An integrated assessment of polycyclic aromatic hydrocarbons (PAHs) and benthic macroinvertebrate communities in Isle Royale National Park

Oliver N. Cox [a,*], William H. Clements [b,1]

[a] Maine Department of Marine Resources, Division of Sea Run Fisheries and Habitat, 650 State Street, Bangor, ME 04401, USA
[b] Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, CO 80523, USA

A R T I C L E   I N F O

A B S T R A C T

Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants that are known carcinogens and mutagens. This research used the sediment quality triad, an integrated weight-of-evidence approach, to evaluate sediment PAH concentrations, sediment toxicity, and benthic community structure at marina and reference sites in Isle Royale National Park, USA. The highest PAH concentrations were measured at marina locations and exceeded threshold effect concentrations (161 µg PAH/g TOC) at one site. Marina locations were dominated by pyrogenic PAHs, indicating anthropogenic sources of these compounds. Survival of the amphipod *Hyalella azteca* was significantly reduced ($p = 0.0320$) when exposed to sediments from marinas. Although macroinvertebrate abundance and species richness were similar at marina and reference sites, results of multivariate analyses showed that composition of benthic communities varied among sites. In particular, abundance of the PAH-sensitive amphipod, *Diporeia* spp. was significantly lower at marina sites compared to reference sites. In contrast to patterns observed for organochlorines (e.g., PCBS, dioxins), biota-sediment accumulation factors for PAHs measured in the burrowing mayfly *Hexagenia limbata* decreased with increasing K_{ow} values, suggesting that the more lipophilic compounds were being metabolized. Increased PAH concentrations, shifts in community composition, low survival of *H. azteca*, and reduced abundances of *Diporeia* spp. at marina sites were consistent with the hypothesis that PAHs impacted these areas; however, across all sites these effects were relatively subtle. These results emphasize the need to use a weight-of-evidence approach when investigating effects of environmental contaminants that occur at relatively low concentrations.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants consisting of 2 to 6 fused aromatic rings that are produced from both natural and anthropogenic sources. Pyrogenic PAHs (4 to 6 rings) are a result of incomplete combustion of organic material at high temperatures (>500 °C) and include combustion of fossil fuels, industry outfalls, emissions from internal combustion engines, forest fires, and volcanic activity (Eisler, 1987; Neff, 1979). Petrogenic PAHs (2 to 3 rings) are derived at low temperatures (~150 °C), under high pressure, over geological time (Neff, 1979), and lead to the formation of fossil fuels. Petrogenic PAHs are usually associated with localized sources (Burgess et al., 2003), are typically found at background concentrations (Gschwend et al., 1983), and are characterized by their acute toxicity to aquatic organisms (Eisler, 1987; Neff, 1979). Polycyclic aromatic hydrocarbons are a major environmental concern due to their carcinogenic and mutagenic effects, their ubiquitous distribution in

aquatic systems, and their hydrophobic properties that result in high sediment concentrations (Eisler, 1987; Neff, 1979). Studies have found that PAHs at extremely low concentrations (5 to 70 ppt) are toxic to many organisms including zooplankton and fish (Giesy et al., 1983; Juttner et al., 1995). A recent historical overview of Great Lakes contaminants found a close association between concentrations of PAHs in sediments and hepatic neoplasia in brown bullhead (Rafferty et al., 2009).

There is a strong positive correlation between the degree of urbanization and the concentration of PAHs in sediments (Buehler et al., 2001; Neff, 1979). Most research on PAHs in sediments has been conducted in heavily contaminated urban and industrialized areas, including wastewater treatment outfalls, storm-water discharges, coking facilities, petroleum depots, and coal storage sites (e.g., Ashley and Baker, 1999; Carr et al., 2000; Crane et al., 1997; Kemble et al., 2000; Zeng and Vista, 1997). Assessing ecological effects of contaminated sediments in these areas is relatively straightforward. While elevated PAHs have been associated with marinas, harbors, fuel docks, and boating activities in heavily populated and highly developed areas (Albers, 2002; Fang et al., 2003), few studies have investigated impacts of PAHs at remote locations.

This two-year study used the sediment quality triad (SQT) (Chapman, 1986; Long and Chapman, 1985) to evaluate effects of PAHs in sediments

* Corresponding author. Tel.: +1 207 941 4487.
 E-mail addresses: oliver.n.cox@maine.gov (O.N. Cox), willc@cnr.colostate.edu (W.H. Clements).
 [1] Tel.: +1 970 491 0690.

on benthic macroinvertebrate communities in Isle Royale National Park, USA. SQT is an approach used to determine the correlation between the bioavailability of mixtures of chemical contaminants in sediments and biological effects (Chapman, 1990). This methodology combines observations of sediment chemistry, benthic community structure, and sediment toxicity to evaluate sediment quality. Sediment quality is determined by combining the response from each assessment to build a weight-of-evidence (WOE). Weight-of-evidence approaches have been classified into three categories, metaphorical, methodological, and theoretical (Weed, 2005). Methodological WOE approaches are often used in environmental risk assessments (Weed, 2005). Integration of the evidence can vary from listing evidence to a formal process of decision analysis and statistical methods (Linkov et al., 2009).

The objectives of the study were to: i) determine the spatial distribution of PAHs in sediments collected from marinas and nearby reference sites; ii) assess potential effects of PAHs on benthic macroinvertebrate communities; iii) measure the toxicity of PAHs to macroinvertebrates in whole sediments; and iv) quantify bioaccumulation of PAHs in the burrowing mayfly *Hexagenia limbata*.

Materials and methods

Study area

Isle Royale National Park is located on a large (535 km^2) remote island in the western basin of Lake Superior. Visitation to the Park is limited, occurring between April and October with approximately 17,000 visitors per year. In contrast, Yellowstone National Park is visited by 3 million people annually. Because we lacked information on PAH concentrations in sediments, sample locations in the Park were identified based on motorboat activity. Motorboats have been documented as a substantial source of PAHs (Kado et al., 2000). Three active marina sites within the Park (Rock Harbor, Washington Harbor, and Mott Island) were paired with 3 nearby reference sites and sampled in July of 2003 and 2004 (Fig. 1). To quantify PAH concentrations in more remote areas of the Park, in 2003 sediments were collected near a small boat dock (McCargoe Cove) and a paired reference site. Finally, to assess background concentrations of PAHs in sediments, we sampled two locations with no motorboat activity (Siskiwit Lake

and Washington Creek). Because of its remote location and minimal input from local sources, previous investigators have used Siskiwit Lake to estimate atmospherically transported PAHs (Gschwend and Hites, 1981; McVeety and Hites, 1988). Duplicate (n = 2) physical and chemical measures were recorded at each sampling location. Water temperature and conductivity were measured with a handheld Thermo Orion model 105 conductivity meter and pH was measured with a handheld Thermo Orion model 210A pH meter. Dissolved oxygen was measured with YSI (model 57) dissolved oxygen meter. All meters were calibrated daily according to the manufacturer's specifications. Secchi depth and bottom depth were also measured.

