

**AN INTEGRATED ASSESSMENT OF POLYCYCLIC AROMATIC
HYDROCARBONS (PAHS) AND BENTHIC MACROINVERTEBRATES OF
ISLE ROYALE NATIONAL PARK, MICHIGAN, USA**

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Isle Royale National Park

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Summary

Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants that are known carcinogens and mutagens. Studies have found that the presence of PAHs at extremely low levels (5-70 parts per trillion) are toxic to many organisms. This research evaluated PAHs from surface sediments and their potential effects on benthic macroinvertebrate communities of Isle Royale National Park, MI, USA, through the use of the sediment quality triad. A weight-of-evidence was produced by contrasting marina locations with reference sites with respect to PAH concentrations in the sediments, toxicity of the sediments, and benthic macroinvertebrate communities to assess pollution-induced degradation. Total concentrations of 39 PAH compounds (Σ PAHs) varied by location; however, marina sites had the greatest levels. The highest Σ PAHs were detected at Rock Harbor Marina, 2438 ng·g⁻¹ in 2003 and 4381 ng·g⁻¹ in 2004. Siskiwit Lake and Washington Creek, background sites, had the lowest concentrations of Σ PAHs (16 and 41 ng·g⁻¹ respectively). All marina locations were influenced by pyrogenic PAH compounds, while the background sites were dominated by petrogenic PAH compounds, indicating that the marina locations have been enriched by anthropogenic inputs of PAHs. Survival of *Hyalella azteca* was negatively correlated with PAH concentration normalized to total organic carbon (Σ PAHnr); however, the only significant difference ($\alpha \leq 0.05$) in survival was between Rock Harbor marina and its reference location. Benthic communities were comprised primarily of *Diporeia* spp. (17.45%), Oligochaeta (15.72%), Cladotanytarsus spp. (15.65%), Sphaeriidae (9.86%), and *Tanytarsus* spp. (9.13%). In 2003, *Diporeia* spp. was statistically ($\alpha = 0.05$) more abundant at the reference sites than the marina sites. In 2004, only Washington Harbor marina had statistically fewer *Diporeia* spp. than its associated reference site. The increased PAH concentrations, decreased survival of *H. azteca* in marina sediments, and reduced abundance of *Diporeia* spp. at the marina sites were consistent with the hypothesis that PAHs impacted these areas. The correlation between Σ PAHnr and *Diporeia* spp. abundance indicated that PAHs are likely influencing the distribution of these organisms. The fact that these patterns were not always statistically significant was not surprising given that PAH concentrations were relatively low and only exceed threshold effect concentrations at Rock Harbor Marina.

We recommend that future monitoring of PAHs in sediments and benthic macroinvertebrate communities should be conducted at marinas and at selected reference locations. This information will be necessary to document potential long-term trends in PAH contamination resulting from Park activities. Because levels of PAHs in sediments at Siskiwit Lake were among the lowest levels reported, this system is an appropriate regional reference site for determination of background PAH concentrations where the only source of contamination is atmospheric deposition.

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INTRODUCTION AND BACKGROUND

Concerns regarding polycyclic aromatic hydrocarbons (PAHs) have risen in recent years due to their carcinogenic and mutagenic effects (DHHS 1990) and their ubiquitous contamination of aquatic systems (Laflamme and Hites 1978; Neff 1979; Eisler 1987; Latimer and Zheng 2003). Studies have found that the presence of PAHs at extremely low levels (5-70 parts per trillion) are toxic to organisms at many trophic levels including zooplankton and fish (Juttner et al. 1995a; Giesy et al. 1983). The U.S. Environmental Protection Agency (EPA) has identified 16 compounds as “priority pollutants” (Table 1). Given their toxicity, carcinogenic and mutagenic potential, and ubiquitous distribution, PAHs are a major environmental concern. This research evaluated the potential degradation of benthic macroinvertebrate communities of Isle Royale National Park, Michigan, USA through the use of the sediment quality triad (Long and Chapman 1985; Chapman 1986; Chapman 1990).

Sediment Quality Triad

In order to identify causal relationships between PAH concentrations and benthic macroinvertebrate communities, an integrated approach is needed (Reice and Wohlenberg 1994). The sediment quality triad (SQT) is an integrated approach that combines the quantification of sediment contamination, toxicology, and *in situ* community composition to develop a weight-of-evidence to assess pollution-induced degradation (Long and Chapman 1985; Chapman 1986; Chapman 1990). Evaluating all aspects of the SQT will demonstrate how communities respond to current abiotic conditions. For example, if there is a shift in community composition and elevated levels of contamination, but no response in the toxicity tests, which would suggest the contaminants are not bioavailable and that the changes in community are due to some other factor.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are a class of persistent organic pollutants with 2-6 fused aromatic rings and molecular weights ranging from 129-278 g/mol. Polycyclic aromatic hydrocarbons result from both natural and anthropogenic sources. Three processes lead to the formation of PAHs: 1) pyrogenic, 2) petrogenic, and 3) diagenetic. Pyrogenic PAHs are a result of incomplete combustion of organic material (e.g., fossil fuel, oils, gasoline, wood, refuse) at high temperatures (~500-2000°C) over a short period of time (Neff 1979) and are distributed on a global scale (Burgess et al. 2003). Pyrogenic PAHs are primarily high molecular weight compounds (4-6 rings) and are known carcinogens (Neff 1979; Eisler 1987). Major anthropogenic sources include the burning of fossil fuels, industry outfall, and emissions from combustion engines. Natural sources include forest fires and volcanic activity (Eisler 1987; Yu 2001). Petrogenic PAHs are derived at low temperatures (~150°C), under high pressure, over geological time (Neff 1979), and lead to the formation of fossil fuels. They are dominated by lower molecular weights compounds (2-3 rings)

and are characterized by their acute toxicity to aquatic organisms (Neff 1979; Eisler 1987). Petrogenic PAHs are usually associated with localized sources (Burgess et al. 2003) and are typically found at background concentrations (Gschwend et al. 1983). Diagenetic PAHs are from biogenic precursors such as plants and terpenes (Hites et al. 1980). Examples include phenanthrene, chrysene, and perylene.

In the environment, PAHs occur as complex mixtures due to the multiple sources and processes of formation. For example, the average 2-stroke motor emits 25-30% of its fuel-oil mixture unburned into the air and water (EPA 1996a). This unburned fraction of the fuel oil mixture would be comprised of petrogenic PAHs and the combusted portion would be dominated by pyrogenic compounds. In aquatic systems, PAHs are partitioned between the water column, sediment, pore water, and organisms. Internal cycling of PAHs in water bodies is a dynamic process dependant on temporal variability, chemical properties, and biological processes (Walker 2001). The density of the water and the molecular weight of PAHs interact to distribute PAHs within the water column (Jeremiason et al. 1998). The chemical properties that control the fate of PAHs in the aquatic environment include solubility and vapor pressure (Neff 1979), both of which decrease as molecular weight increases leading to differential persistence of individual compounds. Polycyclic aromatic hydrocarbons are also known to photo-oxidize. The photo-modified PAHs are more soluble in water and more reactive, resulting in more toxic, bioavailable compounds (Marwood et al. 1999). In addition, some relatively inert PAHs can also be transformed during metabolism to highly toxic, mutagenic, and carcinogenic forms (Eisler 1987).

Sources of PAHs to an aquatic environment include atmospheric deposition, runoff, and direct discharge. Of general concern are mobile compounds ranging in molecular weight from 128 to 300 (Neff 1979). Airborne contaminants associate with dust particles and are transported atmospherically to remote regions where they can contaminate relatively pristine aquatic systems (Quaghebeur et al. 1983; Vilanova et al. 2001). Polycyclic aromatic hydrocarbons can be transported by runoff, through storm water systems, tributaries, and ground water from terrestrial to aquatic systems.

There is a strong positive correlation between urbanization and the level of PAH concentrations in sediments (Neff 1979; Buehler et al. 2001). Elevated PAH levels have been associated with marinas, harbors, fuel docks, and boating activities (Albers 2002; Fang et al. 2003). Outboard motors have been documented as a substantial source of PAHs (Kado et al. 2000) at levels acutely toxic to *Daphnia magna* (Juttner et al. 1995a). Local inputs of PAHs in the waters of Isle Royale National Park may include emissions from motorboat activities, fuel spills, oil spills, and wastewater effluents.

Due to their hydrophobic tendencies, PAHs accumulate to high concentrations in sediments (Neff 1979; Eisler 1987). Studies of sediment cores

from Lake Siskiwit, Isle Royale National Park and the western basin of Lake Superior have revealed increasing PAH contamination from the 1890's to 1980's that were correlated with industrial development (Gschwend and Hites 1981). The fates of PAHs that settle into sediments include burial, ingestion, and desorption. The benthic recycling of 3-4 ring compounds has been demonstrated in Lake Superior; however, large fractions accumulate in sediments (Jeremiason et al. 1998). With increased PAH concentrations in sediments, dietary and pore water exposure to benthos can be substantial in depositional areas such as marinas and harbors.

Uptake of PAHs by benthic macroinvertebrates provides a primary link for contaminants to enter the food web (Menon and Menon 1999; Schuler et al. 2003). It has been demonstrated that benthic macroinvertebrates can bioconcentrate PAHs through dietary or aqueous exposure. Even at low concentrations, PAHs can account for a large fraction of body burden in benthic organisms (Neff 1979; Lamoureux and Brownawell 1999). When concentrations are high organisms can mobilize PAHs, increasing their exposure (Eisler 1987). Selective feeding can further increase dietary exposure. Tissue samples from carp (*Cyprinus carpio*) collected from a polluted lagoon had PAH concentrations in excess of 1000 mg·kg⁻¹ dry weight (Stewart et al. 2003). Landrum et al. (2003) found that concentrations of PAHs in *Diporeia* spp. declined with increased sediment concentrations, most likely a result of changes in the organism's behavior.

Bioaccumulation of Polycyclic Aromatic Hydrocarbons

Bioaccumulation of PAHs depends on an organism's PAH-metabolizing ability, and varies among species. Laboratory studies have demonstrated that bluegill (*Lepomis macrochirus*) and chironomid larvae rapidly biotransformed benzo[a]pyrene and anthracene. In contrast, unionid clams and periphyton did not metabolize these compounds, leading to differences in bioaccumulation (Giesy et al. 1983). Clements et al. (1994) demonstrated that biomagnification of some PAHs does not occur in some aquatic systems. As PAHs move through a food web they are quickly metabolized, resulting in non-detectable concentrations (Burkhard and Lukasewycz 2000). Metabolism of PAHs by organisms can be substantial, resulting in lower concentrations in aquatic systems (Volkerling and Breure 2003). Metabolic activation of PAHs produces intermediate compounds that can covalently bond to macromolecules such as DNA, leading to mutations during DNA replication (Akcha et al. 2003). Elevated levels of PAHs in sediments have been linked to increasing occurrence of tumors in fishes of the Great Lakes and elsewhere (Eisler 1987).

Biota-sediment accumulation factors (BSAF) are used to quantify the passive and active accumulation of PAHs by organisms. The BSAF is the lipid-normalized concentration of a PAH in the organism divided by the organic carbon-normalized concentration in the sediment. Values greater than one would indicate that the compound is being accumulated. Values equal to one

would indicate that the compound is at equilibrium between the organism and the sediments. Values less than one would indicate that the compound is either not bioavailable or the organism is able to metabolize the compound.

Macroinvertebrates as Indicators of Perturbations

Benthic macroinvertebrates are useful for monitoring environmental disturbance given their ubiquitous distribution, sessile nature, large numbers of species, and relatively long life-cycles (Rosenberg and Resh 1993). Measures of biological responses to chemical perturbation reflect integrated exposure. In contrast, chemical measurements only provide a snap-shot in time. The prominent role of benthic macroinvertebrates in food-webs substantiates their relevance as indicators of ecosystem health. Benthic organisms then link sediments to the pelagic region, potentially mobilizing toxins from the sediments to higher trophic levels (Reynoldson 1987). Given that PAHs accumulate to elevated levels in the sediments (Neff 1979; Eisler 1987) and biological effects have been well documented in field and laboratory studies (Payne et al. 2003), benthic macroinvertebrates are useful for assessing ecological impacts.

Community measures can be used to estimate the magnitude of perturbation (Johnson et al. 1993) and provide evidence of differences between impacted and reference locations. Metrics that have been employed include abundance, composition, richness, and indicator species (EPA 1998). Indices that have been used successfully in studies of lakes and reservoirs have relied heavily on chironomids and oligochaetes (EPA 1998). Community level responses are valuable in assessing broad habitat quality because they integrate changes for multiple species; however, community responses may be masked if the habitat is diverse (Hodkinson and Jackson 2005). In such a case, an indicator species may reflect changes in habitat quality. The Great Lakes Water Quality Agreement of 1978 (GLWQA) has suggested the use of *Diporeia* spp (Amphipoda) as an indicator of ecosystem health (IJC 1978).

Toxicity Testing

Invertebrates have a long history in toxicity testing, dating back to the early 1800's (Buikema and Voshell 1993). Toxicity tests are used to evaluate the bioavailability and concentration of chemicals associated with sediments. Invertebrates such as *Hyalella azteca*, *Daphnia magna*, and *Chironomus* spp. have been successfully used in single-species and multi-species toxicity tests of individual PAHs and mixtures of PAHs (e.g., Ingersoll et al. 2001; Schuler et al. 2003). Common endpoints in toxicity tests are survival, growth, and reproduction. The use of toxicity testing provides evidence of a causal relationship between chemicals and macroinvertebrates, as well as concentrations associated with various levels of biological effects. Several researchers have found evidence for additive toxicity of PAHs, thus supporting the use of bulk sediment toxicity tests to assess effects on macroinvertebrates (Landrum et al. 2003; Verrhiest et al. 2001).

Sediment Quality Guidelines

Sediment quality guidelines (SQGs) have been developed for freshwater environments using toxicity tests to help understand and manage associated risks of benthic organisms to PAH exposure (Mount et al. 2003). The approaches for deriving SQGs are based on varying levels of effects and lead to different levels of environmental protection. Mount et al. (2003) describes several different SQGs and the criteria used in their development. Many of the guidelines are developed in similar manners and have subtle differences in their interpretations. In an effort to standardize and simplify SQGs, Swartz (1999) calculated consensus values for PAHs normalized to total organic carbon (TOC) based on the averages of several SQGs. The threshold, median, and extreme effect concentrations for a mixture of PAHs were 290, 1800, and 10,000 $\mu\text{g}\cdot\text{g}^{-1}\text{TOC}$ respectively. Adams et al. (1985) demonstrated that the effective concentration for a given response by an organism varied with substrate composition. When PAH concentrations were normalized to TOC content of the sediments, the relationship between concentration and effect mimic that of interstitial concentrations.

MacDonald et al. (2000) used a similar approach and calculated consensus values based on several freshwater studies. These values are the sum of 10 PAH compounds and represent threshold effect concentrations and median effect concentrations of 161 and 2280 $\mu\text{g}\cdot\text{g}^{-1}\text{TOC}$ respectively. Threshold effect concentration is the concentration at which adverse effects would be expected to occur infrequently. Median effect concentration is the concentration at which adverse effects would be frequently observed.

