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Assessment of the Impact of Highway Runoff on Freshwater Mussels in North Carolina Streams

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16. Abstract

The goal of this study was to assess the effects of road runoff on freshwater mussels in North Carolina streams. We conducted our studies at 20 road crossings in the upper Neuse River Basin above Falls Lake as the study area. Using GIS, we selected 9 agricultural sites and 10 forested sites based on EPA landuse data. A 20th site was selected because of its urban nature and ongoing construction at the site. We surveyed mussels in the 300-meter reaches upstream and downstream of each of these crossings. We used the analysis of hemolymph obtained from the common mussel species *Elliptio complanata* as a non-lethal health assessment technique for studying the health of individual mussels upstream and downstream of these road crossings. Hemolymph analysis was also used to compare agricultural and forested sites. This project was the first field test of this hemolymph technique, and the forested sites were used to develop reference ranges for the various parameters evaluated in *E. complanata* hemolymph. Other health assessments included glycogen analysis, evaluation of the percent of gravid mussels at a site, and presence of parasites. Contaminants were measured in mussel tissue, sediment, and in Passive Sampling Devices (PSDs) deployed at each site.

There tended to be fewer mussels in the first 50 meters downstream of the road crossings; however, there were no differences when the entire 300-meter upstream and downstream reaches were considered. There was no difference in health parameters measured by hemolymph analysis between upstream and downstream mussels. Hemolymph glucose and calcium were significantly different between agricultural and forested sites. Hemolymph reference ranges are presented in this report. Contaminant analyses showed an increase in polycyclic aromatic hydrocarbons (PAHs), and some metals downstream of all road crossings at some sites. This appeared to be directly related to the number of vehicles crossing the bridges. There was, however, no direct correlation between increasing contaminant loads and decreasing mussel abundance. There were no noteworthy differences in contaminant loads between land use types. Passive sampling devices proved to be excellent surrogates for the direct measurement of PAHs in mussel tissue.

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Acknowledgments

Support for this project was provided by the U.S. Department of Transportation and the North Carolina Department of Transportation through the Center for Transportation and the Environment, NC State University. Tim Savidge and other members of the Project Development and Environmental Analysis group of NCDOT provided valuable guidance and advice to direct the study. Tim also provided help in teaching field staff to identify certain species of freshwater mussels. We thank John Alderman on the NCDOT and Judy Ratcliffe of the North Carolina Wildlife Resources Commission for their suggestions, support and assistance in designing the study. For long days spent in the field and lab, we thank Heather Boyette, Leroy Humphries, Chris Wood, April Lee, John Holland, and Shane Hanlon.

Executive Summary

The goal of this study was to assess the effects of road runoff on freshwater mussels in North Carolina streams. We 20 road crossings in the upper Neuse River Basin above Falls Lake as the study area. Using GIS, we selected 9 agricultural sites and 10 forested sites based on EPA landuse data. A 20th site was selected because of its urban nature and ongoing construction at the site. We surveyed mussels in the 300-meter reaches upstream and downstream of each of these crossings. We used hemolymph of the common mussel species *Elliptio complanata* as a non-lethal health assessment of individual mussels upstream and downstream of these road crossings. We used this technique not only to compare upstream and downstream of road crossings but also between agricultural and forested sites. This project was the first field test of this hemolymph technique, and the forested sites were used to develop reference ranges for the various parameters evaluated in the *E. complanata* hemolymph. Other health assessments included glycogen analysis, evaluation of the percent of gravid mussels at a site, and presence of parasites in the mussel. Contaminants were measured in mussel tissue, sediment, and in Passive Sampling Devices (PSDs) deployed at each site.

There tended to be fewer mussels in the first 50 meters downstream of the road crossings; however, there were no differences when the entire 300-meter upstream and downstream reaches were considered. Health evaluations showed no difference between upstream and downstream mussels. Hemolymph glucose and calcium were significantly different between agricultural and forested sites. Hemolymph reference ranges are presented in this report. Contaminant analyses showed an increase in polycyclic aromatic hydrocarbons (PAHs), and some metals downstream of all road crossings. This appeared to be directly related to the number of vehicles crossing the bridges. There was, however, no direct correlation between increasing contaminant loads and decreasing mussel abundance. There were no noteworthy differences in contaminant loads between land use types. Also PSDs proved to be excellent surrogates for the direct measurement of PAHs in mussel tissue.

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Introduction

Transportation agencies promote economic growth through infrastructure development. When road and bridge construction is proposed, an environmental impact assessment is conducted to determine the potential threat that a given project has on sensitive species or ecological areas. Wildlife agencies are especially concerned when construction of road-crossings over streams is proposed because of the variety of adverse effects those activities can have on the aquatic environment. Sedimentation, channelization, and stream bank modifications are potential products of bridge and culvert construction that can be detrimental to local aquatic fauna (Little and Mayer 1993; Forman and Alexander 1998).

Unionids are among the most endangered groups of animals in North America. About 67% of the nearly 300 freshwater mussel species found in North America are considered vulnerable to extinction or already extinct (Bogan 1993; Williams et al. 1993). The decline of mussel populations in North America has occurred steadily since the mid 1800s and has been attributed to construction of dams and impoundments, sedimentation, navigation, pollution, and habitat degradation (Fuller 1974; Bogan 1993; Neves 1997; Brim Box and Mossa 1999; Vaughn and Taylor 1999). The surface waters of North Carolina have historically supported 56 species of unionid mussels (Bogan 2002). Today, 82% of these species are listed as endangered, threatened, or of special concern by the U.S. Fish and Wildlife Service or the State of North Carolina (Code of Federal Regulations 1993; NC Wildlife Resources Commission 2002); several are already extinct. Many of the same human-mediated and environmental factors responsible for the declines of freshwater mussels throughout North America have also contributed to the declines in North Carolina's 17 river basins.

Short-term effects of bridge and culvert construction activities have been documented to impact stream insects (Ogbeibu and Victor 1989) and fish (Barton 1977). Sedimentation, a potential consequence of bridge construction, has been shown to be detrimental to mussel populations (Ellis 1936, Marking and Bills 1979). However, the long-term effect of road-crossings on mussels is poorly documented. During construction storm events can flush construction-related sediments from a site (Taylor and Roff 1986) into adjacent streams. After construction a crossing structure remains in place and serves as a conduit for the movement of road runoff from road surfaces into surface waters, and runoff from paved surfaces has been associated with freshwater mussel declines (Williams et al. 1993).

The goal of this study was to assess the impact of road runoff on mussel populations. Specific objectives were to:

1) **identify the contaminants in road runoff that are entering NC streams,**
2) **develop non-lethal field sampling techniques for assessing the health of freshwater mussel populations, and**
3) **measure the potential impact of contaminants in road runoff on mussel health**

Chapter 1

Study Area

Selection and Description of Study Area

The study area for this project was a subset of sites concurrently sampled to measure the effect of crossing structures on freshwater mussel abundance (HWY-2001-10). In this manner, the study team ensured the relevance of study findings to the broader general concern of mussel population declines. We chose a study area with viable mussel populations and relatively good habitat and water quality to focus our evaluation on the potential effect of road crossings on mussel fauna in exclusion of other factors that could potentially contribute to their decline. In coordination with NCDOT and NCWRC biologists, two areas of the North Carolina piedmont were chosen that met our criteria. To minimize species differences between sites, we kept all sites in the same sub-basin. Areas with federally endangered species were eliminated to avoid damaging sensitive habitats and to avoid the need for special federal permits. Areas with the highest water quality were identified using Basinwide Water Quality Plan of the Neuse River basin (NCDENR 1998). Land use and land cover data for the Neuse River basin were obtained from the Environmental Protection Agency's (EPA) Neuse River Land Use/Land Cover GIS layer. The 30 m resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from October 1998 to March 1999 (EPA, 2000). The chosen study area was in the Neuse River basin and drains into the upper portions of Falls Lake (Fig. 1.1).

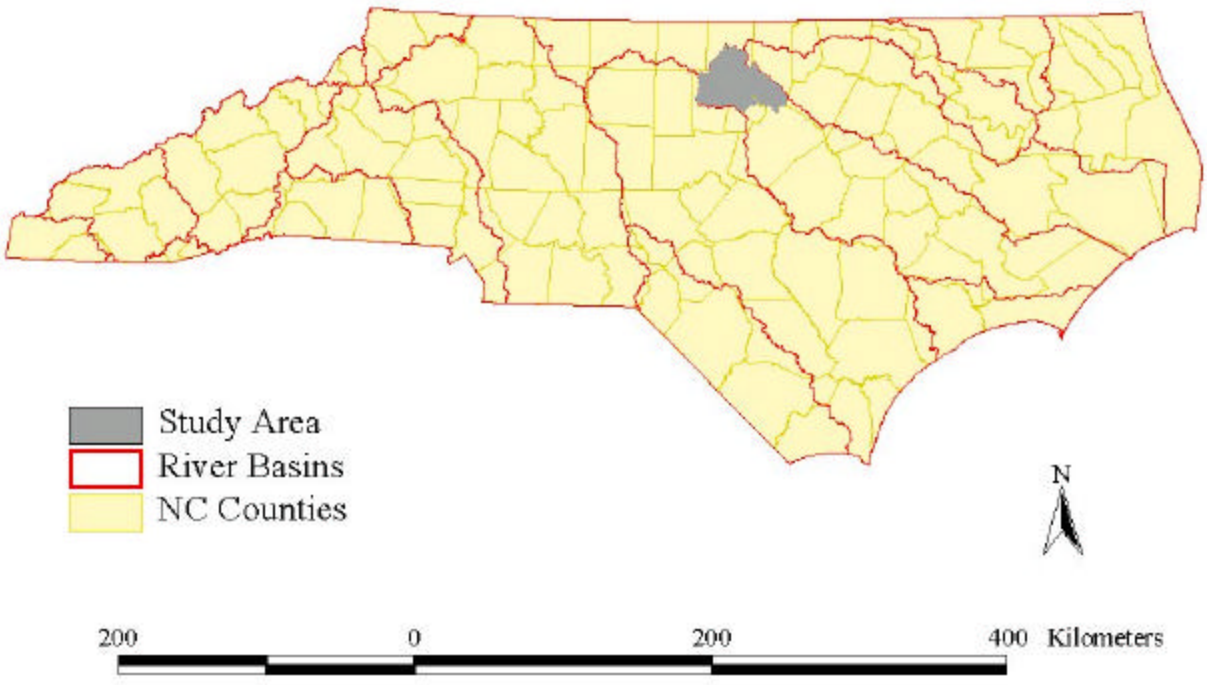


Figure 1.1. *The location of project study area in the Neuse River basin in North Carolina.*

This region is 1685.65 km^2 in area and covers portions of Orange, Durham, Person, Granville, and Wake Counties in North Carolina. The main drainages in the area are the Eno, Little, and Flat River watersheds, but several other smaller watersheds feed directly into Falls Lake from Granville and Wake Counties. The geology in this area results in variety of stream types from rocky to sandy, so a variety of stream channel types are represented in this relatively small portion of the piedmont. Durham, Hillsboro, Creedmoor and Butner are the primary municipalities in the region with Durham being the largest. The dominant land uses within the subbasin included forested (61%), urban (16%), and agriculture (18%). Various wetland types comprised 4% of the land cover, and other land uses (0.2%) were combined and consisted of barren and herbaceous cover types (Figure 1.2).

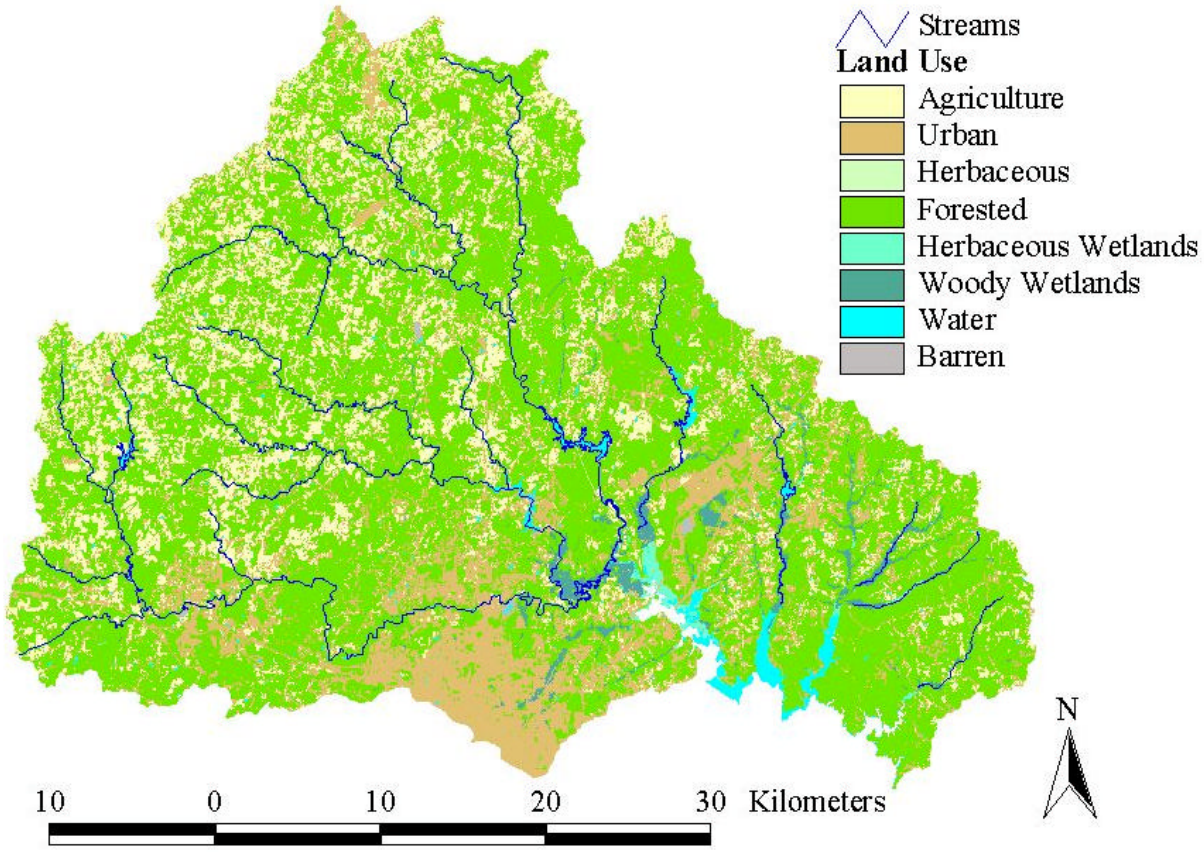


Figure 1.2. *Land use in the Neuse study area as determined by the EPA's Neuse River Land Use/Land Cover data. The 30-meter resolution grid was derived from several Landsat 7 ETM+ scenes ranging in dates from October 1998 to March 1999 (EPA, 2000).*

Selection of Study Sites

To select sites, a GIS data layer of all North Carolina crossing structures was obtained from NCDOT and was clipped according to the study area boundary defined above. We then visited all identified road crossings over streams in the study area to determine if they would serve as viable study sites. In March and April 2001 we visited 123 crossing structures in the Neuse study area and determined that 44 sites (Figure 1.4, Table 1.1) met our criteria to serve as sampling locations for the mussel abundance study. To serve as a study site, a location had to meet the following criteria:

1. The stream and surrounding land had to be accessible to sampling. Access was restricted by the landowner at a few sites.

2. The stream had to be free flowing for 300 meters upstream and downstream of the road crossing. It could not be excessively dammed by humans or beavers.

3. The stream had to have a mussel population. If we found live freshwater mussels in a 30-60 minute search by 2-3 people, the site was considered to meet this criterion.

4. Macrohabitat had to be similar upstream and downstream of the road crossing. Large differences in stream gradient upstream and downstream would likely result in inherent differences in the mussel community and effects of the crossing structure would be difficult to determine.

Landuse was classified at the 44 study sites within a 300-m radius of the road crossing point using the GIS landuse data in order to select a subset of 20 sites for this project (Fig. 1.3, Table 1.1). We then selected the ten sites with the greatest percentage of forest (range: 74 to 98% forested; < 1 to 17% agricultural) and the nine most agriculturally influenced sites (range: 16 to 58% forested; 33 to74% agricultural) (Table 1.2). A tenth agricultural site was not included in the study, because the remainder of potential sites were either too similar to the forested sites or were heavily influenced by urban development. Instead we chose a site with ongoing bridge construction in a relatively urbanized area to serve as the 20th site.

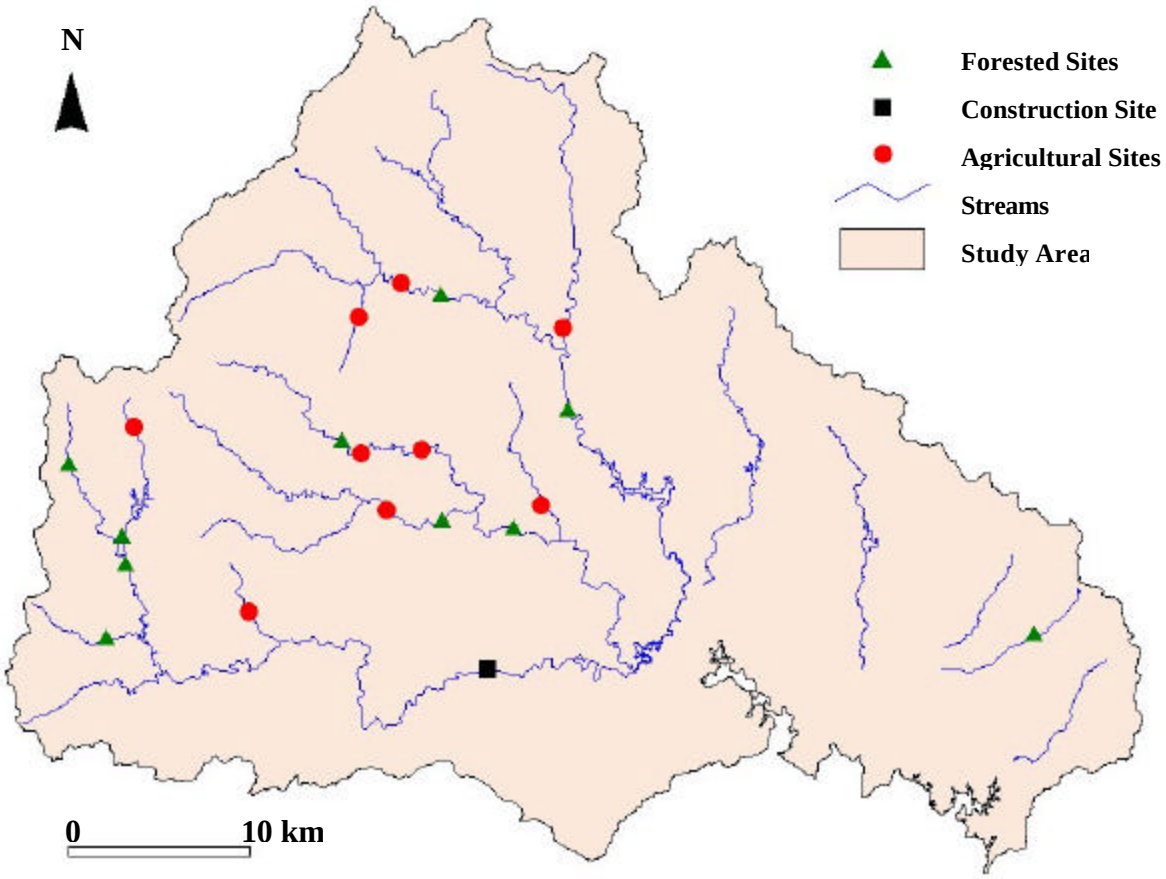


Figure 1.3. *Map of 20 study sites.*

Table 1.1. List of 20 study sites.

County	Bridge Number	Road	Stream	Date Sampled	Site Type
Durham	151	SR 1614	Flat River	6/28/01	Forest
Durham	5	SR 1793	Mountain Creek	6/8/01	Agricultural
Durham	50	NC 157	Eno River	7/11/01	Construction
Durham	56	NC 157	South Fork Little River	6/26/01	Forest
Durham	64	SR 1461	Little River	5/21/01	Forest
Granville	25	SR 1710	Smith Creek	6/18/01	Forest
Orange	11	SR 1536	Eno River	6/27/01	Forest
Orange	12	SR 1332	East Fork Eno River	7/6/01	Forest
Orange	173	SR 1353	East Fork Eno River	5/29/01	Agricultural
Orange	200	SR 1555	Stroud's Creek	5/24/01	Agricultural
Orange	242	SR 1004	West Fork Eno River	7/3/01	Forest
Orange	30	NC 57	North Fork Little River	5/22/01	Forest
Orange	53	SR 1538	North Fork Little River	7/12/01	Agricultural
Orange	54	NC 157	North Fork Little River	6/19/01	Agricultural
Orange	57	SR 1538	South Fork Little River	6/25/01	Agricultural
Orange	67	SR 1324	McGowan Creek	5/30/01	Forest
Person	33	SR 1125	South Flat River	6/20/01	Forest
Person	36	SR 1123	South Flat River	7/5/01	Agricultural
Person	38	SR 1121	Lick Creek	6/13/01	Agricultural
Person	80	SR 1734	Deep Creek	6/11/01	Agricultural

Table 1.2. Percent Land cover within 300 meters of the road crossing at each of the 20 study sites as determined by EPA landuse data (EPA, 2000).

County	Bridge Number	Urban (%)	Agricultural (%)	Forest (%)	Site Type
Durham	151	11.5	0.1	86.8	Forest
Durham	5	3.8	37.6	58.6	Agricultural
Durham	50	39.0	0.0	61.0	Construction
Durham	56	0.9	0.2	98.9	Forest
Durham	64	13.3	0.1	86.6	Forest
Granville	25	0.0	0.1	99.9	Forest
Orange	11	11.5	8.1	80.4	Forest
Orange	12	11.1	11.1	77.8	Forest
Orange	173	3.3	29.5	67.2	Agricultural
Orange	200	9.1	74.0	17.0	Agricultural
Orange	242	8.9	6.7	84.4	Forest
Orange	30	5.4	8.2	86.4	Forest
Orange	53	10.3	26.0	63.7	Agricultural
Orange	54	18.9	45.9	35.2	Agricultural
Orange	57	15.2	30.2	54.6	Agricultural
Orange	67	3.8	7.0	89.3	Forest
Person	33	4.2	1.9	93.8	Forest
Person	36	2.4	54.9	42.7	Agricultural
Person	38	2.4	33.9	63.7	Agricultural
Person	80	12.5	34.5	53.0	Agricultural

There was no significant different in drainage area between agricultural and forested sites in the study (p = 0.462, Mann-Whitney-U test), but 4 of the five sites with the largest drainage areas were classified as forested (Fig. 1.4).

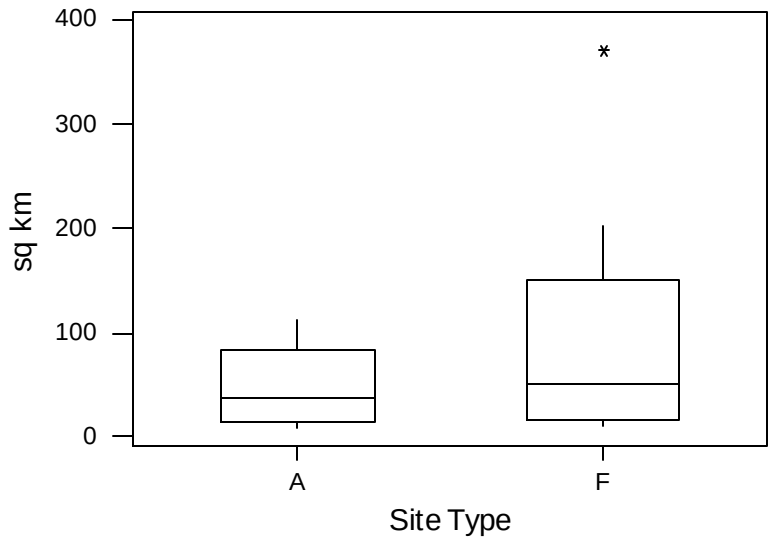


Figure 1.4. *Boxplot of the drainage area of the agricultural (A) and forested (F) sites used in the study.*

Chapter 2

Mussel Surveys

Introduction

Mussel abundance above and below crossing structures was used as a measure of the potential effect of road runoff draining from paved surfaces at crossing structures into adjacent streams. This outcome measure was generated by estimating and comparing mussel abundance and diversity in the 300-meter reach upstream and downstream of the 20 road crossings. Relative abundance and diversity were also compared between agricultural and forested sites.

Methods

Each study site included the 300-meter stream reaches immediately upstream and downstream of the road crossing as well as under the crossing structure itself. The site was divided into 25-meter cross-sections, and the cross-sections were numbered consecutively from downstream to upstream (1-24) (Fig. 2.1).

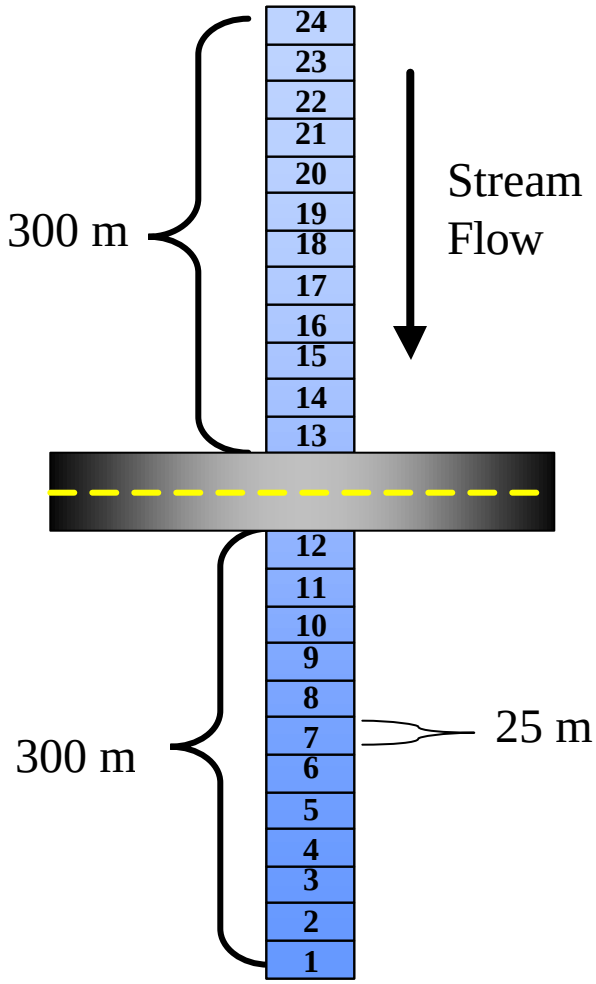


Figure 2.1. *A diagram of a sampling site, which included the 300-meter reaches of stream immediately upstream and downstream of the road crossing.*

Sites were initially assigned a random order to be sampled using a random number generator and surveyed from 21 May – 12 July 2001; however, we deviated from this order slightly as rain events created poor sampling conditions at a few sites on the day that site was to be sampled. Methods used to survey mussels were identical to those used to survey 80 sites mentioned in a prior final report to the NCDOT (Project Hwy# 2003-10)(Levine et al. 2003). At each site, three surveyors each searched 1-meter-wide linear transects (one next to each bank and one in the center of the stream) using view scopes and snorkeling to visually locate mussels. These transects were searched in an upstream direction for the entire 600 meters of stream surveyed at each site and under the crossing structure. The 1-meter width was standardized on each surveyor by measuring against their armspan giving each person a reference point on their body by which to measure, and no mussels were included in the survey that fell outside this 1-meter width. As surveyors moved upstream, the 1-meter transects on each bank were measured from the water's edge using the reference point on their armspan, and the transect in the center of the stream was measured from the centerline of the surveyor's body. The same surveyor surveyed the same linear transect (left bank, middle, or right bank) for an entire site, and a standard rotation was used between sites. In larger, more diverse streams, we used 1-2 extra surveyors to qualitatively search areas between the three linear transects to try to find species not accounted for in the linear transects. The qualitative searches also yielded extra data on sex ratios and gravidity of sexually dimorphic species (Lampsilines). All sites but two were completed on the same day. The two sites that required two days were completed in consecutive days, and no substantial weather changes or rain occurred between those days.

To maximize consistency through time and between surveyors, only visual surveys were done, and no excavation or rock flipping was used to locate mussels. Tactile searching was used occasionally as necessary when murky water, debris piles, or undercut banks made visual searches difficult; however, only mussels felt on the sediment surface were taken. Mussels were identified, and length was measured to the nearest millimeter using calipers on the first 15 of each species collected from each cross-section. We recorded the cross-section number (See Chapter 1) and linear transect (left bank, middle, right bank) in which the mussel was located. Lampsilines were classified as male or female, and we checked for gravidity (presence of mussel larvae) of all known females. Mussels were returned to original life position as soon as data was recorded for each individual.

Two specific measures were taken in the field for quality assurance. Between sites we alternated between starting the survey at two different points within the reach to be sampled. At half of the sites, we started the survey at the most downstream end of the site and moved in an upstream direction to sample the entire reach. At the other sites, we started at the road crossing surveying the upstream reach first then going the downstream end and searching up to the road crossing. This was done to guard against a time bias with respect to the road crossing, so the same portion of stream was not always sampled at the same time of day. Also, a measure of detectability was taken at each site in a predetermined 75-meter reach by removing all mussels found in the bank transects and using a second pass by the field supervisor to locate any mussels missed. This provided a measure of variation in mussel detection between days and between surveyors. Detectability percentage was calculated as the number of mussels found in the first pass divided by the total number found in the first and second passes.