Sediment sample collection

Sediments for benthic macroinvertebrates, PAH analysis, and total organic carbon (TOC) were collected using a stainless steel petite ponar sampler (15.2 cm^2) in July of 2003 and 2004. The sampler was rinsed with A.C.S. spectranalyzed grade acetone between sites. Four replicate samples were collected within 100 m^2 area of the initial sampling location. Sediment (125 mL) from the upper 5 cm of 2 samples was removed for PAH analysis. Sediment samples were placed in 250 mL specially-cleaned glass containers with Teflon® lids, wrapped in foil, stored on ice in the field, and transferred to a freezer within 10 h. We also removed and discarded 125 mL from the remaining two ponar samples so that all benthic samples were comparable. Benthic samples were washed through a 500 μm mesh sieve in the field, stored in a 500 mL polyethylene container, and preserved in 80% ethanol. An additional 1000 mL of sediment was collected in 2004 from each marina and paired reference site for toxicity testing.

Chemical analyses

Polycyclic aromatic hydrocarbon (Table 1) and TOC analyses were conducted at the Texas A&M University Geochemical and Environmental Research Group (College Station, Texas). Sediment samples were oven dried at 40 °C, mixed with anhydrous sodium sulfate, and extracted with methylene chloride in an Automated Solvent Extractor (Qian et al., 1998a). Extract purification was though alumina/silica gel column chromatography. Deuterated PAH surrogate compounds were added
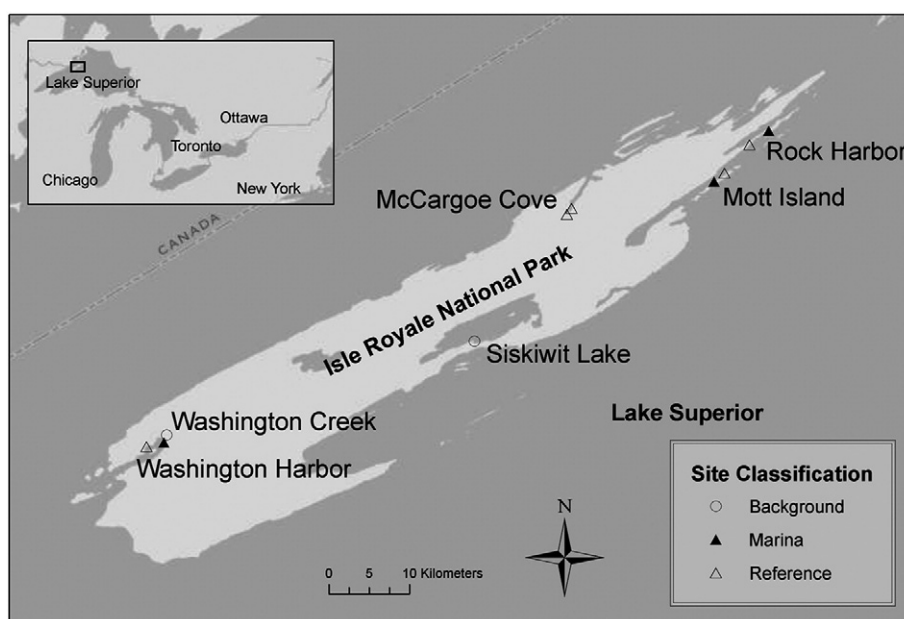

Fig. 1. Location of macroinvertebrate and sediment collection sites at Isle Royale National Park, Michigan, USA. Background sites were locations with no motor boat activity while reference sites had limited motorboat activity relative to marina sites.

before the extraction and were used to calculate analyte concentrations. Quantitative determination of PAHs was conducted using NOAA National Status and Trends Methods (Denoux et al., 1998) on an Agilent 6890/5973 gas chromatograph with a mass selective detector (GC/MS). Quality assurance (procedural blanks, matrix spikes, matrix spike duplicates, and standard reference material) was processed with each batch of samples in a manner identical to the samples. Carbon concentrations were determined on dried sediment using an induction furnace (LECO Model 523-300), an infrared detector, and an integrator. Samples were combusted and carbon dioxide was detected with a Horiba PIR-2000 infrared detector. Percent carbon content was based on an established calibration curve and TOC was determined after sample acidification on dried sediment.

The degree of contamination at marinas was determined by comparing measured concentrations of PAHs to threshold effect concentrations (TECs) for sediments (MacDonald et al., 2000). The TEC is defined as the concentrations below which adverse biological effects are unlikely to occur, thereby providing a high level of protection for aquatic organisms. To distinguish pyrogenic versus petrogenic PAHs in the sediments, the percent of high molecular weight (HMW) compounds (4 to 6 rings) were evaluated (Budzinski et al., 1997; Gschwend and Hites, 1981). Sediments dominated by high molecular weight PAHs were considered to be contaminated by pyrogenic sources. A second method compared the distributions of the parent compound chrysene to alkylated homologs (C1, C2, C3, and C4-chrysenes). Polycyclic aromatic hydrocarbons derived from combustion sources are dominated by non-alkylated parent compounds, whereas petrogenic PAHs are dominated by alkylated homologs (Laflamme and Hites, 1978).