Research Objectives

This two-year study evaluated the potential deleterious effects of PAHs in sediments of Isle Royale National Park and their potential effects on benthic macroinvertebrate community structure. Since benthic communities are spatially variable, if it can be demonstrated that the ecological integrity of reference sites is consistently greater than impacted sites, then evidence that an impact has occurred is strengthened. The objectives of the study were to: i) determine the spatial distribution of PAHs in sediments from selected marinas and coves; ii) evaluate benthic macroinvertebrate community composition; iii) assess the toxicity of PAHs in whole sediments; iv) identify potential sources of PAHs; and v) evaluate the bioavailability of PAHs in the burrowing mayfly *Hexagenia limbata*. Objectives i, ii, and iv were addressed in both years of the study. Impacted and reference sites were selected based on data collected during the first field season. The third and fourth objectives were addressed in the second year of the project. The first three objectives were integrated into the sediment quality triad to evaluate the impact of PAHs on benthic macroinvertebrate communities of Isle Royale National Park.

METHODS

Study Sites

Isle Royale is the largest island in Lake Superior and is located in the western basin, 35 km from Grand Portage, MN and 90 km from the Upper Peninsula of Michigan. The Island is approximately 72 km in length and 8-13 km in width. Lake Superior is the largest freshwater lake in the world with respect to surface area (82,097 km²) and is oligotrophic and monomictic. Other notable properties include a volume of 12,232 km³, maximum depth of 406 m, and a mean depth of 149 m (Hutchinson 1957). The sediments are comprised of sand and mud deposits with some clay and bedrock (ULRG 1976). Isle Royale National Park is open to visitors from April 16th to October 31st and has approximately 20,000 visitors per year. There are three developed areas within the park: Rock Harbor, Washington Harbor, and Mott Island (Park Headquarters).

Without prior knowledge of PAH levels, sample locations were based on the assumption that PAH levels are directly related to motorboat activity. Areas associated with marinas and fuel docks were assumed to be the most impacted sites. Areas of low motor boat activity, away from the marina, were assumed to have low to intermediate levels of PAHs. Locations with no motorboat activity were sampled to provide background concentrations and represent our best approximation for estimating natural PAH concentrations.

Based on the criteria described above, twelve locations were sampled in 2003, of which six locations (3 marinas and 3 marina references) were sampled again in 2004. There were three impacted sites, three reference locations for the marinas, four additional intermediate sites, and two background sites (Figure 1). The focus of additional sampling in 2003 was to provide information regarding the distribution and concentrations of PAHs. Although benthic data were collected at four of the sites, these results were not considered as part of the SQT analysis.

Sampling locations

Sites considered as potentially impacted were Rock Harbor Marina, Mott Island Marina, and Washington Harbor Marina. Marina reference locations were Rock Harbor Reference, Mott Island Reference, and Washington Harbor Reference. Sites sampled in 2003, but not 2004 were Washington Creek Delta, McCargo Cove Boat Dock, McCargo Cove Reference, and Florence Bay: additional background sites sampled in 2003 included Siskiwit Lake and Washington Creek (Figure 1).

Rock Harbor is a popular arrival and departure point for visitors to Isle Royale National Park. Two ferries (Ranger III and Isle Royale Queen III) dock at Rock Harbor twice a week and a third ferry (Voyageur II), based out of Washington Harbor, circumnavigate the island on a regular basis also stopping at Rock Harbor. Mott Island is the residence for most of the park staff, and the vast

majority of motorboat activity in this area is related to Park operations. Washington Harbor is located at the southwest end of Isle Royale National Park and is a protected cove approximately three km in length. McCargoe Cove, located on the northwest side of the Island, is a long narrow inlet (2.5 km) cutting towards the interior of the Island and is influenced by an outlet from Chickenbone Lake. The cove has a small boat dock that accesses a trail head and several camping locations. Florence Bay, located midway along the northwest side of the Island, is a long finger like inlet (1.5 km), paralleling the Island and sheltered from the prevailing winds and currents of Lake Superior. Siskiwit Lake, in the interior of the island, has little to no history of motorboat activity. Washington Creek, flowing into Washington Harbor, is not accessible to motor boats; however, Washington Creek is possibly influenced by seiches, which alter the direction of the current in Washington Harbor.

Site Characterization

Physical and chemical measures were recorded at each sampling location to characterize the sites. Latitude and longitude were recorded at each sampling station using a Global Positioning Unit (GPS, Model IIIPlus, Garmin, Olathe, Kansas). Water temperature and conductivity were measured with a hand held Thermo Orion model 105 conductivity meter and pH was measured with a hand held Thermo Orion model 210A pH meter. Dissolved oxygen was measured with YSI (model 57) dissolved oxygen meter. Secchi depth and bottom depth were also measured. All measurements were recorded as the average of two readings. All meters were calibrated daily according to manufacturer specifications.

Sediment Collection

Sediments for benthic macroinvertebrates, PAHs and total organic carbon (TOC) were collected in July of 2003 and 2004 with an acetone-rinsed (certified A.C.S. spectranalyzed grade), stainless steel petite Ponar grab sampler (15.24 x 15.24 cm Wildco, Saginaw, MI). Four replicate samples were collected within 100 m² area of the initial sampling location. Two benthic samples from each site had 125 mL from the upper 5 cm of sediment removed for PAH analysis. Samples for PAHs and TOC analyses were removed with an acetone-rinsed stainless steel spatula and placed in 250 mL specially cleaned glass containers with Teflon® lids (Eagle Picher, Miami, OK). Sediment samples were transported on ice in the field and then placed in a freezer upon return to Mott Island at the end of each day. Samples were kept frozen until time of analyses. The remaining two grab samples also had 125 mL of the upper 5 cm removed and discarded so that each of the four benthic samples was comparable. Samples were washed through a 500 µm mesh sieve in the field, stored in a 500 mL polyethylene container, and preserved in 80% ethanol.

In July of 2004 additional sediments were collected for toxicity testing. From each marina and marina reference site 1000 mL of sediment was collected in the same manner as previously described for PAHs. Samples were stored in

pre-cleaned glass jars with no headspace with a Teflon® lid, wrapped in foil, put on ice in the field, and placed in a refrigerator (4°C) upon return to Mott Island. The sediment samples remained refrigerated until preparation for toxicity testing, which began on August 2, 2004.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbon and TOC analyses were conducted by Texas A&M University's Geochemical and Environmental Research Group (GERG). Polycyclic aromatic hydrocarbons (Table 1) were extracted from sediment samples following their standard operating procedure (SOP-9805 1998). Quantitative determination of PAHs by gas chromatography/mass spectrometry followed SOP-9733 (1998). Carbon concentrations were determined on dried sediment using an induction furnace, an infrared detector, and an integrator. Total organic carbon (TOC) was determined on dried sediment samples after acidification (SOP-9730 1998).

Polycyclic aromatic hydrocarbons were evaluated based on total concentration and composition of the compounds. The total concentrations of PAHs (Σ PAHs; Table 1) were compared to evaluate patterns based on our assumption that concentrations are related to motorboat activity. Since individual compounds could contribute differentially to the Σ PAHs, PAH composition was evaluated and concentrations were compared to consensus-based sediment quality guidelines.

Given the hydrophobic nature of PAHs, total organic carbon content of the sediments and PAHs are highly correlated in depositional areas (Boehm and Farrington 1984). The relationship between PAHs and TOC was evaluated with regression, since elevated TOC concentrations in the sediments could influence PAH concentration in the sediments. Deviations from a linear relationship have been interpreted as an indication of PAH enrichment (Bouloubassi and Salot 1993).

To distinguish among sites dominated by pyrogenic PAHs versus petrogenic PAHs in the sediments, a combination of ratios and profile plots were used (Gschwend and Hites 1981; Budzinski et al. 1997; Readman et al. 2002; Yunker et al. 2002). Ratios contrasting phenanthrene to anthracene ($>15 \mu\text{g}\cdot\text{g}^{-1}$), methylphenanthrene to phenanthrene ($>2 \mu\text{g}\cdot\text{g}^{-1}$), fluoranthene to pyrene ($<1 \mu\text{g}\cdot\text{g}^{-1}$), and benzo[a]anthracene to chrysene ($\leq 0.4 \mu\text{g}\cdot\text{g}^{-1}$) indicate petrogenic sources. A more subjective method is to compare the relative abundance of low molecular weight compounds (2 and 3 rings) to high molecular weight (4-6 rings) compounds. Greater relative abundance of low molecular weight PAH is characteristic of petrogenic sources. A third method is to compare the distributions of a parent compound to its non-alkylated derivatives. 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). Sauer and Uhler (1994/1995)

demonstrated that enrichment of the parent compound, chrysene, compared to alkylated homologs (C1, C2, C3, and C4-chrysenes) is a characteristic of pyrogenic sources. Thus, the patterns of chrysene and its alkylated homologs will provide additional evidence of PAH sources. A weight-of-evidence is obtained by using a combination of the above methods.

Toxicity Testing

Toxicity tests followed the EPA's test method 100.1 for *Hyalella azteca* (Amphipoda) 10-day survival and growth test for sediments (Tables 2 and 3; EPA 2000). *Hyalella azteca* is an epibenthic detritivore that burrows into the upper (approximately 5 cm) sediment surface (Hargave 1970). Although, *H. azteca* are present at the Isle Royale National Park study sites, *Diporeia* spp. is the most dominant amphipod in the area. Comparative toxicity tests indicate that *Diporeia* are as sensitive to toxicants as most other amphipods if the tests are conducted for a sufficient length of time (as summarized in Landrum and Nalepa 1998). Substituting *H. azteca* for *Diporeia* in 10-day exposures tests reflect the chronic exposure *Diporeia* experience in the field.

Sediments were collected in July of 2004 in conjunction with the benthic community sampling and sediment collection for PAH analysis as previously described. Sediments from each marina were paired with sediments from reference sites. Thus, there were six treatments (3 marina and 3 reference), replicated eight times for a total of 48 beakers. Water for the tests was collected in 20 L carbuys from Horsetooth Reservoir, Fort Collins, Colorado. Each test chamber contained 10, 14-day old *H. azteca*, obtained from Aquatic Bio Systems (Fort Collins, Colorado). The test chambers were maintained in a low temperature incubator (Precision Scientific, Model 181) at $23 \pm 1^\circ\text{C}$ with a photoperiod of 16 light hours and 8 dark hours.

The sediments collected from each site were prepared for the tests 24 hours prior to the start of the experiment. The four replicate sediment samples were combined in an acetone-rinsed (certified A.C.S. spectranalyzed grade), stainless steel beaker and homogenized with an acetone-rinsed stainless steel paint mixer attached to a handheld, variable speed drill. Caution was taken not to over mix the sediments to avoid separation of the matrix. Clumps of aquatic vegetation and organisms that were found during homogenization were removed. One hundred mL of sediments was added to 600 mL Pyrex® beakers and 200 mL of overlying water was added by pouring the water slowly down the side of the beakers to minimize sediment suspension. The test chambers were then placed randomly into the incubator with the constraint that each treatment occurred on a shelf twice.

In addition to the sediments added to the test chambers, 125 mL of homogenized sediments ($n = 2$) from each treatment were placed in 250 mL specially cleaned glass containers for PAH and TOC quantification. Sediment samples were kept frozen until time of analyses by GERG.

Total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia) was measured in all test chambers on day-zero and day-nine prior to renewal of overlaying water. Temperature and dissolved oxygen were measured in two replicates from each treatment prior to renewal of overlaying water on days 1 - 8 and 10. Overlaying water was changed once daily and each chamber received 1 mL of TetraFin® flake food slurry. Temperature and dissolved oxygen were measured with YSI model 100, conductivity was measured with a hand held Thermo Orion model 105 conductivity meter and pH was measured with a hand held Thermo Orion model 210A pH meter. All instruments were calibrated daily. Ammonia was measured in two randomly chosen replicates from each treatment with Quick Dip® aquarium test strips. Water for hardness and alkalinity tests was pooled from four randomly chosen replicates of each test. Overlaying water was renewed by carefully siphoning off approximately 200 mL of existing water with a glass pipette equipped with a 250 µm mesh screen. Between test chambers all equipment and probes were rinsed with deionized water and inspected for organisms

Test organisms were acclimated to Horsetooth water by a series of dilutions. Over a period of four hours three additions of test water were added to the culture/diluted water. After renewal of overlaying water in the test chambers and acclimation of the test organism, 10 *H. azteca* were randomly assigned to test chambers, as well as eight day-zero archived replicates. The archived *H. azteca* were used to obtain day-zero growth and weight estimates.

After 10 day exposure, surviving *H. azteca* were removed from test chambers by first swirling the beakers to suspend the upper layers of sediment and then pouring the contents through a 350 µm mesh sieve. The sieved sediment was then transferred to a Pyrex® pan with water and the surviving *H. azteca* were collected. The remaining sediment in the chamber was then sieved, transferred to the pan and inspected for surviving organisms. Since there is a possibility that any amphipod recovered at the conclusion of the test could be indigenous, recovered *H. azteca* were identified under a compound scope.

All organisms not recovered at the conclusion of the experiment were assumed deceased. Organisms were dried at 65°C to a constant weight, transferred to a desiccator, brought to room temperature, and weighted to the nearest 0.01 mg to obtain a mean weight per survivor per replicate. The same procedure was followed to obtain day-zero weight estimates.

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 (38.1x25.4x5.1 cm), and covered with tap water. Organisms were sorted from detritus and placed in scintillation vials containing 80% ethanol. Invertebrates were identified under a dissecting microscope (Olympus, Melville, New York SZ11 4.8x to 44x) to the lowest practical taxonomic level (genus for most taxa,

class for oligochaetes, family for mollusks, and phylum for nematodes). Midges were sorted to subfamily and grouped by association (Epler 2000) under the binocular scope. A minimum of 10% from each group was mounted on slides and identified with an Olympus compound scope (40x to 100x). 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: Merritt and Cummins (1996); Thorp and Covich, (2001); Wiggins (1996); Epler (2001).

Bioaccumulation of Polycyclic Aromatic Hydrocarbons

The burrowing mayfly, *Hexagenia limbata* were retained for tissue analysis from sediment samples collected at Washington Harbor Marina in 2004. Polycyclic aromatic hydrocarbon and TOC analyses followed GERG SOP-9730 (1998), SOP-9733 (1998), and SOP-9807 (1998). Forty-eight *H. limbata* were collected and placed in a holding chamber equipped with an aerator and depurated for 16 hours. Zimmerman and Wissing (1978) demonstrated that *H. limbata* held at 15°C required 7.44 ± 0.79 h to eliminate their gut contents. Temperature of the chamber was not monitored; however, chamber water was exchanged for cold water (~11°C) to maintain adequate temperature and oxygen procedure. All of the *H. limbata* collected in the field survived the depuration conditions. 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. The tissue samples remained frozen until they were received by GERG.

Data Analyses

One-way analysis of variance (ANOVA) was used to test for differences in PAH concentrations, toxicity test endpoints, and benthic macroinvertebrate data among sites. Tests for normality (QQ-plots) and homogeneity of variance (Levene's test) were performed to check the assumptions of ANOVA. Variables were transformed when appropriate. If the ANOVA was significant ($p \leq 0.05$), Fisher's F Protected least squares difference (LSD) multiple range test was used to compare marinas to their reference site. Fisher's F Protected LSD was used to protect against false negatives (type II error), something that is of concern when protecting natural resources. Additionally, since the goal was to evaluate each marina with its reference site, the concern was to control the experimentwise error rate without over-controlling the comparisonwise error rate. Spearman correlations were used to investigate the relationships between site characteristics, biotic measures, and toxicity test results. All analyses were conducted using The SAS System 9.2 (SAS Institute Inc. Cary, North Carolina). If not explicitly stated, significant differences are based on $\alpha = 0.05$.