All data were tested for normality using a Ryan-Joiner test (Dekker 1986). We then analyzed the data in a variety of ways to assess potential differences in relative mussel abundance and diversity in relation to the road crossing. To equally weight all sites, relative

abundance was calculated as the percent of mussels at a site occurring in a given cross-section. We calculated the Shannon-Weiner Diversity Index (Campbell et al. 1986; Thrush et al. 1998) as a measure of diversity for individual 25-meter cross-sections as well as the entire upstream and downstream reaches at all sites using the following formula:

$$\textbf{Shannon-Weiner Index} = \sum_{n} -\left(\frac{\text{Number of } i^{th} \text{ species found}}{\text{Total mussels found}} \times \log\left(\frac{\text{Number of } i^{th} \text{ species found}}{\text{Total mussels found}}\right)\right)$$

Other measures of diversity used included the number of species found other than *Elliptio complanata* (the most abundant species) and the number of individuals found of these other species. Differences between upstream and downstream were tested with either a paired t-test (normal data) or a Wilcoxon Signed-Rank test (non-normal data). Differences between 25-meter cross-sections were tested with a Kruskal-Wallis test. A proportion was also used to test whether the percentage of bridges with more mussels upstream was significantly different than 50%. Mussel length was also assessed using a Kruskal-Wallis test to compare length between cross-sections and a Mann-Whitney test to compare between the entire upstream and downstream 300-meter reaches and forested with agricultural sites.

Results

The number of mussels found at the construction site (Eno River at NC 157 in Durham County) was quite low compared to other sites in the study. The upstream as well as the downstream reaches were similarly sparse in mussel abundance. For the purposes of this chapter, mussel survey results from this site will not be included in statistical analyses. We found no differences in mussel data between agriculture and forested sites (Appendix I). There was no difference in the number of species found between agricultural (3.1) and forested (3.6) sites ($p = 0.610$, two-tailed t-test). Mean number of mussels found was 1124 at agricultural sites and 1709 at forested sites, ($p = 0.488$, Mann-Whitney). There were no differences in the number of individuals other than the most common species, *Elliptio complanata* ($p = 0.536$, Mann-Whitney) or in the percent of *E. complanata* as gravid between agricultural sites (37.5%) and forested sites (35.1%) ($p = 0.767$, two-tailed t-test). There was also no difference in mean length between agricultural and forested sites ($p = 0.426$). On average, *E. complanata* were slightly but statistically ($p = 0.0009$, Mann-Whitney) longer upstream (72.0 mm) than downstream (71.0 mm). There were also differences in length between 25-meter cross-sections ($p < 0.001$, Kruskal-Wallis), but there were no obvious trends in relation to distance from the road. All measures of diversity showed no differences between cross-sections or between upstream and downstream reaches ($p > 0.9$, Kruskal-Wallis); however, abundance of the most common species *E. complanata* was lower just downstream of the road. On average, both forested and agricultural sites showed a trend toward decreased mussel abundance in approximately the first 50 meters downstream of the road crossing (Figure 2.3); however, this trend was only statistically significant when forested and agricultural sites were analyzed together (Kruskal-Wallis, $p = 0.010$).

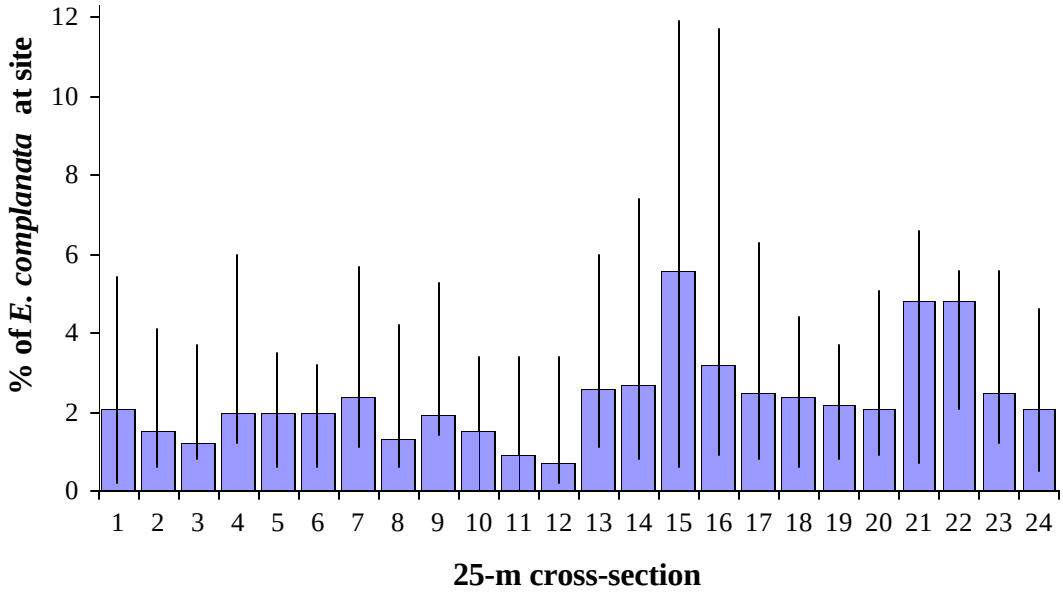


Figure 2.2. *Overall median percentage of Elliptio complanata occurring in a given cross-section. Error bars represent 25 and 75% quartiles. Cross-sections were numbered consecutively from downstream to upstream and the road-crossing was located between 12 and 13.*

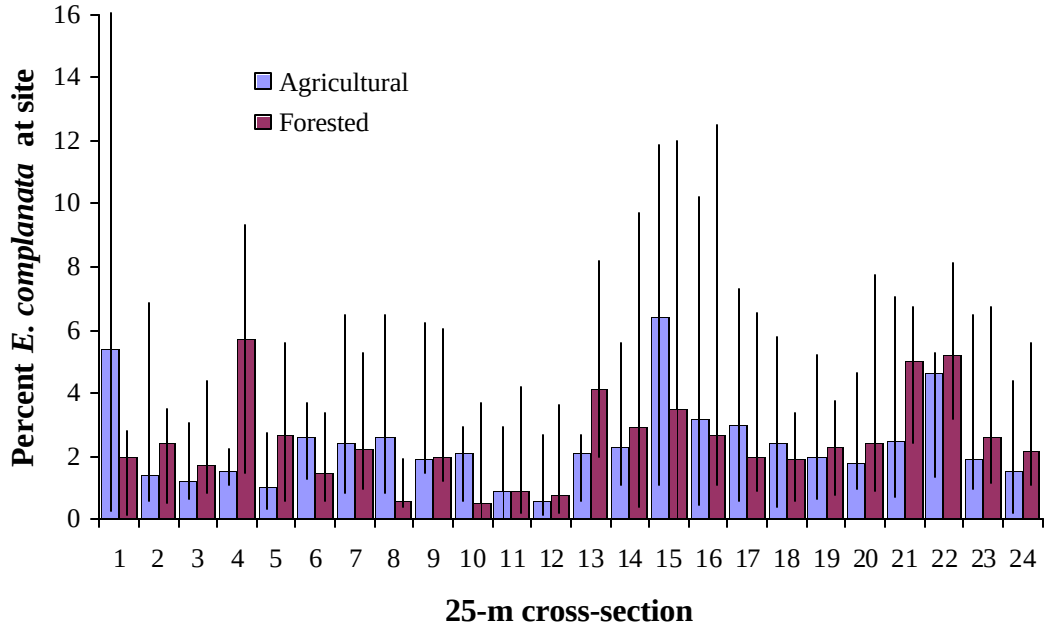


Figure 2.3. *Median percentage of Elliptio complanata occurring in a given cross-section in agricultural and forested sites. Error bars represent 25 and 75% quartiles. Cross-sections were numbered consecutively from downstream to upstream and the road-crossing was located between 12 and 13.*

Discussion

These surveys were done to determine if these road crossing caused a population-level effect on freshwater mussels. The 20 sites surveyed in this study are a subset of the 80 sites described in a previous report submitted to the NCDOT (Levine et al. 2003). Survey results from these sites do not differ from the results reported in the larger group of sites. In both studies, relative abundance of *E. complanata* was statistically lower in the first 50 meters downstream of the road compared to the rest of the site; however, results presented here are somewhat less statistically powerful due to the smaller sample size. By looking at survey data alone, this localized effect seems to indicate that the decrease in mussel abundance is due to physical disturbance from the crossing structure rather than toxicity of road runoff. Any toxic effects would be exhibited more uniformly and much further downstream than what was seen in this study. In the related study, we found that these localized affects were associated with crossing structures built in the 1950s and 1960s, which tended to be poorly designed, and with the most newly constructed crossings (Levine et al. 2003). The poor habitat around many of these structures further implicated physical disturbance as the main cause of the localized effects.

Smith and Kaster (1983) studied rural highway runoff on a road with higher traffic loads than the roads in this study and found the effects on benthic invertebrate communities to be negligible. This is contrary to the findings of Pratt (1977) and Lenat et al. (1979) where benthic communities were affected by urban highway runoff. The low traffic loads and rural nature of our study may have contributed to the absence of detectable runoff-related effect.

Mussel survey data at sites classified as agricultural did not differ significantly from those classified as forested in this study. This is likely because agriculture in the study area was of a low intensity, and vegetated riparian buffers along stream banks remained largely intact, even at sites with the highest percentage of land use as agricultural. In tributaries to the Deep River in the Cape Fear River Basin, we did find agricultural sites to have fewer mussels (Levine et al. 2003). Agriculture landuse in that area is much more intensive, and riparian buffers were much less extensive than in the upper Neuse basin where this study took place.

Summary of Findings

1. There tended to be fewer *Elliptio complanata,* the most common species, in the first 50 meters downstream of the road crossings. This localized decline is likely due more to physical disturbance from the actual crossing structure rather than toxicity of road runoff. This was similar to findings submitted in a previous report on crossing structure effects where 80 sites were studied, of which these 20 were a subset.

2. There were no differences in sites classified as agricultural and those classified as forested. The low intensity of the agriculture and extensive riparian buffers in the study area likely led to this result.

Chapter 3

Development and use of mussel health assessment technique

Introduction

Health assessment of freshwater bivalves traditionally involves lethal tissue collection from animals for histology (Chittick et al., 2001), contaminant (Muncaster et al., 1990; Cope et al., 1999), enzymatic (McMahon, 1991; Doran et al., 2001) or energy analysis (McMahon, 1991; Baker and Hornbach, 2000). These techniques have been most successful at identifying Unionid populations suffering the impacts of dramatic localized habitat degradation (Foe and Knight, 1987; Goudreau et al., 1993) or major infestations of invasive species (Baker and Hornbach, 2000). In these situations a large proportion of a localized population is affected and relatively few animals need to be sacrificed to identify a health effect.

However, many devastating environmental threats are more insidious, with diffuse, less localized impacts. Health effects in these cases may be subtle and initially present a picture of low prevalence. Furthermore, sampling restrictions on endangered or threatened populations limit access to these animals for health assessment or any other purpose. In these situations, lethal sampling of a small number of animals is unlikely to reveal statistically convincing information (Wobeser, 1994; Doran et al, 2001). Suitable nonlethal methods for detection of health problems would increase the feasibility of safely processing sufficient numbers of animals to identify important trends.

Relatively noninvasive techniques, such as mantle (Berg et al., 1995) and foot (Naimo et al., 1998) biopsy for glycogen analysis (Naimo et al., 1998; Patterson et al., 1999), have recently been developed, but a reliable technique for sampling hemolymph from freshwater mussels would greatly expand the potential for establishing a comprehensive protocol for the nonlethal assessment of Unionid health. Blood analysis is a routine component of the evaluation of vertebrate organ health, hydration status, immunologic competence and nutritional status (Willard et al., 1994). Techniques for harvesting hemolymph from marine bivalves are common practice (Fyhn and Costlow, 1975; Ford, 1986; Fisher et al., 1996a,b; Yanick and Heath, 2000), and circulatory fluids have been used in physiologic studies of freshwater mussels (Dietz, 1974; Byrne and McMahon, 1991; Pynnonen, 1994; Pekkarinen, 1997). Hematologic responses to season (Pekkarinen, 1997), dehydration and anoxia (Dietz, 1974; Byrne and McMahon, 1991), transportation (Pekkarinen, 1995) and acidity (Pynnonen, 1994) are described. However, the health impacts of hemolymph collection from freshwater mussels have not been sufficiently evaluated.

Freshwater mussels have an open circulatory system (McMahon, 1991). Hemolymph flows through a series of tissue sinuses. The adductor muscles, readily visualized when the valves are separated, hold particularly large tissue sinuses. We have modified hemolymph collection procedures targeting the adductor muscles in oysters (Fisher et al., 1996a,b) and brackish-water clams (Fyhn and Costlow, 1975) for use in freshwater bivalves, and provide a preliminary evaluation of the techniques' safety when used in freshwater mussels of the common *Elliptio complanata* complex.

Circulatory fluid can reflect an animal's state of health. Blood samples are used in disease surveillance and diagnosis in companion animal, livestock and human populations, at times providing the first indication of abnormalities (Willard et al., 1994). Infections, for example, can alter the numbers and types of circulating blood cells. Nutritional deficiencies or gastrointestinal disease can affect circulating nutrient levels, just as respiratory illness can

change partial pressures of circulating gases. Impaired clearance organs, or exposures to toxic materials, result in increased concentrations of circulating toxicants or waste metabolites. Likewise, enzymes localized to a particular organ can contaminate the bloodstream when organ damage or inflammation causes cellular destruction (Willard et al., 1994).

Hemolymph is the circulatory fluid of freshwater mussels and other invertebrates with open circulatory systems. Hemolymph of freshwater mussels offers the same utility for health assessment as that of blood in vertebrates. Collection from freshwater mussels is simple and relatively non-traumatic (Gustafson et al., in prep). Open circulatory systems hold more fluid than closed systems of similar size; substantial volumes can be collected from these small animals without detriment. Although hemolymph has been used to quantify the presence of selected components (e.g. calcium and glucose) (Pekkarinen and Suoranta, 1995) it is not routinely used as a means of measuring the health status and organ function of freshwater bivalves.

The development of a hemolymph health assessment protocol for freshwater mussels requires: (1) the establishment of reference ranges from populations of healthy individuals and (2) the experimental documentation of hemolymph responses to disease or environmental stressors. The latter is conducive to laboratory study and is readily incorporated into physiological studies of bivalve responses to captivity, translocation, toxicant exposure, or disease. Some of this work has already been done. Studies of freshwater bivalves document alterations in hemolymph calcium and glucose (Pekkarinen and Suoranta, 1995) subsequent to animal collection and transport in water-filled containers. Hemolymph magnesium concentrations of zebra mussels (*Dreissena polymorpha*) decrease after transport in water-filled containers (Martem'yanov, 2000). Emersion (removal from water) times have been correlated with declines in hemolymph pH in *Anodonta grandis* (Byrne and McMahon, 1991) and *Corbicula fluminea* (Byrne et al., 1991), and hemolymph calcium concentrations have been shown to increase when freshwater mussels are exposed to acidified (Pynnonen, 1990 and 1994) or anoxic waters (Dietz, 1974). These studies establish that hemolymph chemistry parameters are affected by conditions that can compromise the health of freshwater mussels. Further research in this arena will provide the information necessary to link hemolymph change with a wider variety of health-related stressors.

Specifically because of the responsiveness of hemolymph chemistry parameters to subtle environmental factors, the establishment of baseline (also called normal) reference ranges is not readily accomplished through experimental study. Reference ranges are best developed through surveys of relatively large numbers of healthy animals, from a number of populations, preferably in their natural environment (Solberg, 1986; Lumsden and Jacobs, 1989). Broad sampling for reference values helps ensure their applicability to a broad range of subject animals and populations.

We had the opportunity to assemble hemolymph data from 380 wild *Elliptio complanata* collected from nineteen stream reaches in a minimally impacted region of North Carolina over late spring and early summer of 2001. We present 95% interpercentile reference ranges from these apparently healthy populations as an initial baseline for clinical interpretation of hemolymph in *Elliptio complanata.* The sites were very similar in landuse/landcover characteristics when evaluated by watershed. However, sites varied somewhat in predominant landuse of the riparian zones immediately adjacent to collection sites. We compare results from populations contiguous to predominantly forested lands versus populations contiguous to more heavily agricultural lands and discuss differences in light of the theory that land use may be a

factor governing freshwater mussel declines. Finally, we present noted correlations of health parameters with general health ratings such as site abundance and diversity rankings, as well as gravidity status and animal size classification.

Methods

Technique Development

Study Animals and Husbandry: *Elliptio complanata*, ranging from 40 to 74 mm in length (median = 54.7, Q1 = 53.5, Q3 = 59.7), were collected over a two-day period in July 2000 from two forested streams northwest of Raleigh, North Carolina. Animals' shells were marked with plastic numbered tags using methacrylate adhesive (Hallprint tags, Holden Hill, Australia). They were then placed in a single large indoor recirculating water system for the duration of the study. Animals were randomly assigned to hemolymph collection and control groups using a random numbers generator (Microsoft Excel 1998). Additional animals were collected for technique development and validation. Animals were given one week to acclimate prior to initiation of the study.

The recirculating water system held approximately 1100 liters of dechlorinated municipal water. Ambient laboratory temperatures were maintained at 21ºC with central air and heating systems. Additional water temperature control was facilitated with titanium heat exchangers [product information]. Temperature modes of 19° C (range 17-24) and 21° C (range 20-21) were maintained for the first and second experiments respectively. Lighting conditions were regulated to 14 light hours and 8 dark each day. Water quality parameters were monitored every 1 to 2 weeks. Dissolved oxygen ranged from 7.5 to 11.9 (mode, 8.5) mg/L, pH ranged from 7.4 to 8.2 (mode, 7.9), hardness ranged from 43 to 98 (mode, 56) mg/L, alkalinity ranged from 24 to 54 (mode, 35) mg/L, total ammonia nitrogen ranged from 0 to 0.23 (mode, 0.02) and nitrate ranged from 0 to 4.6 (mode, 2.8) mg/L. Thirty percent water changes were conducted every 5-10 days. The animals were fed cultured live algae, consisting primarily of *Scenedesmus* sp, 4-5 times per week. The weekly total of fed algal cells averaged 91,000 per ml of water in the animal holding tank.

Hemolymph Sampling Technique: Hemolymph was collected by gently prying the shell open approximately 2-3 mm with a thin knife. The shell was held open with tissue forceps. Under full-spectrum lighting, the anterior adductor muscle was visible between slightly gaping valves as a highly reflective glistening white muscle surface. This muscle mass was gently penetrated with a 25 gauge 5/8 inch needle (Figure 3.1), directed along a line parallel to the anterior edge of the shell valves (Figure 3.2). Hemolymph is best collected slowly with gentle and intermittent suction. A successful collection from a 50 g animal will easily yield approximately 0.5 ml fluid over 30 seconds. If air is drawn, it is often difficult to extract further hemolymph from the animal. In the current study, a draw was considered complete when either the target volume was achieved, or when air was aspirated. The smallest volume collected in this study was 0.3 cc.

Figure 3.1. *Hemolymph collection from the anterior adductor muscle of an E. complanata.*

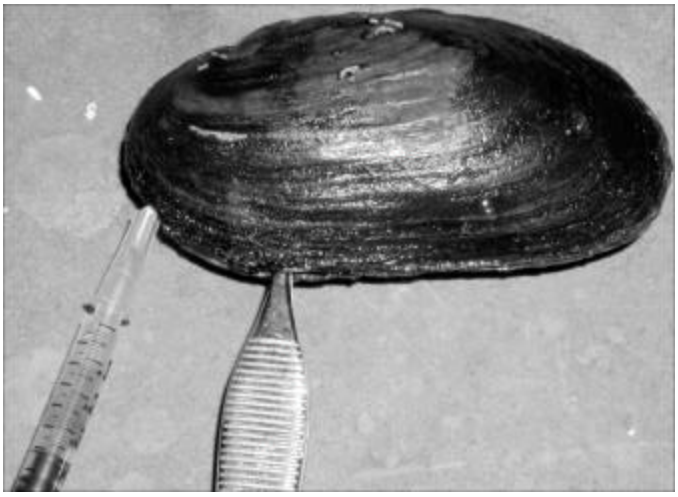


Figure 3.2. *Recommended needle alignment for hemolymph collection from E. complanata.*

Technique Development: Six animals were used in a preliminary study to examine alternative sites for hemolymph collection. Circulatory fluids of different origin can differ in composition and ease of collection. Tissue sinus and ventricular fluids represent circulating hemolymph. Pericardial fluid is routinely harvested from oysters. Pericardial fluid, however, after passing through the cell junctions of the pericardial gland en route to the kidney, represents an ultrafiltrate of hemolymph (McMahon, 1991). We initially attempted to collect fluid from the pericardial sinus or cardiac ventricles, via a hole drilled in the shell and through gaping valves in three animals. The procedures lacked acuity, as it was difficult to ascertain whether a fluid was cardiac or pericardial in origin. We abandoned these approaches due to high mortality (100%). Attempts to collect extrapallial fluid by needle insertion through mantle tissue were made in three additional animals. This approach was rejected, as it was difficult to collect an adequate volume. Our attempts resulted in a maximum of 0.2 cc per draw. In contrast, the anterior

adductor muscle sinus was readily accessed through gaping values in the same animals and provided up to 1.5 cc of fluid for analysis.

Four animals were sacrificed to validate sample integrity. This was accomplished by visualizing the needle track on the cut surface of the adductor muscle of sacrificed animals as a white line running to the center of the muscle mass (Figure 3.3). We also compared the composition of fluids obtained from the adductor muscle and the heart. Ventricular hemolymph was accessed by gently prying (without transecting) the adductor muscles and mantle from their attachments to the shell, removing the upper valve, locating the beating ventricle, and sampling the ventricular fluid with a 25 gauge needle under a dissecting microscope. We analyzed cell count, fluid protein, ammonia, calcium, phosphorus and magnesium levels for each sample. Animals surviving these procedures were sacrificed by transecting the preserved adductor muscles.

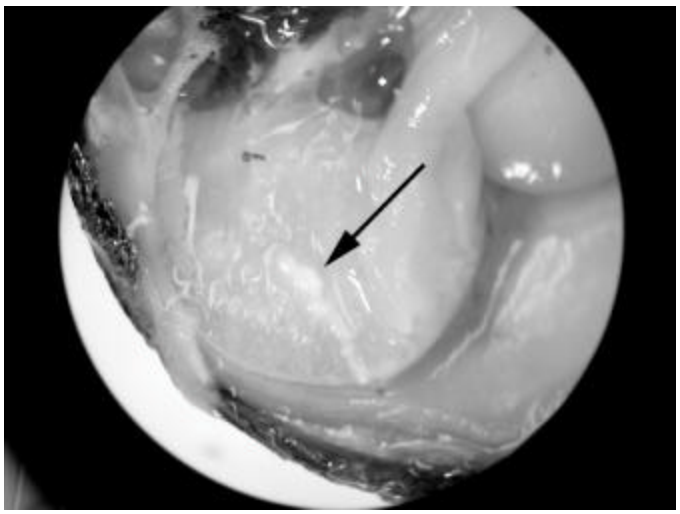


Figure 3.3. *Track left by a 25 g needle in the transected adductor muscle of an E. complanata.*

Safety Studies: Our initial safety study compared survival and growth of a group of 30 animals sampled for a single collection of hemolymph, to that of 30 control animals maintained in identical conditions but not sampled for hemolymph, over a 3-month period (27 July 2000 to 31 Oct 2000). Hemolymph (0.5 cc) was collected from the anterior adductor muscle sinus of each treatment animal once.

The second study (trial B) examined the safety of repeated hemolymph sampling. Growth and survival were compared for 9 animals sampled 3 times at 2-3 month intervals and 9 control animals similarly handled and measured but not hemolymph sampled. This study ended two months after the third hemolymph collection.

A full census, where all animals were physically removed from the tank, counted, weighed to the nearest 0.01 gram in air on a gram scale (Mettler BB 240, Fisher Scientific, Pittsburgh, PA), and measured by Vernier calipers (Fisher Scientific, Pittsburgh, PA) for shell length, width and height to the nearest 0.1 mm, was conducted once a month throughout the

studies. In addition, the holding tank was observed for mortalities multiple times per week in the first 2 months and weekly thereafter.

Data Analysis: Turnbull nonparametric survival analyses were used to evaluate and plot mortality rate differences between cohorts. Kruskal-Wallis analysis was used to identify growth differences between cohorts (Hollander and Wolfe, 1999) with a P-value of 0.05 considered significant.

Establishment of hemolymph reference ranges

Twenty animals (greater than 40 mm in length) were collected at 19 of the 20 sites in the upper Neuse basin: The first 10 found at least 25 meters upstream of the road crossing, and the first 10 found at least 25 meters downstream of the road crossing. The 20th site (Eno River at NC 157) was in an urban area and had ongoing bridge construction, so this was not used in the establishment of reference ranges. Only animals greater than 40 mm in length were included in the study. Study animals ranged in size from 45 to 98 mm in length (median = 72, Q1 = 65, Q3 = 79) and 9.6 to 129.2 g in weight (median = 47.33, Q1 = 35.66, Q3 = 63.80) and did not vary significantly in size between forested and agricultural sites, nor between upstream and downstream of road crossings.

For the purposes of this study, sites with fewer than 200 mussels and/or only one species (*Elliptio complanata*) found in the 600 meters surveyed were considered low abundance/diversity sites. Five of the nineteen sites met this criterion. All other sites were considered substantial in unionid abundance and diversity. Mussel health data was collected on the same day as that surveys were conducted at these sites (Chapter 1). Site sampling dates are listed in Appendix 1b, along with predominant adjacent landcover influence, and water quality parameters and mussel abundance/diversity scores collected at the time of sampling.

Mussel Health Data Collection: Animals were placed in two shaded coolers filled with stream water: one designated for upstream water and animals, the other for downstream water and animals. Animals were processed on site as quickly as possible, usually within 2 hours of collection, using a field lab (microscope, centrifuge, and gram scale) powered by an AC adaptor connected to the electrical system of a field vehicle. In two instances, power supply failure in the field required the transportation of animals to North Carolina State University Veterinary College (1-2 hours driving time) for processing.

During processing, animals were weighed to the nearest 0.1 g (Denver XP600 gram scale, Fisher Scientific, Pittsburgh, PA) and shell dimensions (length, width and height) were measured with Vernier calipers (Fisher Scientific, Pittsburgh, PA) to the nearest mm. Shell length is a common and robust meristic for field estimation of animal size (Molina et al., 2001). The other size parameters were collected to estimate animal weight-to-volume density calculated as mg weight per cubic mm (length x width x height). Gravidity status was determined visually by inspecting the gills for evidence of inflation and whitish coloration. Between 0.5 – 1.0 cc of hemolymph was collected from the anterior adductor muscle using a 25 g needle (Gustafson et al., in prep). A Neubauer hemocytometer (Hausser Scientific, Horsham, PA.) was filled with hemolymph and cell count recorded immediately, including all cells in the corner squares of one chamber. The remaining hemolymph was centrifuged for 3 minutes at 1000g, the cellular fraction removed and the supernatant placed in cryovials (Fisher Scientific, Pittsburgh, PA) on

dry ice packed in regular ice for the duration of the field day. Hemolymph was transferred to a –20ºC freezer at the end of the day, and analyzed on an automated clinical chemistry analyzer (Hitachi 912, Roche Diagnostics, Ingelheim, Germany) within 24 hours for colorimetric determination of total protein, aspartate aminotransferase (AST), ammonia, magnesium, calcium, phosphorus and glucose concentrations.

Fourteen of the 20 animals were returned to the stream after hemolymph collection. The remaining 6, including the first 3 upstream and the first 3 downstream animals processed at each site, were sacrificed for histologic evaluation. Tissues were preserved in Davidson's Fixative and stained with Hematoxylin and Eosin after routine stepwise tissue dehydration, embedding in paraffin and sectioning to 4 microns (Howard and Smith, 1983). Histologic evaluation was used to determine parasite presence and to confirm health status of the study population with standard methods (Chittick et al., 2001).

Glycogen concentration was analyzed on foot tissue of sacrificed animals from a convenience sample of 6 of the forested and 7 of the agricultural sites. Glycogen analysis followed a modified version of the method used by Patterson et al. (1997), Burton et al (1997) and Carr and Neff (1984) in which glycogen is converted to glucose with amyloglucosidase (Sigma-Aldrich Co., St. Louis, MO.) for glycogen determination. Lyophilized mussel foot samples were weighed on an analytical scale (Mettler AE240, Fisher Scientific, Pittsburgh, PA) to the nearest 0.1 mg. The average dry tissue sample weight was 10.4 mg (range, 3.7 – 17.0 mg). Individual tissue samples were each mixed with 0.5 to 1 cc cold trisodium citrate buffer solution (volume recorded). Tissue samples plus buffer were homogenized with a glass mortar and pestle tissue grinder (Fisher Scientific, Pittsburgh, PA). The homogenate was distributed into 2 sterile cryovials (Fisher Scientific, Pittsburgh, PA) in equal volume aliquots, and placed immediately in a boiling water bath for 5 minutes (Carr and Neff, 1984). After cooling, 50 µl amyloglucosidase solution per 0.5 ml tissue homogenate was added to one cryovial of each sample. 50 µl of buffer was added to the second cryovial for use as a blank. All samples (and standards and blanks) were incubated in a 55ºC water bath for two hours. Samples (and blanks) were centrifuged at 5000xg (Eppendorf Centrifuge 5413, Fisher Scientific, Pittsburgh, PA) for 30 minutes and the supernatant collected for glucose determination. Glucose concentrations were analyzed using the hexokinase test (Glucose HK Assay Kit, Sigma Diagnostics, Inc., St. Louis, MO) at a 1:20 sample to reagent ratio and spectrophotometric absorbances recorded at 340 nm (Milton-Roy Spectronic 1201, Ivyland, PA). The spectrophotometric absorbance of the tissue minus the blank was compared to the standard glycogen curve to obtain an estimate of tissue glycogen content (mg glycogen per dL diluent). The final glycogen concentration was calculated as mg glycogen per dL diluent, times dL buffer used in the grinding step, divided by g tissue dry weight. Results are reported as mg glycogen per g foot tissue dry weight.