Laboratory toxicity testing

Sediment toxicity tests conducted in 2004 followed the EPA's test method 100.1 for *Hyalella azteca* (Amphipoda) 10-day survival and growth protocols (Ingersoll et al., 2000). Six treatments (3 marina and 3 reference sites) were replicated eight times for a total of 48 test chambers. Water for the tests was collected from Horsetooth Reservoir, Fort Collins, Colorado. Twenty-four hours prior to the start of the experiment, sediment samples were homogenized in a stainless steel beaker with an acetone-rinsed stainless steel paint mixer attached to a handheld, variable speed drill. Caution was taken to avoid separation of the matrix. Aquatic vegetation and invertebrates found during homogenization were removed. Experiments were conducted in 600 mL beakers containing 100 ml of sediment and 200 mL of overlying water. Each beaker received 10, 14-day old *H. azteca*, obtained from Aquatic

Bio Systems (Fort Collins, Colorado). Amphipods were acclimated in test water over a period of 4 h, after which organisms were randomly assigned to test chambers. Beakers were placed in a low temperature incubator maintained at 23 ± 1 °C with a photoperiod of 16 light hours and 8 dark hours. Water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity) was measured in all beakers on days 0 and 9 prior to renewal of overlaying water. In addition, temperature and dissolved oxygen were monitored in two replicates from each treatment prior to renewal of overlaying water daily. Water for hardness and alkalinity testing was pooled from four randomly chosen replicates of each treatment. Overlying water was renewed daily by siphoning off approximately 200 mL of water with a glass pipette equipped with a 250 μm mesh screen. Temperature and dissolved oxygen were measured with YSI model 100, conductivity was measured with a handheld Thermo Orion model 105 conductivity meter and pH was measured with a handheld Thermo Orion model 210A pH meter. All instruments were calibrated daily. Between test chambers all equipment and probes were rinsed with deionized water and inspected for organisms.

Each chamber received 1 mL of TetraFin ® flake food slurry daily. After 10 day exposure to sediments, surviving *H. azteca* were removed by swirling the beakers to suspend the upper layers of sediment and then pouring the contents through a 350 μm mesh sieve. The sediment was then transferred to a Pyrex ® glass pan with tap water and the surviving *H. azteca* were removed. The remaining sediment was then sieved, transferred to the pan and inspected for surviving organisms. To verify that amphipods removed from sediments were not indigenous, all organisms were identified under a dissecting microscope.

Benthic macroinvertebrate identification

In the laboratory, benthic invertebrate samples were rinsed thoroughly with tap water through a 500 μm sieve, placed in a Pyrex® glass pan, and covered with tap water. Organisms were sorted from detritus and placed in vials containing 80% ethanol. Invertebrates were identified under a dissecting microscope (Olympus, SZ11 4.8× to 44×) to the lowest practical taxonomic level (genus for most taxa; family, class, or phylum for mollusks, oligochaetes, and nematodes, respectively). Midges were sorted to subfamily and grouped by association under a dissecting microscope (Epler, 2001). A minimum of 10% from each group was mounted on slides and identified with a compound scope (Olympus EHS, 40× to 100×). When discrepancies were found within an associated group, additional specimens were mounted and the process was repeated if necessary. The following taxonomic keys were used for identification of macroinvertebrates: Merritt and Cummins (1996), Thorp and Covich (2001), Wiggins (1996), and Epler (2001).

Bioaccumulation of PAHs

To assess bioavailability and uptake of PAHs to benthic macroinvertebrates, in 2004 we collected burrowing mayflies (*H. limbata*) from Washington Harbor marina for tissue analysis. We focused on *Hexagenia* because of their sensitivity to contaminants, importance in Great Lakes food webs and potential to transfer contaminants to higher trophic levels. Forty-eight *H. limbata* were placed in a holding chamber equipped with an aerator and depurated for 16 h. After depuration, *H. limbata* were randomly assigned to three subsamples and placed in 125 mL specially cleaned glass containers with Teflon® lids (Eagle Picher, Miami, OK), covered in deionized water, and frozen. Tissue samples were prepared for PAH extraction using a tissumizer as described in Qian et al. (1998b). Purification of the tissue samples was done with gel permeation chromatography (Qian et al., 1998c) and analyzed using GC/MS as described for sediments. Percent lipid in the tissue of *H. limbata* was determined on the extract before purification (Qian et al., 1998d).

Table 1
Polycyclic aromatic hydrocarbons (PAH) extracted from surface sediments, Isle Royale National Park, Michigan, USA. United States Environmental Protection Agency priority pollutants are indicated in bold and asterisks indicate that the compounds were used to make comparisons with consensus-based sediment quality guidelines.

Compound		
Naphthalene*	**Anthracene***	**Chrysene***
C1-Naphthalenes	C1-Phenanthrenes/Anthracenes	C1-Chrysenes
C2-Naphthalenes	C2-Phenanthrenes/Anthracenes	C2-Chrysenes
C3-Naphthalenes	C3-Phenanthrenes/Anthracenes	C3-Chrysenes
C4-Naphthalenes	C4-Phenanthrenes/Anthracenes	C4-Chrysenes
Biphenyl	Dibenzothiophene	**Benzo(b)fluoranthene**
Acenaphthylene	C1-Dibenzothiophenes	**Benzo(k)fluoranthene**
Acenaphthene	C2-Dibenzothiophenes	Benzo(e)pyrene
Fluorene*	C3-Dibenzothiophenes	**Benzo(a)pyrene***
C1-Fluorenes	**Fluoranthene***	Perylene
C2-Fluorenes	**Pyrene***	Indeno(1,2,3-c,d)pyrene
C3-Fluorenes	C1-Fluoranthenes/Pyrenes	**Dibenzo(a,h) anthracene***
Phenanthrene*	**Benzo(a)anthracene***	**Benzo(g,h,i)perylene**

Table 2
Means and ranges for physicochemical parameters (water temperature, pH, conductivity, dissolved oxygen, depth measured in water and % total organic carbon measured in sediment) at study sites in Isle Royale National Park, Michigan, USA, July 2003 and 2004. NA = not available.