Total PAH concentrations are reported as $\text{ng}\cdot\text{g}^{-1}$ (ppb) of dry weight or ppm when comparing the findings to other studies. When relating PAH concentrations to biota, ΣPAH concentrations were normalized to TOC ($\mu\text{g}\cdot\text{g}^{-1}$ TOC). Principal component analysis (PCA) was used to investigate variation in

PAH composition among sites. Principal component analysis calculates linear combinations of the variables that represent orthogonal indices in order of importance (Manly 2005). Since PAH compositions are highly correlated, PCA should work well to reduce the dimensions of the data (Manly 2005).

Benthic community composition was analyzed to distinguish differences among sites based on community indices (taxa richness, total abundances, Shannon Diversity) and abundance of the amphipod *Diporeia* spp. Indices related to chironomids, including the chironomid genera richness and midge biotic index (MBI), were used as to differentiate among sites. The MBI is based on EPA tolerance values from the upper Midwest (if available) or national values (EPA 1999) and from Bode et al. (1996 and 2002). The MBI was calculated for each sample.

$$\text{MBI} = [\sum (n \cdot t)]/N$$

In this equation, n is the abundance of a given genera, t is the tolerance value, and N is the total number of individuals for that sample. Canonical discriminant analysis (CDA) was used to evaluate differences in community composition between marina and reference sites based on the 15 most abundant taxa. Canonical discriminant analysis generates linear combinations of variables to distinguish among predetermined groups (Manly 2005). The groupings were marina sites ($n = 3$) and marina reference sites ($n = 3$). These analyses were limited to the 15 most abundant taxa.

Polycyclic aromatic hydrocarbon concentrations of the homogenized test sediment were compared to field sediments from the same site with a t -test. The relationship between bulk sediment PAH concentrations and *H. azteca* weight and survival was evaluated by ANOVA.

Biota-sediment accumulation factors (BSAFs) were calculated for *H. limbata* using lipid-normalized and organic carbon-normalized PAH concentrations. The BSAFs were plotted against $\log K_{ow}$ (octanol-water partitioning coefficients) of various polycyclic aromatic hydrocarbons to address the hypothesis that bioaccumulation decreased with hydrophobicity.

RESULTS

Site Characterization

Physical and chemical properties of each sampling site were comparable (Table 4). Mean depth was the most variable, ranging from 1.0 - 10.8 m between years; however, depth was not an important covariate in any of the analyses. Mean water temperature at the Rock Harbor and Mott Island sites were slightly warmer in 2004, while sites at Washington Harbor were cooler. Conductivity was greater at all sites in 2003, ranging from 19.2 - 100.3 μS in 2003 and from 68.3 - 80.1 μS in 2004. Mean dissolved oxygen was greater in 2004 with the exception

of Rock Harbor Reference. Mean pH and TOC were both greater in 2004 than 2003.

Quality Assurance/Quality Control for Sediment and Tissue Analyses

In procedural blanks, no analytes were measured greater than three times the method detection limits. For the duplicate sample, Washington Harbor Marina-1-2003 (site-replicate-year), the average relative percent difference was 10.9% for sediment and 9.8% for tissue, and no individual analytes exceeded quality control. For the duplicate sample (Rock Harbor Marina-1-2004) the average relative percent difference was 10.0% and no individual analytes exceeded quality control. The average recovery for the matrix spike sample (Rock Harbor Marina -1, 2004) for sediment was 105.0% and 80.9% for tissue; however, several individual analytes exceed quality control due to the naturally high concentrations in the sediments. The recovery of analytes with a valid spike met quality control for sediments and tissues.

Polycyclic Aromatic Hydrocarbon Concentrations in Sediments

Total concentrations of 39 PAH compounds (Σ PAHs) extracted from surface sediments varied by location and between years (Figure 2A). Marina locations always had greater PAH concentrations than the associated reference site. Marinas also had greater Σ PAHs in 2004 than 2003. The highest Σ PAHs were detected at Rock Harbor Marina, 2438 ng·g⁻¹ in 2003 and 4381 ng·g⁻¹ in 2004. For each of the marina reference sites the Σ PAHs in year 2004 were less than in year 2003. Total PAH concentrations at the remaining intermediate sites ranged from 99 - 1516 ng·g⁻¹. At the McCargoe Cove boat dock, Σ PAHs measured 1516 ng·g⁻¹, of which 88.9% was the compound perylene. McCargoe Cove reference had lower Σ PAHs (99 ng·g⁻¹). The final intermediate site, Florence Bay also had relatively high concentrations of Σ PAHs (924 ng·g⁻¹). Siskiwit Lake and Washington Creek, background sites, had the lowest concentrations of Σ PAHs (16 and 41 ng·g⁻¹ respectively). Total PAH concentrations with perylene removed (Figure 2B) provided a better understanding of how the sites compared to one another, since perylene is a naturally occurring and highly variable compound. Perylene ranged from 0.32–1348 ng·g⁻¹ and comprised 1.4–88.9% of the total concentration of PAHs at a site.

The first two principal components of the PCA supported the distinction between marina and marina reference sites and that there was little distinction between the marina reference sites and the additional reference locations (Figure 3). The groups separated on the presence of all PAHs (factor score 1). The first factor explained 87.5% and the second factor explained 3.8% of the variability. Once again, Florence Bay was distinguished from the other sites along factor 2 because of high concentrations of C1-Fluoranthenes/Pyrenes.

Total Organic Carbon

The percent TOC in sediment samples was highly variable, ranging from 0.07% at Siskiwit Lake to 2.50% at the McCargoe Cove boat dock. The percent

TOC was greater at marina sites relative to reference sites, especially in 2004 (Figure 4); however, the greatest percent TOC occurred at McCargoe cove Boat Dock and Florence Bay (Table 4). Total PAHs concentrations were significantly correlated with TOC concentrations for background and intermediate sites ($R^2 = 0.64$, $p < 0.0001$), but the marina sites deviated from that relationship (Figure 5).

Source Identification of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbon ratios (Figure 6) were calculated to identify sites influenced by pyrogenic PAHs which are known to be produced at higher rates during the combustion of organic matter. The ratio of phenanthrene to anthracene ranged from 1.60 at Mott Island Reference to 14.03 at Siskiwit Lake. Siskiwit Lake and Washington Creek were the only two locations where the source of PAHs could be considered petrogenic. The remaining sites were dominated by combustion sources of PAHs. The ratio of methylphenanthrene to phenanthrene ranged from 0.08 at Rock Harbor Marina to 0.44 at Florence Bay, indicating that all sites were comprised of pyrogenic PAHs. The ratio of fluoranthene to pyrene ranged from 1.0 at Florence Bay to 1.76 at Washington Harbor Reference, indicating that all sites, with the exception of Florence Bay, were influenced by pyrogenic compounds. The ratio of benzo[a]anthracene to chrysene ranged from 0.53 at Washington Harbor Reference to 1.87 at Florence Bay, indicating that all sites were dominated by pyrogenic compounds.

Profile plots of PAH composition allowed for the comparison of low molecular weight compounds (petrogenic) to high molecular weight compound (pyrogenic). All marina sites and marina reference sites had profiles with greater relative abundances of high molecular weight compounds. In contrast, Siskiwit Lake and Washington Creek both had signatures that were clearly dominated by low molecular weight compounds. The percent of high molecular weight compounds ranged from 67.8 to 74.2% at marina sites and for from 50.7 to 67.0% for reference sites. The percent of high molecular weight compounds at background sites, Siskiwit Lake and Washington Creek, was 19.0 and 10.8%, respectively. McCargoe Cove boat dock and its reference had high molecular weight compounds that were 30.7 and 45.8% of total PAHs, respectively. The percent of high molecular weight compounds at Washington Creek Delta was 51.1%. Florence Bay was also dominated by high molecular weight compounds (66.6%) it lacked the characteristic enrichment of fluoranthene and pyrene as seen in the marina profiles. Additionally, profiles of the chrysene homologous series shows that all of the marina and marina reference sites are dominated by pyrogenic sources (Sauer and Uhler (1994/1995)). In contrast, Siskiwit Lake, Washington Creek, Florence Bay, and both McCargoe Cove sites were dominated by pyrogenic.

Toxicity Testing

Toxicity test conditions were within acceptable ranges for temperature, dissolved oxygen, conductivity, and pH (Table 5; EPA 2000). The average temperature over the duration of the experiment was 22.5°C and the

instantaneous temperatures ranged from 20.0 - 24.4°C. Dissolved oxygen ranged from 3.54 - 7.03 mg·L⁻¹. No measurements of conductivity, hardness, and alkalinity differed by more than 50% from day-0 to day-9. Ammonia remained at safe concentrations (≤ 0.25 mg·L⁻¹) throughout the experiment.

The test sediments had similar concentrations of PAHs to the field sediments with the exception of Washington Harbor Marina (Figure 7A). The total concentration of PAHs for each marina location was significantly greater than the PAH concentrations measured from the respective reference sites ($p < 0.0001$; Figure 7A). Sediment concentrations of PAHs from Washington Harbor Marina used in the toxicity test differed from the PAH concentrations from the field sediments ($p = 0.018$); however, differences in PAH concentrations were minimal when corrected for TOC (Figure 7B). When PAH concentrations were adjusted for TOC, the same relationship between test sediments and field sediments remained ($p \leq 0.0027$; Figure 7B).

Survival of *H. azteca* ranged from 0 to 100 %. Survival in Rock Harbor Marina sediments ranged from 0 - 80% (mean = 30%) where as survival in reference sediments ranged from 20 - 80% (mean = 60%). Mott Harbor also showed the same pattern, with organisms from the marina treatment having a mean survival of 31% (0 - 80%) and the reference sediments had a mean survival of 60% (0 - 100%). In contrast to these results survival of *H. azteca* in Washington Harbor sediments was low 10% for each. Significant differences existed with regards to survival of *H. azteca* among the treatments ($p = 0.0056$; Figure 8). Rock Harbor Marina had significantly lower survival of *H. azteca* than its reference site ($\alpha \leq 0.05$). Growth of organisms in all of the treatments, with the exception of Washington Harbor Reference, was statistically different from the day-0 (Figure 9; $p = 0.0006$) and there was no statistical difference in growth among any of the other treatments. Given the very low survival and the lack of growth of *H. azteca* in the Washington Harbor Reference, it is apparent that the control failed. This treatment was dropped from further analyses.

Benthic Macroinvertebrate Community Composition

A total of 11,700 individuals consisting of 69 taxa collected from 6 sites across years were collected and identified (Appendices IV and V). Average benthic invertebrate densities in 2003 ranged from 763/m² at Washington Harbor Marina to 3025/m² at Rock Harbor Reference (Figure 10). Average benthic invertebrate densities were greater in 2004 and ranged from 1805/m² at Washington Harbor Marina to 4718/m² at Rock Harbor Marina. Benthic communities were comprised primarily of chironomids, oligochaetes, and amphipods. The most common taxa were *Diporeia* spp. (17.45%), Oligochaeta (15.72%), *Cladotanytarsus* spp. (15.65%), Sphaeriidae (9.86%), and *Tanytarsus* spp. (9.13%). There were 10 other taxa that accounted for $\geq 1\%$ of the total abundance. The remaining taxa accounted for an average of 13.9% of total abundance. Coefficients of variation for total abundances varied from year to

year and among sites, ranging from 12.6 to 52.8% in 2003 and from 16.3 to 45.9% in 2004.

Mean oligochaeta abundance was the lowest at Washington Harbor Marina in 2003 and 2004 (115 and 5/m², respectively) and greatest at Rock Harbor Marina in 2003 and 2004 (985 and 1538/m², respectively). Grab samples at Rock Harbor Marina also had the greatest amount of aquatic vegetation. Mean abundance of the indicator species *Diporeia* spp. (Figure 11) was greatest at Mott Island Reference in 2003 (613/m²) and Washington Harbor Reference in 2004 (940/m²), ranging from 4.92 to 38.37%. The fewest *Diporeia* were collected at Washington Harbor Marina in 2003 (mean = 38/m²) and in 2004 (mean = 130/m²). In 2003, *Diporeia* spp. was statistically ($\alpha = 0.05$) less abundant at all marina sites relative its reference site (Figure 11). In 2004, only Washington Harbor Marina had statistically fewer *Diporeia* spp. ($\alpha = 0.05$) than its reference site. Mean Chironomidae abundance was greatest in 2003 at Rock Harbor Reference (1680/m²) and 2004 at Mott Island Marina (2058/m²), while the fewest were recorded at Washington Harbor Marina (330/m²) in 2003 and Rock Harbor Marina (575/m²) in 2004.

Midges accounted for 41.83% of all taxa. Of the 5154 midges, 3063 (59.43%) were mounted. On average, 69.9% (28.6 – 100%) of the midges were mounted and identified per sample. The midge communities consisted of 41 genera in six subfamilies (Chironomini, Diamesinae, Tanypodinae, Tanytarsini, Orthocladiinae, and Prodiamesinae). Rock Harbor Reference had a total of 32 chironomid genera, while Washington Harbor Marina and Mott Island Reference each had 16 genera (Appendices IV and V). The most dominant midge at Rock Harbor Marina was *Pagastiella* spp. (Chironomini), a moderately tolerant midge (EPA 1990; EPA 1999). Rock Harbor Marina also had the greatest abundances of *Cricotopus* (Orthocladiinae), a species considered to be tolerant to organic enrichment (EPA 1990; EPA 1999). *Cladotanytarsus* spp. and *Tanytarsus* spp. were the most dominant midges at all sites, with the exception of Rock Harbor Marina and Washington Harbor Marina in 2003. *Heterotrissocladius* spp., a midge associated with oligotrophic habitats (Johnson et al. 1993) was collected from every site in 2003, but not 2004 (Figure 12) and appears to be more abundant at the reference locations. No mentum deformities were found in any midges. There were instances of worn mentums as well as a few broken teeth; however, these observations were rare and could not be attributed to any particular level of PAH contamination.

There were significant differences in numerical abundance, taxa richness, and midge biotic index among sites ($\alpha \leq 0.05$); however, the directions were opposite of what was expected for each matched pairs of sites. Numerical abundance was greater at the reference sites when compared to their associated marina site in 2003, but that trend was not observed in 2004 (Figure 10). Mean taxa richness ranged from 17.5 at Mott Island Reference in 2004 to a high of 29.8 at Rock Harbor Marina in 2003 (Table 6). The midge biotic index ranged from

6.07 at Rock Harbor Reference in 2003 to 7.03 at Washington Harbor Marina in 2004 (Table 6). Again, the marina sites did not consistently score higher than the paired reference sites. Shannon diversity was significantly different ($\alpha \leq 0.05$) among sites in 2003, but did not differ among sites in 2004 (Table 6). Again, there was no consistent difference between marina sites and their paired reference sites.

Community composition, based on the 15 most abundant taxa, was investigated with canonical discriminant analysis (Figure 13). Marina and reference sites were separated based on the presence of *Pagastiella* spp. in the direction of the marinas and *Diporeia* spp. in the direction of the reference sites. Canonical factor one was significant at $\alpha = 0.05$ ($p < 0.0001$).