Fresh ice-cold 0.1 M trisodium citrate (pH 5.0) buffer solution was used in the preparation of glycogen standards, tissue homogenates and amyloglucosidase (0.5%) solution. Glycogen standards were prepared daily by dilution of commerical *Mytilus edulis* glycogen (type VII, Sigma-Aldrich Co., St. Louis, MO) to 10, 50, 100, 150, 200 and 250 mg/dL. An in-house reference standard was prepared from biopsied *Elliptio complanata* foot tissues from fifteen non-experimental animals, which were lyophilized and ground to a fine powder. The powder was stored at –20° and homogenates prepared daily as reference standards. Quality control (APHA et al., 1995) included daily measurement of 5 concentrations of glycogen standard and multiple procedural, 4 sets of triplicate in-house reference standards and known additions, and duplicate analysis of 33% of the tissue samples. Tissue glycogen samples were analyzed in a single batch.

The glycogen standard curves for the tissue samples were linear in the test range described (10-250 mg/dL) with R-squared (coefficient of determination) values of 99.7% and 99.6% respectively. The average % difference of duplicate samples was 12.23% (range, 1.27 – 24.36). The mean RSD (relative standard deviation) of triplicate in-house standards was 8.13% (range, 5.44 – 11.61). Recovery of known additions averaged 91.08% (range, 62.38 – 109.32).

Statistical Analysis: Reference intervals and associated confidence limits for hemolymph parameters were calculated from the group of 380 animals using nonparametric determination of the central 95% intervals (Solberg, 1986). The nonparametric procedures, based on ranks, alleviated analytical problems associated with data sets bounded by lower limits of detection and allowed out-of-bound data to retain full significance.

The results for each parameter were sorted in ascending order and assigned a rank number from 1 to n. The bounds of the central 95% reference interval were then defined as the values corresponding to rank numbers equal to 0.025(n+1) and 0.975(n+1). If the calculated rank numbers were not integers, then values were interpolated from the two closest ranks. The 90% confidence intervals were calculated from the binomial distribution (Solberg, 1986). For datasets of n = 373-380, the 90% confidence interval for the lower 2.5 percentile corresponded to rank numbers 5 and 16. The 90% CI for the upper 97.5 percentile corresponded to rank numbers (n+1)-16 and (n+1)-5. For datasets of n = 364-372, the 90% confidence interval for the lower 2.5 percentile corresponded to rank numbers 5 and 15, and the 90% CI for the upper 97.5 percentile corresponded to rank numbers (n+1)-15 and (n+1)-5. Out-of-bound data were not an issue for glycogen results, and presumed normality was not rejected by the Kolmogorov-Smirnov test at a 0.05 significance level ($p > 0.15$). Consequently, parametric reference ranges, providing narrower distributions, are reported for glycogen.
After ruling out conditional dependence between land-use designation and gravidity status or shell length, we used Spearman's Rank correlation coefficient on the full data set of 380 animals to identify correlations between animal length and potential health parameters. We evaluated associations between potential health parameters and gravidity status logistic regression. Associations between site abundance/diversity rank and site prevalence of unusual health measures were established using Chi-square tests and logistic regression.

Bayesian likelihood ratios are presented as measures of strength of association (Rothman and Greenland, 1998) between variables that may hold predictive value for future diagnosis of compromised unionid populations. A Bayesian likelihood ratio represents the prevalence of a characteristic among a compromised (or test) group relative to the prevalence of the same characteristic among the healthy (or control) group (Gustafson et al., 1998).

Effects of road crossings and landuse

We used the nonparametric Kruskal-Wallis test to evaluate differences in health parameter medians between landuse types (n = 10 forested and 9 agricultural sites), and between upstream (above bridge) and downstream (below bridge) collection locations (n = 19 sites).

Results

Technique Development

Hemolymph Composition and Technique Verification: The hemolymph samples collected from the adductor muscle and the ventricle were similar in composition in three of the four animals examined (Table 3.1). In one animal, ammonia and cell count differed substantially by fluid type.

Table 3.1. Hemolymph parameters from the heart ventricle and adductor muscle sinus of four *Ellipto complanata.*

Animal	Collection Site	Cells /µL	Phos, mg/dL	Ca, mg/dL	Mg, mg/dL	NH3, µmol/L	Protein, mg/dL
A	Adductor	2640	0.9	19.3	2.7	44.1	73.3
A	Ventricle	2020	0.8	21.7	2.9	37.7	73.5
B	Adductor	960	0.8	17.9	2.7	26.4	41.9
B	Ventricle	860	0.7	19.0	2.8	35.3	40.1
C	Adductor	1490	0.9	17.0	2.8	26.8	72.6
C	Ventricle	250	1.0	18.8	2.8	193.8	75
D	Adductor	460	0.8	17.7	2.3	36.1	66.1
D	Ventricle	420	0.8	18.3	2.3	46.9	60.4

Safety of One Time Sampling: There were no significant differences in survival rates between the control group and the hemolymph-sampled group. Both cohorts showed 97% survival at one month and 90% survival at three months.

We found no meaningful difference in 3-month weight gain between the hemolymph-sampled and the control cohorts (Kruskal-Wallis test, P value = 0.079, n = 27, H = 3.08, D.F. = 1). Weight change for the control animals ranged from –1.83 to 0.89 g (median = -0.21 g, Q1 = -0.98 g, Q3 = 0.35 g). Weight change for the hemolymph-sampled group ranged from -2.6 to 2.22 g (median = 0.34 g, Q1 = -0.45, Q3 = 0.71). Though not significant at an alpha level of 0.05, the hemolymph-sampled group (rather than the control group) demonstrated greater weight gains.

Shell growth over three months was not significantly different between the hemolymph-sampled and the control cohorts (Kruskal-Wallis test, P value = 0.789, n = 27, H = 0.07, D.F. = 1). Neither group showed appreciable changes in shell size. The change in shell size (estimated by the cubic root of shell length x width x height) for the control animals ranged from –1.15 to 1.05 mm (median = 0.19, Q1 = -0.11, Q3 = 0.48). The change in shell size for the hemolymph-sampled group ranged from -2.65 to 0.78 mm (median = 0.25, Q1 = -0.16, Q3 = 0.52).

Safety of Repeated Sampling: There was no significant difference in survival rates between animals sampled repeatedly and the control group, as there were no mortalities in either cohort. Furthermore, there were no significant differences in weight gain (Kruskal-Wallis test, P value = 0.354, n = 9, H = 0.86, D.F. = 1) or shell growth (Kruskal-Wallis test, P value = 0.895, n = 9, H = 0.02, D.F. = 1) between repeatedly sampled animals and controls over the subsequent 7 month (Oct 2000 to May 2001) study period. The change in animal weight ranged from -0.74 to 2.48 g for the treatment group (median = -0.03, Q1 = -0.31, Q3 = 0.72) and from -0.38 to 1.65 g for the

controls (median = 0.34, Q1 = 0.13, Q3 = 0.89). The change in shell size ranged from -0.49 to 3.25 mm for the treatment group (median = 0.00, Q1 = -0.22, Q3 = 0.34) and from -1.07 to 0.32 mm for the controls (median = 0.00, Q1 = -0.16, Q3 = 0.12).

Establishment of hemolymph reference ranges

Reference Ranges: The 95% reference intervals and associated 90% confidence intervals (Table 3.2) for health parameters collected on animals from the forested sites are reported for *Elliptio complanata.* Animal weights and shell lengths are provided to describe the reference population.

Table 3.2. Hemolymph and tissue parameter 95% reference limits and 90% confidence intervals for *Elliptio complanata* from 19 stream reaches in North Carolina. Weights, lengths and weight-to-volume indices were measured in air on intact animals. Glucose, phosphorus, magnesium, AST, ammonia, bicarbonate, protein and cell count are hemolymph parameters. Glycogen and $d^{15}N$ are foot tissue parameters.

Parameter	n	Lower Reference Limit	Lower Limit 90% CI	Upper Reference Limit	Upper Limit 90% CI
Weight	380	18.8 g	(17.0, 21.5)	104.6 g	(96.0, 113.3)
Length	380	54 mm	(52, 55)	94 mm	(90, 95)
Weight-to-Volume	380	0.58 mg/mm3	(0.51, 0.59)	0.77 mg/mm3	(0.75, 0.79)
Glucose	372	<2 mg/dL	(<2, <2)	4 mg/dL	(4, 5)
Phosphorus	374	<0.3 mg/dL	(<0.3, <0.3)	0.9 mg/dL	(0.9, 1.0)
Calcium	375	13.1 mg/dL	(12.5, 13.8)	23.7 mg/dL	(22.7, 25.0)
Magnesium	374	1.6 mg/dL	(1.5, 1.9)	3.8 mg/dL	(3.7, 4.0)
AST	374	<4 U/L	(<4, <4)	38 U/L	(27, 42)
Ammonia	380	<10 µmol/L	(<10, <10)	138 µmol/L	(111.2, 198.8)
Bicarbonate	375	<5 mmol/L	(<5, 5)	12 mmol/L	(11, 13)
Protein	378	19.5 mg/dL	(13.3, 22.5)	142.8 mg/dL	(130.1, 160.1)
Cell Count	377	250 /µL	(170, 300)	2300 /µl	(2020, 2900)
$d^{15}N$	379	4.96	(4.72, 4.97)	9.63	(9.26, 10.62)
Glycogen	78 (13 sites)	47 mg/g	(36, 57)	176 mg/g	(155, 187)

Health Measures Correlate with Gravidity and Shell Length: Hemolymph glucose, AST and ALT, and foot tissue $d^{15}N$ were negatively correlated with shell length, while hemolymph calcium and bicarbonate showed positive correlations.

Gravidity status was statistically correlated with foot glycogen, hemolymph protein and trematode presence (Table 3.3). However, only trematode presence showed substantial predictive strength for gravidity status with an odds ratio of 0.25 (Table 3.3). Adding stream site to the model did not improve its resolution.

Table 3.3. Logistic regression of trematode status, foot glycogen concentration and hemolymph protein concentration on gravidity status in *Elliptio complanata* from 19 stream reaches.

Predictor	Coefficient	St Dev	Z	P value	Odds Ratio	Lower 90% C.I	Upper 90% C.I.
Constant	0.988	1.148	0.86	0.389			
Trematode	-1.402	0.57	-2.46	0.014	0.25	0.08	0.75
Glycogen	-0.019	0.009	-2.02	0.044	0.98	0.96	1.00
Protein	0.021	0.009	2.33	0.020	1.02	1.00	1.04

Glycogen values were lower in animals that were gravid (median = 99.61 mg/g dry tissue weight, range 0 – 173.60, Q1 = 77.82, Q3 = 125.26), relative to those that were not gravid (median = 114.32 mg/g dry tissue weight, range = 57.95 – 211.07, Q1 = 100.68, Q3 = 138.03), at the time of hemolymph collection. Protein values were slightly higher in animals that were gravid (median = 67 mg/dL, range = 24.7 – 174.6, Q1 = 43.38, Q3 = 105.27) compared with those that were not gravid (median = 55.0 mg/dL, range = 5.4 – 159.5, Q1 = 34.6, Q3 = 73.3). Forty-seven percent of the non-parasitized animals and only 24% of the parasitized animals examined were gravid. One of the animals examined histologically was hermaphroditic: spermatogenic tissue was much more common than oogenic tissue (Figures 3.4 and 3.5), but both were present. This animal was not gravid.

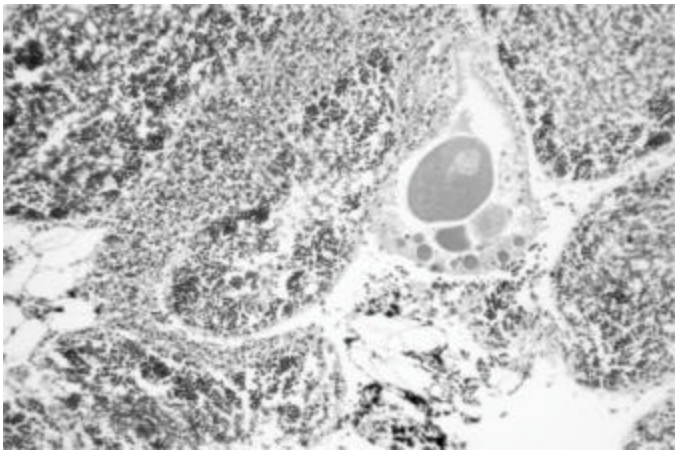


Figure 3.4. *Elliptio complanata hermaphrodite with predominantly spermatogenic, and a locus of oogenic, gonadal tissue. (H&E, magnification 10x18)*

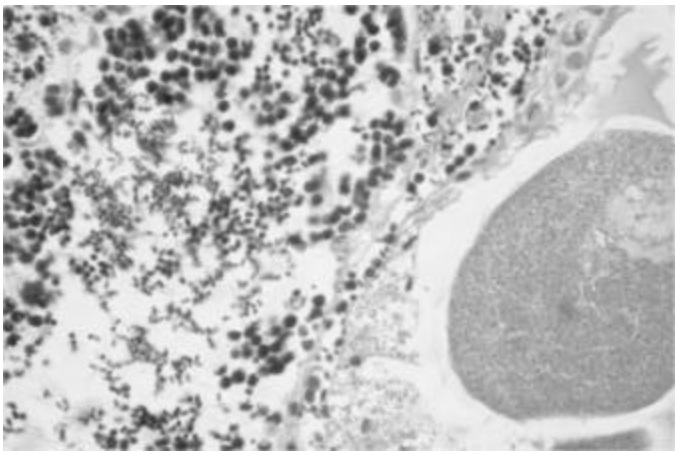


Figure 3.5. *Higher magnification of spermatogenic (left side and center) and oogenic (right side) tissues from the gonad of an Elliptio complanata (H&E, 40x18).*

Linear regression by site found that a site's percentage of parasitized animals collected was inversely correlated with its percentage of gravid animals (tremadode percent = 0.485 – 0.216 gravidity percent; F = 8.82, regression DF = 1, residual error DF = 17, P = 0.009; R2 = 34.2%). Larval forms of the trematode parasite, possibly *Homalometron armatum* (Chittick et al., 2001), were found in tissues of 39% of the animals studied histologically and 65% of the sample sites. Trematode parasites were seen primarily in the foot (Figure 3.6) and mantle tissues of the mussels, though were also found in the gills, gonad and digestive gland (Figure 3.7). Sections varied in appearance from metacercarial forms encysted within smooth eosinophilic capsules (Figures 4,5), to encysted forms packed with eosinophilic spheres (Figure 3.6), to sporocyst or redia specimens encapsulated within cellular teguments (Figure 3.7). It was not determined whether these variations in form represent different species or multiple life stages and histologic cross-sections of a single species. Little evidence of host inflammatory response accompanied the parasites, other than possibly a very slight increase in cellular infiltrate in some of the more heavily parasitized specimens. The gonadal tissue of a single animal was almost entirely obliterated by trematode metacercariae (Figure 3.7). The host *Elliptio* had an elevated hematocyte count.

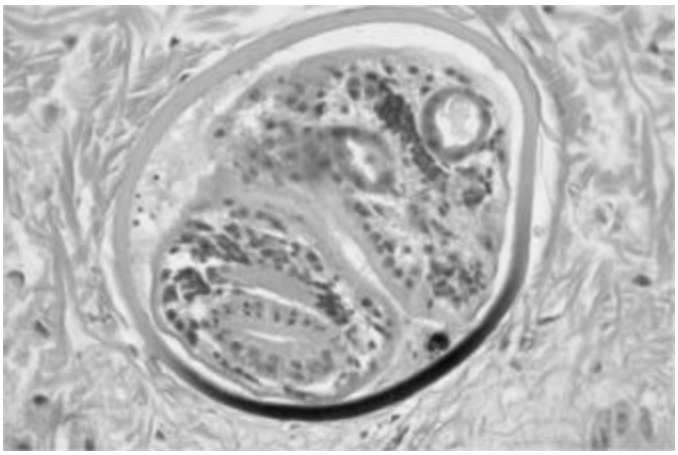


Figure 3.6. *Encysted cercaria of a trematode in foot tissue of an Elliptio complanata (H&E, 40x18).*

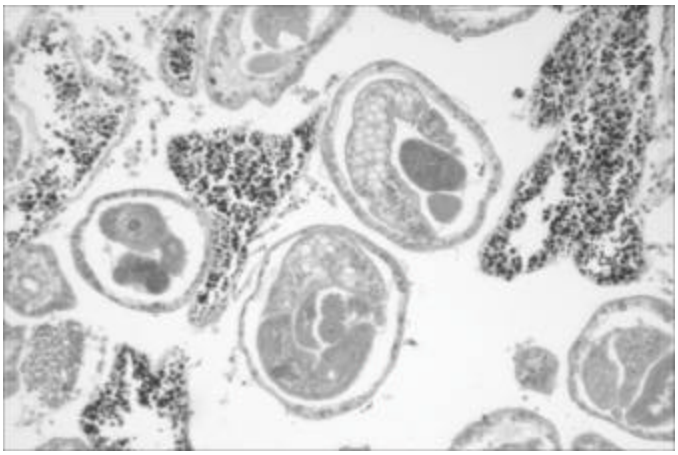


Figure 3.7. *Cercaria of a trematode parasite (right center) in the digestive gland of an Elliptio complanata (H&E, 10x18)*

Parasitized animals were much less likely to be gravid than those that were not parasitized (Chi-square = 7.594, DF = 1, P-value = 0.006). The Bayesian likelihood ratio for parasitized animals was 2.04 against gravidity. The Bayesian likelihood ratio for parasite-free animals was 0.66 (or 1 / 1.52) favoring gravidity. Parasite prevalence did not correlate with any other individual health parameters.

Health Measure Correlates with Mussel Abundance and Diversity: Parasitized individuals were more common in low mussel abundance/diversity sites. Similarly, gravid animals were less prevalent in low abundance/diversity sites. However, neither association was statistically significant at an alpha level of 0.05 in univariate (chi-square) and logistic regression analyses.

Effects of road crossings and landuse

All parameters varied significantly by stream site ($p < 0.05$, Kruskal-Wallis). Therefore, geographic location and/or sampling date appear to play a role in parameter variability. Because the field season was relatively short (21 May – 12 July), little temporal variation was expected. However, because individual sites were visited only once, temporal variability cannot be tested with this data set. There were no overall significant differences in hemolymph parameters between upstream and downstream of road crossings. None of the health parameters varied in a consistent manner between upstream and downstream of road crossings (n = 20 sites, $p > 0.05$, Kruskal-Wallis).

There were also very few statistically significant differences between data collected from animals near agricultural settings and those residing along forested settings. Kruskal-Wallis tests identified only glucose and calcium as significant variants by contiguous land-use designation (Table 3.4). Glucose values were slightly lower on average in forested compared to agricultural settings. Calcium also tended to run slightly lower in animals from forested streams. The animals collected from forested sites were similar in size and weight to those collected from agricultural sites (Table 3.5).

Table 3.4. Results from Kruskal-Wallis tests for differences in median parameter values between populations contiguous to forested and agricultural settings.

Parameter	#Forested / #Agricultural Sites	Forested Site Median	Agricultural Site Median	Kruskal-Wallis H	DF	P
Glucose	10 / 9	<2 mg/dL	<2 mg/dL	5.28	1	0.022
Calcium	10 / 9	16.7 mg/dL	17.65 mg/dL	4.51	1	0.034
Phosphorus	10 / 9	0.4 mg/dL	0.5 mg/dL	2.77	1	0.096
Protein	10 / 9	58.83 mg/dL	67.05 mg/dL	2.16	1	0.142
Magnesium	10 / 9	2.7 mg/dL	2.8 mg/dL	1.06	1	0.303
Foot Tissue Glycogen	6 / 7	113.9 mg/g	106.5 mg/g	1.00	1	0.317
Bicarbonate	10 / 9	7.5 mmol/L	8.0 mmol/L	0.92	1	0.337
Cell Count	10 / 9	1018 cells/µL	1010 cells/µL	0.60	1	0.438
AST	10 / 9	5.75 U/L	6.0 U/L	0.24	1	0.622
Length	10 / 9	71.75 mm	71.5 mm	0.24	1	0.624
Weight-to-Volume	10 / 9	0.6766 mg/mm^3	0.6703 mg/mm^3	0.17	1	0.683
Parasite Prevalence	10 / 9	0.2977	0.4286	0.08	1	0.775
Ammonia	10 / 9	21.5 µmol/L	18.35 µmol/L	0.06	1	0.806
Weight	10 / 9	47.82 g	47.34 g	0.00	1	1.000
ALT	10 / 9	<4 U/L	<4 U/L	0.00	1	1.000
Tissue $d^{15}N$	10 / 9	7.626	7.326	0.00	1	1.000

Table 3.5. Size and weights of *Elliptio complanata* by contiguous land-use classification.

Meristic	Forest Median (n=200)	Forest 95% Interpercentile	Agricultural Median (n=180)	Agricultural 95% Interpercentile	Kruskal-Wallis P value
Weight, g	46.67	(17.5 - 102.1)	47.77	(21.9 – 104.6)	0.273
Shell Length, mm	71	(53 - 92)	72	(54 – 94)	0.078
Wt/Vol, mg/mm^3	0.67	(0.57 – 0.76)	0.67	(0.58 – 0.77)	0.777

Discussion

Technique development

Technique Development and Appraisal: The chemical attributes of hemolymph collected from the adductor muscle sinus were very similar to those of hemolymph collected from the ventricle of the heart in three of the four animals examined. The differences seen in ammonia and cell count in one of the four animals tested may have resulted from in-apparent mixing of pericardial or intestinal fluids with the ventricular sample. Sources of both of these fluids are in close proximity to the site of collection of ventricular hemolymph. Ventricular hemolymph was difficult to collect without injury to the animal. Furthermore, discerning whether the fluid was ventricular or pericardial in origin required the use of a dissecting microscope and even then was difficult. The anterior adductor muscle sinus, in contrast, was easy to access, produced a sizable quantity of fluid and collection resulted in no obvious harm to the animal. Consequently, we chose to focus on the anterior adductor muscle sinus for hemolymph collection safety studies.

Safety Analyses: A limited number of studies have examined the impacts of hemolymph collection in marine (Ford, 1986; Yanick and Heath, 2000) or brackish-water (Fyhn and Costlow, 1975) bivalve species and have found no adverse effects on survival. Our results on a freshwater species corroborate the earlier findings. We found that hemolymph sampling from the anterior adductor muscle sinus impacted neither survival nor growth in *Elliptio complanata.* Surprisingly, the singly-sampled group displayed greater weight gain than the controls, though the difference was not statistically significant. Shell growth in this time frame and under our husbandry conditions was not appreciable in either cohort.

Our findings of very little growth over time, in both the treatment and control groups, are consistent with other studies of adult freshwater mussels in captivity. Attempts to house adult freshwater mussels in captivity for any length of time have met with limited success (Gatenby et al., 1999; Naimo et al., 2000). This is presumed to reflect inadequate diet. Survival rates were not affected by hemolymph sampling, even under the imperfect conditions of prolonged captivity. Consequently, unless there is increased visibility to predators following handling, it is likely that the procedure's safety will transfer to the context of the field environment.

Utility of Hemolymph for Ecologists and Health Specialists: Freshwater bivalves inhabit a wide range of aquatic habitats, are sensitive to habitat disturbance and are fairly simple to collect (Hauer and Lamberti, 1996). Therefore, they are potentially important sentinel species for biomonitoring aquatic changes. They are also currently one of the most imperiled faunal groups in North America (Williams et al., 1993). The better their health and habitat requirements are understood, the more clearly we can target interventions to improve population and ecosystem recovery efforts. Our studies found that 0.5 cc is a safe collection target volume for *Elliptio complanata* measuring 4-7 cm in shell length. With modification of target volumes and perhaps needle gauge, this technique is likely to be adaptable to different size classes and species of freshwater mussels. Circulatory fluid analysis may provide a nonlethal avenue for freshwater bivalve health assessment that is both simple to perform and minimally intrusive to populations under study. This may also open to the door to use of hemolymph for other assays such as genetic composition analysis.

Establishment of hemolymph reference ranges

Reference Ranges: The utility of freshwater mussel hemolymph as a diagnostic tool is dependent on the development of standardized reference ranges that can guide the interpretation of hemolymph samples. The reference ranges provided by this study are an initial step towards developing hemolymph analysis as a viable diagnostic aid for assessing the health of unionids. The *Elliptio complanata* used in these studies were obtained from streams in a region with relatively low environmental impacts. By sampling 380 animals from nineteen different populations we have attempted to ensure that these reference ranges are relatively robust to site and animal differences. Histologic examination of a portion of the harvested animals confirmed the general health of the sampled populations.

Our efforts were concentrated in early summer months to coincide with fieldwork for a related project. The current reference ranges are most suited for use on *Elliptio complanata* collected during early summers in the piedmont region of North Carolina. Bivalve hemolymph parameters are likely to change with species, season and habitat type (Holopainen, 1987; Pekkarinen, 1997) and knowledge of the extent and probability of these variations is also essential to appropriate interpretation of this type of data. Results may also vary somewhat with different laboratories, personnel and methods of analysis. The extent and implications of expected differences by season, geography, laboratory and species have not yet been established and need further study.

Our sites were fairly homogeneous at the level of the watershed. Approximately 70% of the contributing watershed was classified as forested, and approximately 30% as agricultural in the vast majority of sites. However, classification of the riparian zone immediately proximate to our collection sites did show more variability, with half of the sites adjacent to predominantly forested settings and half adjacent to mild to moderate agricultural activity (including land designated for crop or animal production, whether active or currently fallow). We found few differences in health parameters relative to adjacent land-use patterns. However, given the current and ongoing declines of many sensitive freshwater mussel species (Bogan, 1993), it is important to examine these findings in relation to our expectations regarding land-use.

Influence of Parasite Burden, Gravidity and Animal Size: We found that trematode prevalence was heaviest in sites with low animal abundance or low species diversity. Little histologic evidence of inflammation accompanied the parasite burden, however, and hematologic parameters, on average, did not show any statistical relationship to parasite burden. This suggests that the mussels are perhaps fairly well adapted to the parasite, and that different hematological measures are needed to support nonlethal detection. However, in seemingly related correlations, gravidity was less prevalent in parasitized animals, and also less prevalent in the low abundance/diversity sites. These findings, in concert with observations of mild to severe trematode presence in gonadal tissues, raises the concern that trematode infestation may directly diminish a population's reproductive potential. Alternatively, parasite infestation may be a corollary of, or secondary to, habitat quality (Chittick et al., 2001). Further research to establish the population effects of this relatively common freshwater mussel parasite is recommended.

Animals that were gravid at the time of collection had significantly lower median glycogen values than the other animals. This may reflect the energy required to maintain or produce glochidia. Gravid animals also had significantly higher hemolymph protein values than their non-gravid counterparts. It is possible that the osmoregulatory function of the gill is impacted somewhat by brooding glochidia, affecting hemolymph protein either by compensatory or direct response. It is also possible that the lower protein values correspond to a metabolic shift away from carbohydrates in glycogen-depleted conditions. Given the subtle nature of impacts at our study sites, we recommend that parasite burden, glycogen, gravidity, and hemolymph protein, calcium and glucose receive future attention as possible early warning indicators of habitat demise.

Animal size, represented by shell length, also showed some statistical association with certain measured parameters. The strengths of these correlations were relatively weak (small correlation coefficients). However, given the relative homogeneity of animal size may be important to future work. Delta ^{15}N and hemolymph glucose, AST and ALT, for example, did show linear dependence on shell length, with the highest values in the smallest animals. Hemolymph calcium and bicarbonate, in contrast, were positively correlated with shell length, showing the highest values in the largest animals.

Effects of road crossings and landuse

Road crossings appeared to have no effect on hemolymph chemistry because no trends were seen in measured parameters between upstream and downstream samples. This result may support the notion that the greatest threat crossing structures pose is in their initial construction phase and in their hydraulic influence (Levine et al. 2003). However Agricultural land-use has been implicated as a detrimental factor governing freshwater mussel declines (Bogan, 1993). Soil erosion and bank instabilities can lead to siltation and turbidity of streams. Both fertilizers in runoff and loss of shading contribute to an increase in nuisance algal growth, and potentially hypoxic conditions (Hauer and Lamberti, 1996). Furthermore, heavy metals, petroleum by-products, solvents and other toxic materials found in conjunction with machinery and industrialization, can wash into streams during rain events (Hoffman et al., 1985).

Contaminants may be of greatest consequence in disturbed riparian zones. Agricultural and industrial influences can diminish a streams' natural capacity to filter and remove toxicants

prior to recharge of the stream (Outwater, 1996). The healthy riparian zone is a network of intertwining roots, soil particles and rocks through which overland water is filtered and detained en route to the water table (Hauer and Lamberti, 1996). Beaver ponds create natural holding ponds slowing water recharge (Outwater, 1996). Changes that accompany urban and agricultural development, such as channelization, beaver dam disturbance, soil homogeneity, and removal of trees and roots, reduce the natural filtration capacity of the riparian banks. Consequently, physical and chemical impacts to a waterway may be multiplicative in their combined impacts on flora and fauna.

The goal of our study was to establish reference ranges for *Elliptio complanata* and to examine their robustness to slight variations in habitat use. Though relatively homogenous at the watershed level, our sites did vary in the predominant character of surrounding riparian lands. We chose only sites with known populations of unionids, thus none were experiencing acknowledged or gross population declines. In this light however, differences seen between study groups, no matter how small, become interesting to discuss. Small differences in parameters may reflect subtle differences in habitat health. It is particularly important to be able to distinguish populations in early stages of decline for successful mitigation.