	Rock Harbor reference	Rock Harbor marina	Mott Island Reference	Mott Island Marina	Washington Harbor Reference	Washington Harbor Marina	McCargoe Cove Reference	McCargoe Cove Dock	Washington Creek	Siskiwit Lake
n	4	4	4	4	4	4	2	2	2	2
Water temperature (°C)	8.5 (7.3–9.7)	8.6 (6.6–10.6)	9.3 (8.3–10.3)	10.0 (8.6–11.4)	8.9 (7.0–10.8)	8.4 (6.8–10.0)	9.8 (9.8–9.8)	11.6 (11.6–11.6)	NA	NA
pH	7.4 (7.0–7.9)	7.4 (7.0–7.9)	7.5 (7.0–8.0)	7.6 (7.3–8.0)	7.7 (7.3–8.1)	7.6 (6.9–8.2)	7.7 (7.8–7.9)	7.7 (7.6–7.7)	NA	NA
Conductivity (µS)	88.9 (79.1–98.3)	89.3 (77.6–100.3)	87.6 (77.7–97.4)	89.6 (80.6–98.1)	84.6 (67.1–100.1)	85.7 (66.8–103.1)	84.7 (84.6–84.7)	87.3 (87.0–87.6)	NA	NA
Dissolved oxygen (mg/L)	11.2 (10.2–12)	11.4 (10.9–11.6)	11.7 (11.3–12.1)	11.0 (10.5–11.2)	12.4 (11.4–13.3)	12.0 (11.5–12.6)	14.5 (14.4–14.6)	11.9 (11.8–12.0)	NA	NA
Depth (m)	9.4 (8.0–11.0)	4.6 (4.3–5.0)	4.9 (3.0–6.6)	3.2 (3.0–3.5)	4.6 (4.0–5.5)	2.4 (2.0–3.0)	1.8 (1.8–1.8)	4.1 (4.1–4.1)	1.0 (1.0–1.0)	1.0 (1.0–1.0)
Total organic carbon %	0.64 (0.49–0.85)	1.12 (0.77–1.53)	0.98 (0.82–1.17)	1.24 (0.89–1.78)	0.99 (0.67–1.39)	1.56 (1.18–2.31)	2.39 (2.27–2.5)	0.41 (0.35–0.47)	0.09 (0.08–0.1)	0.08 (0.07–0.08)

Data analyses

We compared total PAH concentrations (µg/g TOC), concentrations of compounds used to calculate consensus-based sediment quality guidelines (Table 1), benthic macroinvertebrate metrics (total abundance, taxa richness and abundance of the amphipod *Diporeia* spp.), and sediment toxicity between reference and marina sites. To account for the influence of TOC on PAH bioavailability and toxicity, PAH concentrations were normalized to TOC levels in sediment. One-way analysis of variance (ANOVA) was used to test for differences in PAH concentrations, sediment toxicity, and benthic macroinvertebrate metrics among sites. Variables were log-transformed when necessary to satisfy assumptions of parametric statistics. All analyses were conducted using The SAS System 9.2 (SAS Institute Inc., Cary, North Carolina). A multivariate technique, canonical discriminant analysis, was used to evaluate differences among sites based on PAH composition and macroinvertebrate community structure. Canonical discriminant analysis generates linear combinations of variables to distinguish among predetermined groups.

Biota-sediment accumulation factors (BSAFs), defined as the lipid-normalized concentration of contaminants in an organism divided by the organic carbon-normalized concentration in sediment (Gewurtz et al., 2000), were calculated for *H. limbata*. In theory, assuming that the capacity of lipids and organic carbon to sorb contaminants is equal, BSAF values at equilibrium should approach 1.0 (Di Toro et al., 1991). Values that are considerably less than 1.0 suggest that a contaminant is being metabolized, whereas values > 1.0 suggest biomagnification. BSAFs were plotted against known octanol–water partitioning coefficients (log K_{ow}) of various PAHs to test the hypothesis that uptake was related to hydrophobicity.

Results

Site characterization

Routine physicochemical characteristics that we measured were similar among sites and showed little differences between marinas and reference areas with the exception of total organic carbon (Table 2). Mean percent TOC in sediment was variable (0.08% to 2.39%) but was greater at each marina sites compared to the paired reference site, although there was overlap in the ranges observed. Mean depth was also variable among sites, ranging from 1.8 to 10.8 m. Mean conductivity ranged from 84.6 µS to 89.6 µS.

PAHs in sediments

PAH quality assurance was conducted on 4 procedural blanks. No analytes were measured greater than three times the method detection limits in the procedural blanks. No individual analytes exceeded the quality control criteria (QC). Lab matrix spike sample recoveries were 105.0%, 115.0%, 108.75, and 95.3%. The average relative percent difference for the three duplicate samples was 10.1%, 10.0%, and 14.2%. Several individual analytes exceeded the QC criteria in one matrix spike from Rock Harbor Marine due to the high natural concentration in the samples. Recovery of analytes with valid spike amounts met quality control criteria. Recoveries for the standard reference material (SRM) 1944 met the QC criteria of 35% limits. The SRM was evaluated for all certified and non-certified analytes. Relative accuracy was computed by comparing the observed concentration against the certified concentrations and its 95% confidence interval. The concentration determined should be within +/−35% of the upper and lower confidence interval on average for all analytes. The overall average for PAHs was within the QC criteria.

PAH concentrations and composition (Table 3) differed among sites and between treatments (reference versus marina). Mean concentrations of total PAHs at marina sites were approximately 10× greater than at reference sites. The highest concentrations were consistently

Table 3
Mean (±1 standard error, n=4) concentration of polycyclic aromatic hydrocarbons (ng/g) measured in sediments at reference, marina and background sites in Isle Royale National Park, Michigan, USA, July 2003 and 2004. See Map Fig 1. for location of sites.