Several indices and taxa abundances were correlated with site characteristics (Table 7); however, the variation explained by the correlations was relatively low. *Diporeia* spp. was the only macroinvertebrate metric negatively correlated with Σ PAHs or Σ PAHnr. Taxa richness and Chironomidae genera richness were negatively correlated with TOC and MBI was positively correlated with TOC, suggesting that enrichment was also influencing community composition.

Bioaccumulation of Polycyclic Aromatic Hydrocarbons

The mean body burden of Σ PAHs measured in tissue samples of *H. limbata* was $2355 \text{ ng}\cdot\text{g}^{-1}$ compared to $2581 \text{ ng}\cdot\text{g}^{-1}$ for WH1 sediments. Biota-sediment accumulation factors for lipid-normalized tissue concentrations and organic carbon-normalized sediment concentrations ranged from 0 to 1.0674 (Table 8). Many of the BSAFs were below equilibrium values, with an average BSAF of 0.25. There was a significant inverse relationship between the log BSAF and log K_{ow} values (Figure 14; $p = 0.0002$).

DISCUSSION

The goal of this study was to assess the affect PAHs associated with surface sediments in the marinas of Isle Royale National Park was having on the benthic community. To evaluate this goal three research components (Sediment concentrations of PAHs, toxicity of surface sediments, and community composition) were evaluated. In addition, the sources of PAHs and the bioavailability of the PAHs to burrowing mayfly *Hexagenia limbata* were evaluated. The results of this study indicate that elevated PAHs, from combustion sources, are contributing to the degradation of the benthic community in Rock Harbor and that polycyclic aromatic hydrocarbons derived from sources of combustion (4-6 ring structures) do not bioaccumulate in *Hexagenia limbata*.

Polycyclic Aromatic Hydrocarbons Concentrations in Sediments

Polycyclic aromatic hydrocarbons concentrations in sediments from Isle Royale National Park were significantly greater at all marina sites compared to background and intermediate sites. The greatest PAH concentrations were recorded from Rock Harbor Marina and were at the edge of toxicity. The lowest concentrations measured were from Siskiwit Lake and Washington Creek, sites with no history of motorboat activity. At these two sites many compounds were below method detection limits; suggesting that Siskiwit Lake and Washington Creek provided reliable estimates of background concentrations. In fact, previous studies have used Siskiwit Lake to estimate atmospherically transported PAHs because of its remote location (Gschwend and Hites 1981; McVeety and Hites 1988). Polycyclic aromatic hydrocarbon concentrations were always higher at each marina site relative to the paired reference site. For example, total PAH concentrations were 14 times greater at Rock Harbor Marina compared to the reference site. This large decrease in concentrations indicates that the PAHs are locally associated with the marinas, a finding supported by Johnson et al. (1985) and Marcus et al. (1988). The same trend in PAH decline was observed in McCargoe Cove when comparing the boat dock area with a secondary location in the cove. The decline is presumably due to motorboats since there were no other observed sources of PAHs in that area. The increase PAH concentrations at the marinas cannot be contributed to greater levels of TOC. Bouloubassi and Saliot (1993) found that there was no relationship between PAHs and TOC when the PAHs were derived from pyrogenic sources (Figure 5). In addition to increased PAH sediment concentrations at the marina sites, there is the potential for increased exposure to PAHs by organisms due to increased mobilization by benthic organisms (Clements et al. 1994) and turbulence from motorboat activity (Albers 2002).

It is clear from the sediment analysis that PAH concentrations at marina sites are above background levels and that pyrogenic compounds dominate the PAH composition. The most obvious source of PAHs in this system is the contribution from motorboat engine exhaust. However, since the areas with the highest concentrations of motorboats are also the most developed, one cannot eliminate contributions from other local sources. Polycyclic aromatic hydrocarbon concentrations at Rock Harbor Marina in 2003 and 2004 exceeded threshold effect concentration (MacDonald et al. 2000), which is of concern. A recent study of contaminant hotspots in the Duluth/Superior Harbor area of Lake Superior revealed PAH concentrations in surface sediment ranging from 0.43 to 89 ppm, with 72% having concentrations exceeding lowest effect levels (Crane et al. 1997). Polycyclic aromatic hydrocarbon concentrations in sediment cores from the Milwaukee Harbor Estuary ranged from 17 to 138 ppm on average (Li et al. 1998). In Lake Erie PAH concentrations as high as 8 ppm were measured near Cleveland Ohio (Marvin et al. 2004). While there is concern for the PAH levels at Rock Harbor Marina (4.4 ppm in 2004), the concentration at Siskiwit Lake (0.02 ppm) were less than sediments from other areas of the Great Lakes.

Sediment quality guidelines have been developed for single PAHs as well as mixtures. The EPA's guidelines for acenaphthene, phenanthrene and fluoranthene are 230, 240, 300 $\mu\text{g}\cdot\text{g}^{-1}$ TOC respectively. In our study, concentrations of these compounds (8, 32, and 44 $\mu\text{g}\cdot\text{g}^{-1}$ TOC respectively) at Rock Harbor Marina were well below EPA guidelines. Sediment quality guidelines based on mixtures of PAHs are considerably more conservative. For instance, MacDonald et al. (2000) calculated a consensus-based threshold effect concentration (TEC) of 161 $\mu\text{g}\cdot\text{g}^{-1}$ TOC and median effect concentration (MEC) of 22801 $\mu\text{g}\cdot\text{g}^{-1}$ TOC, incorporating 10 parent PAH compounds. These TEC and MEC values have been used to establish sediment quality targets for the protection of benthic organisms for the St. Louis River Area of Concern (AOC, Crane et al. 2002). The St. Louis River AOC is one of 43 sites for the Great Lakes, designated by the International Joint Commission, because of concerns regarding environmental quality (IJC 1989). Furthermore, Crane et al. (2002) tested the predictability of the Level I and Level II guidelines for the St. Louis AOC, other regions of the Great Lakes, and in North America for 10- to 14-day amphipod toxicity tests and found the predictability of the sediment quality targets to be similar across regions. Crane and MacDonald (2003) also note that the use of the sediment quality targets is most applicable for soft sediments in depositional areas. Thus, adopting MacDonald et al. (2000) as a benchmark for sediment quality is reasonable. When comparing the same 10 PAHs used to establish TEC and MEC, Rock Harbor Marina exceeded the TEC in 2003 (176 $\mu\text{g}\cdot\text{g}^{-1}$ TOC) and 2004 (163 $\mu\text{g}\cdot\text{g}^{-1}$ TOC). Mott Island Marina had the next highest value at 110 $\mu\text{g}\cdot\text{g}^{-1}$ TOC. No sites approached MEC. These results suggest that adverse effects are possible at Rock Harbor Marina, but there is little indication of negative effects due to PAHs for the remaining study sites.

Sources of PAH inputs were evaluated based on the relative concentrations of low and high molecular weight compounds, patterns of non-alkylated and alkylated chrysene, and a series of ratios. The profile plots indicate that pyrogenic sources of PAHs contributed a large portion of the total concentrations at marina sites. This pattern is consistent with the increased levels of motorboat activity at those locations. Although PAH concentrations were lower at marina reference sites, the same profiles were observed, suggesting that those locations were also influenced by pyrogenic PAHs. The ratios of selected compounds tend to indicate that all sites were influenced by pyrogenic compounds with a few exceptions. For example, the ratio of phenanthrene to anthracene indicated that Siskiwit Lake is predominantly influenced by petrogenic sources. This is in agreement with its profile plot. However, the use of the ratios alone is not recommended since they were not able to consistently classify the sites. Zeng and Vista (1997) also noted that the use of ratios to identify sources of PAHs was not reliable for areas with low concentrations of PAHs. Interestingly, based on ratios of phenanthrene/anthracene, phenanthrene/C1-phenanthrene, and benzo(e)pyrene/benzo(a)pyrene, Gschwend and Hites (1981) reported that Siskiwit Lake was dominated by petrogenic sources. In the current study,

inspection of these same ratios indicated an increase in pyrogenic inputs since 1981. Generally, when all of the ratios are taken into consideration simultaneously, it appears that all of the study sites have been influenced by pyrogenic PAHs to various degrees.

Toxicity Testing

The decreased survival of *H. azteca* at Rock Harbor and Mott Island Marinas was positively correlated with the increased PAH concentrations in sediments. Although differences between Mott Island Marina and Reference sites were not statistically significant, survival was 25% lower at the marina. Growth of *H. azteca* was not significantly different among treatments; however, growth as an endpoint is not valid given the differential survival (Gelber et al. 1985). Thus, it was not surprising that there is no relationship between growth and PAH concentration.

The PAH concentrations in the toxicity test sediments were comparable to bulk sediments from the field. However, when Σ PAHs were normalized for TOC, test sediments for the marinas had reduced values. This slight decrease in the toxicity potential of the test sediment may have lead to increased survival or greater variation with respect to the test endpoints. Thus, the true toxicity of the marina sediments may have been underrepresented in the test sediments and therefore survival estimates for the marina treatment should be considered conservative.

The toxicity tests suggest that there was greater toxicity at the marinas compared to reference sites. Those differences were strongest at Rock Harbor (30%) as indicated by the significant difference in *H. azteca* survival ($\alpha \leq 0.05$). The fact that the differences observed between marina sites and reference sites were not always statistically significant is explained, in part, by the moderate levels of contamination at the sites.

The failure of the Washington Harbor control makes it difficult to assess toxicity at that marina. However, based on the very low survival it would appear that the sediment from the Washington Harbor Marina site was toxic to *H. azteca*. The pattern of *H. azteca* survival in the toxicity test was very similar to the pattern of *Diporeia* spp. abundances in the field, suggesting that some unmeasured characteristic of Washington Harbor Marina might have limited the survival of amphipods. One characteristic of the Washington Harbor Marina tests was that the overlaying water remained cloudy throughout the duration of the test. It is possible that the fine silt contributed to the increased mortality; however, it has been noted that *H. azteca* are tolerant to a wide range of particle sizes and organic carbon content (Kemble et al. 2000; EPA 2000). As for the low survival at WH3, it remains unclear as to possible explanations.

Benthic Macroinvertebrate Community Composition

Metrics of community response were not consistent with the hypothesis that PAHs impacted macroinvertebrates at Isle Royale National Park. It was expected that as PAHs increased, taxa richness would decrease. However, taxa richness was greater at Rock Harbor and Mott Island marina sites compared to reference sites. Midge biotic index was always greater at the marina sites compared to their paired reference site and there were no differences among Shannon Diversity values. Of all of the community metrics evaluated none of the patterns were negatively associated with increasing PAH concentrations. Because of the relatively low levels of PAH contamination, community level responses were not negatively correlated with either Σ PAH or Σ PAHnr. Since taxa richness declined and MBI increased with TOC it is likely that the communities were responding to organic enrichment.

At the population level, the indicator species, *Diporeia* spp. was consistently more abundant at reference sites when compared to the paired marina site (Figure 11). This pattern was significant in 2003 and very strong in 2004. No other taxa responded in the same manner across both years. Marzolf (1965) found no relationship between *Diporeia* spp. abundance and substrate size or organic carbon content; however, in laboratory studies *Diporeia* spp. did prefer substrate with enriched surface layers, suggesting that organic enrichment at marina sites should result in greater abundances of *Diporeia* spp. Thus, some other factor limited the success of *Diporeia* spp. at the marina locations.

It is evident that *Diporeia* spp. is responding to differences between the marina and reference sites; however, those differences are not strong enough to produce differences among the various community metrics. Subtle differences in community composition were driven by *Diporeia* spp. as indicated by CDA (Figure 13). *Diporeia* spp. is the most dominant component of the benthic community with respect to abundance and biomass and it is a very important in Lake Superior food webs (Cook and Johnson 1974).

The GLWQA has suggested that *Diporeia* spp. be used as an indicator of ecosystem health, stating that at depth of less than 100 m², densities should range from 220 to 320/m² (IJC 1978). *Diporeia* spp. populations have been declining or extirpated throughout the lower Great Lakes; however, populations in Lake Superior appear to be stable (Scharold et al. 2004). In studies of benthic communities along the Keweenaw Peninsula, Lake Superior, *Diporeia* spp. was the most abundant taxa and accounted for 48% of the invertebrate community (Auer and Kahn 2004). *Diporeia* spp. densities were below that of the GLWQA guidelines at all marina locations in 2003 and at WH1 in 2004. At marina reference locations *Diporeia* spp. exceeded the GLWQA objective across years suggesting that the sediments around marinas were toxic to these organisms.

Bioaccumulation of Polycyclic Aromatic Hydrocarbons

Hexagenia limbata bioaccumulated many PAH compounds, but only concentrated C1-Fluorenes. The relationship among BSAF and log K_{ow} indicated that *H. limbata* were accumulating greater concentrations of the more water soluble compounds. The more hydrophobic compounds may be less bioavailable and therefore do not accumulate. Gewurts et al. (2000) found a similar relationship between *Hexagenia* spp. and PAH accumulation; however, the BSAFs were consistently larger for compounds with log $K_{ow} > 5.0$. In that study, PAH concentrations in sediments and *Hexagenia* were considerably greater than in the present study.

CONCLUSIONS

The result of the sediment quality triad demonstrates that marinas are contaminated and that Rock Harbor Marina is impaired. The observed pattern of increased PAH concentrations, decreased survival of *H. azteca* in toxicity tests, and reduced abundance of *Diporeia* spp. at the marina sites was consistent, although not always statistically significant (Table 9). Although the community composition indices showed no response to PAHs there was modest impairment of the benthic communities in the marinas of Isle Royale National Park. Polycyclic aromatic hydrocarbon concentrations normalized to total organic carbon were negatively correlation with *Diporeia* spp. indicating that PAHs were likely influencing the distribution of these organisms.

A review of the literature indicated that studies of PAH contamination most often evaluated more heavily contaminated sediments, such as, urbanized and industrialized areas, wastewater treatment outfalls, storm-water outfall, coking facilities, petroleum depots, and coal storage sites (e.g., Crane et al. 1997; Zeng and Vista 1997; Li et al. 1998; Ashley and Baker 1999; Carr et al. 2000; Kemble et al. 2000). In contrast, sediment contamination of Isle Royale resulted primarily from motorboat activity. Of the studies that have focused on PAHs in rural and remote areas most have examined atmospheric deposition of PAHs and did not include benthic community or sediment toxicity analysis components (e.g., McVeety and Hites, 1988; Buehler et al. 2001; Vilanova et al. 2001). Thus, the use of the sediment quality triad approach to evaluate PAH contamination by motorboats in a remote setting is unique.

The current study is important because it provides a baseline of PAH concentrations and benthic community composition for areas around the Park with an emphasis on the marinas. This information is critical to evaluating any future impairment (e.g., fuel spills) or shifts in management practices related to motorboat activity. New government regulations for emission standards of gasoline-fueled outboards and inboards have been and are continuing to be implemented (EPA 1996b; EPA 2002; EPA 2004). The new regulations will be fully implemented by 2007 with a goal of reducing hydrocarbon emissions by

70% in the year 2008 (EPA 2002). The typical two-stroke gasoline engine produces hydrocarbons that are an order of magnitude greater than a similar four-stroke gasoline engine (Juttner et al. 1995b; Kado et al. 2000) and diesel engines produce 2.5% of the hydrocarbons of a comparable two-stroke engine (Jemma et al. 1995). Juttner et al. (1995a) determined that the emission of two-stroke gasoline engines into the water were more acutely toxic than emissions from a comparable four-stroke engine to *Daphnia*. Thus, as engine technology is updated to meet EPA standard and the use the engines become more prevalent, PAH concentrations in the sediment and toxicity of those sediments should be reduced. This expectation will not be observed right away since regulations do not apply to existing two-stroke engines. The Park may want to consider ways to facilitate the updating of two-stroke outboards associated with concessionaires at Rock Harbor.

