Sediments in some areas of the harbour contain extreme levels of contaminants including polycyclic aromatic hydrocarbons (PAH) (Marvin *et al.* 1992, Murphy *et al.* 1990). Since the early 1990's, dreissenid mussels (*Dreissena polymorpha*, zebra mussel and *Dreissena bugensis*, quagga mussel) have become firmly established in Hamilton Harbour. The population densities in some areas of the harbour have since increased to prolific levels. Dreissenids have become a food source for some species of fish and wildlife in the harbour, including brown bullhead and lesser scaup.

Dreissenid mussels located in other areas of the Great Lakes have been shown to be accumulating contaminants in their tissues (Secor *et al.* 1993, Mills *et al.* 1994). We have previously investigated the utility of dreissenids as bioindicators of PAH contamination and genotoxic contamination in the water column in Hamilton Harbour (Marvin *et al.* 1994). Dreissenids are filter feeders and their sedentary nature provides time-integrated data of contaminant levels in particulate and water fluxes. Dreissenids also provide samples representing bioconcentration of xenobiotics by living residents of the harbour ecosystem. Grab sampling or centrifugation of water permits the evaluation of contaminant concentrations during relatively short time intervals, and sampling of suspended sediments using sediment traps requires long periods of deployment and potentially prohibitive infrastructure requirements.

The suspension and transport of material from areas of severe contamination of sediment by coal tar has been implicated as a major source of chemical and genotoxic contamination of the water column and sediments in other areas of Hamilton Harbour. In this preliminary work, we illustrate the comparisons of the chemical contaminant profiles of extracts of coal tar-contaminated sediments, suspended particulates from sediment traps, dreissenids, and particulates associated with aggregates of dreissenids. We have extended the scope of the study to include the analyses of extracts of gammarid amphipods (*Gammarus fasciatus*) coexisting with dreissenids in Hamilton Harbour. As with dreissenids, the accumulation of contaminants by amphipods has ramifications for higher trophic levels. Amphipods coexisting with dreissenids are potentially exposed to, and accumulating, greater quantities of contaminants through the detrital food chain than amphipods living in areas devoid of dreissenid mussels.

METHODS

Aggregates of dreissenid mussels were sampled from the top 1 m of the water column in late November of 1994 from a floating tire boom on the north shore of Hamilton Harbour at the Lasalle Marina (station 1, Figure 1), and from a marine buoy in the Randle Reef area of the harbour (station 2). Samples were gently rinsed and placed in containers with harbour water for transport to the laboratory. Gammarid amphipods were elutriated during separation of mussel aggregates into individual animals. Material remaining after separation of amphipods and dreissenids was filtered to yield a particulate fraction. Samples were then freeze-dried for 48 hours. The dried dreissenids were mechanically ground to an approximate 45 μm mesh size. The sediment trap sample (station 53, Figure 1) was kindly provided by Mr. Murray Charlton of the Canada Centre for Inland Waters, Burlington, Ontario. The trap was deployed approximately 1 m below surface for a six month period.

Dry samples were extracted using a Fisher 300 watt sonic dismembrator (ultrasonicator) apparatus. Samples were suspended in 50 mL of dichloromethane in a beaker, and subjected to four consecutive 30 second pulses with the ultrasonicator at full power. The beaker was immersed in ice, and a time interval of 1 minute was maintained between pulses to minimize solvent heating. The solvent was removed by filtration. The solvent extracts from the

ultrasonic extraction procedures were then subjected to a combined alumina/Sephadex LH-20 clean-up procedure, which has been described in detail previously (Marvin *et al.* 1992). Alumina (3 g, Brockman activity 1, 80-200 mesh, Fisher Scientific) was added to the solvent extracts. Following solvent evaporation, the sample adsorbed to alumina was applied to the top of fresh alumina (6 g) contained in a glass column (1 cm X 30 cm). Hexane (60 mL) was added to the column to elute aliphatic compounds. The polycyclic aromatic compounds (PAC) were eluted by the sequential addition of benzene (50 mL), and dichloromethane (70 mL). The PAC fraction was then subjected to a Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) gel column to remove the remaining aliphatic compounds (mobile phase: hexane/methanol/ dichloromethane (6:4:3 v/v), flow rate 3 mL/min).