Compound	Rock Harbor reference	Rock Harbor marina	Mott Island reference	Mott Island marina	Washington Harbor reference	Washington Harbor marina	McCargoe Cove reference	McCargoe Cove dock	Washington Creek	Siskiwit Lake
Naphthalene	6.21 ± 0.76	24.86 ± 3.43	5.75 ± 0.55	17.92 ± 1.03	5.10 ± 0.29	19.41 ± 2.08	2.71 ± 1.15	9.16 ± 3.46	1.16 ± 0.22	1.36 ± 0.46
Biphenyl	1.62 ± 0.08	4.88 ± 0.99	1.52 ± 0.13	4.30 ± 0.46	1.54 ± 0.22	4.26 ± 1.38	0.87 ± 0.43	3.54 ± 1.00	0.55 ± 0.10	0.57 ± 0.17
Acenaphthylene	2.58 ± 1.08	15.62 ± 5.88	1.37 ± 0.10	15.82 ± 4.68	1.29 ± 0.33	11.76 ± 2.28	0.15 ± 0.03	0.50 ± 0.03	0.05 ± 0.00	0.06 ± 0.01
Acenaphthene	1.71 ± 0.54	50.87 ± 8.92	0.67 ± 0.09	24.21 ± 4.18	0.74 ± 0.03	9.75 ± 0.90	0.40 ± 0.13	1.17 ± 0.39	0.22 ± 0.04	0.28 ± 0.09
Fluorene	4.22 ± 2.04	55.01 ± 10.67	1.87 ± 0.29	27.71 ± 3.43	1.91 ± 0.47	16.49 ± 0.20	0.63 ± 0.28	3.90 ± 1.48	0.23 ± 0.05	0.23 ± 0.02
Phenanthrene	20.24 ± 10.01	254.68 ± 46.10	7.37 ± 1.05	140.59 ± 17.73	7.37 ± 1.03	96.38 ± 2.57	2.21 ± 0.99	8.10 ± 2.76	0.74 ± 0.09	0.94 ± 0.35
Anthracene	6.72 ± 2.77	129.16 ± 26.82	2.03 ± 0.16	67.95 ± 7.41	2.02 ± 0.36	49.63 ± 3.04	0.30 ± 0.09	1.54 ± 0.27	0.08 ± 0.02	0.08 ± 0.02
Dibenzothiophen	1.21 ± 0.52	12.80 ± 2.15	0.59 ± 0.06	8.57 ± 1.10	0.62 ± 0.09	6.55 ± 0.92	0.16 ± 0.05	0.55 ± 0.11	0.08 ± 0.02	0.07 ± 0.01
Fluoranthene	31.22 ± 12.93	406.63 ± 75.26	13.33 ± 2.18	250.81 ± 25.90	13.29 ± 1.51	234.15 ± 4.96	1.98 ± 0.39	4.70 ± 0.87	0.41 ± 0.04	0.39 ± 0.18
Pyrene	26.77 ± 10.24	338.1 ± 63.82	9.00 ± 1.40	210.19 ± 23.03	8.00 ± 0.91	214.88 ± 6.39	1.22 ± 0.31	3.15 ± 0.70	0.29 ± 0.00	0.32 ± 0.07
Benzo(a) anthracene	14.57 ± 6.02	194.04 ± 24.88	5.11 ± 0.57	127.37 ± 18.39	4.57 ± 0.50	129.10 ± 6.09	0.74 ± 0.17	1.90 ± 0.11	0.22 ± 0.06	0.11 ± 0.01
Chrysene	16.24 ± 5.54	206.25 ± 32.40	7.42 ± 1.14	128.29 ± 15.08	7.29 ± 0.64	113.38 ± 3.86	1.29 ± 0.25	2.98 ± 0.46	0.20 ± 0.03	0.17 ± 0.10
Benzo(b) fluoranthene	22.07 ± 6.08	286.72 ± 53.92	10.68 ± 1.14	183.91 ± 25.18	10.66 ± 1.21	149.63 ± 3.51	1.93 ± 0.44	4.43 ± 1.11	0.20 ± 0.04	0.27 ± 0.12
Benzo(k) fluoranthene	7.49 ± 2.40	82.07 ± 8.98	3.25 ± 0.63	54.61 ± 7.73	3.23 ± 0.40	44.56 ± 3.21	0.70 ± 0.07	2.23 ± 0.37	0.11 ± 0.02	0.13 ± 0.04
Benzo(e)pyrene	11.83 ± 3.84	134.44 ± 28.04	4.60 ± 0.45	86.76 ± 13.51	4.50 ± 0.51	70.62 ± 3.74	0.95 ± 0.19	1.57 ± 0.22	0.12 ± 0.02	0.20 ± 0.08
Benzo(a)pyrene	19.71 ± 6.97	238.62 ± 39.32	5.73 ± 0.55	146.42 ± 20.93	4.99 ± 0.66	131.04 ± 2.73	0.71 ± 0.13	1.67 ± 0.84	0.13 ± 0.05	0.25 ± 0.01
Indeno (1,2,3c,d)-pyrene	14.60 ± 4.03	144.80 ± 32.11	7.97 ± 0.67	97.31 ± 14.78	8.23 ± 1.14	76.92 ± 5.81	1.36 ± 0.31	2.21 ± 0.34	0.12 ± 0.01	0.14 ± 0.02
Dibenzo (a,h)-anthracene	2.20 ± 0.68	28.03 ± 7.63	0.95 ± 0.05	17.31 ± 3.24	1.07 ± 0.20	14.88 ± 2.31	0.17 ± 0.05	0.18 ± 0.12	0.02 ± 0.01	0.03 ± 0.01
Benzo(g,h,i)perylene	11.29 ± 3.31	108.92 ± 21.96	5.52 ± 0.57	74.61 ± 11.37	5.65 ± 0.67	60.58 ± 4.02	1.15 ± 0.21	1.66 ± 0.30	0.13 ± 0.02	0.14 ± 0.04
Total PAHs	346.3 ± 97.4	3410.2 ± 602.0	180.6 ± 17.6	2221.0 ± 229.4	254.3 ± 29.9	2426.5 ± 105.2	99.4 ± 27.6	1516.3 ± 26.0	41.8 ± 22.0	16.6 ± 2.7

detected at Rock Harbor marina and the lowest concentrations were measured at the 2 background sites (Siskiwit Lake and Washington Creek). This pattern was the same for consensus-based PAHs used to calculate TOC-adjusted sediment quality guidelines (Fig. 2). Total concentration of the 10 consensus-based PAHs was greatest at Rock Harbor marina, which was the only site to exceed the estimated threshold effect concentration. There were also important differences between pyrogenic and petrogenic PAHs among sites. High molecular weight compounds accounted for approximately 67 to 74% of all PAHs at marina sites and 51 to 66% at reference sites. In contrast, these high molecular weight compounds accounted for only 20% of total PAHs at Siskiwit Lake and Washington Creek, the two background sites with no motorboat activity. The parent compound chrysene dominated the chrysene series at all of the marina and marina reference sites indicating pyrogenic sources. Chysene, C1 was the dominant constituent at Siskiwit Lake and Washington Creek indicating petrogenic sources.
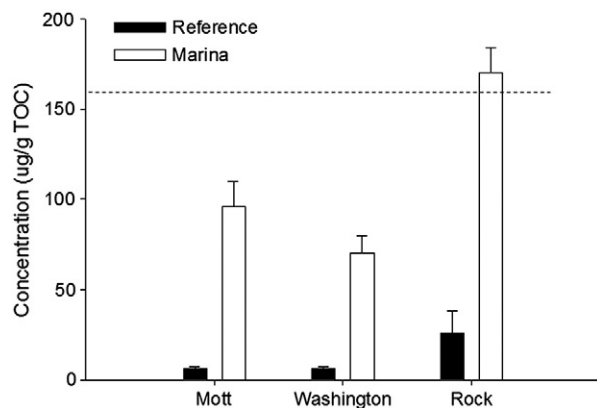
Results of canonical discriminant analysis based on TOC-adjusted concentrations of the U.S. EPA priority pollutants showed that reference sites were very similar to each other but distinct from all marina sites (Fig. 3). Separation of reference sites from Rock Harbor marina
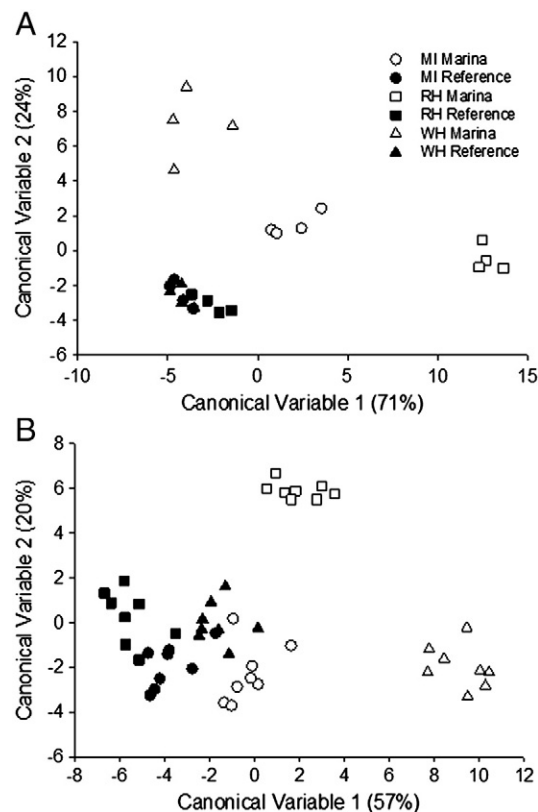
Fig. 3. Canonical discriminant analysis showing separation of marina and reference sites based on concentrations of polycyclic aromatic hydrocarbons in sediments (A) and benthic macroinvertebrate community composition (B) at Isle Royale National Park, 2003–2004.