Our investigations found that calcium and glucose showed significant differences between populations residing along primarily forested versus agricultural riparian tracts. On average, both calcium and glucose ran higher at the agricultural sites. Hemolymph glucose is a measure of the carbohydrate energy released and circulating in the bloodstream. Many, if not all, bivalve tissues and organs are capable of breaking glycogen, the principal storage form of carbohydrate, into glucose (Gabbott, 1983). Circulating glucose levels may reflect the animal's ability or readiness to respond to immediate nutritional needs. Heightened circulating glucose may be a response to weather extremes, handling stress (Pekkarinen and Suoranta, 1995) or any recent environmental stress perceived to require extra fuel. It is also possible that simple differences in habitat could account for these effects. For example, shading and riparian zones associated with the forested streams may help buffer changes in weather (sun, rain, wind and temperature changes) that seem more intense in the open environment of the agricultural habitats. In this sense, the agricultural animals may, on average, truly experience greater fluctuations in environmental condition than their similarly treated forest counterparts.

Calcium is a circulating ion important in shell formation, acid-base regulation and respiratory health (McMahon, 1991). Calcium, phosphates, silica and magnesium solutes in streams originate primarily from weathering of soils and rocks (Webster and Ehrman, 1996). Differences in calcium may reflect differences in habitat health. Hemolymph calcium is known to increase in response to anoxic or hypoxic conditions (Pynnonen, 1990 and 1994). Calcium is exchanged for hydrogen ions, allowing for homeostasis of ionic equilibrium while helping to balance blood pH (crucial to enzyme regulation and animal survival) (McMahon, 1991). While many of these streams were relatively shallow and apparently well oxygenated, small differences in oxygen availability related to nutrient enrichment might affect calcium values. Siltation, potentially clogging gill membranes or impeding gill function, might also affect oxygenation and result in slight differences in calcium levels, though both hypotheses need experimental confirmation.

Summary of Findings

1. We recommend a target hemolymph volume of 0.5 cc drawn from the anterior adductor muscle of 4-8-cm *Elliptio complanata.* No affects to growth or survival were seen with our method

2. We present reference ranges for Elliptio complanata in NC taken in the summer for the following hemolymph parameters:

Glucose	Ammonia
Phosphorus	Bicarbonate
Calcium	Protein
Magnesium	Cell Count
AST	$d^{15}N$

3. Hemolymph glucose, AST and ALT, and foot tissue $d^{15}N$ were negatively correlated with shell length, while hemolymph calcium and bicarbonate showed positive correlations.

4. Gravidity status was statistically correlated with foot glycogen, hemolymph protein and trematode presence; however, only trematode presence showed substantial predictive strength for gravidity status with an odds ratio of 0.25 (Table 2.3).

5. Road crossings had no overall effect on hemolymph parameters

6. Hemolymph calcium and glucose were significantly higher at agricultural sites compared to forested sites. These differences may reflect a true environmental stressor to these mussels from the surrounding landuse, but the true cause is unknown.

Chapter 4

Contaminant Assessment

Introduction

The purpose of this chapter is to present findings related to chemical exposure associated with road runoff. The specific objectives of this study on chemical exposure were to:

1. **Determine whether road crossings contribute to chemical body burdens in freshwater mussels**

2. **Assess the bioavailability of sediment-bound chemicals to the freshwater mussels**

3. **Evaluate the utility of passive sampling devices to serve as a surrogate measurement for mussel body burdens**

Freshwater mussels and sediment were collected at 20 road crossing sites within the larger number of sites studied by Levine et al. (2003). In addition, passive sampling devices designed to accumulate hydrophobic and persistent organic contaminants were deployed at these sites. Samples were analyzed quantitatively for approximately 150 chemicals and qualitatively for another 100,000 chemicals (Table 4.1). Our findings are presented below.

Table 4.1. List of chemicals analyzed in this study.

Current Use Pesticides

Defoliants
tribufos

Herbicides
2,4-D
acifluorfen
alachlor
atrazine
bentazon
butylate
cyanazine
diuron
EPTC
ethalfluralin
fluometuron
linuron
metolachlor
metribuzin
molinate
napropamide
norflurazon
pebulate
pendimethalin
prometryn
pronamide
propachlor
propanil
simazine
tebuthiuron
terbacil
thiobencarb
triallate
trifluralin

Herbicide Metabolites
2,6-diethylaniline
desethylatrazine
deisopropylatrazine
3,4-dichloroaniline

Insecticides
azinphos methyl
carbaryl
carbofuran
chlorpyrifos
diazinon
dimethoate
disulfoton
ethoprop
fonofos
malathion
methyl parathion
phorate
profenofos
propargite
terbufos

Polycyclic Aromatic Hydrocarbons

Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthylene
Acenaphthylene
Acenaphthene
Dibenzofuran
2,3,5-Trimethylnaphthalene
C1 - Naphthalenes
C2 - Naphthalenes
C3 - Naphthalenes
C4 - Naphthalenes
Fluorene
1-Methylfluorene
C1 - Fluorenes
C2 - Fluorenes
C3 - Fluorenes
Dibenzothiophene
C1 - Dibenzothiophenes
C2 - Dibenzothiophene
C3 - Dibenzothiophene
Phenanthrene
Anthracene
1-Methylphenanthrene
C1 - Phenanthrenes/Anthracenes
C2 - Phenanthrenes/Anthracenes
C3 - Phenanthrenes/Anthracenes
C4 - Phenanthrenes/Anthracenes
Fluoranthene
Pyrene
C1 - Fluoranthenes/Pyrenes
Retene
Benz[a]anthracene
Chrysene
C1 - Chrysenes
C2 - Chrysenes
C3 - Chrysenes
C4 - Chrysenes
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[e]pyrene
Benzo[a]pyrene
Perylene
Indeno[1,2,3-c,d]perylene
Dibenz[a,h]anthracene
Benzo[g,h,i]perylene
Coronene

Other Organic Chemicals

benzothiazole
full scan GC/MS (~100,000 chemicals)

Chlorinated Pesticides

alpha BHC
beta BHC
gamma-BHC (lindane)
delta BHC
hexachlorobenzene
heptachlor
heptachlor epoxide
alpha chlordane
gamma chlordane
trans-nonachlor
aldrin
dieldrin
alpha endosulfan
beta endosulfan
endosulfan sulfate
endrin
endrin aldehyde
endrin ketone
methoxychlor
mirex
4,4'-DDT
4,4'-DDD
4,4'-DDE
2,4'-DDT
2,4'-DDD
2,4'-DDE

PCBs

PCB 8
PCB 18
PCB 28
PCB 52
PCB 44
PCB 66
PCB 101
PCB 77
PCB 118
PCB 153
PCB 105
PCB 138
PCB 126
PCB 187
PCB 128
PCB 180
PCB 170
PCB 195
PCB 206
PCB 209

Metals

Cu, Cd, Pb, Ni, Pt, Pd

Methods

Sample Collection and Handling

Mussels (*Elliptio complanata*) were taken from an area within a 50-m reach upstream and downstream of each bridge crossing, resulting in two sample locations within each study site. Five mussels were randomly selected among those collected for chemical analysis, placed in zip-lock bags and on ice, and transported to the laboratory. Within 24 hrs of collection, mussels were gently pried open with a clam knife or spatula. Tweezers were then inserted sideways to hold the shell open. Hemolymph was collected from the adductor mussel using a 1-mL insulin syringe with a 25G needle. Approximately 1 mL of hemolymph was collected and then dispensed into a 1-mL cryo-vial. The samples were stored at -80 ºC. Soft tissue from the five mussels were scraped from the shell, composited, freeze dried, homogenized, and then aliquots were taken for extraction and analysis. Shells were measured and archived.

Surficial (top 2-3 cm) sediment samples were taken by collecting equal portions of sediment from five randomly chosen locations within each study site. An effort was made to collect only fine-grained sediment inhabited by the mussels, so as to avoid sand and gravel. Sediment was placed on ice, transported to the laboratory, and frozen at -20 ºC. Sediment was freeze dried, homogenized, and then aliquots were taken for extraction and analysis.

Passive sampling devices (PSDs) were constructed using approximately 12.7-µm thick low-density polyethylene (PE) tubing, containing no plasticizers or additives. The PE tubing (5 cm X 30 cm, surface area of 300 cm^2) was pre-extracted with hexane for 48 hours prior to use and fixed inside a protective polyethylene cage. Two PSDs were placed in each cage and deployed within the 50-m zones upstream and downstream from the bridge crossing and retrieved approximately 30 days later. Previous work has demonstrated that a 30-day deployment time allows the 12.7-µm PE to reach equilibrium with water. Following deployment, one of the PSDs was archived at -20 ºC and the second was cleaned with de-ionized water and a brush, followed by a quick rinse in acetone to remove material from the surface of the LDPE prior to extraction.

Sample Extraction and Preparation

Mussel, sediment, and PSD samples were extracted for organic analysis as described by Thorsen et al. (2004) and Luellen and Shea (2002). Samples were shaker-extracted (200 rpm) for 24 h using dichloromethane (DCM) for mussels and PSDs and using DCM:acetone (1:1) for sediment samples. Concentrated extracts were fractionated using high performance gel permeation chromatography to remove high molecular weight matrix components (e.g., lipids, polyethylene waxes). The extracts were solvent exchanged into hexane and then further purified on a 3-g silica column. Mussel lipid content was determined by passing extracts through a gel permeation chromatography (GPC) column, collecting the lipid fraction, evaporating and weighing. For metals analysis, freeze-dried tissue was digested with HNO_3 using a microwave procedure described by Zimmermann et al. (2002).

Instrumental Analysis

The purified extracts were analyzed for PAH and current use pesticides (including benzothiazole) on two separate chromatographic runs using an Agilent 6890 gas chromatograph (GC) connected to an Agilent 5973N MSD utilizing a Restek 30m x 0.25mm Rtx-5 (film thickness 0.25 µm) MS w/Integra-Guard column. The pressure was ramped to 40 psi before injection with a 1-min hold time. The flow was then dropped to give a constant flow of 1 mL/min for the duration of the run. The temperature program for PAH analysis was as follows: initial temperature 40 °C for 1 min with a ramp of 6 °C /min to 290 °C and a final hold time of 30 min; injector temperature 300 °C, detector temperature 280 °C. Selected ion monitoring (SIM) was used for analysis. The full scan GC/MS analysis was performed using the same instrument and run conditions, but operating the MS in the full scan mode and using both the NIST and Agilent pesticide libraries to search for mass spectra and identify peaks.

Polychlorinated biphenyls and chlorinated pesticides were analyzed by GC with electron capture detection (ECD) using a dual-column (30 m x 0.25-mm i.d., 0.25 um film, DB-1 and DB-17; J&W Scientific, Folsom, CA) dual ECD (Hewlett-Packard 5890 Series II, Avondale, PA) for confirmation. The GC temperature program was initial 60 ºC (1 min hold) to 160 ºC at 20 ºC/min, held for 10 min, and ramped to 260 ºC at 2 ºC/min with a final hold of 20 min. Injector and detector temperatures were 260 and 280 ºC, respectively. Samples were quantified from the DB-1 chromatograms and confirmed using the DB-17 chromatograms.

Metals were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). The mass fraction of organic carbon (OC) in sediment was determined by Carbon, Hydrogen, Nitrogen (CHN) analysis with an elemental analyzer.

Results and Discussion

Contaminant Concentrations in Mussels

A summary of chemical contaminant concentrations found in the mussels is provided in Table 3. A complete list of the data is provided in the Appendix. All organic contaminant data for mussels are expressed on a lipid-normalized basis (microgram per gram lipid) because it is well established that the accumulation of hydrophobic, non-ionic chemicals in most organisms is highly dependent on the lipid mass fraction in that organism. This has recently been confirmed for *E. complanata* by Thorsen et al. (2004). Trace metal concentrations are expressed on a dry weight basis because their accumulation is dependent on factors in addition to lipid content. The Appendix provides organic contaminant data on both dry and lipid basis and provides lipid and dry fractions to allow conversion among these normalizations.

Polycyclic Aromatic Hydrocarbons (PAHs): Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment, produced primarily as a result of anthropogenic activities, and are known to cause adverse human and ecological health effects (Neff 1979; Page et al. 1999; Baumard et al. 1998; Dickerson et al. 1994; Trust et al. 1994; Ward et al. 1984). PAHs can be broadly separated into three non-exclusive categories based on their source (Page et al. 1999): biogenic, petrogenic, and pyrogenic PAHs. Biogenic PAH are formed from natural biological processes including diagenesis; petrogenic PAH are derived from petroleum and usually enter the aquatic environment dissolved in water, air, or a cosolvent such as motor oil; and pyrogenic PAH are formed as a result of incomplete combustion of fuels, and largely enter the environment tightly sorbed to particulate matrices (Neff 1979). Pyrogenic PAH are also produced in tandem with combustion products such as soot (Neff 1979; Goldberg 1985; Broman and Naf 1990). Petrogenic PAHs include the unsubstituted parent and alkyl homologues of naphthalenes, fluorenes, phenanthrenes, dibenzothiophenes and chrysenes; where the alkyl homologues are more abundant than the parent PAH (Page et al. 1999). Pyrogenic PAHs are generally represented by greater abundances of parent compounds, and a predominance of the 3 to 5 ring PAHs such as fluoranthene and benzo(a)pyrene (Page et al. 1999). This simple classification is often useful in discussions of PAH source and fate, but in reality PAH source is complicated by overlap among the 3 classes and by different PAH sources having varying relative abundances of individual PAHs.

PAHs were found at relatively low but measurable concentrations at all sites (Table 4.2). Four different summaries of PAHs are reported: the sum of all 48 PAH analytes measured (see Table 4.1 for list), the sum of the 16 EPA Priority Pollutant PAHs (EPA PAHs), the sum of the petrogenic or petroleum-related PAH, and the sum of the pyrogenic or combustion-related PAH. The sum of the EPA Priority Pollutant PAHs ranged from 0.63 – 6.96 µg/g lipid in samples collected upstream of road crossings and ranged from 2.24 – 12.64 µg/g lipid at sites downstream from crossings. The sum of all 48 PAHs ranged from 1.54 – 41.85 µg/g lipid upstream and 4.58 – 55.97 µg/g lipid downstream. These PAH concentrations are similar to those we have found in *E. complanata* in other rural areas of North Carolina and about 1-2 orders of magnitude lower than those found at more urban sites (Shea, unpublished data).

Table 4.2a. Summary of Contaminant Concentrations in Mussels.										
Upstream	**Agricultural Sites**									
	5	17	200	53	54	57	36	38	80	50
PAHs (ug/g lipid)										
Sum of PAH	2.4	5.8	42	3.2	1.5	5.1	2.1	10	3.4	3.5
Sum of 16 PP PAH	0.63	1.9	7.0	1.1	0.71	1.7	0.68	1.5	1.8	1.2
Petrogenic PAH	1.5	3.4	30	1.9	0.6	2.9	1.2	7.7	1.5	2.1
Pyrogenic PAH	0.51	1.5	6.0	0.62	0.47	1.3	0.42	0.98	1.4	0.87
Other Organics (ng/g lipid)										
PCB 153	13	11	15	<2	13	5.5	12	<2	<2	9.0
DDE	<2	<2	65	61	<2	97	123	125	216	36
Total Chlordane	52	64	181	90	<2	94	75	<2	<2	190
Metals (ug/g dry)										
Cu	18	15	14	16	12	13	15	15	19	17
Cd	3.1	2.8	1.7	2.4	2	2.8	1.9	2.4	1.6	2.4
Pb	0.62	0.41	0.38	0.55	0.62	0.39	0.35	0.44	0.57	0.66
Ni	6.2	4.5	8.6	4.7	5.2	4.9	2.6	5.7	6.8	5.5
Pt (ng/g dry)	0.39	0.09	0.24	0.31	0.26	0.25	0.16	0.17	0.11	0.48
Pd (ng/g dry)	1.3	0.87	0.81	0.86	1.12	1.3	1.5	0.82	0.85	1.4
Downstream	**Agricultural Sites**									
	5	173	200	53	54	57	36	38	80	50
PAHs (ug/g lipid)										
Sum of PAH	9.3	6.9	56	8.3	6.4	9.6	6.9	14	4.6	36
Sum of 16 PP PAH	2.8	2.3	8.6	2.7	2.8	3.5	2.4	2.4	2.2	13
Petrogenic PAH	5.3	4.1	39	4.8	2.9	5.1	3.9	10	2.2	21
Pyrogenic PAH	2.1	1.8	7.4	1.6	1.6	2.6	1.4	1.6	1.8	9.2
Other Organics (ng/g lipid)										
PCB 153	16	6.17	24	<2	8.6	14	19	<2	<2	15
DDE	<2	<2	98	77	<2	79	176	182	287	45
Total Chlordane	35	82	137	69	<2	84	87	<2	<2	201
Metals (ug/g dry)										
Cu	41	18	36	29	38	23	29	19	18	52
Cd	3.2	2.7	1.9	3.7	2.4	3.1	3.3	2.9	1.4	4.4
Pb	0.96	0.73	1.04	0.76	1.38	0.87	0.64	0.57	0.48	1.5
Ni	5.4	5.8	5.3	6.1	5.6	4.9	5.4	5.9	5.8	6.0
Pt (ng/g dry)	0.62	0.14	0.34	0.51	0.39	0.4	0.26	0.16	0.17	1.2
Pd (ng/g dry)	3.1	0.61	1.4	2.3	3.2	1.9	2.5	0.76	0.95	5.1
Traffic Count (vehicles/day)	2900	60	1600	1700	2100	900	1600	130	100	16000

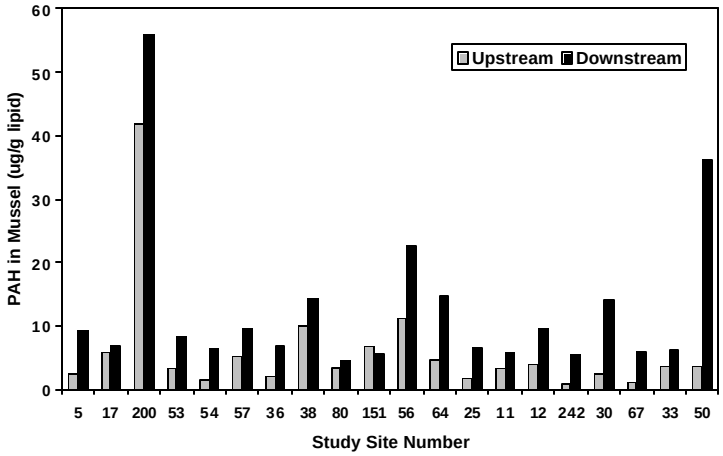
Table 4.2b. Summary of Contaminant Concentrations in Mussels.										
Upstream	**Forested Sites**									
	151	56	64	25	11	12	242	30	67	33
PAHs (ug/g lipid)										
Sum of PAH	6.7	11	4.6	1.8	3.3	3.9	0.86	2.5	1.0	3.7
Sum of 16 PP PAH	1.9	2.8	0.94	0.42	1.0	1.2	0.25	0.54	0.52	1.1
Petrogenic PAH	3.9	7.5	3.4	1.0	1.8	2.4	0.53	1.8	0.42	2.0
Pyrogenic PAH	1.3	1.8	0.74	0.26	0.47	0.76	0.16	0.34	0.31	0.71
Other Organics (ng/g lipid)										
PCB 153	6.9	<2	<2	<2	<2	5.1	<2	<2	<2	15
DDE	139	114	74	<2	27	79	178	829	124	253
Total Chlordane	35	210	147	<2	75	34	84	156	227	89
Metals (ug/g dry)										
Cu	14	12	18	18	15	18	14	17	12	11
Cd	1.5	2.1	1.8	2.8	2.6	2.2	1.9	1.8	2.3	2.9
Pb	0.71	0.49	0.43	0.61	0.35	0.64	0.55	0.41	0.44	0.56
Ni	4.6	3.8	2.5	5.6	3.1	4.3	5.5	4.1	4.4	5.5
Pt (ng/g dry)	0.15	0.36	0.17	0.29	0.22	0.14	0.32	0.38	0.36	0.23
Pd (ng/g dry)	0.88	1.3	0.96	1.0	0.95	1.0	1.1	1.2	1.0	0.95
Downstream	**Forested Sites**									
	151	56	64	25	11	12	242	30	67	33
PAHs (ug/g lipid)										
Sum of PAH	5.7	23	15	6.6	5.8	9.6	5.4	14	6.0	6.3
Sum of 16 PP PAH	1.8	6.3	3.4	1.7	1.9	2.9	1.5	3.7	3.0	2.1
Petrogenic PAH	3.3	14	11	3.5	3.1	5.8	3.4	9.5	2.6	3.3
Pyrogenic PAH	1.2	4.0	2.6	1.0	0.88	1.9	1.0	2.3	2.0	1.4
Other Organics (ng/g lipid)										
PCB 153	10	<2	<2	3.7	<2	8.4	<2	<2	<2	8.8
DDE	91	78	57	<2	31	229	216	657	151	205
Total Chlordane	44	252	134	<2	61	51	102	123	345	63
Metals (ug/g dry)										
Cu	16	30	15	24	17	15	27	36	30	18
Cd	1.5	2.4	2.9	2.8	2	2.2	2.6	2.9	3	2.7
Pb	0.72	1.1	0.41	0.8	0.81	0.57	0.95	1.1	0.77	0.82
Ni	6.2	5.7	5.2	6.4	5.4	6.0	5.0	5.1	5.5	5.2
Pt (ng/g dry)	0.13	0.66	0.16	0.49	0.26	0.19	0.57	0.88	0.46	0.38
Pd (ng/g dry)	0.64	3.4	1.5	2.1	1.4	1.1	2.4	3.5	2.2	1.5
Traffic Count (vehicles/day)	50	3700	490	910	260	580	1800	3300	1000	690

A comparison of the upstream and downstream PAH concentrations is shown for each study site in Figure 1 for the sum of all 48 PAH, and the petrogenic and pyrogenic PAH. On average, the downstream mussels had a little more than twice the concentration of PAHs compared to upstream mussels. A few sites exhibited very little difference (e.g., Sites 17, 80, 151) and a few had larger differences (e.g., Sites 54, 64, 25, 242, 30, 67, 50). Site 50 had the largest difference, with more than a 10-fold increase in PAHs downstream compared to upstream; this was also the site with construction activity. Sites 242, 30, and 67 were all 6- to 7-fold higher downstream compared to upstream. Regression analysis of upstream versus downstream PAH yielded excellent correlations with slopes near or slightly above one and positive intercepts (Figures 4.2 and 4.3). These regressions quantify the PAH enrichment downstream that was illustrated in Figure 4.1.

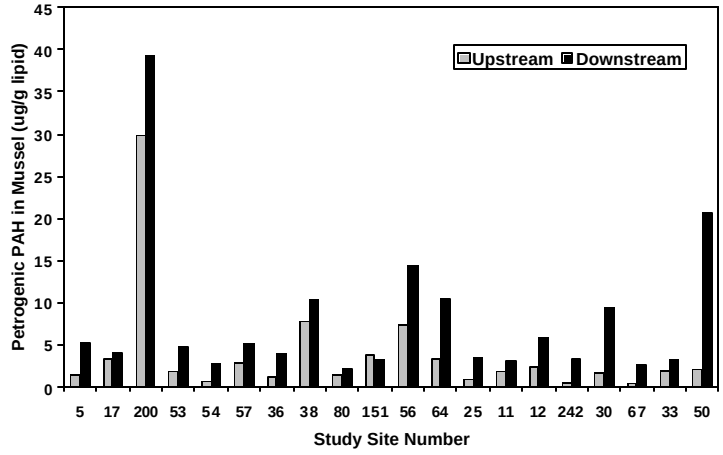
The slightly higher slope and y-intercept for petrogenic versus pyrogenic PAH did not result in an enrichment of petrogenic PAH because the greater abundance of petrogenic PAH in all samples (compare y-axis scales in Figure 4.3). There was no significant difference in the ratio of petrogenic to pyrogenic PAH with mean ratios of 3.16 ± 1.50 upstream and 3.05 ± 1.28 downstream. This indicates that sources of PAH to all sites, both upstream and downstream, are similar in composition. This is somewhat surprising as one would expect the upstream samples to be more weathered if the road crossing was serving as a source of PAH. The fresher PAH signature of recent exhaust and oil leaks that one might expect downstream is not evident in these data. The 16 EPA PAHs consistently make up about 1/3 of all the PAHs measured; site location does not appear to influence this ratio with nearly identical mean ratios at upstream (0.31 ± 0.10) and downstream (0.32 ± 0.09) sites.

The consistency in the relative abundance of individual PAH from upstream to downstream is shown more clearly in Figure 4.4, where the PAH concentrations are normalized to the C1-phenanthrene (P1) concentration. The relative abundance at Site 200 is typical for the sites investigated, with a greater amount of 3-ring and 4-ring aromatics relative to other PAHs. The phenanthrenes (P, P1, P2, P3, P4) and fluoranthene (FL) are enriched relative to the naphthalenes (N, N1, N2, N3, N4). This is a fairly typical signature of runoff that contains weathered oil and automotive exhaust. In contrast, Site 50 is enriched in the lower molecular weight PAH, particularly the naphthalenes. Despite the differences between these two sites, both sites display remarkable consistency between upstream and downstream PAH signatures. This indicates that although road crossings appear to be a source of PAHs to the mussels, the type of PAH is very similar to that coming from upstream.

A



B



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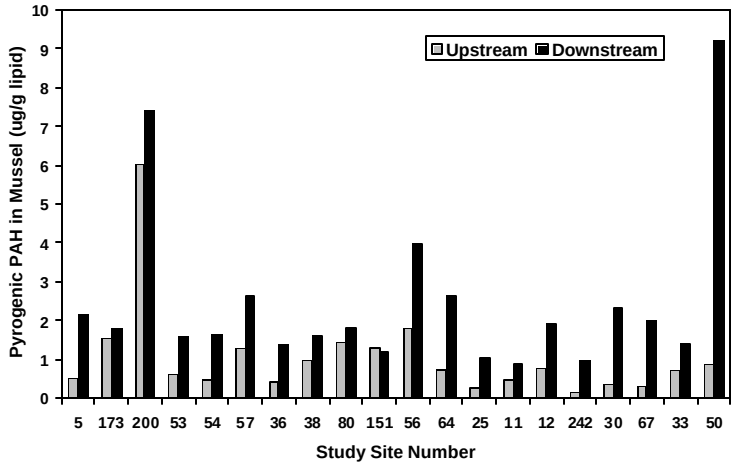
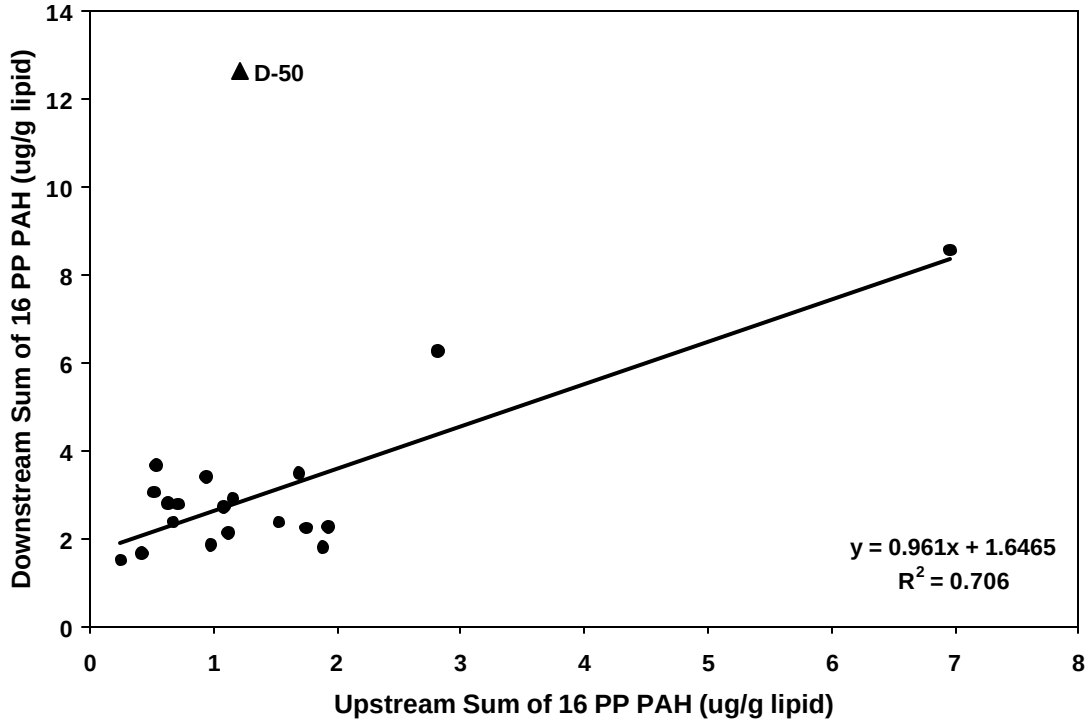


Figure 4.1. *Concentrations of PAH in mussel tissue (µg/g lipid) for the sum of all 48 PAH (A), the petrogenic PAH (B), and the pyrogenic PAH (C).*