Gas chromatography-mass spectrometry (GC-MS) analyses were performed using a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with an on-column injector and a Hewlett-Packard Model 5971A mass selective detector. The following temperature program was used: 130°C to 300°C at 1.6°C/min; final time at 300°C, 30 min. The linear flow velocity of the helium carrier gas was 26.4 cm/sec. The column was a 60 m X 0.25 mm i.d. DB-5MS with a 0.25 micron stationary phase film coating (J and W Scientific, Folsom, CA).

RESULTS AND DISCUSSION

The sampling sites for biota, sediments, and suspended sediments in Hamilton Harbour are shown in Figure 1. Aggregates of mussels were sampled approximately 1 m below surface in late November from a floating tire boom at a marina on the north shore of the harbour (station 1), and from a marine buoy located at Randle Reef on the south shore of the harbour (station 2). The Randle Reef site was chosen to maximize dreissenid exposure to coal tar-contaminated material resuspended from an area east of the sample site. The marina sampling site on the harbour's north shore was chosen to reflect conditions of relatively low bottom sediment contamination. The north shore site is also exposed to suspended particulates circulating in the harbour water column. The shell lengths of the dreissenids sampled ranged from 0.5 cm to 3.5 cm. The size distributions were bimodal with maxima at 1.5 cm and 2.5 cm.

Figure 2 shows the GC-MS selected ion monitoring (SIM) chromatograms from the analyses of the PAC fractions of the extracts of dreissenids, amphipods, and particulates associated with aggregates of dreissenids sampled at station 2. The chromatograms afforded facile quantitation of compounds, and illustrate the effectiveness of the clean-up procedure as evidenced by the relatively low number of interfering compounds. Table 1 lists the PAC determined in extracts of dreissenids, amphipods, and particulates from each site. The individual compound concentrations for the dreissenid and amphipod extracts are expressed in nanograms/gram (ng/g) dry weight of sample, and as the total weight of the contaminant (ng) in the sample extracts. As expected, the highest contaminant burdens were associated with samples from the Randle Reef area. Total PAH concentrations, expressed as the

summation of the concentrations of the individual PAH, were 338 ng/g for the extract of dreissenids sampled at station 1 and 64,400 ng/g for the dreissenids sampled at station 2. Total PAH concentrations in amphipod extracts were 750 ng/g at station 1 and 18,000 ng/g at station 2. At both sampling sites, the total PAH contaminant burdens were roughly 1,000-fold greater in the dreissenid mussels when compared with the amphipods (Table 1). The total PAH values are a measure of the absolute quantities of contaminants associated with each sample matrix, and were calculated by multiplying the PAH concentrations (ng/g) by the total dry weights of the samples.

The GC-MS total ion chromatogram profiles of the extracts of the dreissenids, amphipods, and particulates sampled from Randle Reef (station 2, Figure 2) were similar to profiles we observed in the analyses of extracts of bottom sediments sampled from an area east of Randle Reef (station 3, Figure 1), and suspended particulates collected in sediment traps deployed in the east end of the harbour (station 53, Figure 1). The sediment trap samples provided an approximate parallel to the filter feeding mechanism by which dreissenids are exposed to chemical contamination. The suspension of PAH-contaminated benthic sediments, in addition to industrial and municipal discharges, result in total PAH concentrations in suspended sediments in some areas of the harbour that are comparable to those of the bottom sediments. Mayer and Nagy (1992) determined total PAH concentrations (sum of 16 priority pollutant compounds) in suspended sediments as high as 106 $\mu\text{g/g}$ near the station 53 sediment trap site. We determined the total PAH concentration (sum of 16 priority pollutant compounds) of the station 53 sediment trap sample extract to be 90 $\mu\text{g/g}$. High concentrations of PAH have also been determined in Hamilton Harbour water (Ontario Ministry of the Environment 1985).