Total organic carbon is an additional factor that affects the toxicity of the sediments. As TOC increased the bioavailability of PAHs is reduced. For example the Σ PAH sediment concentrations at Rock Harbor in 2004 were 1.8 times greater than in 2003 and TOC concentration in the sediment was 1.9 times lower. The result is that the PAH concentrations in 2003 were potentially more toxic. Thus, any efforts to control organic inputs into the water may inadvertently affect the PAH toxicity of the sediment.

Future actions should be taken to monitor PAH levels in sediments of the marinas and selected reference locations. This could be done on a three-five year schedule. It should also be noted that the levels of PAHs in sediments at Siskiwit Lake are some of the lowest recorded levels and might serve as a regional reference site for background PAH concentrations where the only source is atmospheric deposition. This study emphasized how sensitive our aquatic systems are to even low levels of PAH contamination. Efforts should be made to minimize local inputs of PAHs.

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Table 1. List of polycyclic aromatic hydrocarbons (PAH) extracted from surface sediments, Isle Royale National Park, Michigan, USA during, July of 2003 and 2004. United States Environmental Protection Agency priority pollutants are indicated in bold. Asterisks (*) indicate that the compound was not included in the calculation of total PAH concentrations.

Compound	Abbreviation	Compound	Abbreviation
Naphthalene	NAPH	Fluoranthene	FLUORAN
C1-Naphthalenes	C1NAPH	Pyrene	PYRENE
C2-Naphthalenes	C2NAPH	C1-Fluoranthenes/Pyrenes	C1FLPY
C3-Naphthalenes	C3NAPH	Benzo(a)anthracene	BENAAAN
C4-Naphthalenes	C4NAPH	Chrysene	CHRYSEN
Biphenyl	BIPHEN	C1-Chrysenes	C1CHRY
Acenaphthylene	ACENAPL	C2-Chrysenes	C2CHRY
Acenaphthene	ACENAPH	C3-Chrysenes	C3CHRY
Fluorene	FLUOREN	C4-Chrysenes	C4CHRY
C1-Fluorenes	C1FLUOR	Benzo(b)fluoranthene	BENBFLU
C2-Fluorenes	C2FLUOR	Benzo(k)fluoranthene	BENKFLU
C3-Fluorenes	C3FLUOR	Benzo(e)pyrene	BENEPYR
Phenanthrene	PHENAN	Benzo(a)pyrene	BENAPRY
Anthracene	ANTHRAC	Perylene	PERYLENE
C1-Phenanthrenes/Anthracenes	C1PHAN	Indeno(1,2,3-c,d)pyrene	I123CDP
C2-Phenanthrenes/Anthracenes	C2PHAN	Dibenzo(a,h)anthracene	DBAHANT
C3-Phenanthrenes/Anthracenes	C3PHAN	Benzo(g,h,i)perylene	BGHIPER
C4-Phenanthrenes/Anthracenes	C4PHAN	*2-Methylnaphthalene	M2NAPH
Dibenzothiophene	DIBEN	*1-Methylnaphthalene	M1NAPH
C1-Dibenzothiophenes	C1DIBEN	*2,6-Dimethylnaphthalene	D26NAPH
C2-Dibenzothiophenes	C2DIBEN	*1,6,7-Trimethylnaphthalene	T167NAP
C3-Dibenzothiophenes	C3DIBEN	*1-Methylphenanthrene	M1PHEN

Table 2. Schedule of activity for 10-day sediment toxicity tests conducted with *Hyalella azteca*. Based on EPA (2000).

Day	Activity
-1	Added sediments to test chambers and placed chambers into exposure system.
0	Measured water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia). Renewed overlaying water. Acclimated <i>H. azteca</i> to test water. Transferred 10, 14-day old amphipods into each test chamber. Archived 80 <i>H. azteca</i> for dry weight determination. Added 1.0 mL of prepared Tetra-Fin® flake food. Observed behavior of <i>H. azteca</i> .
1 to 8	Measured temperature and dissolved oxygen. Renewed overlaying water. Added 1.0 mL of prepared Tetra-Fin® flake food. Observed behavior of <i>H. azteca</i> .
9	Measured total water quality. Renewed overlaying water. Added 1.0 mL of prepared Tetra-Fin® flake food. Observed behavior of <i>H. azteca</i> .
10	Measured temperature and dissolved oxygen. Ended tests by collecting the <i>H. azteca</i> with a 250 µm sieve. Enumerated survivors and prepared organisms for dry weight measurements.

Table 3. Test conditions for 10-day sediment toxicity test conducted with *Hyalella azteca*. Based on EPA (2000).

Parameter	Conditions
Test type:	Whole-sediment toxicity tests with renewal of overlying water
Temperature:	23 ± 1°C
Light quality:	Wide-spectrum fluorescent lights
Photo period:	16 hours of light and 8 hours of dark
Test Chamber:	600 mL beaker
Sediment volume:	100 mL
Overlying water volume:	200 mL
Renewal of overlying water:	1 volume addition/day
Age of <i>H. azteca</i> :	14-day old at start of the test (2-day range)
Number of <i>H. azteca</i> per chamber:	10
Number of replicates/treatment:	8
Feeding:	1.0 mL of ground Tetra-Fin® flake food slurry
Aeration:	Continuous
Overlying water quality:	Surface water from Horsetooth Reservoir, Fort Collins, Colorado
Test duration	10 days
Endpoints:	Survival and growth (dry weight)

Table 5. Physical and chemical characteristics of toxicity test sediments collected from various sites in Isle Royale National Park, Michigan, USA. Temperature and dissolved oxygen (DO) were measured daily on two replicates. Conductivity (Cond) and pH were measured on two replicates on day-0 and day-9. Hardness (Hrdns) and alkalinity (Alk) were measured by pooling the overlaying water of all 8 replicates on day-0 and again on day-9.

	Temperature (°C)	DO (mg/L)	Cond (µS)	pH	Hrdns (mg/L)	Alk (mg/L)
Site	Mean (Range)	Mean (Range)	d-0/d-9	d-0/d-9	d-0/d-9	d-0/d-9
Rock Harbor Marina	22.4 (20.6-23.3)	6.1 (5.4-6.8)	119/125	7.5/7.9	46/52	45/54
Rock Harbor Reference	22.7 (21.0-23.9)	6.4 (5.4-7.0)	75/111	7.1/7.9	36/46	33/-46
Mott Island Marina	22.8 (21.8-24.1)	6.0 (3.5-6.6)	78/108	7.1/7.9	32/46	37/51
Mott Island Reference	22.6 (20.1-24.4)	6.3 (4.9-6.9)	86/111	7.2/7.9	38/30	39/49
Washington Harbor Marina	22.6 (20.0-24.2)	5.9 (4.9-7.0)	173/168	7.4/8.1	102/86	98/79
Washington Harbor Reference	22.7 (20.7-24.0)	6.1 (5.7-6.8)	76/103	7.4/7.9	30/44	36/42

Table 6. Summary of benthic macroinvertebrate community metrics for surface sediment samples collected from Isle Royale National Park, Michigan, USA, July 2003 and 2004. Lettering represents homogeneous groupings (Fisher's F-protected least significant difference; $\alpha \leq 0.05$).

Metric	Taxa Richness		Shannon Diversity		Midge Biotic Index	
	2003	2004	2003	2004	2003	2004
P value	0.0012	0.0177	0.0081	0.0571	0.0001	0.0001
Rock Harbor Marina	28.5 A	27.8 A	1.07 AB	0.89	6.65 A	6.59 BC
Rock Harbor Reference	29.8 A	18.3 B	1.15 A	0.88	6.07 B	6.08 D
Mott Island Marina	21.8 B	19.8 B	0.88 C	1.01	6.89 A	6.68 BC
Mott Island Reference	21.0 B	17.5 B	0.90 BC	0.85	6.77 A	6.83 AB
Washington Harbor Marina	19.8 B	18.3 B	1.08 A	0.99	6.69 A	7.03 A
Washington Harbor Reference	17.8 B	21.5 B	0.99 BC	0.89	6.08 B	6.44 C

Table 7. Spearman correlations (n = 12) of benthic macroinvertebrate data versus abiotic site characteristics from Isle Royale National Park, Michigan, USA, July 2003 and 2004.

	Total Abundance	Taxa Richness	Shannon Diversity	Chironomidae Richness	Midge Biotic Index	Diporeia	Oligochaeta	Bivalva
ΣPAHs	0.36*	0.30*	0.12	0.24	0.18	-0.16	0.61**	0.60**
ΣPAHnr	0.22	0.35*	0.22	0.29*	0.04	-0.32*	0.61**	0.44**
TOC	0.11	-0.32*	-0.26	-0.36*	0.52**	-0.10	-0.03	0.04
Cond	-0.23	0.15	0.19	0.06	-0.28	-0.07	0.02	0.29*
DO	-0.05	-0.06	0.08	0.09	0.07	0.16	-0.28	0.22
pH	0.08	0.04	-0.48**	-0.30*	0.13	0.34*	-0.07	0.13
Depth	0.35*	0.47**	0.03	0.50**	-0.57**	0.51	0.43*	0.29*

* Statistically significant at $p \leq 0.05$.

** Statistically significant at $p \leq 0.01$.

Table 8. Biota-sediment accumulation factors (BSAF) normalized to lipid-organic carbon for *Hexagenia limbata* and log K_{ow} (octanol-water partitioning coefficients for various polycyclic aromatic hydrocarbons). Sediments and *H. limbata* were collected from Washington Harbor Marina, Isle Royale National Park, Michigan, USA, during July 2004. Means ($n=2$) $\pm$ 1 standard deviation (Stdev)

		<i>Hexagenia limbata</i>			Sediment		BSAF
		log K_{ow} ^a	Mean	Stdev	Mean	Stdev	
Lipid/Organic Carbon Content			0.0876 $\pm$ 0.0055		0.0194 $\pm$ 0.0052		
PAH Compound (ug/g)							
Naphthalene	3.8		0.6964 $\pm$ 0.2702		0.9677 $\pm$ 0.3486		0.7196
C1-Naphthalenes			0.3983 $\pm$ 0.1861		1.5952 $\pm$ 0.6065		0.2497
C2-Naphthalenes			0.4519 $\pm$ 0.1750		1.2879 $\pm$ 0.2186		0.3509
C3-Naphthalenes			0.5291 $\pm$ 0.1133		1.0697 $\pm$ 0.4193		0.4946
C4-Naphthalenes			---	---	0.7192 $\pm$ 0.2499		---
Biphenyl			0.1999 $\pm$ 0.1119		0.2918 $\pm$ 0.1985		0.6849
Acenaphthylene			0.0576 $\pm$ 0.0249		0.8003 $\pm$ 0.1162		0.0720
Acenaphthene			0.1468 $\pm$ 0.0790		0.4284 $\pm$ 0.0594		0.3428
Fluorene	4.2		0.4089 $\pm$ 0.1076		0.8652 $\pm$ 0.0157		0.4726
C1-Fluorenes			1.6318 $\pm$ 0.3255		1.5287 $\pm$ 0.2918		1.0674
C2-Fluorenes			0.8226 $\pm$ 0.7651		1.2351 $\pm$ 0.1599		0.6660
C3-Fluorenes			---	---	2.1091 $\pm$ 0.3235		---
Phenanthrene	4.2		1.5432 $\pm$ 0.1998		4.8337 $\pm$ 0.3650		0.3193
Anthracene	4.1		0.2767 $\pm$ 0.0669		2.8130 $\pm$ 0.1133		0.0983
C1-Phenanthrenes/Anthracenes			1.2722 $\pm$ 0.1881		3.3083 $\pm$ 0.5639		0.3845
C2-Phenanthrenes/Anthracenes			1.1203 $\pm$ 0.0131		2.9184 $\pm$ 0.4830		0.3839
C3-Phenanthrenes/Anthracenes			0.7483 $\pm$ 0.0627		4.0175 $\pm$ 0.6065		0.1863
C4-Phenanthrenes/Anthracenes			---	---	3.7308 $\pm$ 1.6196		---
Dibenzothiophene			0.1291 $\pm$ 0.0205		0.3774 $\pm$ 0.1432		0.3420
C1-Dibenzothiophenes			0.3020 $\pm$ 0.0442		0.5770 $\pm$ 0.2462		0.5234
C2-Dibenzothiophenes			0.5378 $\pm$ 0.0427		0.9530 $\pm$ 0.3278		0.5643
C3-Dibenzothiophenes			0.5119 $\pm$ 0.0914		1.0416 $\pm$ 0.2586		0.4914
Fluoranthene	4.8		4.4376 $\pm$ 1.0245		12.3684 $\pm$ 0.4036		0.3588
Pyrene	4.8		5.5227 $\pm$ 0.7956		11.5581 $\pm$ 0.4109		0.4778
C1-Fluoranthenes/Pyrenes			1.1473 $\pm$ 0.0805		7.1563 $\pm$ 0.9351		0.1603
Benzo(a)anthracene	5.5		0.1279 $\pm$ 0.0212		6.2527 $\pm$ 0.2189		0.0204
Chrysene	5.4		0.7754 $\pm$ 0.0492		6.0773 $\pm$ 0.4218		0.1276
C1-Chrysenes			0.1995 $\pm$ 0.0573		3.7359 $\pm$ 0.0444		0.0534
C2-Chrysenes			---	---	1.3999 $\pm$ 0.0353		---
C3-Chrysenes			---	---	0.4296 $\pm$ 0.0189		---
C4-Chrysenes			---	---	0.0842 $\pm$ 0.0076		---
Benzo(b)fluoranthene	5.8		0.1776 $\pm$ 0.0112		7.9435 $\pm$ 0.0069		0.0224
Benzo(k)fluoranthene			0.0722 $\pm$ 0.0118		2.0751 $\pm$ 0.3475		0.0348
Benzo(e)pyrene			0.2129 $\pm$ 0.0302		3.9376 $\pm$ 0.2452		0.0541
Benzo(a)pyrene	6.1		0.0701 $\pm$ 0.0119		6.8622 $\pm$ 0.3901		0.0102
Perylene			2.5285 $\pm$ 0.1923		16.7120 $\pm$ 7.4614		0.1513
Indeno(1,2,3-c,d)pyrene	6.5		0.0459 $\pm$ 0.0095		4.4546 $\pm$ 0.3206		0.0103
Dibenzo(a,h)anthracene	6.8		---	---	0.9628 $\pm$ 0.1231		---
Benzo(g,h,i)perylene	6.5		0.0743 $\pm$ 0.0185		3.4773 $\pm$ 0.0809		0.0214
2-Methylnaphthalene			0.2418 $\pm$ 0.1285		1.0208 $\pm$ 0.4579		0.2369
1-Methylnaphthalene			0.1565 $\pm$ 0.0608		0.5744 $\pm$ 0.1486		0.2724
2,6-Dimethylnaphthalene			0.0665 $\pm$ 0.0696		0.8129 $\pm$ 0.1137		0.0818
1,6,7-Trimethylnaphthalene			0.1434 $\pm$ 0.1531		0.3707 $\pm$ 0.1220		0.3869
1-Methylphenanthrene			0.3089 $\pm$ 0.0513		0.9865 $\pm$ 0.1421		0.3132

^a Log K_{ow} values (Karickhoff 1981; Mackay 1992).