A



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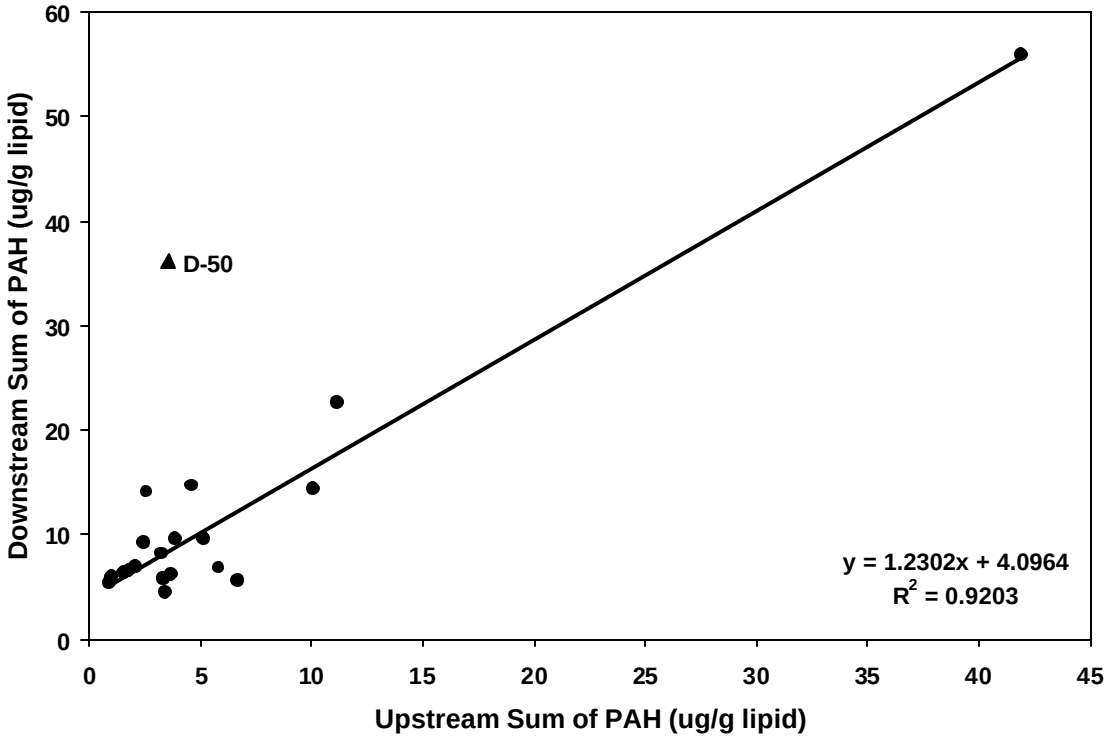
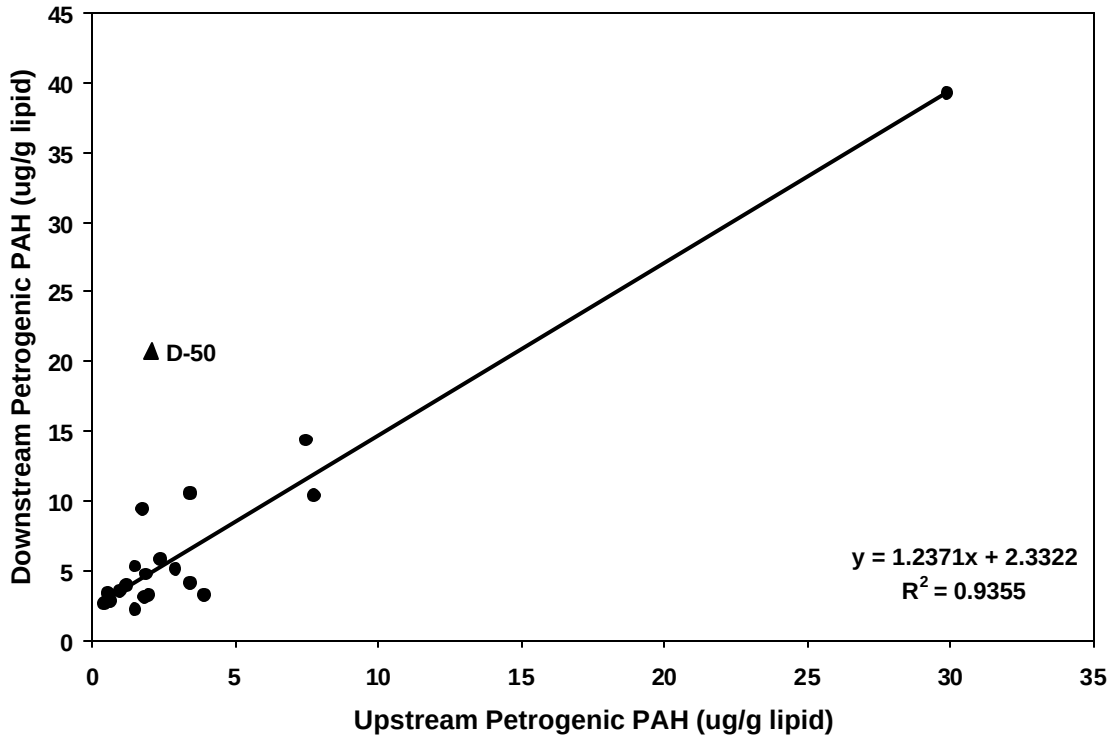


Figure 4.2. *Regression of upstream versus downstream PAH; sum of the 16 EPA Priority Pollutant PAH (A) and sum of the 48 PAH (B). The construction site (D-50) was excluded from the regression.*

A



B

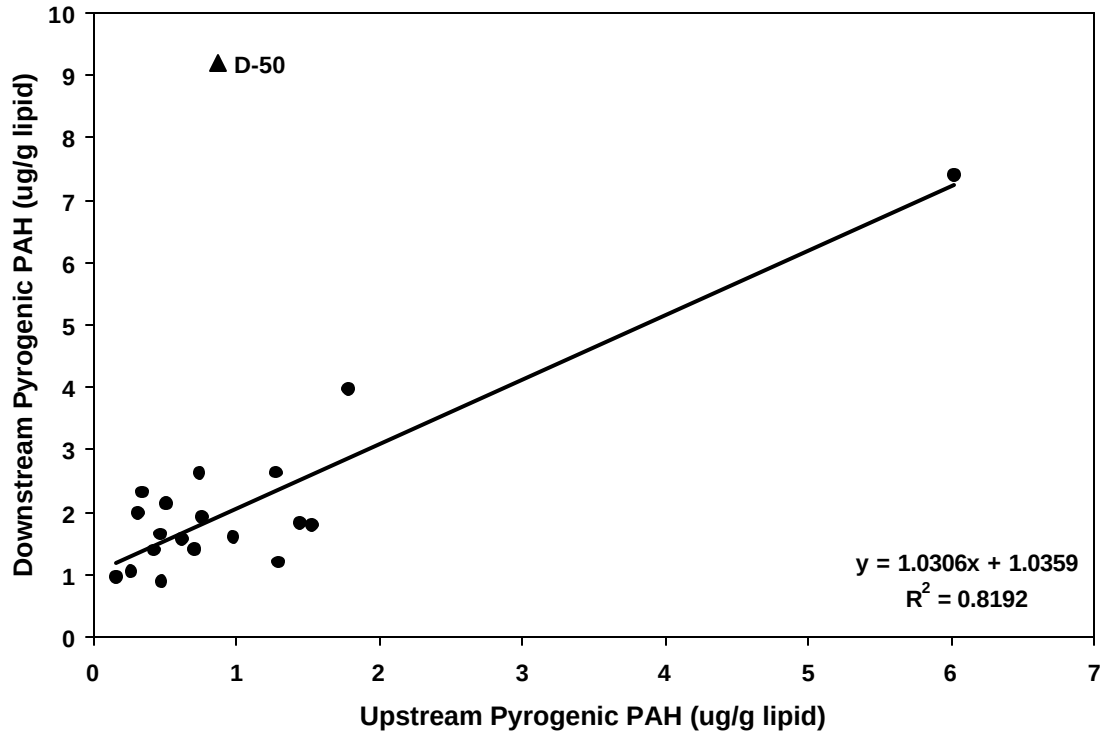
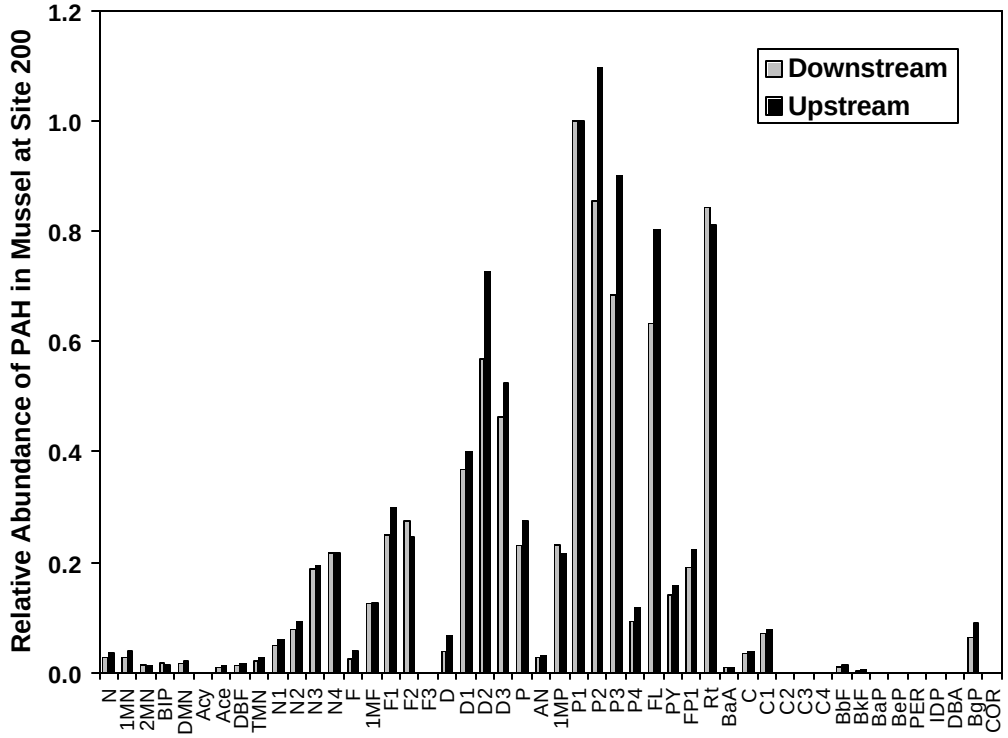


Figure 4.3. *Regression of upstream versus downstream PAH; Petrogenic PAH (A) and Pyrogenic PAH (B). The construction site (D-50) was excluded from the regression.*

A



B

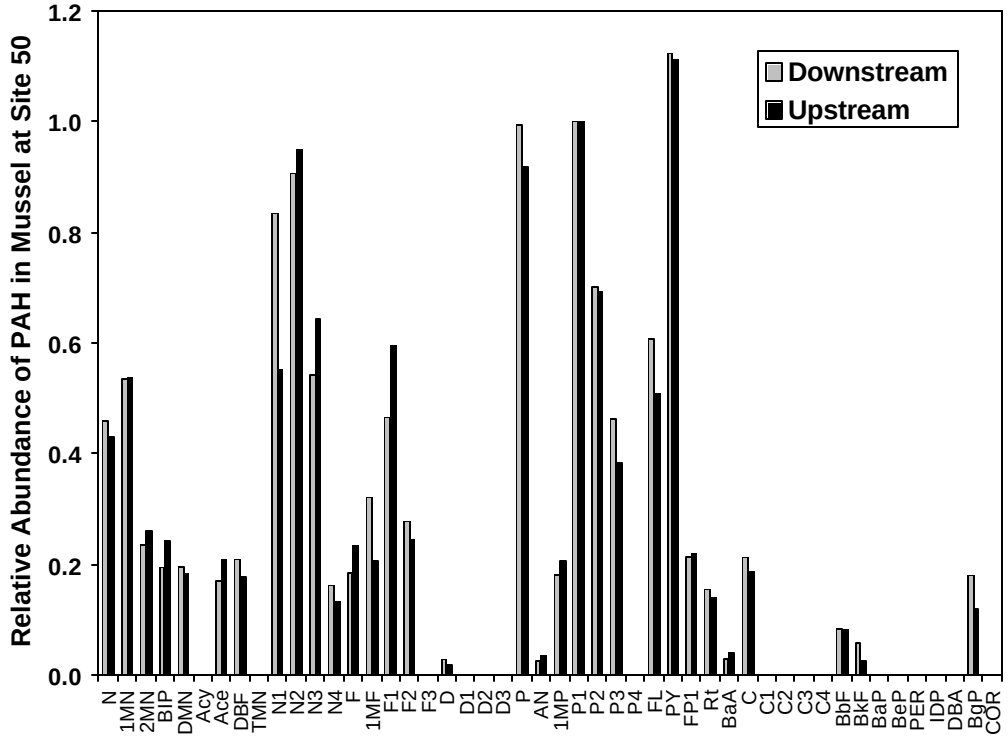


Figure 4.4. *Relative abundance of PAH in mussels normalized to C1-phenanthrene (P1) for Site 200 (A) and Site 50 (B).*

PCBs, Pesticides, and Other Organic Chemicals: Concentrations of polychlorinated biphenyls (PCBs), chlorinated pesticides, current use pesticides, and other organic chemicals identified using full scan GC/MS were either very low or not detectable throughout the study area. All of the organic chemicals detected are listed in Table 4.2. PCB congener 153 was the only PCB detected above 2 ng/g lipid in the mussels, while DDE and chlordane were the only chlorinated pesticides detected. Current use pesticides and benzothiazole (used in tire rubber) were not detected in any samples. The full scan GC/MS analysis also did not reveal the presence of any other chemicals except those known to be naturally occurring (e.g., plant-derived chemicals). Note that the full scan analysis had detection limits about 100-1000 times higher than that for PAHs, PCBs, and pesticides.

Unlike for the PAHs, there was no difference between upstream and downstream concentrations of these other organic chemicals (Figure 4.5) nor was there any pattern to differences among sites. Mean PCB 153 concentrations were 10 and 11 ng/g lipid for upstream and downstream, respectively. Chlordane concentrations were 112 and 116 ng/g lipid and DDE concentrations were 158 and 166 ng/g lipid for upstream and downstream, respectively. These are remarkably similar mean values and indicate that there is no input from the road that differs from upstream sources. Plots of upstream versus downstream concentrations result in good correlations and slopes near unity (Figure 4.6). The following regression equations were obtained:

DDE	$y = 0.7781x + 0.9140$	$R^2 = 0.8422$
Chlordane	$y = 1.1129x - 0.1850$	$R^2 = 0.8122$
PCB	$y = 0.8234x + 0.0949$	$R^2 = 0.2098$

Removing the 3 PCB data points that appear below the regression line yields a much better correlation ($y = 1.5282x + 0.0258$, $R^2 = 0.8193$), but there is no statistical or observational justification for this censoring of the PCB data. Overall, these regressions indicate that PCBs, DDE, and chlordane exposure are not coming from the road crossings because there is no difference between upstream and downstream concentrations (i.e. slope is near unity and the intercept near zero).

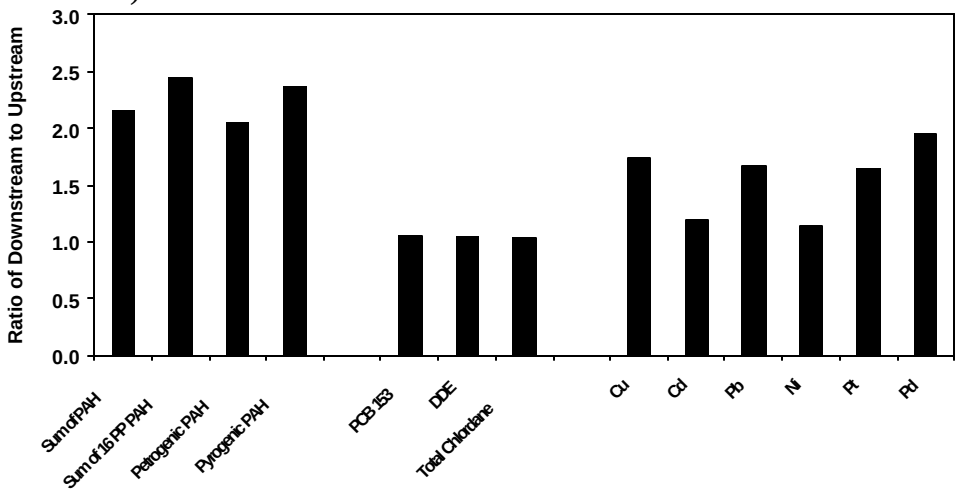


Figure 4.5. *Concentrations of chemicals in mussels expressed as ratio of downstream to upstream.*

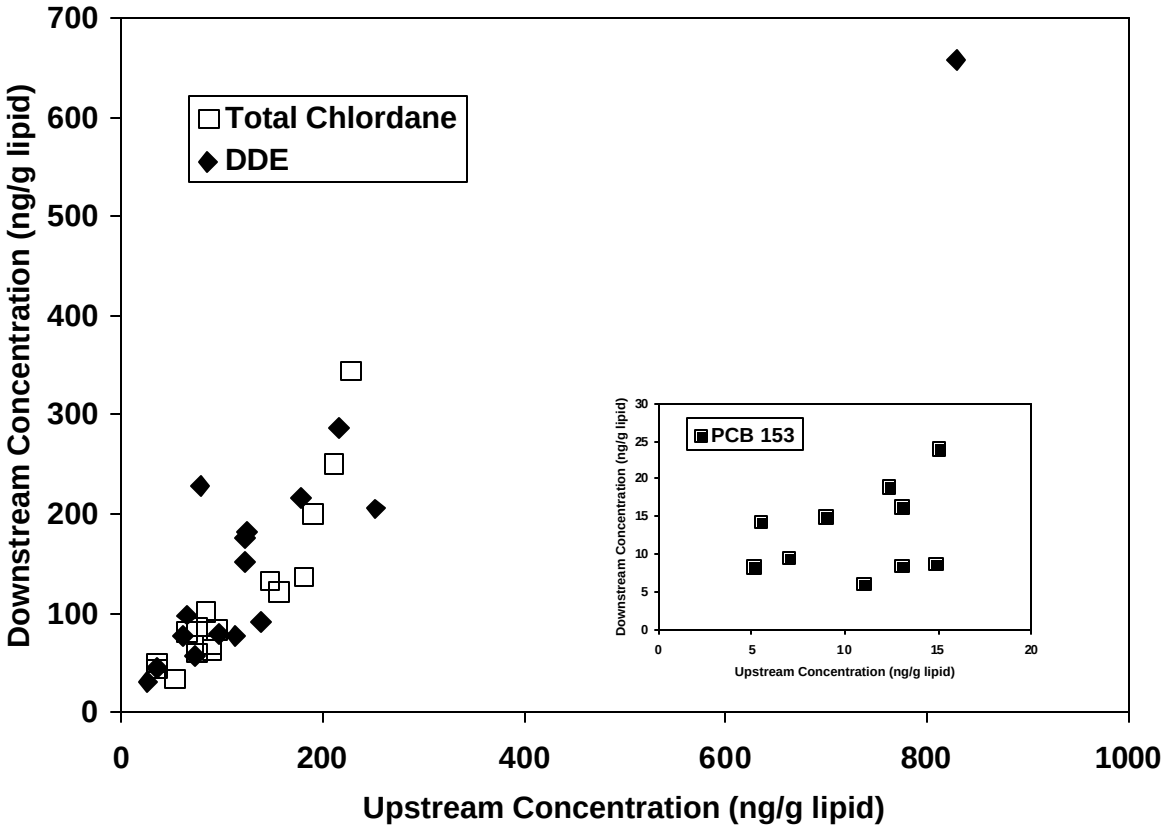


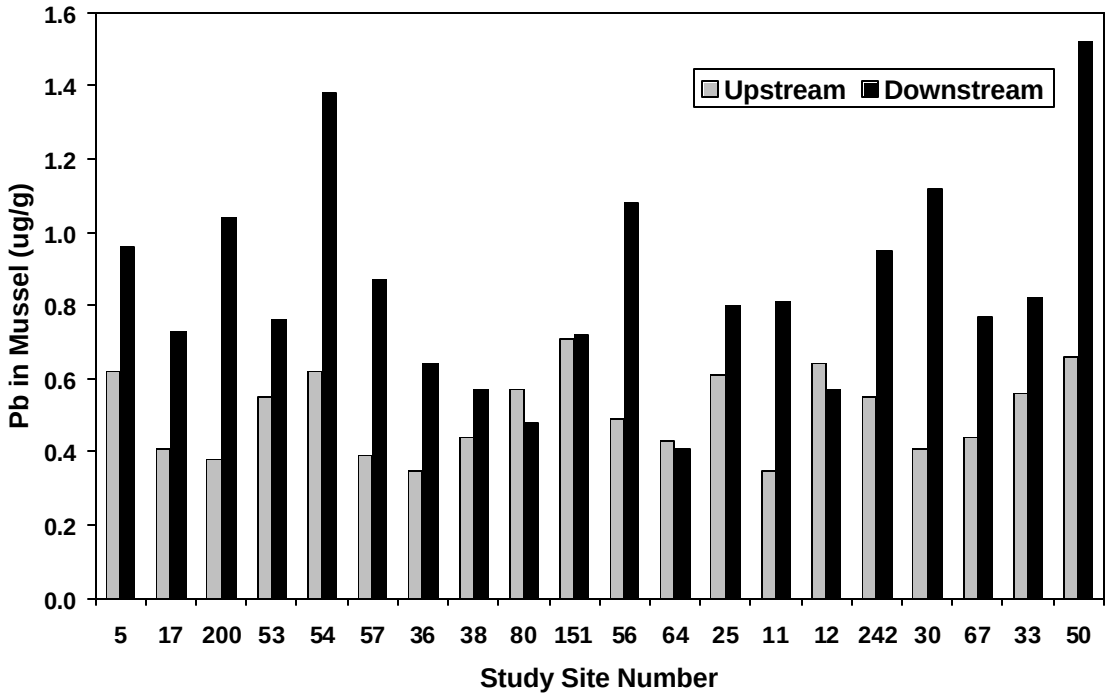
Figure 4.6. *Upstream versus downstream concentrations of DDE, chlordane, and PCB 153 (inset). Regressions are listed in the text.*

Metals: Concentrations of metals in mussels were quite low (Table 4.2), with a 1.5 to 2-fold increase in Cu, Pb, Pt, and Pd downstream and only a very slight increase of Cd and Ni downstream (Figure 4.5). Upstream and downstream concentrations are shown in Figure 7 for the metals that exhibited the largest increase\(Cu, Pb, Pt, and Pd). Traffic-related sources of these metals are possible, for example, historic use of leaded fuel (Pb), tires (Cd), brake linings (Cu), corrosion of metals (Cu, Cd, Ni, Pb), and exhaust from catalytic converters (Pt, Pd). Despite the consistent increase downstream for most of these metals, the magnitude of increase is quite small.

Summary of Mussel Data

A summary of the ratio of upstream and downstream concentrations of chemicals in mussels is shown in Figure 4.8, along with the ratio for agricultural versus forested sites. There is a consistent small increase downstream of road crossing for traffic-related chemicals (PAHs and most metals), but no increase for the chemicals unrelated to vehicles (PCBs and pesticides). Agricultural sites had slightly higher concentrations of most chemicals compared to forested sites, and this was particularly evident for upstream PAH concentrations (Figure 4.8B).

A



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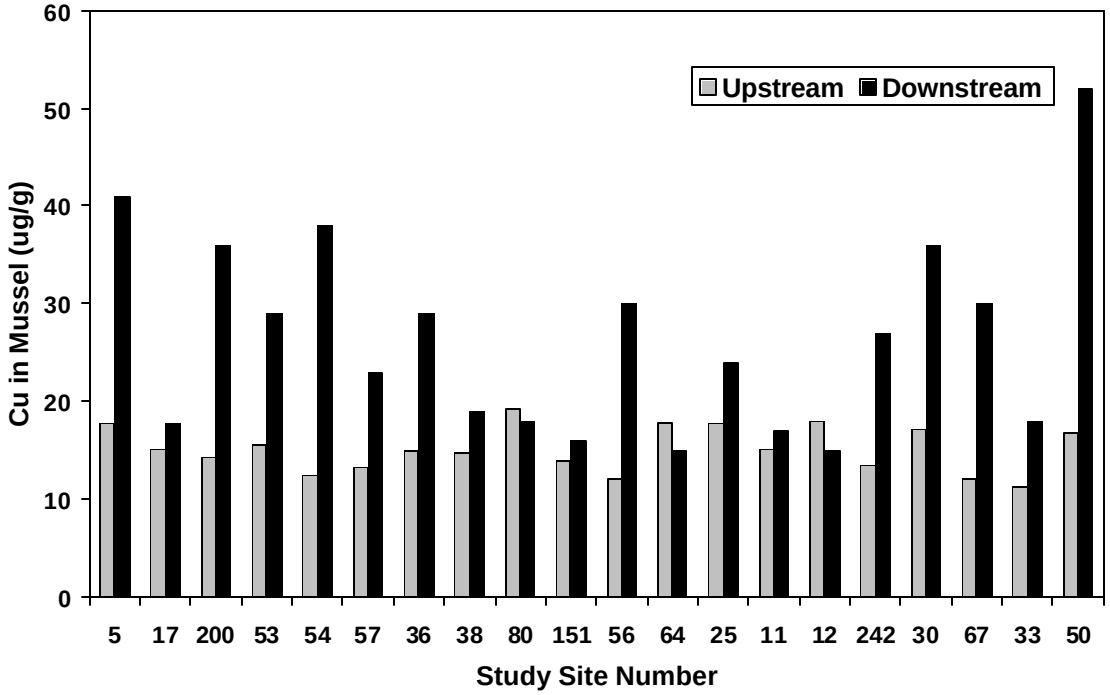
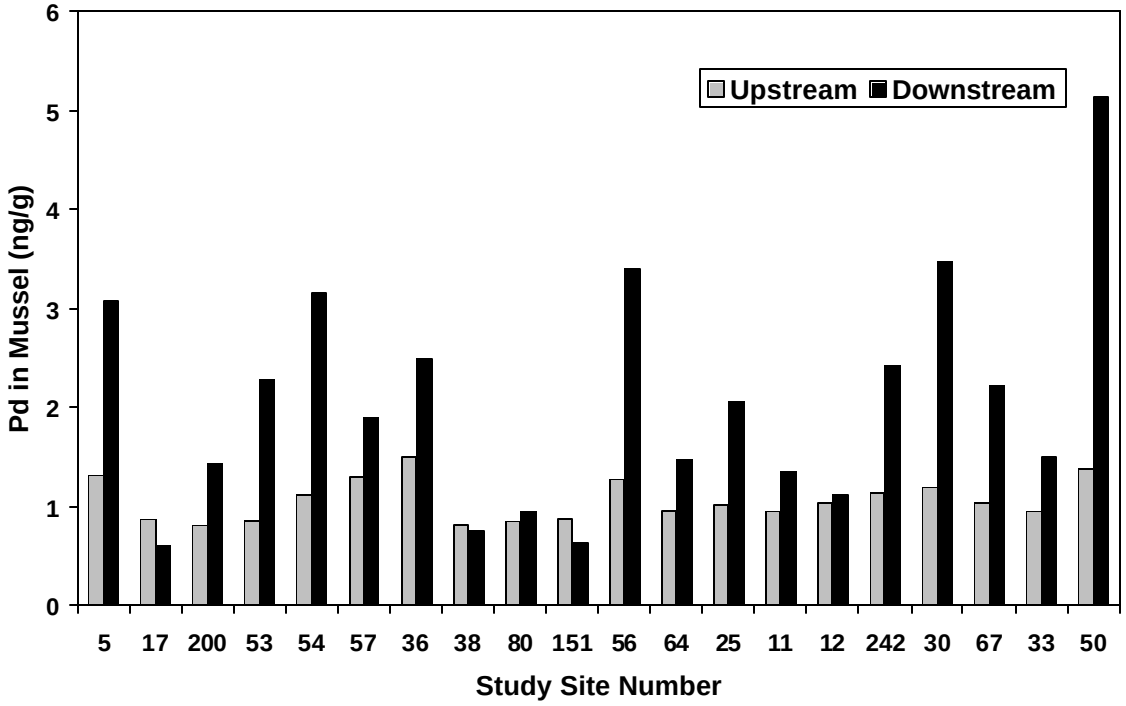


Figure 4.7a. *Upstream and downstream concentrations of Pb (A) and Cu (B) in mussels.*