The similarities in the chromatographic profiles of the sample extracts are illustrated in Figure 3, which shows the concentrations of select PAH normalized to the concentration of benzo[e]pyrene in each sample. Figure 3A is a comparison of the benzo[e]pyrene-normalized profiles of the Lasalle Marina (station 1) dreissenid, amphipod, and particulate extracts, with extracts of coal tar-contaminated bottom sediments from the area east of Randle Reef (station 3), and suspended particulates from the station 53 sediment trap. With the exception of the PAH with molecular weights 202 and lower (phenanthrene, anthracene, fluoranthene and pyrene), the chromatographic profiles are generally similar and are characteristic of coal tar-contaminated material. We have previously observed lower ratios of compounds of molecular weight 202 and lower, when comparing profiles of sediment trap extracts with coal tar-contaminated sediment from the Randle Reef area. We speculate that there are losses of the relatively more volatile lower molecular weight PAH during suspension and transport of particulates. The profiles of extracts from the Randle Reef site (station 2), and extracts of suspended particulates and coal tar-contaminated sediment are strikingly similar (Figure 3B).

A statistical comparison of the contaminant profiles of dreissenids and amphipods from both sites was not possible due to the limited amount of data. We are currently accumulating more occurrence data, and sampling amphipods from areas of the harbour devoid of dreissenids to enable the generation of comparative amphipod contaminant profile and contaminant

burden data. These data will allow us to test the hypothesis that amphipods coexisting with dreissenids are potentially accumulating greater quantities of contaminants through the detrital food chain than amphipods living in areas devoid of dreissenid mussels.

CONCLUSIONS

The ultrasonic extraction coupled with the alumina/Sephadex LH-20 clean-up methodology afforded extracts of dreissenids and gammarid amphipods that were relatively free of interfering compounds, and allowed the accurate quantitation of PAH. All of the extracts analyzed in the study contained detectable levels of PAH contamination. These results indicate that dreissenid mussels and gammarid amphipods in Hamilton Harbour are being exposed to, and accumulating organic contaminants with possible ramifications for higher trophic levels.

ACKNOWLEDGEMENTS

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Table 1. Polycyclic aromatic hydrocarbons determined in Hamilton Harbour sample extracts. nd denotes compounds that were not detected. unr denotes compounds that were unresolved from the previously eluting compound.

PAH	Station 2 (Randle Reef)					Station 1 (LaSalle)				
	Amphipods		Dreissenids		Particulates	Amphipods		Dreissenids		Particulates
	ng/gram	Total ng	ng/gram	Total ng	Total ng	ng/gram	Total ng	ng/gram	Total ng	Total ng
phenanthrene	757	264	228	23900	43000	17	1	4	442	792
anthracene	nd	nd	3500	367000	7800	nd	nd	nd	nd	nd
fluoranthene	2890	1010	385	40300	115000	86	4	20	2260	2159
pyrene	2560	895	10300	1080000	87300	79	3	34	3890	1965
benzo[a]fluorene	540	189	8220	861000	12100	14	1	5	516	237
benzo[b]fluorene	732	256	1180	124000	13300	15	1	6	689	201
benz[a]anthracene	1480	517	1370	144000	48900	38	2	33	3670	1208
chrysene	2050	716	4950	519000	47400	182	8	37	4130	1748
benzo[b]fluoranthene	1880	655	5850	613000	46900	53	2	45	5060	2208
benzo[k]fluoranthene	unr	unr	4870	510000	27100	38	2	26	2940	nd
benzo[e]pyrene	1140	398	2790	292000	37900	52	2	47	5280	2118
benzo[a]pyrene	1390	487	3880	407000	55900	43	2	28	3110	2203
perylene	377	132	5520	578000	16100	13	1	8	848	589
indeno[1,2,3-cd]pyrene	1240	432	1610	169000	52600	52	2	16	1860	2432
dibenz[ac/ah]anthracene	unr	unr	5220	547000	9200	33	1	4	491	535
picene	51	18	879	92100	8800	11	0.5	nd	nd	564
benzo[ghi]perylene	905	316	3670	385000	34200	23	1	18	2030	2385
Total PAH (ng)	18000	6300	64400	6750000	663000	750	33	338	38100	21343