Table 9. Summary of results from the sediment quality triad contrasting differences in sediment concentrations of polycyclic aromatic hydrocarbon (PAHs), survival of *Hyalella azteca* in 10-day sediment toxicity tests, and benthic community composition for marinas of Isle Royale National Park, Michigan, USA. ++ indicates a statistical difference between the marina and its reference site in the negative direction, + indicates a consistent difference in the negative direction, and – indicates that differences were not observed or they were in the positive direction.

	PAHs		Community Composition		Diporeia spp.		Sediment Toxicity
	2003	2004	2003	2004	2003	2004	2004
Rock Harbor Marina	++ ^a	++ ^a	-	-	++	+	++
Mott Island Marina	++	++	-	-	++	+	+
Washington Harbor Marina	++	++	+	-	++	++	++ ^b

^a Polycyclic aromatic hydrocarbon concentrations exceed threshold effect level.

^b Significantly different from Rock Harbor and Mott Island reference sites.

ISLE ROYALE NATIONAL PARK

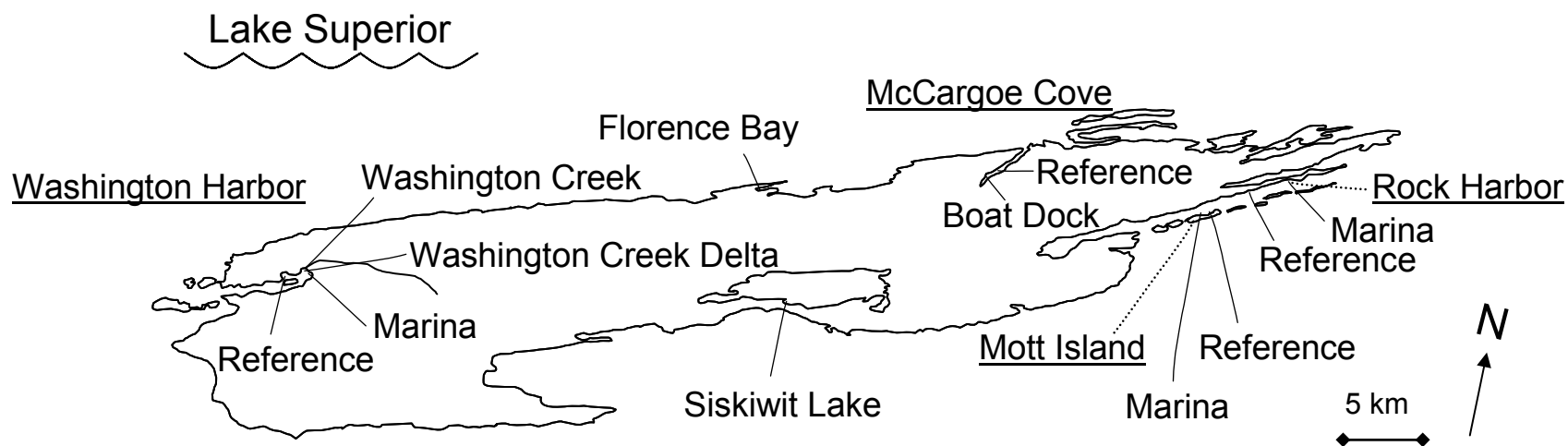


Figure 1. Location of polycyclic aromatic hydrocarbon (PAH) surface sediment samples, collected during July of 2003 and 2004, from Isle Royale National Park, Michigan, USA.

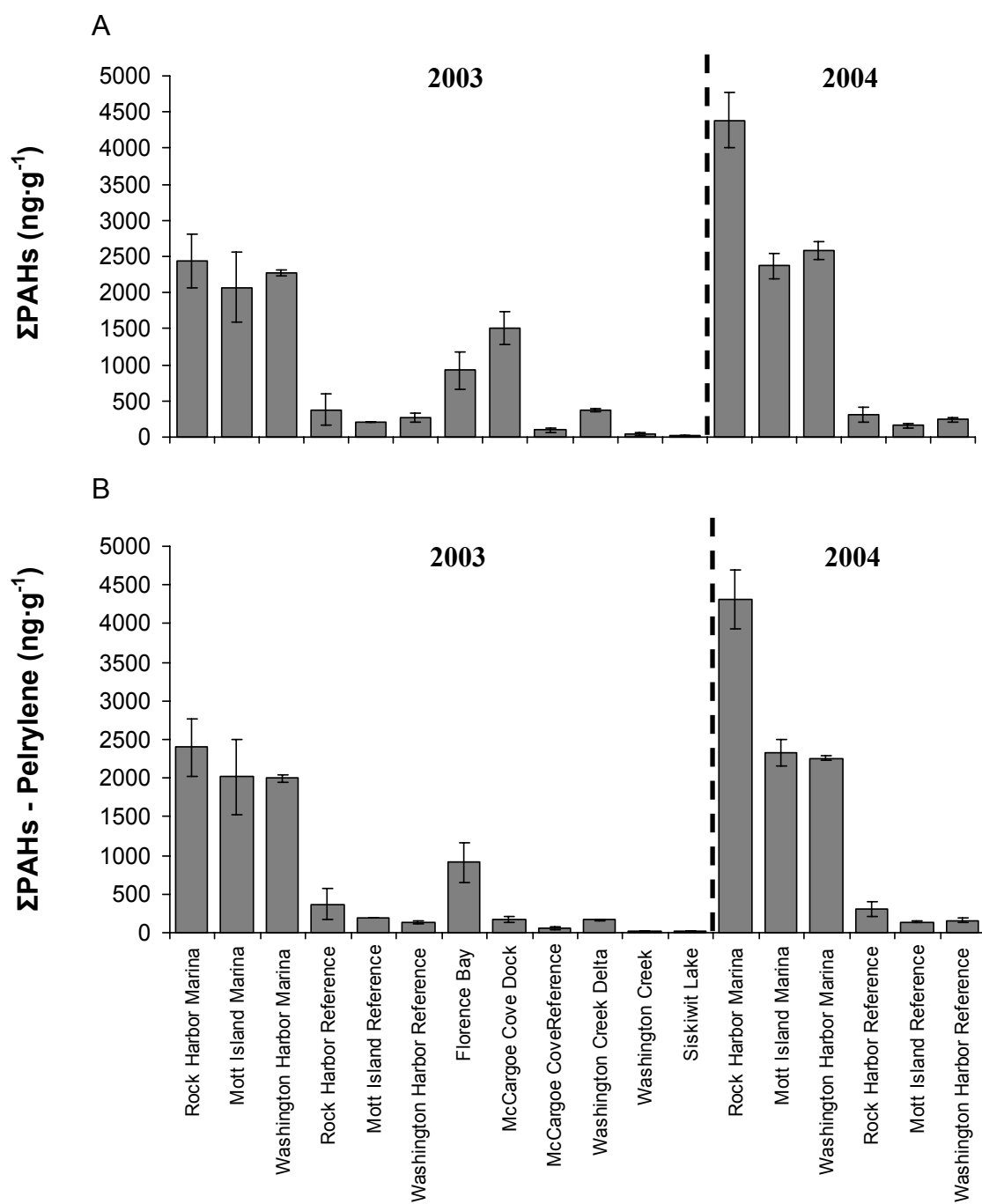


Figure 2. Mean ($n = 2$) concentrations of polycyclic aromatic hydrocarbons (PAHs) with (A) and without (B) the compound perylene from surface sediments, collected during July of 2003 and 2004, from Isle Royale National Park, Michigan, USA. Error-bars represent ± 1 standard error.

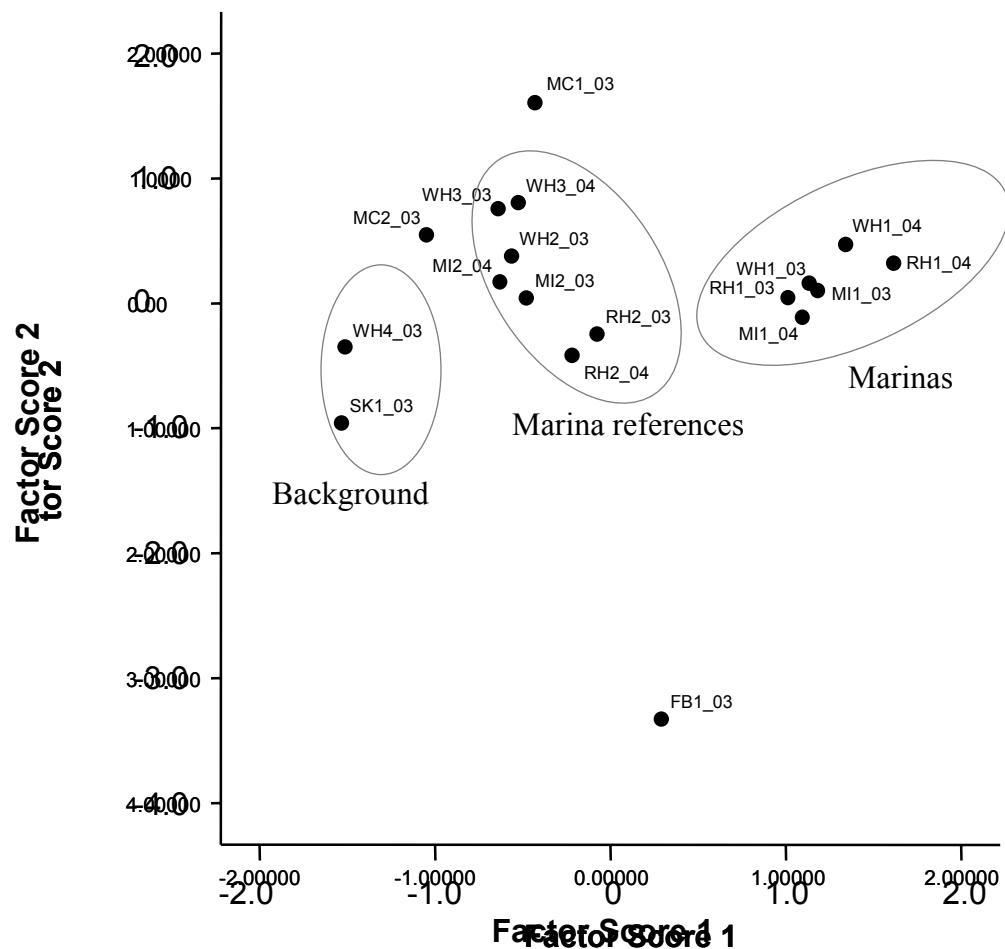


Figure 3. Plot of first and second principal component factor scores of 44 polycyclic aromatic hydrocarbons (PAHs) measured in surface sediments, collected during July of 2003 and 2004, from Isle Royale National Park, Michigan, USA. Factor 1 (presence of all PAHs) explains 87.5% of the variation and factor 2 explains 3.8%. Ovals indicate marina, marina reference, and background sites. Points not included in any ovals were additional site with limited motorboat activity.

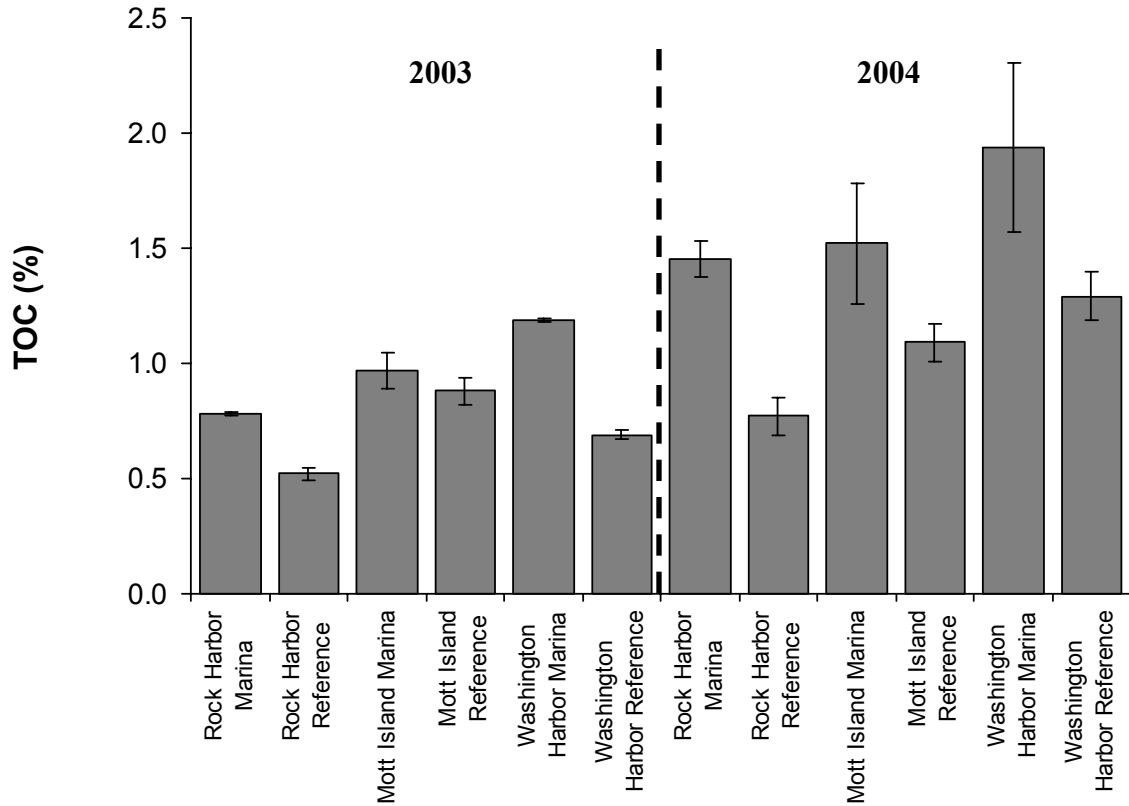


Figure 4. Mean (n=2) percent total organic carbon (TOC) measured from bulk surface sediments. All paired site-by-year combinations are significantly different from each other (Fisher's F-protected least significant difference; $p < 0.0001$). Sediments were collected from Isle Royale National Park, Michigan, USA, during July 2003 and 2004. Error-bars represent ± 1 standard error.