C



D

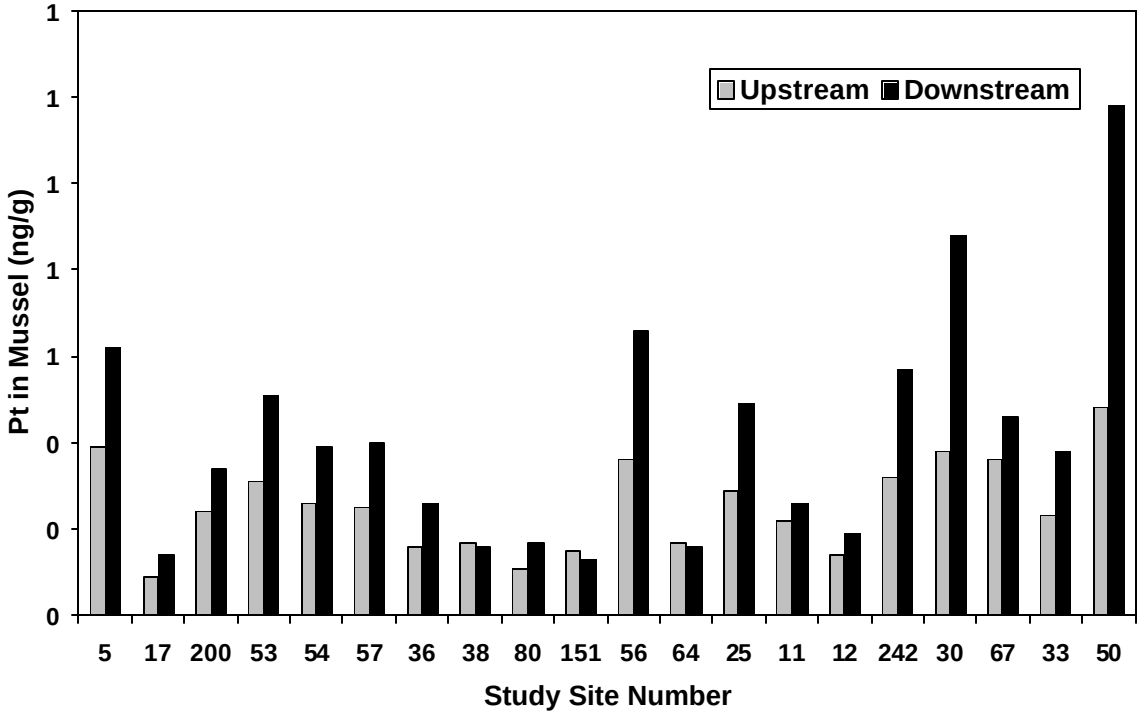
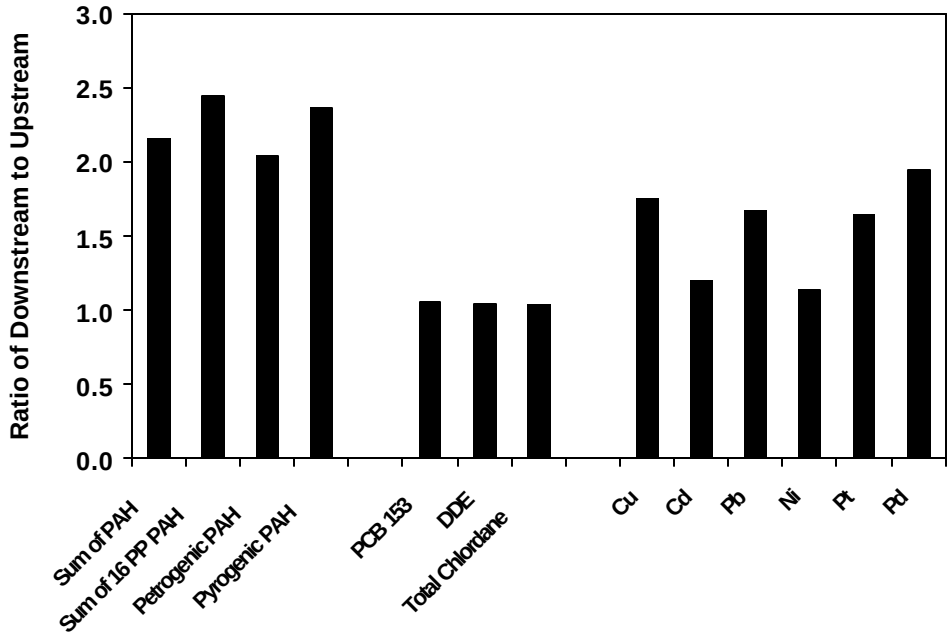


Figure 4.7b. *Upstream and downstream concentrations of Pd (C) and Pt (D) in mussels.*

A



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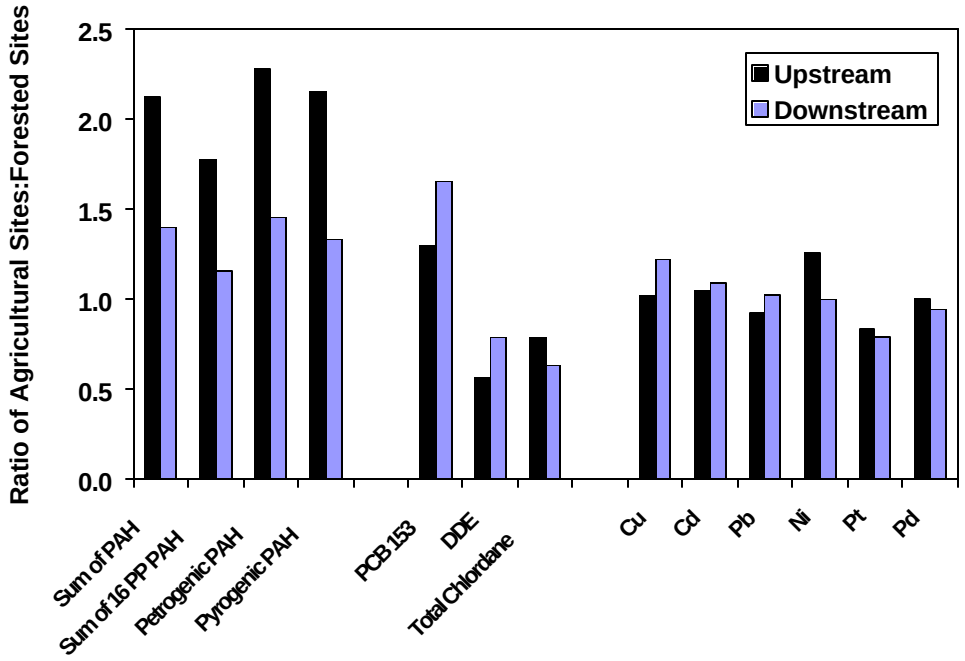
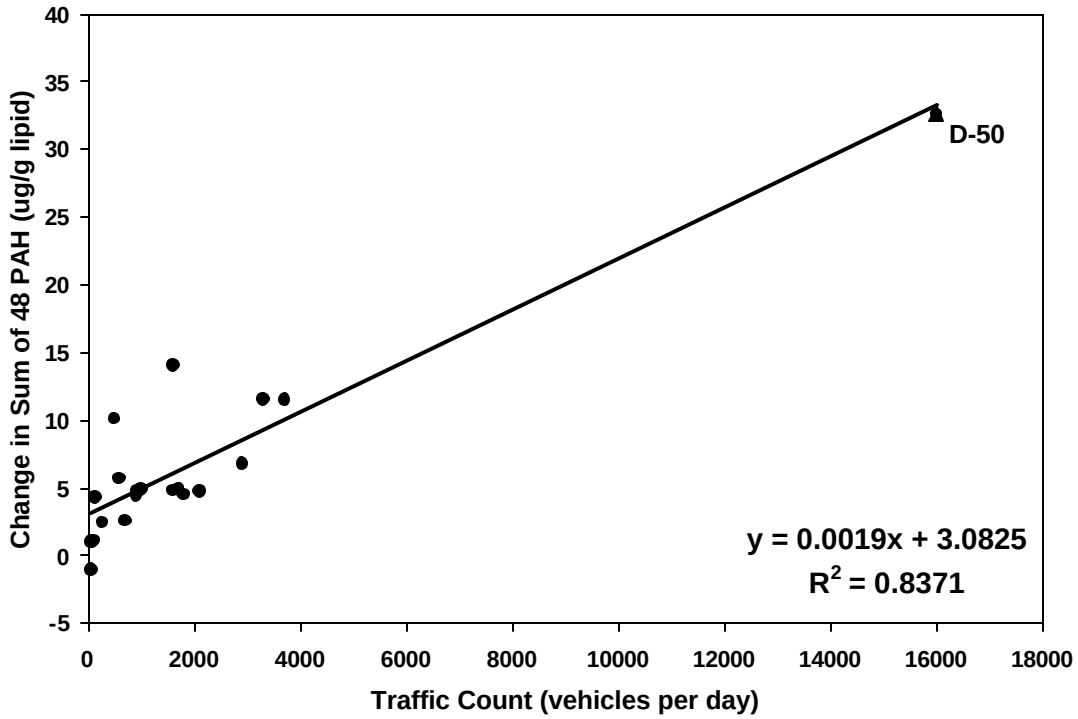


Figure 4.8. *Ratio of downstream to upstream concentrations of chemicals in mussels (A) and ratio of chemical concentrations at agricultural sites to those at forested sites (B).*

Influence of Traffic on the Accumulation of Chemicals in Mussels

Given the relatively consistent increases in traffic-related chemicals downstream from bridge crossings, we explored whether these increases could be quantitatively related to the number of vehicles crossing each bridge (Figures 4.9 - 4.11). For the PAHs, there is a very good correlation between the change in PAH concentrations (downstream – upstream) with the traffic count – when one includes the construction site (Site 50 in Durham County) that also had the highest traffic count (Figures 4.9 and 4.10). However, as shown in the figure captions, the correlation is not nearly as good when one excludes this single point. Unfortunately, we do not have data at sites with intermediate traffic counts. Nonetheless, there is a strong indication that observed increases in PAH concentrations in mussels downstream from road crossings are caused by increased traffic at those sites. This is further supported by similar plots of metal concentrations as a function of traffic counts (Figure 4.11), where site 50 was excluded as an outlier. Very good correlations were obtained for Pb, Cu, Pd, and Pt.

A



B

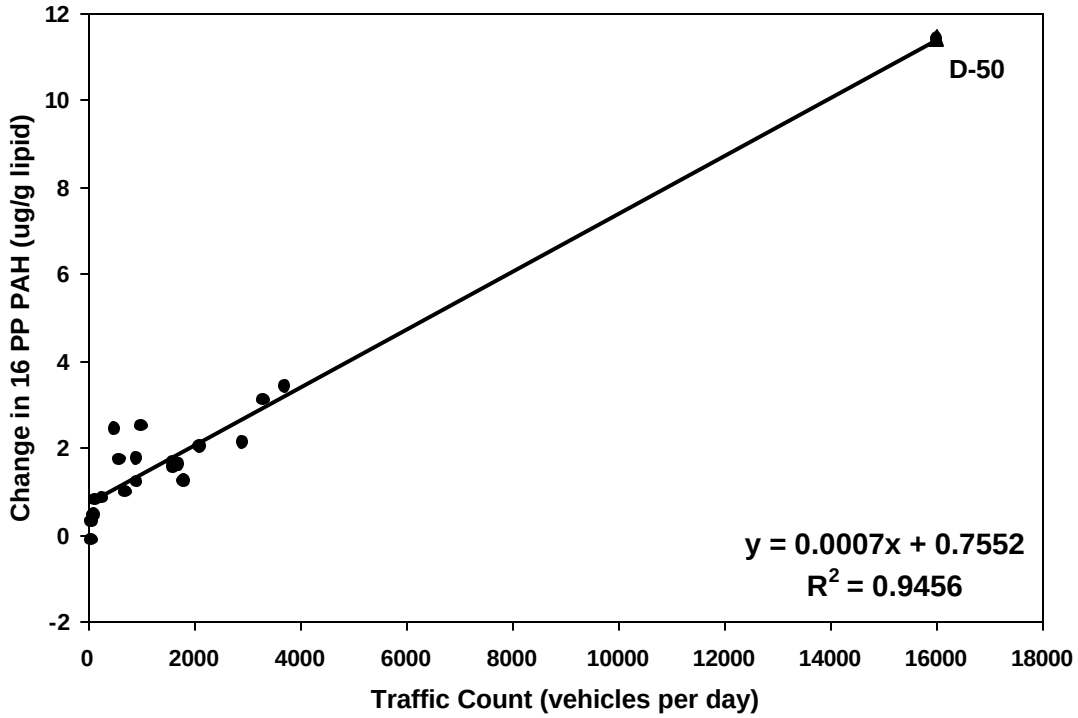
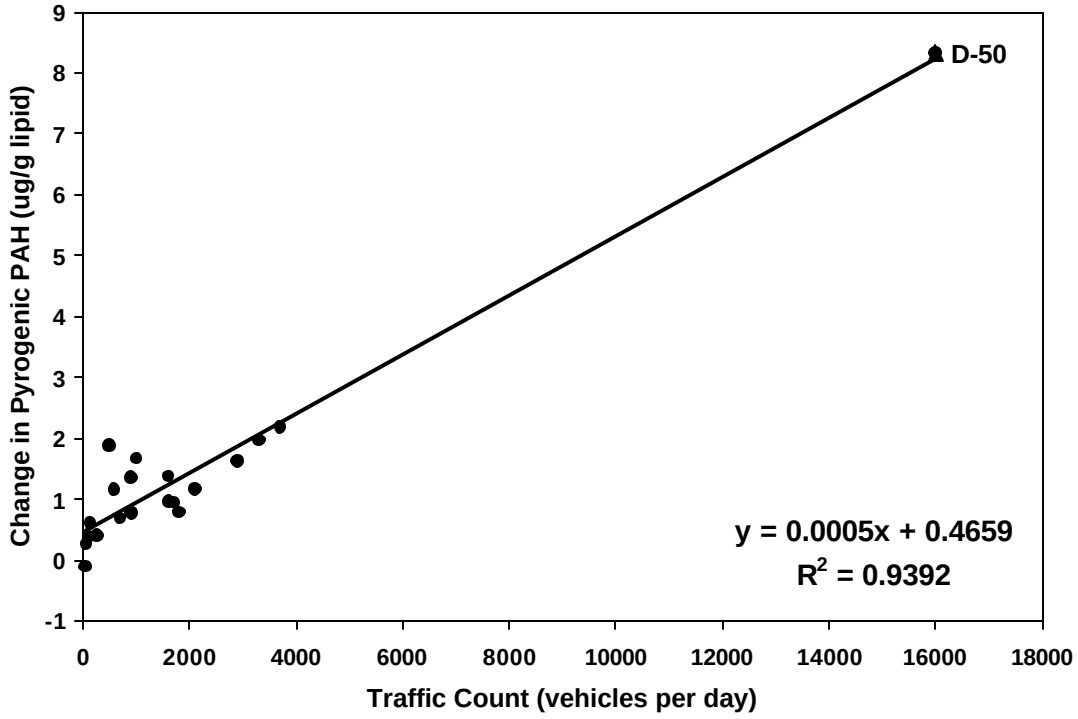


Figure 4.9. *Change in the sum of all 48 PAH (A) and sum of 16 Priority Pollutant PAH (B) in mussels as a function of traffic count. Regression without Site D-50 is (A): y = 0.0023x + 2.58 R^2 = 0.4383 and (B): 0.0006x + 0.773 R^2 = 0.6198.*

A



B

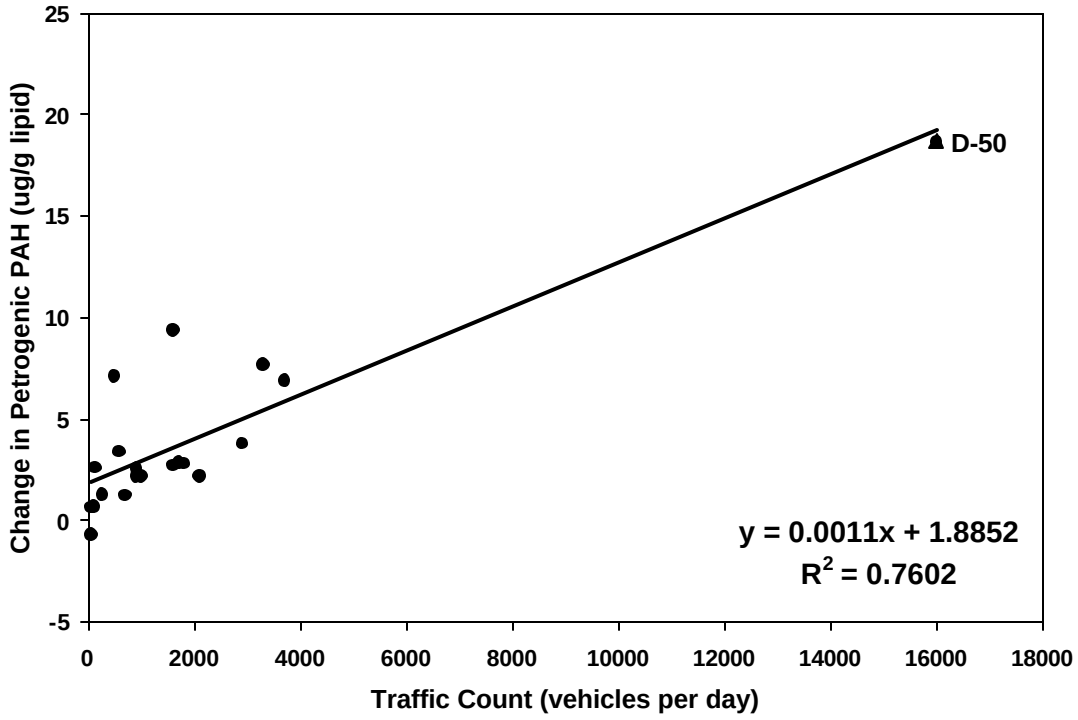


Figure 4.10. *Change in the sum of pyrogenic PAH (A) and sum of petrogenic PAH (B) in mussels as a function of traffic count. Regression without Site D-50 is (A): 0.0004x + 0.556* R^2 *= 0.5289 and (B): y = 0.0014x + 1.49* R^2 *= 0.3560.*

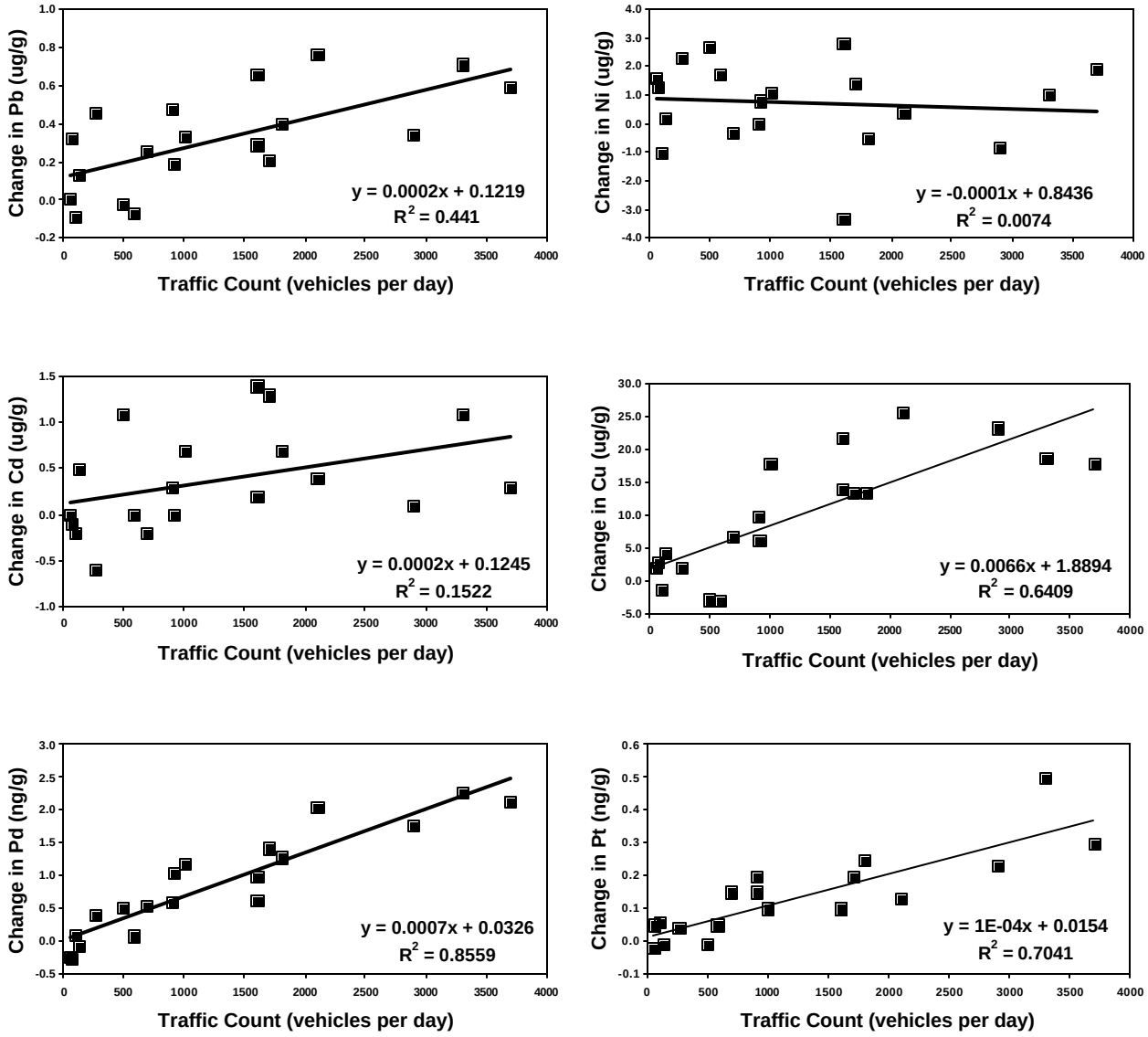


Figure 4.11. *Change in metal concentrations in mussels as a function of traffic count, excluding data point from Site 50.*

Implications of Exposure to Mussel Health and Relative Abundance

The concentrations of chemicals measured in the mussels were relatively low compared to what we and others have found at urban sites, or sites otherwise considered *polluted*. The concentrations also are low relative to concentrations known to adversely impact the health of adult bivalves (Neff 1979). However, we have very little information on the effects of these chemicals on freshwater mussels, and *E. complanata* in particular, and we have no information on the potential health effects of these chemicals on juvenile mussels or glochidia. Levine et al. (2003) reported decreased relative abundance of mussels downstream of the same bridge crossings studied here. However, there is no quantitative relationship between those decreased mussel abundances and the concentrations of chemicals we found in the mussels. This is illustrated in Figure 12, where change in the relative abundance is plotted as a function of the change in PAH concentration. There is essentially no relationship between the two data sets. Therefore, at this point we have no data to support the hypothesis that chemicals in road runoff are adversely impacting mussel populations at bridge crossings. There may be effects of chemicals we did not measure or could not detect, or there may be effects on juveniles or glochidia that are not evident from our data. These questions remain unanswered.

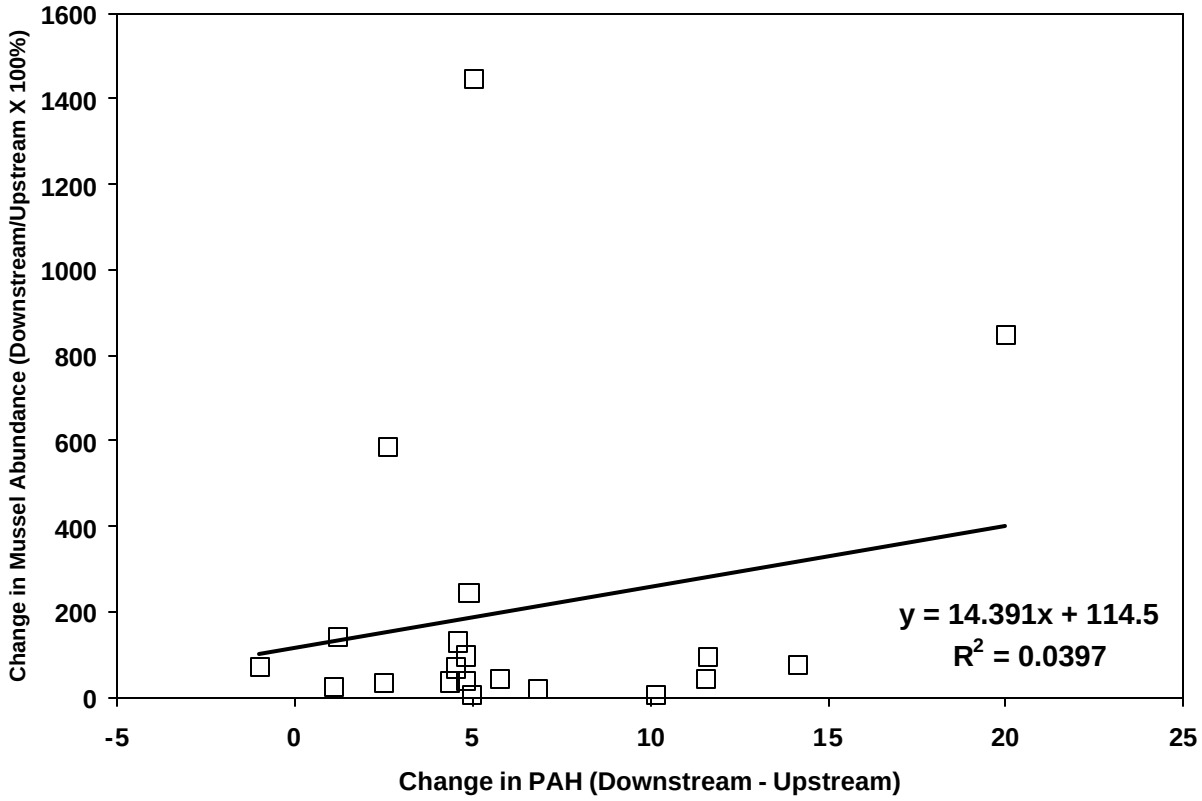


Figure 4.12. *Change in relative abundance of mussels as a function of change in PAH concentrations.*

Bioavailability of Sediment-Bound PAH

Due to their hydrophobic nature, all PAHs are preferentially associated with carbon phases of particles and thus adverse health effects resulting from PAH contamination are often evident in the sediment. The extent to which PAHs accumulate in a sediment-dwelling organism depends primarily on the ratio of the PAH uptake rate to the depuration rate, the capacity of the organism to metabolize PAHs, the mobility of the organism, and on various physical-chemical properties of the individual compounds. For example, bivalves are frequently used as sentinel organisms because of their low capability to metabolize PAHs (Neff 1979; Varanasi 1989), and their relatively sessile character, thereby providing a time-integrated measurement of PAH contamination. Bioaccumulation potential also depends on the desorption rate of the PAH from the sediment or particle matrix (Chung and Alexander 1999; Alexander 2000; Landrum 1989; Landrum et al. 1994; Ferarro et al. 1990). If desorption kinetics are fast relative to the co-occurrence of the PAH and the organism, the PAH will be available for uptake. However, if desorption rates are slow, the contaminant may be less available for uptake. Therefore, it is important to consider the bioavailability of PAHs when assessing their potential adverse effects.

Others have reported that PAHs may exhibit low chemical and biological availability (Ferarro et al. 1990; Krauss et al. 2000; Alexander 1995; Luthy et al. 1997; Wong et al. 2001; Thorsen et al. 2004). This low availability has been described by field-derived solid-water partition coefficient (K_D) values being greater than predicted (Bucheli and Gustafsson, 2000; Accardi-Dey and Gschwend, 2002; Gustafsson et al. 1997; Boese et al. 1996), as fractions available for equilibrium partitioning (AEP) being less than predicted (McGroddy et al. 1996), and culminates in toxicities that are less than predicted based on equilibrium partitioning theory. When identifying the potential for toxicity of sediment to aquatic organisms, determining sediment PAH concentration is only one aspect of evaluation. Particularly when investigating PAHs, it is important to assess the availability of individual PAHs to organisms. High total sediment PAH concentrations may not confer toxic levels to organisms if individual PAHs are sequestered, or tightly sorbed, and are unavailable for desorption and uptake into the organism (Alexander 1995). One way to assess the bioavailability of PAHs in the environment is to compare individual PAH concentrations in benthic organisms to individual PAH concentrations in sediment. This approach is described as the Biota-Sediment Accumulation Factor (BSAF) Model (Boese et al. 1996; Ferarro et al. 1990):

$$BSAF = (C_m/f_L)/(C_s/f_{oc}) \qquad (1)$$

where C_m is the individual PAH concentration in mussel tissue (ng PAH/g mussel dry weight), f_L is the organism lipid fraction (g lipid/g mussel dry weight), C_s is the individual PAH concentration in sediment (ng PAH/g sediment dry weight.), and f_{oc} is the mass fraction of organic carbon (g organic C/g sediment dry weight). The BSAF models the partitioning of PAHs between the hydrophobic (sorptive) phases present in a benthic organism and sediment. These sorptive phases are traditionally the lipid fraction in the organism and the organic carbon fraction in sediment. One must consider specific assumptions when using the BSAF model, including:

1) The organism possesses minimal capability to metabolize PAHs (BSAF values of <1 may suggest metabolism has occurred)

2) Sorption/desorption kinetics are fast relative to uptake kinetics so that PAHs are bioavailable (BSAF values may be <1 if bioavailability is decreased)
3) The affinity of PAHs for organism lipid and sediment organic carbon are equivalent (i.e., octanol-water partition coefficient (K_{ow}) is equal to organic carbon normalized partition coefficient (K_{oc}))
4) Organic carbon (OC) is the only sorptive phase present in sediment

Given these assumptions, if the PAHs are in equilibrium with the OC then BSAF values should be close to one. Often, BSAF values slightly greater than one (1.5 – 2.5) are observed (Wong et al. 2001), perhaps due to $K_{ow} > K_{oc}$ (i.e., assumption 3 is not correct). Alternatively, BSAF values are sometimes less than one (Thorsen et al. 2004), indicating a decreased bioavailability. This decreased bioavailability may be a result of a secondary sorptive phase that is not accounted for in equation 1. While the traditional form of the BSAF model considers only OC as the sorptive phase, others have recently suggested that soot carbon (SC) may provide an additional sorptive phase for PAHs and other planar compounds (Bucheli and Gustafsson 2000; Accardi-Dey and Gschwend 2002; Gustafsson et al. 1997). We recently demonstrated the effect of soot carbon on the bioavailability of sediment-bound PAH to the freshwater mussel *E. complanata* used in this study and also to the marine clam Mya arenaria (Thorsen et al. 2004). In this other study, we also found that BSAF values for most petrogenic PAH were 3 – 5 times higher than those for most pyrogenic PAH.