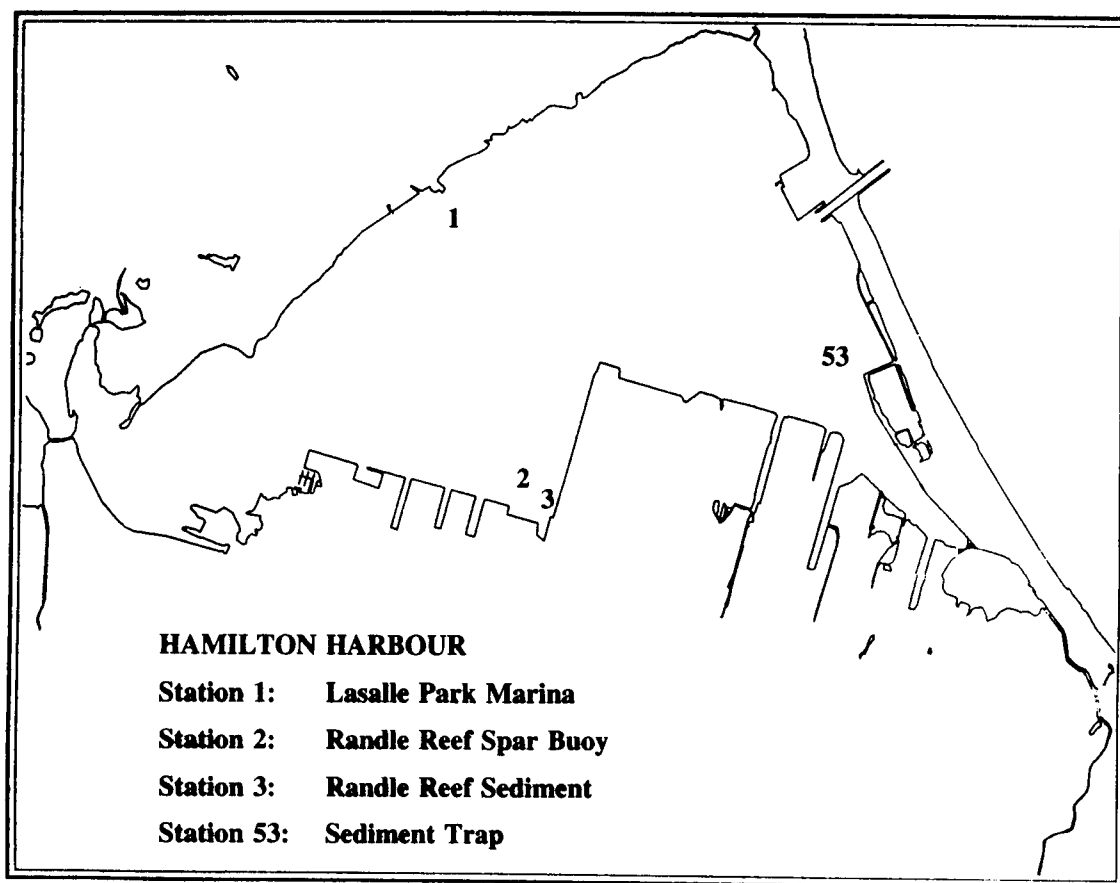


Figure 1. Map of Hamilton Harbour sample sites.