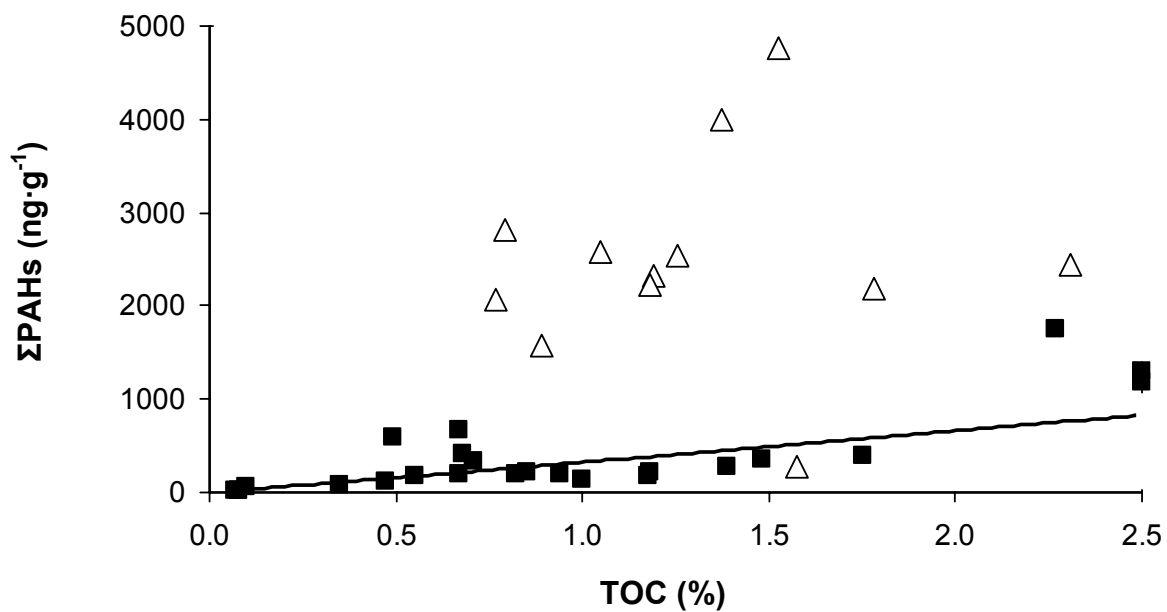


Figure 5. Polycyclic aromatic hydrocarbons (PAHs) and total organic carbon (TOC) measured from surface sediments collected in July of 2003 and 2004 from Isle Royale National Park, Michigan, USA. Squares represent intermediate and background sample locations, considered to be unimpaired, and triangles represent marina locations. The regression line is for the intermediate and background locations only ($R^2 = 0.64$; $p < 0.0001$).

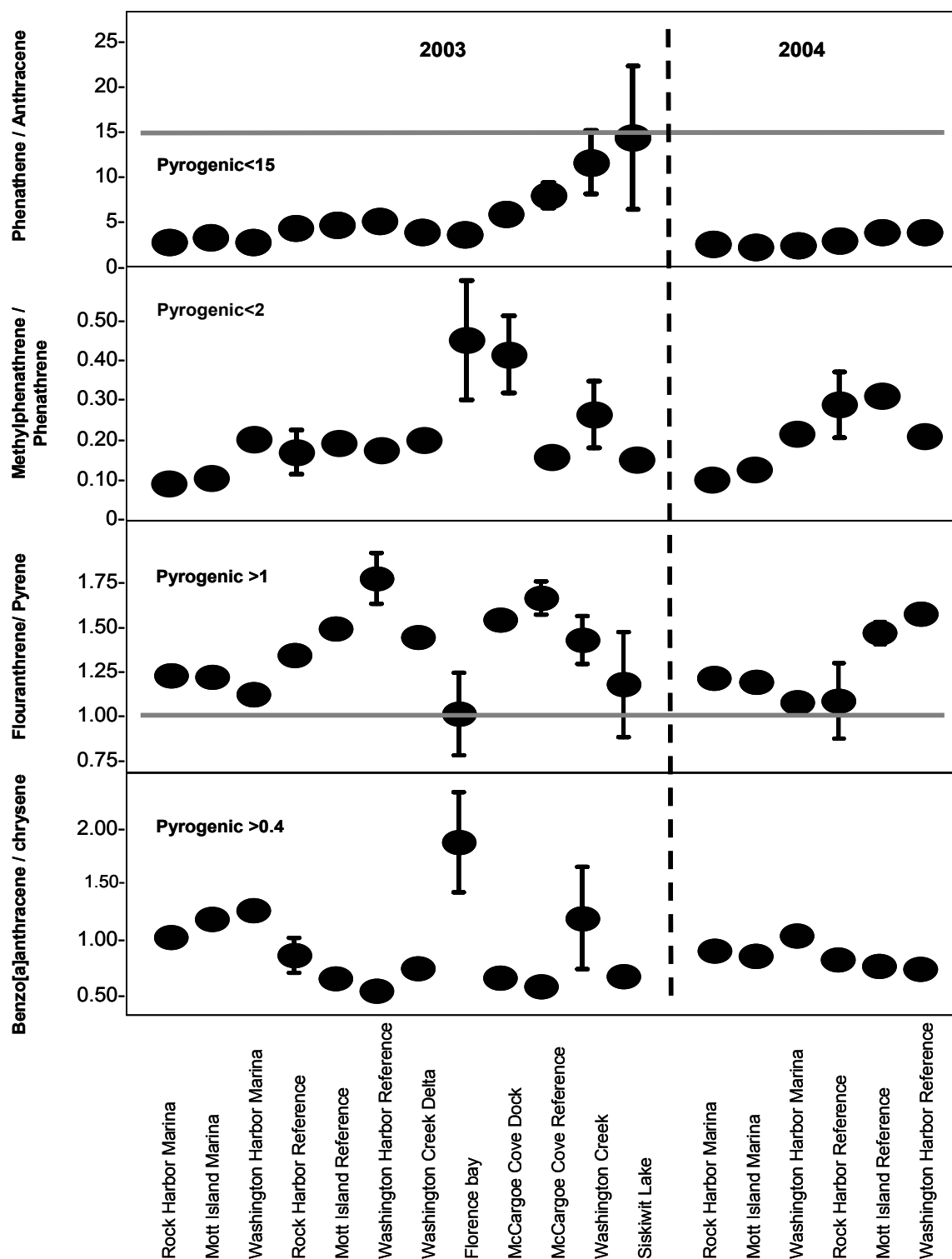


Figure 6. Ratios of selected polycyclic aromatic hydrocarbons (PAHs) measured from surface sediments collected from Isle Royale National Park, Michigan, USA during July of 2003 and 2004. Sites below the indicated cutoff line are considered to be influenced by inputs of pyrogenic PAHs and site above would be dominated by petrogenic PAHs. Error-bars represent ± 1 standard error.

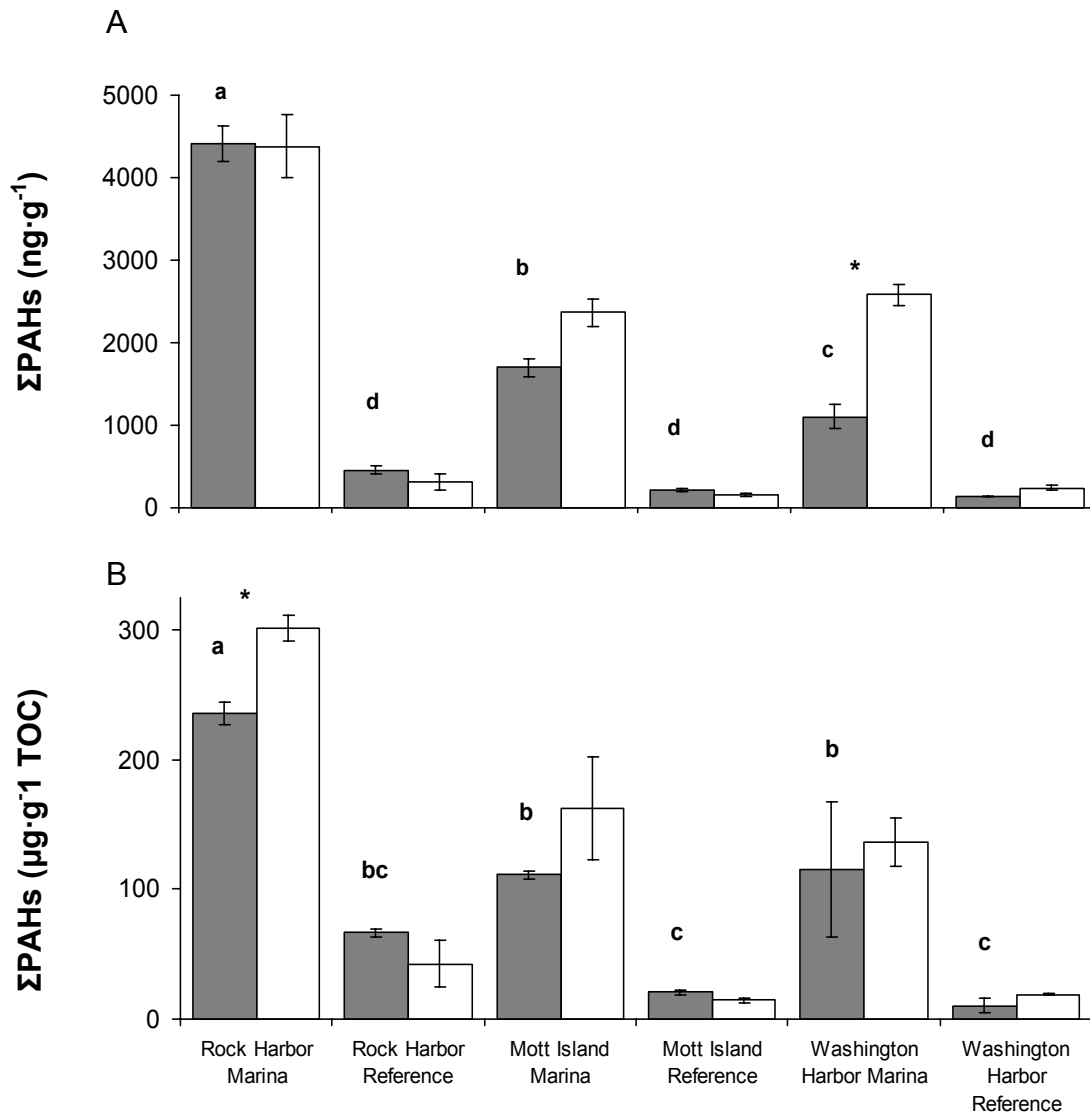


Figure 7. Mean ($n = 2$) concentrations of 39 polycyclic aromatic hydrocarbons (PAHs) before (A) and after (B) adjusting for total organic carbon (TOC). PAHs were measured in homogenized surface sediments used for toxicity tests (grey bars) and in field collected surface sediments (white bars) from Isle Royale National Park, Michigan, USA during July 2004. Surface sediments from each site were sampled simultaneously. Asterisks indicate a significant difference was detected in PAH concentrations for between the toxicity test sediments and the field sediments and lettering above grey bars indicates homogeneous groups based on Fisher's F-protected least significant difference ($\alpha \leq 0.05$, $p < 0.0027$). Error-bars represent ± 1 standard error.

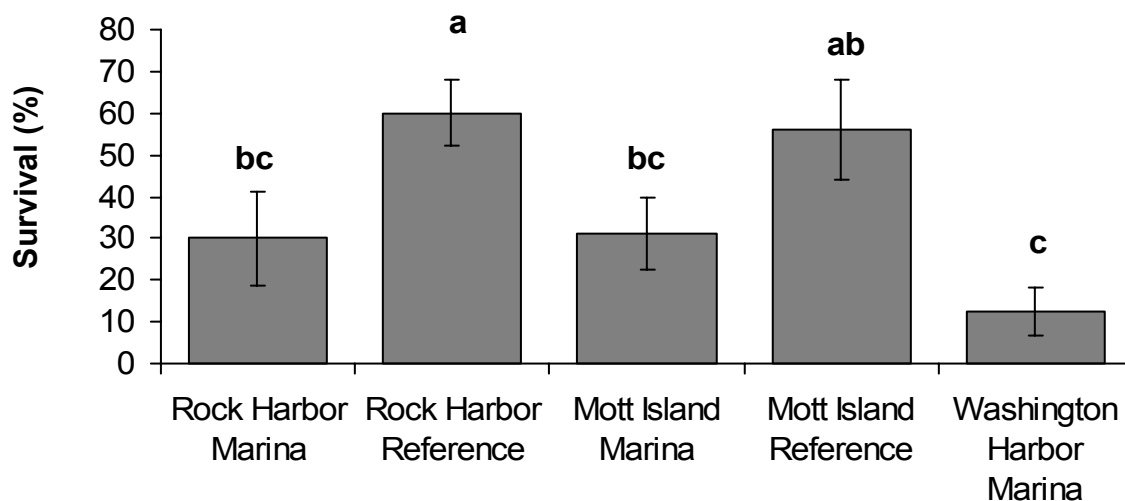


Figure 8. Mean percent survival of *Hyalella azteca* from 10-day sediment toxicity test (ANOVA $p < 0.0056$). Sediments were collected during July of 2004 from Isle Royale National Park, Michigan, USA. Lettering above columns indicates homogeneous groups based on Fisher's F-protected least significant difference ($\alpha \leq 0.05$). Error-bars represent ± 1 standard error.

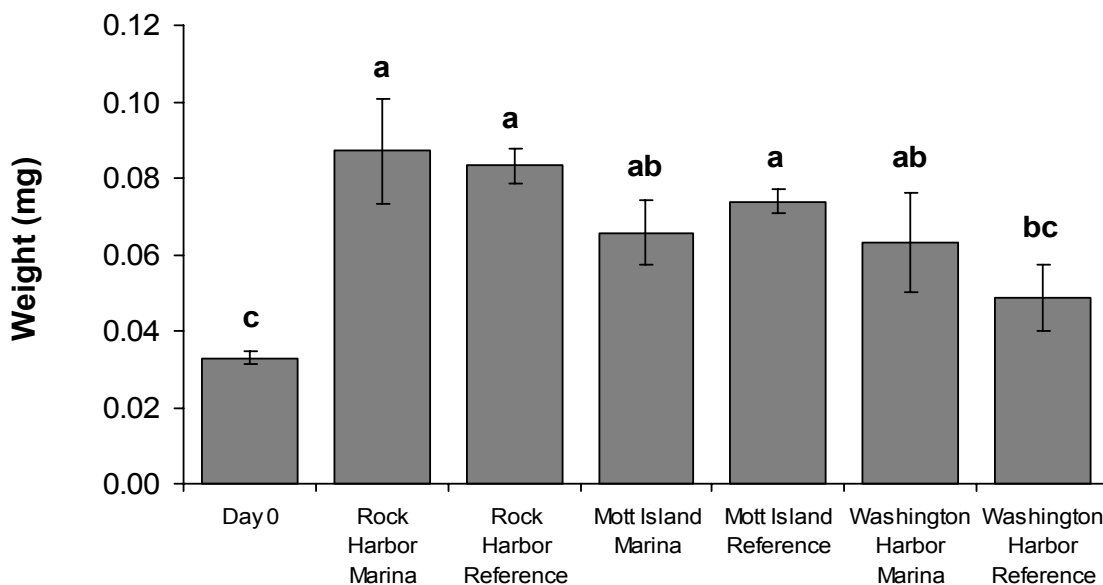


Figure 9. Weight (mg) of *Hyalella azteca* during 10-day sediment toxicity tests (ANOVA $p < 0.0006$). Sediments were collected during July of 2004 from Isle Royale National Park, Michigan, USA. Lettering above columns indicates homogeneous groups based on Fisher's F-protected least significant difference ($\alpha \leq 0.05$). Error-bars represent ± 1 standard error.