Fig. 2. Mean (±1 standard error) concentrations of consensus-based polycyclic aromatic hydrocarbons (PAHs) normalized to total organic carbon (TOC) collected from surface sediments at Isle Royale National Park, 2003–2004. Dashed horizontal line is the threshold effect concentration (MacDonald et al., 2000).

along canonical variable 1, which explained 71% of the variation among groups, resulted from higher concentrations of all PAHs in marina sediments. Washington Harbor marina was separated from reference sites along canonical variable 2 because of high concentrations of pyrene, chrysene and benzo-a-pyrene.

Benthic macroinvertebrate community composition

A total of 14,492 individuals consisting of 73 taxa were collected and identified in 2003 and 2004. Benthic invertebrate densities ranged from $763/m^2$ to $4718/m^2$ and three major groups (chironomids, oligochaetes, and amphipods) accounted for 77% of total macroinvertebrate abundance. Across all sites, the five most common taxa were *Cladotantytarsus* spp. (18.4% of total abundance), *Diporeia* spp. (15.7%), Oligochaeta (14.9%), *Tanytarsus* spp. (9.9%), and *Pisidium* spp. (9.3%). Total macroinvertebrate abundance ($F_{1, 46} = 0.05$, $p = 0.8210$) and taxa richness ($F_{1, 46} = 0.00$, $p = 0.9523$) were similar among sites and did not differ between marinas and reference sites (Fig. 4). In contrast to these results, overall abundance of the sensitive amphipod *Diporeia* spp. was significantly reduced ($F_{1, 46} = 17.78$, $p < 0.0001$) at marina sites compared to reference sites.
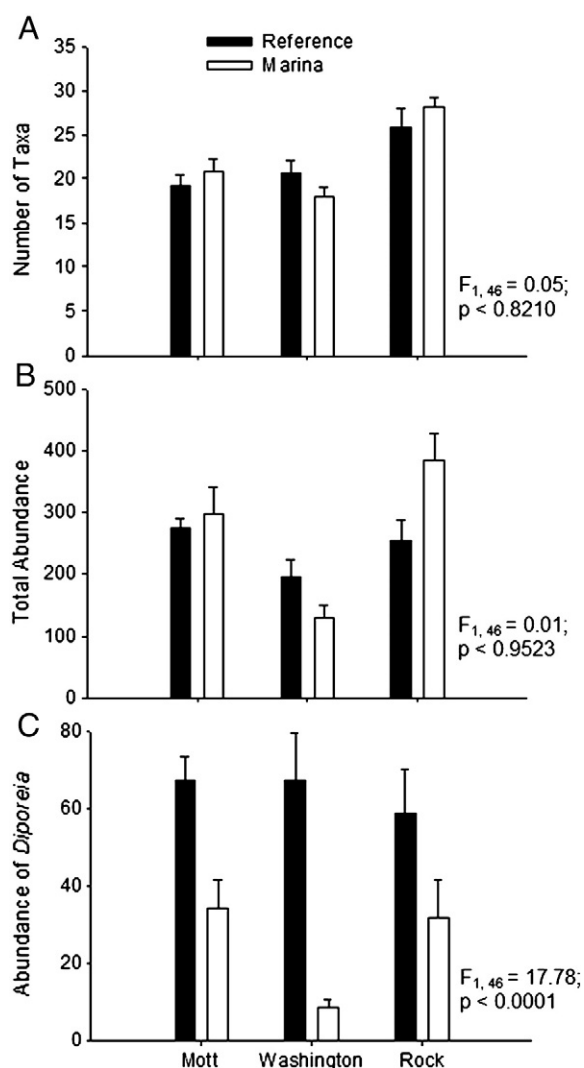
Differences in community composition among sites based on canonical discriminant analysis of the 15 dominant taxa were highly significant (Wilks' Lambda, $F = 13.9$, $p < 0.0001$). Although the 3 reference sites grouped together and overlapped slightly with Mott Island marina (Fig. 3), benthic communities at Rock Harbor and Washington Harbor marinas were unique and distinctly separated from other sites. Canonical variable one, which explained 57% of the variation among groups, separated Washington Harbor marina from reference sites based primarily on lower abundance of *Diporeia* spp., *Pisidium* spp., and *Larsia* spp. and greater abundance of *Pagastiella* spp. and *Paralauterborniella* spp. Benthic communities at Rock Harbor marina were separated from other sites along canonical variable 2 primarily due to greater abundance of *Pagastiella* spp. and the isopod *Caecidotea* spp.