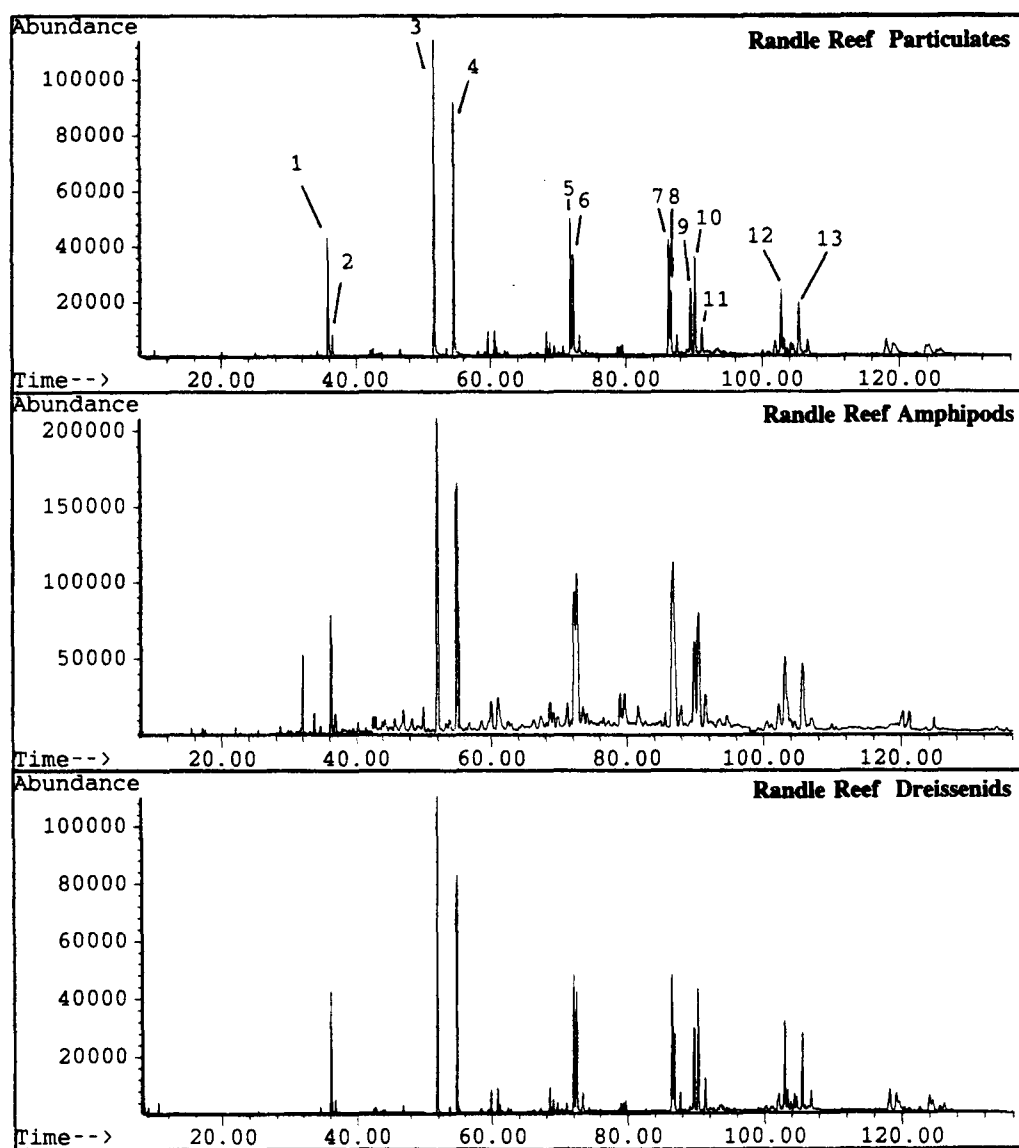


Figure 2. GC-MS selected ion monitoring chromatograms of the PAC fractions of the extracts of dreissenid mussels, gammarid amphipods, and particulates associated with aggregates of dreissenids, sampled from the Randle Reef site (station 2). The sample was processed using the described sample preparation scheme. The peak numbers identify some of the PAH determined in the sample extracts.