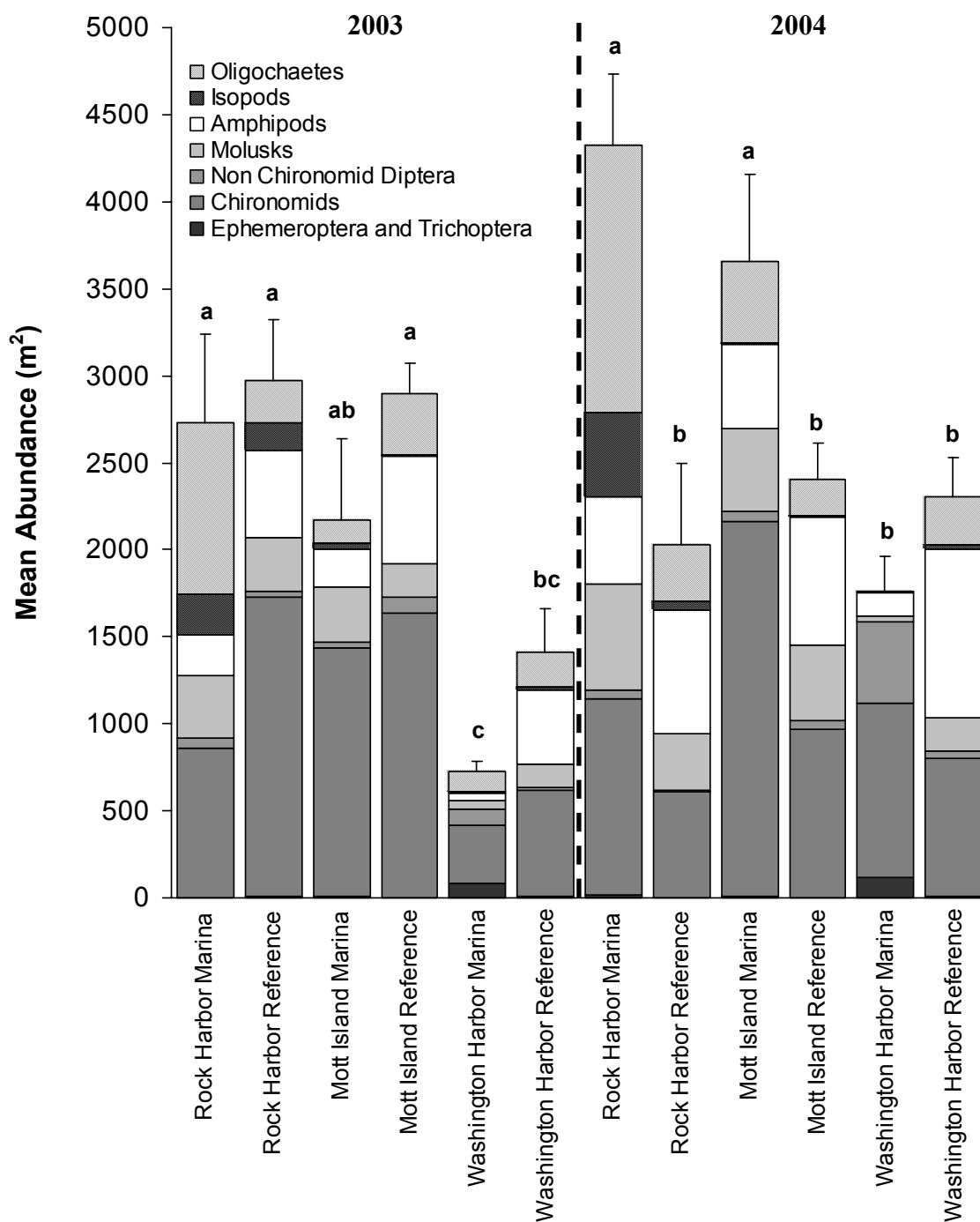


Figure 10. Community composition of benthic macroinvertebrates collected in Ponar samples, during July 2003 and 2004, from Isle Royale National Park, Michigan, USA. (n = 4). Lettering above columns indicates homogeneous groups with respect to total abundance, based on Fisher's F-protected least significant difference ($\alpha \leq 0.05$). Error-bars represent 1 standard error for total abundance.

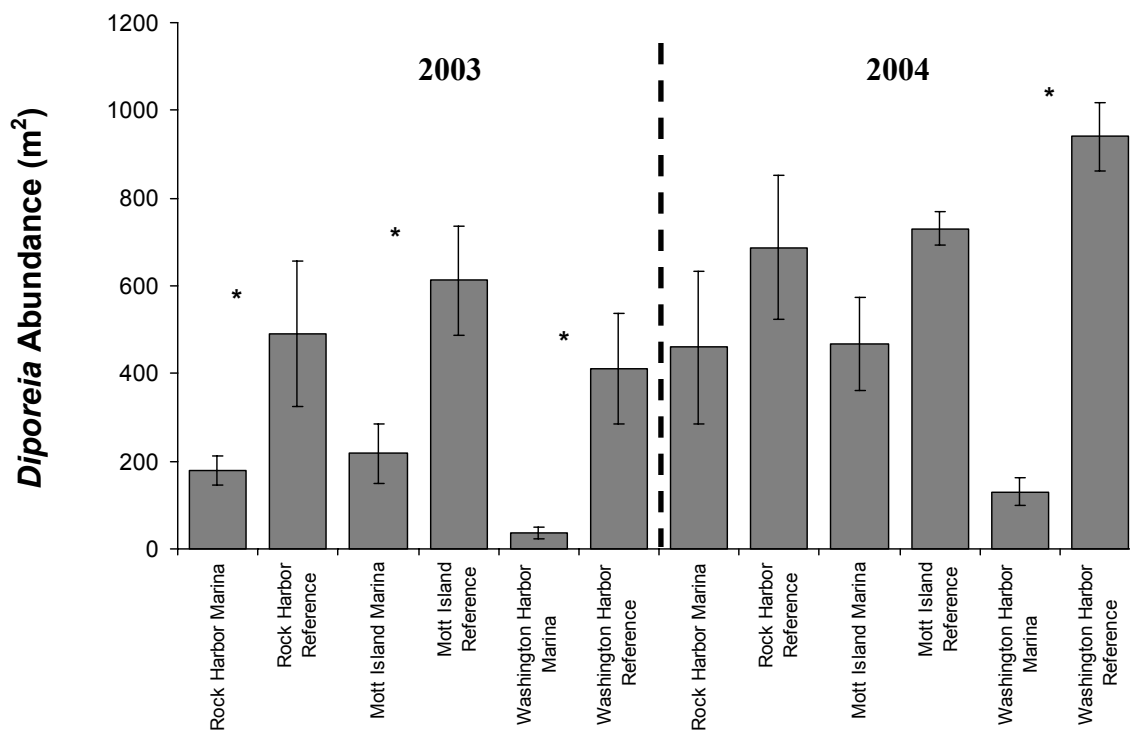


Figure 11. Mean ($n = 4$) abundance of the amphipod, *Diporeia* spp. in Ponar samples from Isle Royale National Park, Michigan, USA. Asterisks indicate a significant difference ($\alpha \leq 0.05$) between the Marina and Reference sites within years based on Fisher's F-protected least significant difference. Error-bars represent ± 1 standard error.

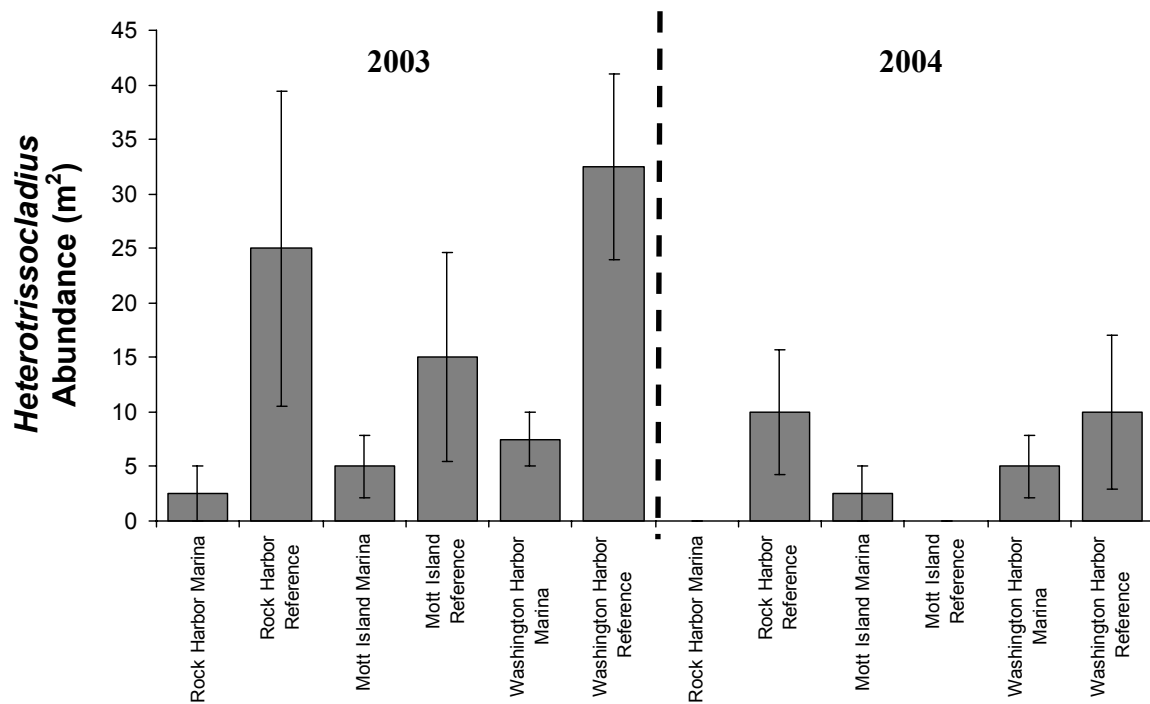


Figure 12. Mean ($n = 4$) abundance of chironomid, *Heterotrissocladius* spp. in Ponar samples from Isle Royale National Park, Michigan, USA. Error-bars represent ± 1 standard error.

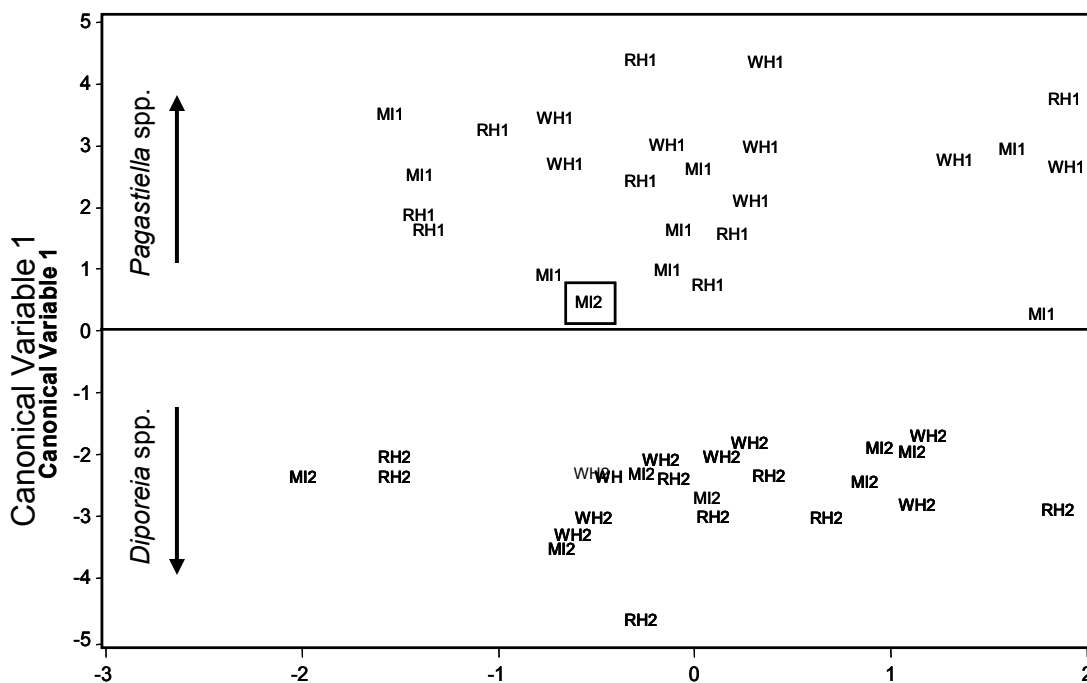


Figure 13. Canonical discriminant function analysis distinguished marina (RH1, MI1, and WH1) and reference (RH2, MI2, and WH2) sites based on the presence of *Diporeia* spp. in the direction of reference sites and *Pagastrella* spp. in the direction of marina sites. Canonical variable 1 is significant ($p < 0.0001$). Data are from benthic macroinvertebrate samples collected from Isle Royale National Park, MI, USA, during July of 2003 and 2004.

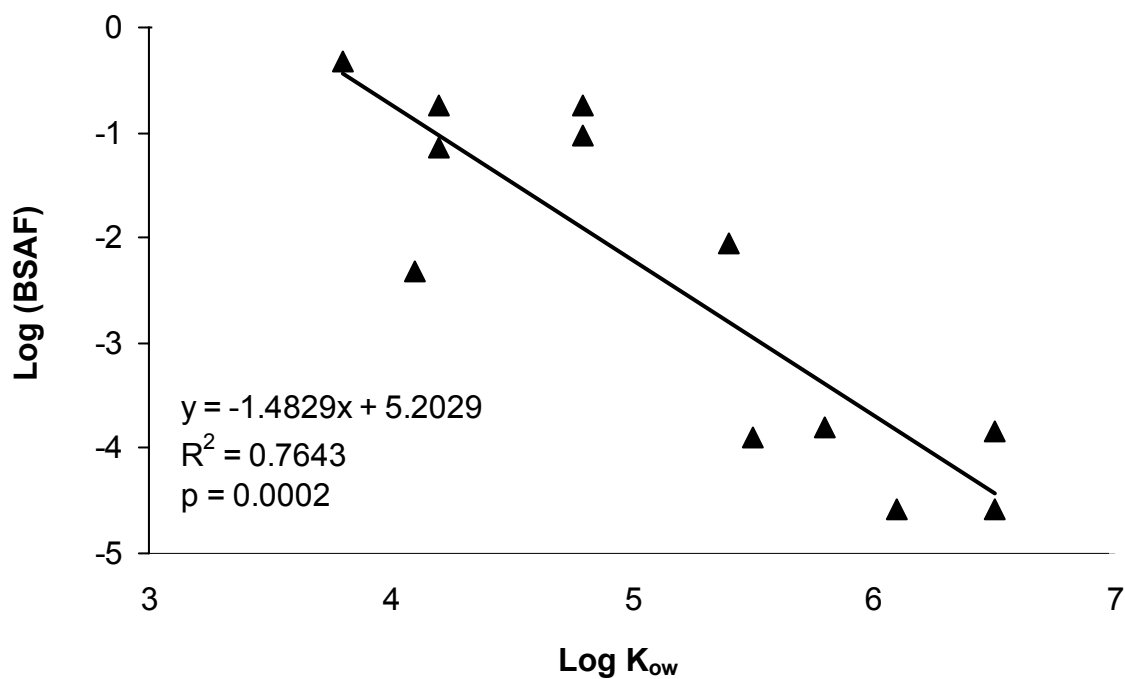


Figure 14. Relationship between $\log k_{ow}$ (octanol-water partitioning coefficients for various polycyclic aromatic hydrocarbons) and lipid-organic carbon normalized biota-sediment accumulation factors (BSAF). Bulk sediments and *Hexagenia limbata* (Ephemeroptera) were collected July 2004 from Washington Harbor Marina, Isle Royale National Park, Michigan, USA.