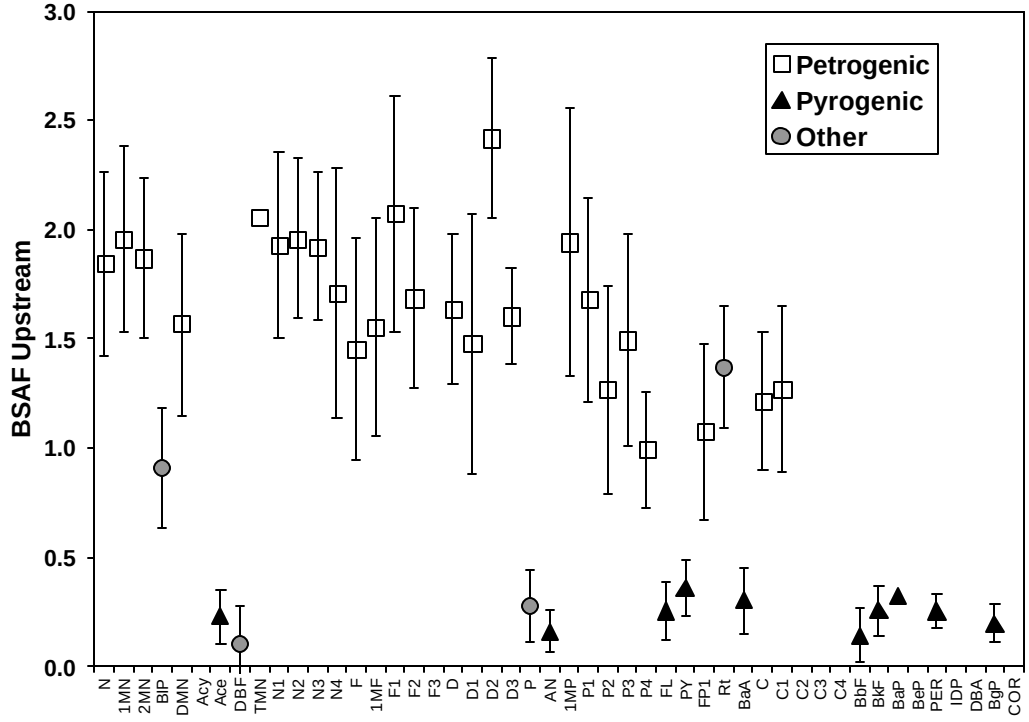
BSAF values are shown in Figure 13 for individual PAH at upstream and downstream locations. Both plots exhibit very consistent BSAF values between upstream and downstream locations and also exhibit higher BSAF values for petrogenic PAH compared to pyrogenic PAH. This last observation is consistent with the recent report by Thorsen et al. (2004). The mean BSAF values are given in Table 4 and indicate that petrogenic PAH are more available to mussels than pyrogenic PAH. Based on the work of Thorsen et al. (2004), we believe this difference is caused by the stronger adsorption of pyrogenic PAH to soot carbon compared to the petrogenic PAH. The petrogenic PAH are largely associated with the amorphous organic carbon in the sediment, while much of the pyrogenic PAH is associated with soot carbon and is probably native to that soot. That is, the pyrogenic PAH was generated along with the soot carbon, whereas the petrogenic PAH come mostly from petroleum products and later sorb into the sediment organic carbon.

The difference in apparent availability of the pyrogenic and petrogenic PAH is about a factor of four (Table 4.3), though this can be as much as a factor of 15 for some individual PAH (see data in Appendix). It is also noteworthy that the mean BSAF value for the 16 EPA Priority Pollutant PAH is much lower than that for the sum of the 48 PAH. This reflects the dominance of pyrogenic PAH in the list of EPA Priority Pollutant PAH, while the broader range of PAH that actually exists in nature is enriched in the petrogenic PAH.

Table 4.3. BSAF values estimated from mussel and sediment data using equation 1 (mean ± SD).

PAH Classification	Upstream BSAF	Downstream BSAF
Sum of all 48 PAH	1.19 ± 0.103	1.17 ± 0.074
Sum of 16 EPA PP PAH	0.63 ± 0.102	0.75 ± 0.110
Petrogenic PAH	1.70 ± 0.096	1.61 ± 0.112
Pyrogenic PAH	0.39 ± 0.072	0.48 ± 0.073

A



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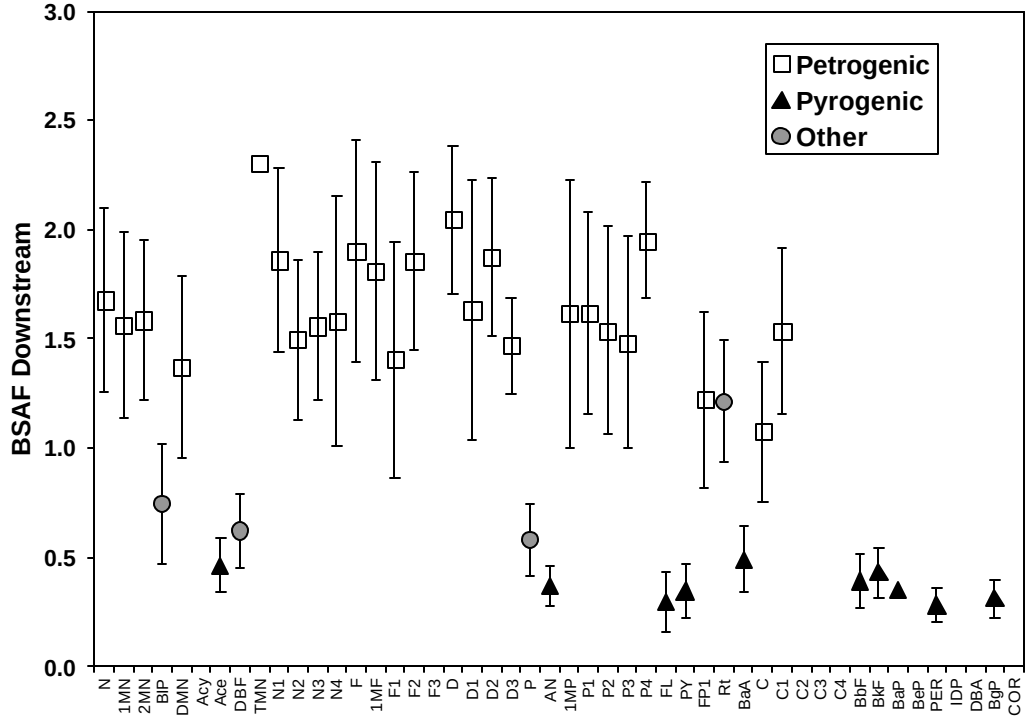

Figure 4.13. *BSAF values upstream (A) and downstream (B) of bridge crossing.*

Passive Sampling Devices as Surrogates for Measuring Exposure to Mussels

The final objective of this chemical exposure study was to assess the utility of using polyethylene (PE) passive sampling devices as a surrogate for measuring organic chemicals in mussels. If a reliable surrogate measurement is possible, then accumulation of chemicals in mussels could be estimated without sampling of mussels and locations that do not support mussels could still be assessed to determine whether organic contaminants might be a possible cause of low mussel abundance. We have previously reported on the use of various passive sampling devices to monitor contaminant exposure in aquatic systems and to estimate accumulation in marine and freshwater bivalves (Hofelt and Shea 1997; Luellen and Shea 2002; Luellen and Shea 2003). In this study, we used a thin polyethylene membrane that we previously determined would reach equilibrium with aqueous PAH within a 30-day deployment period. This time to reach equilibrium is a little longer than the 3-10 days we found it that it took *E. complanata* to reach equilibrium, but it is much shorter than is required for thicker PE membranes or the commonly used semi-permeable membrane device (SPMD).

A comparison of the sum of all 48 PAH in mussels with that of the PE passive sampling devices is shown in Figure 4.14. There is excellent agreement between the two, indicating that PE passive sampling devices can serve as a surrogate for measuring PAH concentrations in mussels. This is particularly noteworthy given the poor correlation of sediment PAH with mussels owing to the difference in bioavailability among the PAH. The PE passive sampling device is equilibrating with PAH that are dissolved in the water, just as the mussels do, allowing the PE to be a good measure of the bioavailable fraction of PAH bound to the sediment. Note also that the slope of this plot is about 0.3, indicating that the PE does not have as high of a capacity to accumulate PAHs as mussel lipid. Nonetheless, the regression in Figure 4.14 would allow passive sampling devices to be used to predict concentrations of PAHs in mussels.

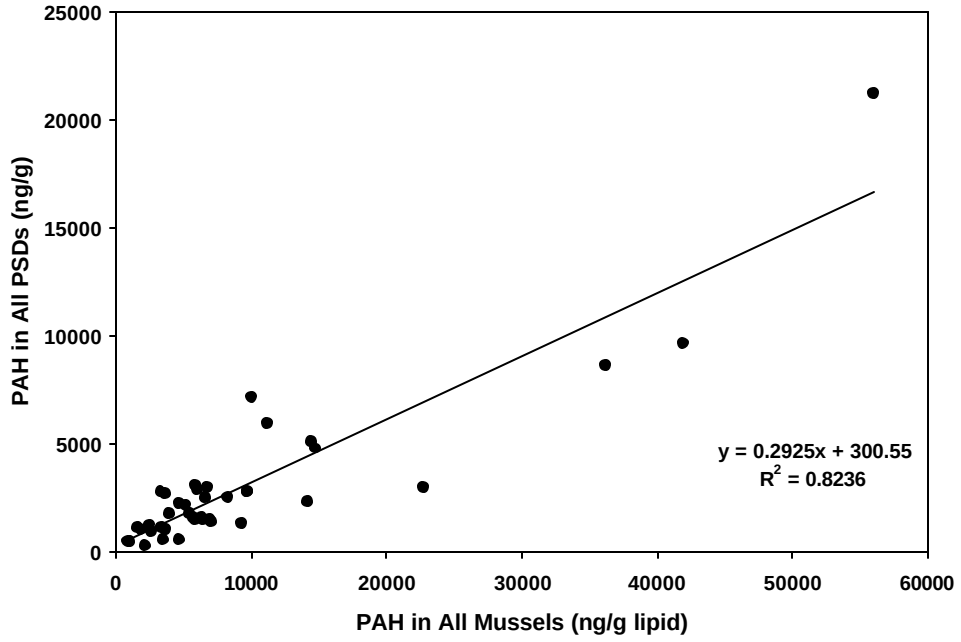

Figure 4.14. *Relationship between PAH accumulation mussels and that of polyethylene passive sampling devices (PSDs).*

Summary of Findings

1. Concentrations of all chemicals measured in mussels were low or not detectable. It is unlikely that these low concentrations would have direct adverse effects on adult mussels, but we have insufficient information on how these chemical mixtures might affect mussels or how even low exposures could adversely affect juveniles or glochidia.

2. Polycyclic aromatic hydrocarbons (PAHs) and several metals (Cu, Pb, Pt, and Pd) increased in mussels downstream from road crossings and these increases appear to be directly related to the number of vehicles crossing the bridges.

3. Although the relative abundance of mussels decreased downstream of road crossings, there was no correlation between this decrease and the increase in chemical exposure.

4. There were no noteworthy differences between the agricultural and forested sites. One site was undergoing construction and had the highest concentrations of many contaminants, but this site also had the highest traffic count.

5. Using biota-sediment accumulation factors (BSAF), we demonstrated that pyrogenic PAH were less bioavailable than petrogenic PAH, by as much as a factor of 15.

6. Passive sampling devices (PSDs) constructed of polyethylene can serve as excellent surrogates for the direct measurement of PAHs in mussel tissue. This has important implications to monitoring chemical exposure and to assessing chemical risk to mussel health and populations.

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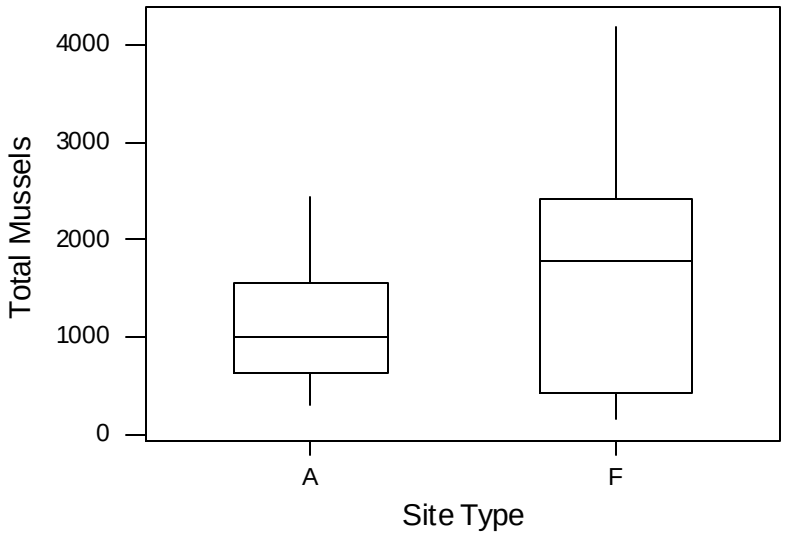
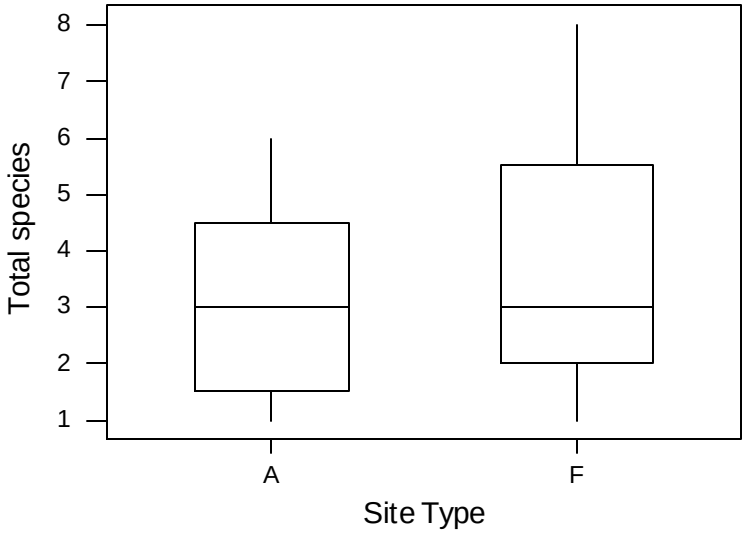
Appendix I

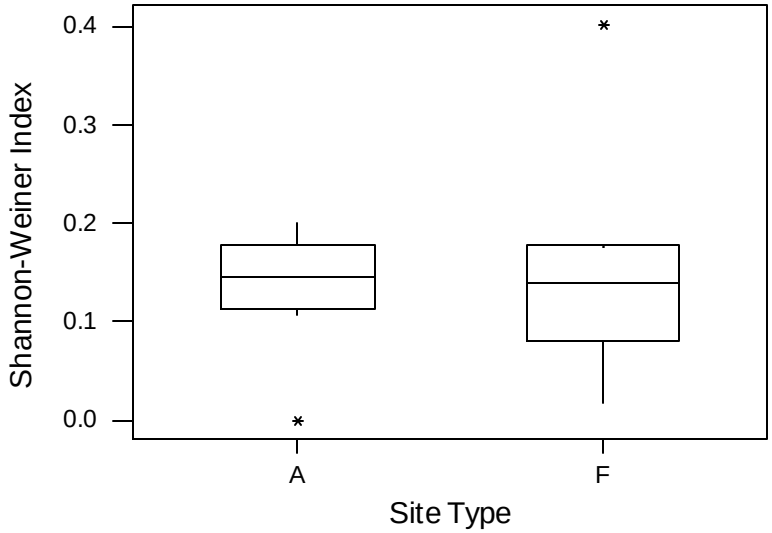
Mussel Survey Data Appendices



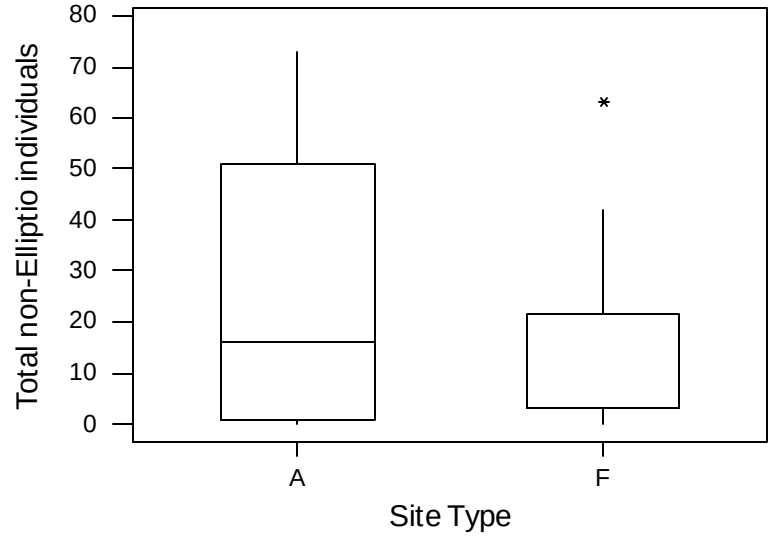
Appendix I-1. *Boxplot of the total number of mussels found at agricultural (A) and forested (F) sites.*



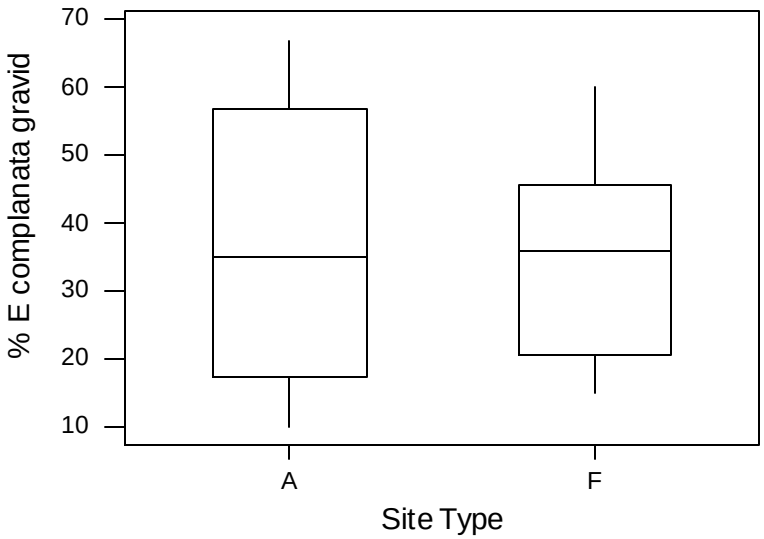
Appendix I-2. *Boxplot of the total number of species found at agricultural (A) and forested (F) sites.*



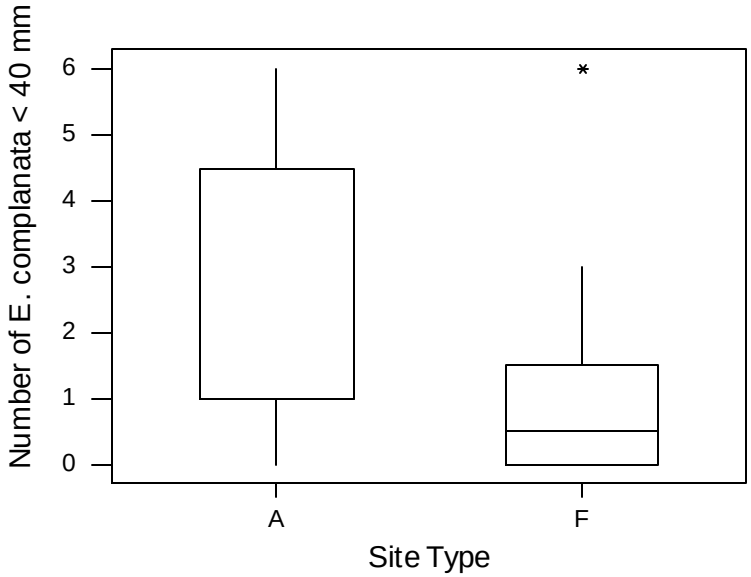
Appendix I-3. *Boxplot of the Shannon-Weiner Diversity Index for agricultural (A) and forested (F) sites.*



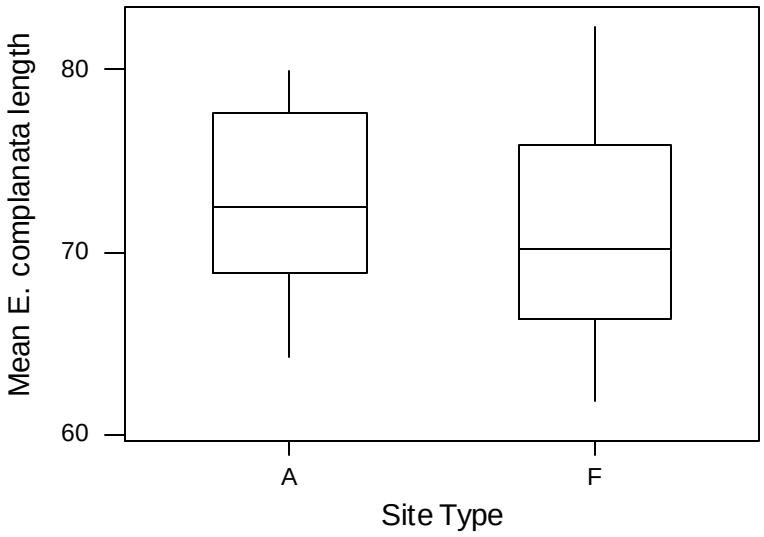
Appendix I-4. *Boxplot of the total number of non-Elliptio species found at agricultural (A) and forested (F) sites.*



Appendix I-5. *The percentage of E. complanata sampled found to be gravid at agricultural (A) and forested (F) sites.*



Appendix I-6. *The number of individuals found <40 mm long at agricultural (A) and forested (F) sites.*



Appendix I-7. *Mean length of Elliptio complanata at agricultural (A) and forested (F) sites.*

Appendix II

Contaminant Assessment Appendices

Table II-1. Summary of sampling for chemical exposure study.

Bridge number	Co.	Sample ID	Stream	Location	Stream Size	Site Type	Mussel Sampling Date	Sediment/ PSD Sampling Date	PSD Recovery Date
36	P	P36U-P	South Flat	Dick Holeman Rd.	Large	AG	07/05/01	07/12/01	08/16/01
36	P	P36D-P	South Flat	Dick Holeman Rd.	Large	AG	07/05/01	07/12/01	08/16/01
54	O	O54U-P	NF Little	Hwy 157	Large	AG	06/19/01	07/11/01	08/17/01
54	O	O54D-P	NF Little	Hwy 157	Large	AG	06/19/01	07/11/01	08/17/01
5	D	D5U-P	Mountain Cr.	Bahama Rd.	Small	AG		07/17/01	08/16/01
5	D	D5D-P	Mountain Cr.	Bahama Rd.	Small	AG		07/17/01	08/16/01
38	P	P38U-P	Lick Cr.	Willie Gray Rd.	Small	AG	06/13/01	07/12/01	08/16/01
38	P	P38D-P	Lick Cr.	Willie Gray Rd.	Small	AG	06/13/01	07/12/01	08/16/01
80	P	P80U-P	Deep Cr.	Smith Rd.	Small	AG	06/11/01	07/12/01	08/16/01
80	P	P80D-P	Deep Cr.	Smith Rd.	Small	AG	06/11/01	07/12/01	08/16/01
173	O	O173U-P	EF Eno	Compton Rd.	Small	AG		07/12/01	08/17/01
173	O	O173D-P	EF Eno	Compton Rd.	Small	AG		07/12/01	08/17/01
200	O	O200U-P	Stroud's Creek	Miller Rd.	Small	AG	05/24/01	07/17/01	08/17/01
200	O	O200D-P	Stroud's Creek	Miller Rd.	Small	AG	05/24/01	07/17/01	08/17/01
53	O	O53U-P	NF Little	Gates Rd.	Large	BRIDGE		07/11/01	08/17/01
53	O	O53D-P	NF Little	Gates Rd.	Large	BRIDGE		07/11/01	08/17/01
57	O	O57U-P	SF Little	Johnson's Mill Rd.	Large	BRIDGE	06/25/01	07/11/01	08/17/01
57	O	O57D-P	SF Little	Johnson's Mill Rd.	Large	BRIDGE	06/25/01	07/11/01	08/17/01
33	P	P33U-P	South Flat	Ned Moore Rd.	Large	FOREST	06/20/01	07/12/01	08/16/01
33	P	P33D-P	South Flat	Ned Moore Rd.	Large	FOREST	06/20/01	07/12/01	08/16/01
56	D	D56U-P	SF Little	Hwy 157	Large	FOREST	06/26/01	07/11/01	08/17/01
56	D	D56D-P	SF Little	Hwy 157	Large	FOREST	06/26/01	07/11/01	08/17/01
64	D	D64U-P	Little River	Johnson's Mill Rd.	Large	FOREST	05/21/01	07/11/01	08/16/01
64	D	D64D-P	Little River	Johnson's Mill Rd.	Large	FOREST	05/21/01	07/11/01	08/16/01
151	D	D151U-P	Flat	Unnamed Road off of Moore's Mill Rd. near US 501	Large	FOREST	06/28/01	07/12/01	08/16/01
151	D	D151D-P	Flat	Unnamed Road off of Moore's Mill Rd. near US 501	Large	FOREST	06/28/01	07/12/01	08/16/01
11	O	O11U-P	Eno	unnamed road near Faucette Rd.	Small	FOREST	06/27/01	07/17/01	08/17/01
11	O	O11D-P	Eno	unnamed road near Faucette Rd.	Small	FOREST	06/27/01	07/17/01	08/17/01
25	G	G25U-P	Smith Cr	Lawrence Rd.	Small	FOREST	06/18/01	07/13/01	08/15/01
25	G	G25D-P	Smith Cr	Lawrence Rd.	Small	FOREST	06/18/01	07/13/01	08/15/01
67	O	O67U-P	McGowan Cr.	Clark Rd.	Small	FOREST		07/17/01	08/17/01
67	O	O67D-P	McGowan Cr.	Clark Rd.	Small	FOREST		07/17/01	08/17/01
242	O	O242U-P	WF Eno	Cedar Grove Rd.	Small	FOREST	07/03/01	07/12/01	08/17/01
242	O	O242D-P	WF Eno	Cedar Grove Rd.	Small	FOREST	07/03/01	07/12/01	08/17/01
12	O	O12U-P	EF Eno	Old Hillsborough Rd.	Small	FOREST-ALT1	07/06/01	07/12/01	08/17/01
12	O	O12D-P	EF Eno	Old Hillsborough Rd.	Small	FOREST-ALT1	07/06/01	07/12/01	08/17/01
50	D	D50U-P	Eno	HWY 157				07/17/01	08/16/01
50	D	D50D-P	Eno	HWY 157				07/17/01	08/16/01
30	O	O30U-P	NF Little	Hwy 57	Small	FOREST	05/22/01	07/20/01	08/17/01
30	O	O30D-P	NF Little	Hwy 57	Small	FOREST	05/22/01	07/20/01	08/17/01

Table II-2. Summary of mussel physical measurements.

Site	Date	Location	Animal ID	Weight	Length	Height	Width	Gravid	Blood, ml	Bld Draw	Cut Adduct
D64	5/21/2001	upstream	A	38.2	62	37	22	*	0.9	*	yes
D64	5/21/2001	upstream	B	40.8	69	36	22.5	*	1	*	yes
D64	5/21/2001	upstream	C	35.2	67	38	21	*	0.65	*	yes
D64	5/21/2001	upstream	D	36.3	63	38	21	*	0		yes
D64	5/21/2001	upstream	E	25.3	60	33	18	*	0		yes
D64	5/21/2001	downstream	F	26	61	34	18	*	0.35	*	yes
D64	5/21/2001	downstream	G	29.85	65	34	19	*	0.55	*	yes
D64	5/21/2001	downstream	H	33.65	66	34	21	*	0.9	*	yes
D64	5/21/2001	downstream	I	27.95	60	33	20	*	0		yes
D64	5/21/2001	downstream	J	32	62	36	20	*	0		yes
O30	5/22/2001	upstream	A	70.65	84	44	26	*	0.7	*	yes
O30	5/22/2001	upstream	B	59.6	82	41	23	*	0.5	*	yes
O30	5/22/2001	upstream	C	52.45	74	44	24	*	0.8	*	yes
O30	5/22/2001	upstream	D	48.6	78	42	23	*	0		?
O30	5/22/2001	upstream	E	51.9	80	43	24	*	0		?
O30	5/22/2001	downstream	F	56.15	76	41	24	*	1	*	yes
O30	5/22/2001	downstream	G	62.65	82	44	25	*	0.7	*	yes
O30	5/22/2001	downstream	H	32.1	62	36	22	*	0.5	*	yes
O30	5/22/2001	downstream	I	28.7	63	34	20	*	0		?
O30	5/22/2001	downstream	J	23.3	62	32	19	*	0		?
O200	5/24/2001	upstream	A	69.55	82	47	28	*	1	*	no
O200	5/24/2001	upstream	B	40.55	70	39	22	*	1	*	no
O200	5/24/2001	upstream	C	52.05	70	42	26	*	0		no
O200	5/24/2001	upstream	D	26.1	60	36	19	*	1	*	no
O200	5/24/2001	upstream	E	34.95	65	39	27	*	0		no
O200	5/24/2001	downstream	F	51.5	74	40	25	yes	0.75	*	no
O200	5/24/2001	downstream	G	53.8	73	43	26	*	1	*	no
O200	5/24/2001	downstream	H	47.5	68	42	23	yes	0.77	*	no
O200	5/24/2001	downstream	I	39.6	67	37	23	*	0		no
O200	5/24/2001	downstream	J	62.35	76	43	28	*	0		no
O173	5/29/2001	upstream	A	46.95	72	43	24	no	0.95	*	no
O173	5/29/2001	upstream	B	87.3	82	50	30	no	1	*	no
O173	5/29/2001	upstream	C	77.45	85	45	21	yes	1	*	no
O173	5/29/2001	upstream	D	106.4	100	56	33	*	0		no
O173	5/29/2001	upstream	E	40.1	69	40	22	*	0		no
O173	5/29/2001	downstream	F	111.2	93	56	32	no	0.6	*	no
O173	5/29/2001	downstream	G	70.45	84	47	26	*	0.55	*	no
O173	5/29/2001	downstream	H	100.55	93	49	33	no	0.95	*	no
O173	5/29/2001	downstream	I	81.75	89	51	29	*	0		no
O173	5/29/2001	downstream	J	71.6	82	47	28	*	0		no
O67	5/30/2001	upstream	A	48.2	72	39	23	*	0.87	*	no
O67	5/30/2001	upstream	B	50.7	74	42	23	yes	0.85	*	no
O67	5/30/2001	upstream	C	43.5	61	40	26	no	0.93	*	no
O67	5/30/2001	upstream	D	32.8	63	37	23	*	0		no
O67	5/30/2001	upstream	E	24.8	57	37	19	*	0		no
O67	5/30/2001	downstream	F	49.6	65	40	29	yes	1	*	no
O67	5/30/2001	downstream	G	69.15	80	47	27	no	1	*	no
O67	5/30/2001	downstream	H	46.5	65	43	25	no	1	*	no
O67	5/30/2001	downstream	I	25.6	56	34	20	*	0		no
O67	5/30/2001	downstream	J	14.3	47	29	16	*	0		no
O67	5/30/2001	upstream	AA	6.6	39						no
O67	5/30/2001	upstream	BB								no
O67	5/30/2001	upstream	CC								no
O67	5/30/2001	downstream	FF	2.55	31	17	9				no

Table II-2 (continued). Summary of mussel physical measurements.