Sediment toxicity

Toxicity test conditions were within acceptable ranges for temperature, dissolved oxygen, conductivity and pH throughout the experiments (Ingersoll et al., 2000). Across all sites, amphipod survival was significantly lower ($F_{1, 46} = 4.89$, $p = 0.0320$) in marina sediments compared to reference sediments (Fig. 5). However, this difference was a result of greater toxicity of Rock Harbor and Mott Island sediments. Survival of *H. azteca* in sediments collected from Washington Harbor marina and the reference site was low (approximately 10%). Slightly higher turbidity was noted in the Washington Harbor toxicity tests and that the turbidity persisted for the duration of the test. With the exception of the Washington Harbor reference site, amphipod growth was similar among treatments.

Bioaccumulation of PAHs by Hexagenia

Concentrations of PAHs in the burrowing mayfly *Hexagenia* collected from Washington Harbor marina varied among constituents (Fig. 6A), but were significantly related to concentrations in sediments ($R^2 = 0.53$; $p = 0.0003$). Pyrene and fluoranthene were the dominant PAHs measured in both sediments and mayflies. Although concentrations of several constituents were greater in mayflies compared to sediments, patterns of bioaccumulation were quite different when these data were adjusted to account for bioavailability. Biota sediment accumulation factors (BSAFs) based on lipid-normalized tissue and organic carbon-normalized sediment concentrations were relatively low and less than equilibrium (<1.0) for all constituents, with most values <0.5. The inverse relationship between BSAF and K_{ow} was highly significant ($R^2 = 0.79$; $p = 0.0002$), indicating that *Hexagenia* primarily accumulated water soluble (less lipophilic) PAHs (Fig. 6B).
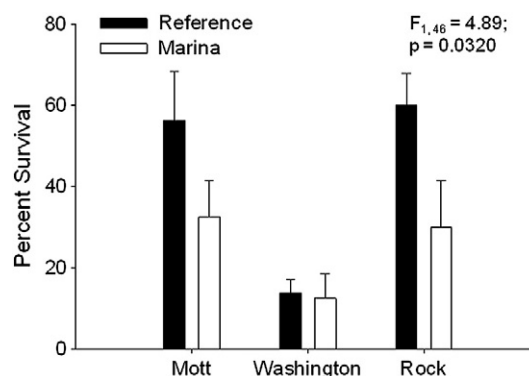

Fig. 4. Mean (± 1 standard error) taxa richness (A), macroinvertebrate abundance (B) and abundance of the amphipod, *Diporeia* spp. (C) per ponar sample (15.2 cm^2) for paired marina and reference site from Isle Royale National Park, 2003–2004. Results of one-way ANOVA testing for differences between reference and marina sites are also shown.

Fig. 5. Mean (± 1 standard error) percent survival of *Hyalella azteca* exposed to sediments collected from marina and reference sites at Isle Royale National Park, 2004. Results of one-way ANOVA testing for differences between reference and marina sites are also shown.
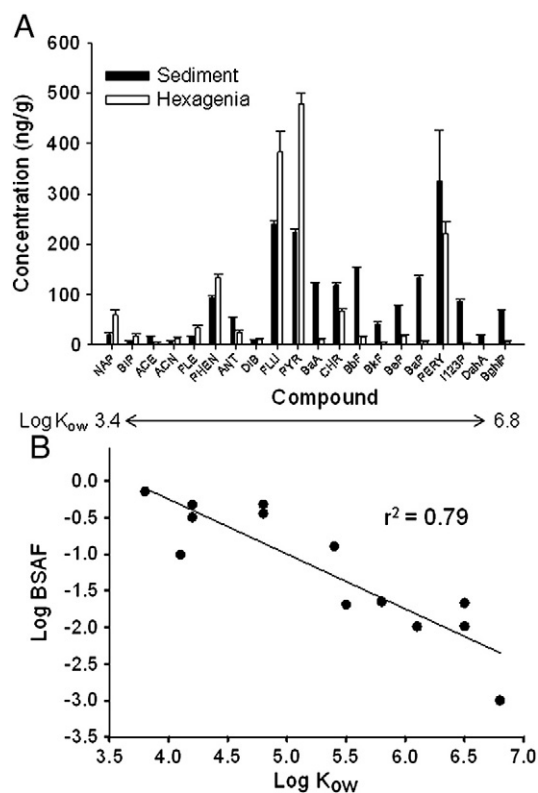
Fig. 6. Bioaccumulation of PAHs by the mayfly *Hexagenia* collected from Washington Harbor marina at Isle Royale National Park, 2004. Concentrations of PAHs in sediments and mayflies along a gradient from low to high K_{ow} values (where known) or molecular weights (A). The relationship between biota sediment accumulation factors (BSAF) and K_{ow} values for PAHs measured in *Hexagenia* (B). NAP = Naphthalene; BIP = Biphenyl; ACE = Acenaphthylene; ACN = Acenaphthene; FLE = Fluorene; PHEN = Phenanthrene; ANT = Anthracene; DIB = Dibenzothiophen; FLU = Fluoranthene; PYR = Pyrene; BaA = Benzo(a)anthracene; CHR = Chrysene; BbF = Benzo(b)fluoranthene; BkF = Benzo(k) fluoranthene; BeP = Benzo(e)pyrene; BaP = Benzo(a)pyrene; I123P = Indeno(1,2,3-c,d) pyrene; DahA = Dibenzo(a,h)anthracene; and BghiP = Benzo(g,h,i)perylene.

Discussion

Although concentrations of PAHs and other legacy contaminants are generally lower in Lake Superior compared to the other Great Lakes (Gschwend and Hites, 1981), elevated levels of these contaminants do occur in developed areas. Consistent with research conducted in marine ecosystems (Johnson et al., 1985), PAH concentrations in surface sediments were greatly elevated at marinas compared to reference and background sites. The highest PAH concentrations were measured at Rock Harbor marina, and sediments collected from both Rock Harbor and Mott Island marinas were toxic to amphipods. The lowest PAH concentrations were measured at Siskiwit Lake and Washington Creek, suggesting that these sites provided reasonable estimates of background conditions. In contrast to patterns observed at background sites, PAHs in sediments collected from marinas were dominated by pyrogenic compounds that are generally associated with combustion of fossil fuels. These results suggest that the most obvious anthropogenic source of PAHs in Isle Royale National Park was from motorboat activities.

The highest concentrations of TOC were documented at the marina sites and McCargoe Cove. The observed TOC concentrations at marina sites were typical of sediments in many areas of Lake Superior where values generally ranged from 1 to 3% (Johnson et al., 1982). The low variation in TOC also means that the observed pattern in PAHs and normalized PAHs were similar.