1. phenanthrene 2. anthracene 3. fluoranthene 4. pyrene 5. benzo[a]anthracene 6. chrysene
7. benzo[b and j]fluoranthenes 8. benzo[k]fluoranthene 9. benzo[e]pyrene 10. benzo[a]pyrene
11. perylene 12. indeno[1,2,3-cd]pyrene 13. benzo[ghi]perylene.

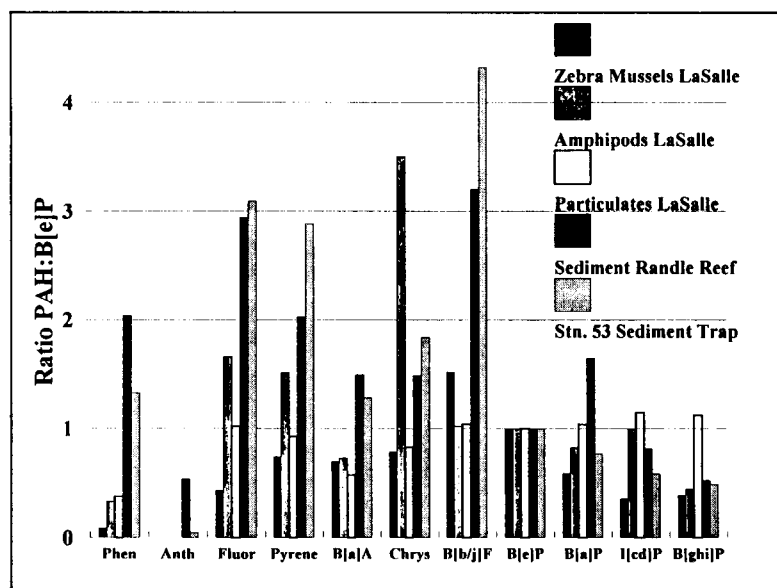


Figure 3a. Histograms comparing relative PAH levels in extracts of dreissenids, amphipods, and particulates (3a; north shore station 1, 3b; Randle Reef station 2) with coal tar-contaminated sediment sampled from an area east of Randle Reef (station 3) and suspended particulates from the station 53 sediment trap. The concentrations of the individual PAH were normalized to the concentration of benzo[e]pyrene (given the value of 1) in each of the extracts.

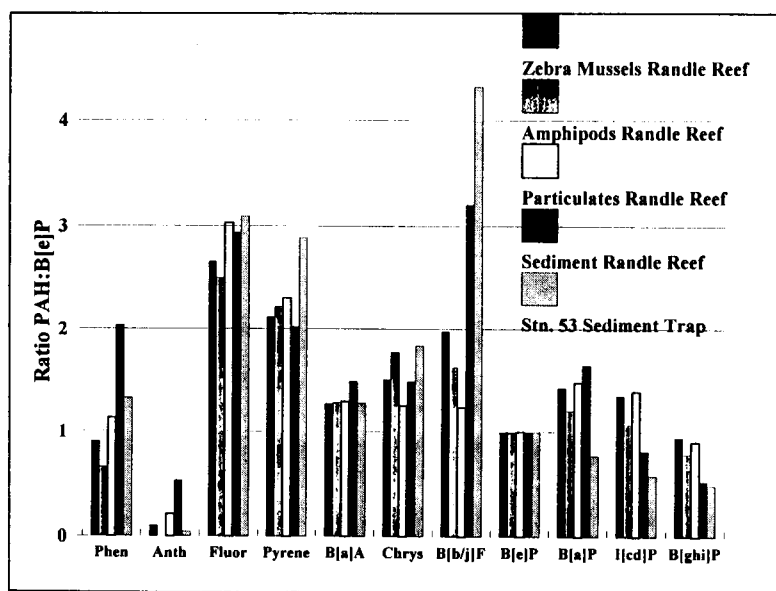


Figure 3b.