MacDonald et al. (2000) calculated a consensus-based TEC of 161 μg/g TOC and probable effect concentration (PEC) of 2280 μg/g TOC for PAHs. These values have been used to establish sediment

quality guidelines for the protection of benthic organisms in other areas (Crane et al., 2002). The TEC estimated by MacDonald et al. (2000) is a reasonable benchmark for sediment quality at a national park such as Isle Royale because it provides a high level of protection for macroinvertebrate communities. Sediments from Rock Harbor marina exceeded the TEC in 2003 (176 μg/g TOC) and 2004 (163 μg/g TOC), and concentrations at Mott Island marina approached the TEC. However, no sites at Isle Royale National Park approached PEC. A study of PAH contamination in the Duluth area of Lake Superior measured total PAHs ranging from 0.290 to 17,000 μg/g TOC (Crane et al., 1997). In other words, PAHs at Isle Royale National Park are very low compared to an industrial marina. While there is some concern regarding elevated PAHs at Rock Harbor, our results suggest that adverse effects of PAHs are generally limited to areas within the immediate vicinity of the marina.

Lower survival of *H. azteca* in sediments from Rock Harbor and Mott Island marinas and reduced abundance of the amphipod *Diporeia* spp. at marinas relative to reference sites support the hypothesis that marina sediments were toxic to macroinvertebrates. Although the results of multivariate analyses showed that macroinvertebrate community composition differed between reference and marina sites, abundance and taxa richness showed no consistent variation between treatments. While patterns of macroinvertebrate abundance, species richness, and community composition are valuable for assessing broad habitat quality, community responses to anthropogenic stressors may be obscured if the habitat is diverse (Hodkinson and Jackson, 2005). This is especially true at locations such as Isle Royale where levels of contamination are relatively low. In these situations an indicator species may better reflect changes in sediment quality. The Great Lakes Water Quality Agreement of 1978 suggested the use of *Diporeia* spp. as an indicator of ecosystem health (GLWQA, 1978), and our results support this general recommendation. *Diporeia* spp. densities were significantly lower at marinas (p < 0.0001), suggesting that conditions were generally unfavorable for these organisms. It is possible that factors other than PAHs may explain spatial variation in *Diporeia* spp. populations. For example, density of *Diporeia* spp. increases with depth in Lake Superior, with maximum densities occurring from 40 to 80 m (Auer et al., 2009). However, at depths more similar to those in our study (<20 m), Auer et al. (2009) did not observe differences among sites. Although our results do not demonstrate that PAHs were the only cause of reduced *Diporeia* spp. abundance at marinas, elevated levels of several highly toxic constituents and the observed toxicity of marina sediments to *Hyalella* are consistent with this explanation.

In addition to their use as an indicator of ecosystem health, *Diporeia* spp. are an important component of the Great Lakes food web. Since 1990, populations of *Diporeia* spp. have been declining or disappearing in Lakes Michigan, Huron, and Ontario. Although populations in Lake Superior have not exhibited the same declining temporal trend (Nalepa et al., 2006), our study did document significantly lower abundance of *Diporeia* spp. associated with the presence of PAHs on a localized scale.

It has been reported that *Hexagenia* can obtain 90% of their contaminant body burden from sediment-associated contaminants (Landrum and Poore, 1988). The distribution of PAHs in mayfly tissue samples generally reflected patterns observed in sediments and was dominated by pyrogenic compounds. BSAF values for most PAHs were considerably less than 1.0, suggesting relatively low bioavailability or that mayflies metabolized these compounds, particularly higher K_{ow} constituents. In contrast to patterns observed for organochlorines (e.g., PCBs, dioxin), there was an inverse relationship between BSAFs and K_{ow} values. Gewurtz et al. (2000) reported similar results for *Hexagenia* collected from Lake Erie (BSAF ranging from 0.1 to 1.0), concluding that mayflies accumulated most of the middle to high K_{ow} compounds from sediments, whereas water was the major route of exposure for lower K_{ow} compounds. Although concentrations of PAHs in *Hexagenia* collected from Washington Harbor were considerably less than those in

sediments, our data indicate that mayflies bioaccumulate PAHs and are therefore capable of transferring these contaminants to higher trophic levels. Furthermore, the inverse relationship between BSAFs and K_{ow} values demonstrate that simple equilibrium models based on hydrophobicity may not be appropriate for predicting uptake and food chain transfer of PAHs (Gewurtz et al., 2000).

Weight-of-evidence approaches such as the sediment quality triad have been employed extensively in quantitative ecological risk assessment and to support causal arguments (Linkov et al., 2009, 2012; Weed, 2005). In this study, we statistically evaluated the measurable difference of each assessment tool (sediment toxicity, sediment chemistry, and benthic community) between paired reference and marina sites. Conclusions were based on a qualitative integration of the three assessments tools and the pattern of measurable differences between marina and reference sites. The use of canonical discriminant analysis to describe the relationship between PAHs and community composition and the comparison of sediment chemistry to an establish threshold effect level to evaluate sediment quality were used to further support the risk assessment.

Conclusions

The observed patterns of increased PAH contamination in sediments, decreased survival of *H. azteca*, reduced abundance of *Diporeia* spp. and bioaccumulation of PAHs by *H. limbata* suggested moderate impairment of benthic communities in marinas at Isle Royale National Park. Levels of PAHs measured in the mayfly *H. limbata* demonstrated that some constituents, especially low molecular weight compounds, were bioavailable to macroinvertebrates. Measuring the effects of PAHs on benthic communities in lakes associated with industrialized or urbanized watersheds is relatively straightforward. However, assessing PAH effects in moderately contaminated sediments collected from remote and relatively undeveloped areas such as Isle Royale National Park is considerably more challenging. A weight-of-evidence approach was necessary to detect deleterious effects of PAHs on benthic communities. Since PAH concentrations at marinas were generally near or below the TEC, measures of sediment contamination alone would have been insufficient to demonstrate potential adverse effects. The addition of benthic community assessments, bioaccumulation analysis, and toxicity tests provided additional information to suggest a link between moderate levels of PAH contamination and responses of macroinvertebrates. Weight-of-evidence approaches such as the sediment quality triad will be useful for evaluating adverse impacts of other anthropogenic stressors in remote ecosystems, especially in situations where contaminant concentrations are low and effects are expected to be relatively subtle.

Acknowledgments

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