Site	Date	Location	Animal ID	Weight	Length	Height	Width	Gravid	Blood, ml	Bld Draw	Cut Adduct
D5	6/7/2001	upstream	A	41	66	42	23	yes	0.8	*	no
D5	6/7/2001	upstream	B	56.05	80	43	24	no	0.94	*	no
D5	6/7/2001	upstream	C	21	56	32	18	yes	0.65	*	no
D5	6/7/2001	upstream	D	33.55	64	37	21	*	0		no
D5	6/7/2001	upstream	E	22.6	59	34	18	*	0		no
D5	6/7/2001	downstream	F	65.35	78	46	26	yes	1	*	no
D5	6/7/2001	downstream	G	49.95	72	41	24	yes	1	*	no
D5	6/7/2001	downstream	H	39.35	66	39	22	no	0.85	*	no
D5	6/7/2001	downstream	I	23.15	61	35	17	*	0		no
D5	6/7/2001	downstream	J	25.85	60	35	19	*	0		no
D5	6/7/2001	upstream	AA	3.7	35	19	9	*	0		no
D5	6/7/2001	downstream	FF	5.6	39	22	11	*	0		no
D5	6/7/2001	downstream	GG	3.6	35	18	8	*	0		no
D5	6/7/2001	downstream	HH	7.2	41	23	12	no	0		no
D5	6/7/2001	downstream	II	3.45				*	0		no
P80	6/11/2001	upstream	A	48.55	74	42	21	yes	0.97	*	no
P80	6/11/2001	upstream	B	34.6	66	39	22	yes	0.83	*	no
P80	6/11/2001	upstream	C	50.15	75	44	24	yes	0.62	*	no
P80	6/11/2001	upstream	D	53.05	72	45	24	*	0		no
P80	6/11/2001	upstream	E	20.35	57	32	18	*	0		no
P80	6/11/2001	downstream	F	24.95	60	33	20	*	0.65	*	no
P80	6/11/2001	downstream	G	57.5	79	50	27	*	0.95	*	no
P80	6/11/2001	downstream	H	47.6	73	44	22	yes	0.94	*	no
P80	6/11/2001	downstream	I	30.7	62	37	21	*	0		no
P80	6/11/2001	downstream	J	52.35	73	42	26	*	0		no
P80	6/11/2001	upstream	AA	6.1	41			*	0		no
P80	6/11/2001	upstream	BB	4.9	38			*	0		no
P80	6/11/2001	upstream	CC	3.85	37			*	0		no
P80	6/11/2001	upstream	DD	4.25	35			*	0		no
P80	6/11/2001	upstream	EE	1.65	27			*	0		no
P38	6/13/2001	upstream	A	22.6	57	35	18	*	0.75	perfect	no
P38	6/13/2001	upstream	B	63.7	81	45	27	*	1	perfect	no
P38	6/13/2001	upstream	C	17.7	57	31	16	*	0.7	good	no
P38	6/13/2001	upstream	D	88	91	52	30	*	0		no
P38	6/13/2001	upstream	E	11.45	53	27	15	*	0		no
P38	6/13/2001	downstream	F	93.5	87	52	30	*	1.1	perfect	no
P38	6/13/2001	downstream	G	19.25	57	32	18	*	0.34	good	no
P38	6/13/2001	downstream	H	49.45	75	42	24	*	0.45	fair	no
P38	6/13/2001	downstream	I	18.65	57	32	15	*	0		no
P38	6/13/2001	downstream	J	29.1	64	38	20	*	0		no
G25	6/18/2001	upstream	A	86.64	92	50	29	*	1.1	perfect	no
G25	6/18/2001	upstream	B	61.78	72	47	23	no	1.1	perfect	no
G25	6/18/2001	upstream	C	29.34	67	37	20	yes	0.8	perfect	no
G25	6/18/2001	upstream	D	41.7	74	42	22	*	0		no
G25	6/18/2001	upstream	E	41.4	66	41	23	*	0		no
G25	6/18/2001	downstream	F	44.1	78	44	24	yes	0.9	fair	no
G25	6/18/2001	downstream	G	77.98	86	54	28	yes	1.2	good	no
G25	6/18/2001	downstream	H	31.72	62	35	21	yes	1.1	poor	no
G25	6/18/2001	downstream	I	12.34	50	27	14	*	0		no
G25	6/18/2001	downstream	J	34.82	71	38	18	*	0		no

Table II-2 (continued). Summary of mussel physical measurements.

Site	Date	Location	Animal ID	Weight	Length	Height	Width	Gravid	Blood, ml	Bld Draw	Cut Adduct
O54	6/19/2001	upstream	A	42.76	70	38	23	no	0.8	perfect	no
O54	6/19/2001	upstream	B	43.92	74	39	22	*	1	perfect	no
O54	6/19/2001	upstream	C	35	70	31	20	*	0.5	good	no
O54	6/19/2001	upstream	D	66.28	84	46	26	*			no
O54	6/19/2001	upstream	E	49.86	79	44	22	*			no
O54	6/19/2001	downstream	F	76	85	49	27	*	1.15	perfect	no
O54	6/19/2001	downstream	G	24.12	66	34	18	*	0.75	fair	no
O54	6/19/2001	downstream	H	64.1	85	44	25	*	1.1	good	no
O54	6/19/2001	downstream	I	13.9	51	27	15	*	0		no
O54	6/19/2001	downstream	J	93.42	94	50	30	*	0		no
P33	6/20/2001	upstream	A	40.84	66	40	22	yes	1	perfect	no
P33	6/20/2001	upstream	B	54.52	75	44	25	no	1.02	perfect	no
P33	6/20/2001	upstream	C	57.22	80	45	24	yes	1.02	perfect	no
P33	6/20/2001	upstream	D	45.84	71	40	23	*	0		no
P33	6/20/2001	upstream	E	86.08	82	52	30	*	0		no
P33	6/20/2001	downstream	F	79.52	78	53	28	no	1.1	perfect	no
P33	6/20/2001	downstream	G	68.44	79	47	28	no	0.98	perfect	no
P33	6/20/2001	downstream	H	72.66	78	47	26	no	1.1		no
P33	6/20/2001	downstream	I	69.42	80	46	28	*	0		no
P33	6/20/2001	downstream	J	59.14	73	45	28	*	0		no
O57	6/25/2001	upstream	A	38.2	65	40	21	no	0.55	good	no
O57	6/25/2001	upstream	B	38.5	67	39	22	no	0.65	good	no
O57	6/25/2001	upstream	C	35.96	69	40	21	no	0.8	perfect	no
O57	6/25/2001	upstream	D	17.26	56	31	11	yes	0		no
O57	6/25/2001	upstream	E	39.59	69	40	22	yes	0		no
O57	6/25/2001	downstream	F	34.04	64	39	21	yes	1.05	perfect	no
O57	6/25/2001	downstream	G	38	37	39	21	yes	*		no
O57	6/25/2001	downstream	H	40.34	63	39	22	yes	*		no
O57	6/25/2001	downstream	I	24.08	61	32	19	yes	0		no
O57	6/25/2001	downstream	J	24.58	58	35	19	no	0		no
D56	6/26/2001	upstream	A	23.7	57	34	18	no	0.83	good	no
D56	6/26/2001	upstream	B	40.18	67	38	21	no	0.8	good	no
D56	6/26/2001	upstream	C	14.94	48	28	15	no	0.45	good	no
D56	6/26/2001	upstream	D	36.66	66	41	20	no	0		no
D56	6/26/2001	upstream	E	32.44	65	37	20	no	0		no
D56	6/26/2001	downstream	F	21.14	52	30	19	no	1.05	perfect	no
D56	6/26/2001	downstream	G	42.8	67	42	24	no	0.68	good	no
D56	6/26/2001	downstream	H	18	54	29	16	no	0.6	good	no
D56	6/26/2001	downstream	I	25	56	33	20	no	0		no
D56	6/26/2001	downstream	J	13.14	48	27	14	yes	0		no
O11	6/27/2001	upstream	A	27.54	59	35	19	no	1.05	perfect	no
O11	6/27/2001	upstream	B	86.92	86	48	29	no	1.02	perfect	no
O11	6/27/2001	upstream	C	49.42	70	41	24	no	0.95	perfect	no
O11	6/27/2001	upstream	D	66.94	75	44	27	no	0		no
O11	6/27/2001	upstream	E	38.6	67	38	21	no	0		no
O11	6/27/2001	downstream	F	54.84	78	45	25	no	1.03	perfect	no
O11	6/27/2001	downstream	G	51.02	75	44	26	no	1.02	perfect	no
O11	6/27/2001	downstream	H	34.68	61	37	20	no	1	perfect	no
O11	6/27/2001	downstream	I	27.74	60	34	20	no	0		no
O11	6/27/2001	downstream	J	42.74	69	40	22	no	0		no
D151	6/28/2001	upstream	A	63.02	77	46	26	yes	0.6	poor	no
D151	6/28/2001	upstream	B	48.82	69	40	26	no	1.01	perfect	no
D151	6/28/2001	upstream	C	67.28	76	44	30	no	0.56	poor	no
D151	6/28/2001	upstream	D	57.86	70	42	28	no	0		no
D151	6/28/2001	upstream	E	34.56	59	34	24	no	0		no
D151	6/28/2001	downstream	F	22.96	59	32	19	yes	0.6	good	no
D151	6/28/2001	downstream	G	39.38	68	41	23	no	1.02	perfect	no
D151	6/28/2001	downstream	H	37.42	65	37	22	no	0.55	good	no
D151	6/28/2001	downstream	I	43.36	66	39	23	no	0		no

Table II-2 (continued). Summary of mussel physical measurements.

Site	Date	Location	Animal ID	Weight	Length	Height	Width	Gravid	Blood, ml	Bld Draw	Cut Adduct
D151	6/28/2001	downstream	J	49.3	69	41	26	no	0		no
O242	7/3/2001	downstream	A	92.56	81	52	33	yes	0.99	perfect	no
O242	7/3/2001	downstream	B	55.08	75	44	25	no	1.02	perfect	no
O242	7/3/2001	downstream	C	52.88	75	44	25	no	1.03	perfect	no
O242	7/3/2001	downstream	D	66.72	81	47	28	yes	0		no
O242	7/3/2001	downstream	E	57.98	82	48	24	no	0		no
O242	7/3/2001	upstream	F	70.28	81	46	27	no	1.02	perfect	no
O242	7/3/2001	upstream	G	30.68	69	39	20	no	0.5	fair	no
O242	7/3/2001	upstream	H	100.26	93	57	29	no	0.69	fair	no
O242	7/3/2001	upstream	I	63.3	81	47	26	no	0		no
O242	7/3/2001	upstream	J	90.72	90	53	34	yes	0		no
P36	7/5/2001	upstream	A	54.48	76	42	23	yes	1.02	perfect	no
P36	7/5/2001	upstream	B	46.26	75	43	23	no	0.5	good	no
P36	7/5/2001	upstream	C	47.22	71	40	22	yes	0.85	good	no
P36	7/5/2001	upstream	D	27.1	59	34	19	no	0		no
P36	7/5/2001	upstream	E	22.06	60	33	17	no	0		no
P36	7/5/2001	downstream	F	65.6	80	45	26	no	1.02	perfect	no
P36	7/5/2001	downstream	G	41.08	70	39	22	no	0.54	poor	no
P36	7/5/2001	downstream	H	66.7	82	49	25	no	1.03	perfect	no
P36	7/5/2001	downstream	I	59.74	80	43	25	yes	0		no
P36	7/5/2001	downstream	J	46.44	70	42	24	no	0		no
O12	7/6/2001	upstream	A	51.04	70	42	26	no	1.1	perfect	no
O12	7/6/2001	upstream	B	40.8	64	38	23	no	1	perfect	no
O12	7/6/2001	upstream	C	35.42	63	38	23	yes?	1.02	perfect	no
O12	7/6/2001	upstream	D	50.84	70	42	24	no	0		no
O12	7/6/2001	upstream	E	26.48	60	35	20	no	0		no
O12	7/6/2001	downstream	F	45.88	70	41	23	no	1.05	perfect	no
O12	7/6/2001	downstream	G	49.5	69	43	23	no	1.1	perfect	no
O12	7/6/2001	downstream	H	47.88	69	41	26	*	1.03	good	no
O12	7/6/2001	downstream	I	32.74	59	36	23	no	0		no
O12	7/6/2001	downstream	J	46.74	73	42	26	no	0		no
D50	7/11/2001	upstream	A	20.36	56	32	17	no	0.55	good	no
D50	7/11/2001	upstream	B	20.1	54	20	17	no	0.52	good	no
D50	7/11/2001	upstream	C	17.65	53	30	17	*	0.35	poor	no
D50	7/11/2001	upstream	D	9.18	43	25	13	no	0		no
D50	7/11/2001	upstream	E	*	*	*	*	*	*		*
D50	7/11/2001	downstream	F	25	57	35	19	no	0.55	good	no
D50	7/11/2001	downstream	G	29.94	60	36	19	no	0.72	good	no
D50	7/11/2001	downstream	H	39.6	63	38	25	no	1	good	no
D50	7/11/2001	downstream	I	27.5	59	34	21	no	0		no
D50	7/11/2001	downstream	J	30.52	60	35	20	*	0		no
O53	7/12/2001	upstream	A	50.1	73	42	24	no	1.01	good	no
O53	7/12/2001	upstream	B	89.1	91	50	21	no	0.9	good	no
O53	7/12/2001	upstream	C	43.8	70	41	22	no	0.85	good	no
O53	7/12/2001	upstream	D	67.45	84	47	25	no	0		no
O53	7/12/2001	upstream	E	46.4	72	43	23	no	0		no
O53	7/12/2001	downstream	F	46.7	69	40	25	no	0.5	poor	no
O53	7/12/2001	downstream	G	51.2	77	43	23	yes	0.9	fair	no
O53	7/12/2001	downstream	H	49.15	73	40	24	yes	1	perfect	no
O53	7/12/2001	downstream	I	42.05	69	40	24	no	0		no
O53	7/12/2001	downstream	J	62.5	78	44	27	no	0		no

Table II-3. PAHs in Downstream Mussels (ng/g dry weight).										
Bridge Number	5	173	200	53	54	57	36	38	80	151
dry wt (%)	9.59	10.16	9.67	9.52	9.01	8.82	8.39	10.51	10.03	6.85
lipid (%)	2.09	2.27	2.66	1.80	1.98	2.02	1.94	2.49	2.55	1.87
naphthalene	5.96	3.99	5.39	5.51	3.06	5.21	6.10	3.02	3.34	5.97
2-methylnaphthalene	9.29	3.57	5.27	7.01	2.73	5.71	6.26	2.25	3.08	5.87
1-methylnaphthalene	4.34	1.86	2.79	3.29	1.44	2.69	3.01	1.28	1.62	2.96
biphenyl	3.47	2.47	3.38	2.22	2.63	2.39	2.62	2.04	2.44	2.91
2,6-dimethylnaphthalene	3.95	0.00	3.10	2.53	2.12	2.45	2.42	0.00	0.00	2.55
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	2.61	0.00	1.69	1.87	0.00	2.31	1.82	0.00	0.00	2.15
dibenzofuran	3.55	0.00	2.47	2.47	2.09	2.96	2.51	0.00	0.00	2.34
2,3,5-trimethylnaphthalene	0.00	0.00	4.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	16.07	6.43	9.16	10.44	5.65	8.71	10.05	5.03	5.88	9.06
C2-naphthalenes	16.95	9.61	14.72	10.62	9.28	10.90	10.93	9.27	9.30	11.57
C3-naphthalenes	14.53	20.24	35.55	6.74	10.96	7.38	7.81	21.15	10.59	0.00
C4-naphthalenes	3.66	3.64	40.90	1.89	3.13	2.23	2.24	10.18	3.60	1.34
fluorene	2.97	2.03	4.95	2.06	2.08	2.45	2.09	2.72	2.05	1.67
1-methylfluorene	2.40	2.58	23.43	3.35	1.87	4.26	3.06	5.82	2.33	3.02
C1-fluorenes	10.74	7.96	46.96	5.35	6.16	7.23	4.98	16.23	7.37	4.64
C2-fluorenes	0.00	0.00	51.78	3.39	0.00	6.67	0.00	0.00	0.00	0.00
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	0.00	0.00	7.10	0.48	0.85	0.54	0.00	2.74	0.00	0.00
C1-dibenzothiophenes	7.83	0.00	68.96	2.91	0.00	3.10	0.00	20.07	0.00	0.00
C2-dibenzothiophenes	0.00	0.00	106.78	2.78	0.00	0.00	0.00	37.34	0.00	0.00
C3-dibenzothiophenes	0.00	0.00	87.14	0.00	0.00	0.00	0.00	31.66	0.00	0.00
phenanthrene	10.52	10.21	43.41	10.76	8.51	10.29	12.07	14.71	10.68	7.36
anthracene	1.22	0.91	5.20	0.24	0.00	0.45	0.00	1.90	1.89	0.00
1-methylphenanthrene	2.87	3.30	43.72	2.07	0.00	2.75	2.07	4.98	0.00	1.42
C1-phenanthrenes/anthracenes	12.73	10.60	187.62	11.62	0.00	14.68	11.23	26.79	0.00	9.21
C2-phenanthrenes/anthracenes	6.49	11.40	160.49	8.76	0.00	13.68	7.93	23.82	0.00	7.44
C3-phenanthrenes/anthracenes	2.15	5.90	128.50	6.02	0.00	10.08	5.19	12.67	0.00	4.73
C4-phenanthrenes/anthracenes	0.00	0.00	17.40	0.00	0.00	0.00	0.00	2.98	0.00	0.00
fluoranthene	10.43	10.67	118.75	6.24	9.57	12.99	6.65	10.74	14.43	5.00
pyrene	15.56	18.16	26.64	10.64	10.34	18.74	9.88	11.02	18.28	10.13
C1-fluoranthenes/pyrenes	5.41	6.49	35.83	1.89	4.00	5.39	2.16	9.47	4.31	1.68
retene	8.38	7.80	158.13	3.67	8.82	6.57	4.60	37.09	4.28	1.51
benz[a]anthracene	0.71	0.57	1.69	0.37	2.00	1.39	0.00	0.74	0.29	0.21
chrysene	3.84	2.78	6.56	1.50	3.58	4.75	1.85	3.83	3.33	1.31
C1-chrysenes	0.00	0.00	13.40	0.00	7.01	0.00	0.00	15.77	4.46	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	1.93	0.92	2.12	0.70	1.91	1.77	0.00	1.12	1.05	0.00
benzo[k]fluoranthene	0.80	0.47	0.69	0.41	0.91	1.07	0.00	0.33	0.53	0.00
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	2.43	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	8.15	14.98	7.81	4.77	6.60	0.00	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	2.33	1.31	11.90	1.05	0.00	2.13	0.82	2.85	1.46	0.00
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	194	156	1488	149	126	194	135	358	117	106
Sum of 16 PP PAH	58	51	227	49	55	70	46	59	57	34
Petrogenic PAH	111	93	1044	86	56	103	77	258	56	61
Pyrogenic PAH	45	41	197	28	33	53	27	40	47	22
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table II-3 (continued). PAHs in Downstream Mussels (ng/g dry weight).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	8.52	9.58	8.54	11.81	5.63	10.03	13.58	10.89	8.11	7.27
lipid (%)	1.84	2.45	2.17	1.47	1.78	2.08	1.70	2.02	1.82	2.12
naphthalene	13.88	6.31	6.94	4.64	9.16	5.89	5.17	2.92	5.01	30.06
2-methylnaphthalene	18.33	5.78	9.96	5.92	8.69	7.22	8.04	1.70	6.02	35.05
1-methylnaphthalene	8.50	3.12	4.56	2.81	4.18	3.39	3.64	0.99	2.95	15.43
biphenyl	6.82	3.77	3.39	1.91	4.14	2.14	3.12	2.47	2.34	12.61
2,6-dimethylnaphthalene	7.12	3.39	4.00	2.07	4.06	2.59	3.31	0.00	2.67	12.73
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	4.57	0.00	2.58	1.74	3.53	1.84	2.19	0.00	0.00	11.14
dibenzofuran	6.32	2.50	3.69	1.71	4.49	2.04	2.75	0.00	2.78	13.65
2,3,5-trimethylnaphthalene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	27.87	11.03	17.13	8.98	12.89	10.96	13.41	4.76	9.05	54.52
C2-naphthalenes	32.85	14.09	15.08	8.86	17.08	11.19	13.71	7.63	11.91	59.27
C3-naphthalenes	19.98	26.05	12.44	4.97	11.74	4.78	16.55	15.01	0.00	35.45
C4-naphthalenes	5.92	5.61	4.63	1.05	2.68	1.83	3.50	2.54	0.00	10.56
fluorene	5.05	2.72	3.02	1.13	3.48	1.52	2.36	1.97	2.06	12.12
1-methylfluorene	11.85	5.64	2.67	1.61	3.21	2.17	3.37	2.50	3.51	20.97
C1-fluorenes	17.28	13.88	9.78	2.37	5.55	4.24	9.26	8.82	4.97	30.37
C2-fluorenes	11.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.09
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	1.00	1.87	0.00	0.00	0.00	0.00	1.14	1.13	0.00	1.74
C1-dibenzothiophenes	4.97	12.29	0.00	0.00	0.00	0.00	8.60	0.00	0.00	0.00
C2-dibenzothiophenes	0.00	29.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-dibenzothiophenes	0.00	23.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
phenanthrene	33.03	20.73	7.38	2.79	10.67	7.97	12.50	10.66	6.38	65.08
anthracene	0.80	1.41	0.89	0.10	0.00	0.00	1.14	3.72	0.18	1.68
1-methylphenanthrene	8.07	8.51	0.00	0.65	2.22	1.61	0.00	0.00	1.36	11.85
C1-phenanthrenes/anthracenes	45.82	41.26	0.00	3.46	12.93	8.47	0.00	0.00	7.89	65.39
C2-phenanthrenes/anthracenes	36.68	37.92	0.00	4.00	10.48	7.56	76.65	0.00	7.75	45.95
C3-phenanthrenes/anthracenes	18.92	16.28	0.00	3.96	9.06	7.09	0.00	0.00	5.77	30.24
C4-phenanthrenes/anthracenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fluoranthene	14.50	14.95	5.71	3.03	8.51	4.53	7.93	9.37	6.14	39.77
pyrene	27.83	29.08	6.83	5.49	12.54	8.54	16.00	16.55	12.39	73.53
C1-fluoranthenes/pyrenes	5.75	5.04	3.94	1.21	3.29	2.57	4.35	3.42	1.94	13.96
retene	5.40	5.61	14.31	2.51	2.07	1.61	6.61	7.76	3.31	10.10
benz[a]anthracene	0.84	0.57	0.36	0.25	0.40	0.00	0.46	0.22	1.08	1.86
chrysene	2.53	2.31	1.73	0.97	2.07	0.99	1.70	2.83	1.30	13.84
C1-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	1.49	1.41	0.00	0.00	0.65	0.00	1.03	1.09	0.00	5.45
benzo[k]fluoranthene	0.87	0.58	0.00	0.00	0.28	0.00	0.38	0.41	0.00	3.83
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	7.32	0.00	0.00	7.38	0.00	0.00	9.47	11.44	4.06	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	3.28	3.71	0.88	0.00	0.96	0.00	2.40	0.72	1.26	11.69
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	417	361	142	86	171	113	241	121	114	768
Sum of 16 PP PAH	115	83	36	27	52	31	62	62	39	268
Petrogenic PAH	264	258	77	46	104	70	161	54	60	440
Pyrogenic PAH	73	64	23	13	34	20	39	40	26	195
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table II-4. PAHs in Upstream Mussels (ng/g dry weight).										
Bridge Number	5	17	200	53	54	57	36	38	80	151
dry wt (%)	10.38	10.36	9.06	9.65	10.62	9.92	9.51	9.44	11.15	10.60
lipid (%)	2.39	1.97	2.06	2.14	1.87	2.19	2.20	2.40	2.35	2.24
naphthalene	0.99	2.36	3.45	2.29	0.85	1.91	1.84	2.42	2.82	4.96
2-methylnaphthalene	1.86	3.73	3.78	3.13	0.90	3.76	1.43	1.26	2.58	6.02
1-methylnaphthalene	1.51	1.09	1.27	1.48	0.28	1.27	0.81	0.96	1.11	4.74
biphenyl	1.32	1.86	1.42	0.92	0.67	1.09	0.90	1.49	2.20	4.57
2,6-dimethylnaphthalene	1.30	0.00	2.09	1.36	0.41	1.65	0.89	0.00	0.00	4.87
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	0.85	0.00	1.18	0.90	0.00	1.41	0.70	0.00	0.00	3.07
dibenzofuran	1.36	0.00	1.60	1.24	0.46	1.20	0.80	0.00	0.00	3.23
2,3,5-trimethylnaphthalene	0.00	0.00	2.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	6.30	4.97	5.70	5.82	1.02	6.73	4.08	2.67	4.46	11.38
C2-naphthalenes	5.95	8.34	8.86	2.65	2.04	7.43	4.55	6.25	5.42	15.68
C3-naphthalenes	5.63	11.29	18.51	3.30	1.60	4.57	2.27	17.44	5.36	0.00
C4-naphthalenes	0.80	2.17	20.77	0.82	0.68	1.44	0.45	6.28	2.95	1.14
fluorene	0.76	1.75	3.80	0.70	0.33	1.13	0.55	2.10	1.69	2.40
1-methylfluorene	0.58	2.04	12.12	1.73	0.46	3.38	1.25	4.12	1.67	4.25
C1-fluorenes	3.46	5.81	28.48	2.46	1.36	5.79	1.88	10.39	3.07	6.40
C2-fluorenes	0.00	0.00	23.55	1.25	0.00	4.27	0.00	0.00	0.00	0.00
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	0.00	0.00	6.48	0.18	0.22	0.36	0.00	1.73	0.00	0.00
C1-dibenzothiophenes	2.44	0.00	38.22	1.24	0.00	1.77	0.00	10.64	0.00	0.00
C2-dibenzothiophenes	0.00	0.00	69.22	1.45	0.00	0.00	0.00	35.19	0.00	0.00
C3-dibenzothiophenes	0.00	0.00	50.09	0.00	0.00	0.00	0.00	24.38	0.00	0.00
phenanthrene	2.69	8.54	26.21	5.97	2.27	7.92	3.09	11.24	6.04	12.09
anthracene	0.43	0.94	2.97	0.12	0.00	0.23	0.00	1.24	1.40	0.00
1-methylphenanthrene	0.99	1.87	20.52	0.97	0.00	1.48	0.80	2.45	0.00	2.47
C1-phenanthrenes/anthracenes	4.55	7.71	95.19	5.18	0.00	7.82	4.36	18.73	0.00	17.54
C2-phenanthrenes/anthracenes	1.70	8.60	104.33	5.35	0.00	9.37	2.42	13.03	0.00	11.35
C3-phenanthrenes/anthracenes	0.50	4.06	85.70	3.38	0.00	3.76	1.65	12.34	0.00	8.27
C4-phenanthrenes/anthracenes	0.00	0.00	11.35	0.00	0.00	0.00	0.00	1.85	0.00	0.00
fluoranthene	2.45	6.68	76.38	2.98	2.79	5.61	2.33	4.60	9.57	7.02
pyrene	4.20	13.44	15.07	4.66	2.69	9.11	3.95	6.95	15.64	10.77
C1-fluoranthenes/pyrenes	1.20	6.08	21.32	0.81	1.12	3.15	0.70	6.57	2.54	2.46
retene	1.75	6.20	77.24	1.25	2.40	2.68	1.38	17.77	3.04	2.66
benz[a]anthracene	0.14	0.42	0.96	0.11	0.40	0.84	0.00	0.52	0.23	0.22
chrysene	1.22	2.40	3.74	0.58	1.00	2.52	0.44	2.04	1.89	1.88
C1-chrysenes	0.00	0.00	7.41	0.00	1.46	0.00	0.00	7.95	3.72	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	0.54	0.70	1.43	0.33	0.59	0.95	0.00	0.74	0.67	0.00
benzo[k]fluoranthene	0.27	0.29	0.50	0.19	0.16	0.58	0.00	0.27	0.32	0.00
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	1.39	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	4.05	2.65	4.38	1.64	3.66	0.00	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	0.70	0.94	8.66	0.35	0.00	1.29	0.31	1.46	1.10	0.00
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	58	114	862	69	29	112	45	241	80	149
Sum of 16 PP PAH	15	38	143	23	13	37	15	37	41	42
Petrogenic PAH	36	67	616	40	12	63	26	186	35	88
Pyrogenic PAH	12	30	124	13	9	28	9	23	34	29
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table II-4 (continued). PAHs in Upstream Mussels (ng/g dry weight).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	10.18	11.24	9.70	9.25	9.10	9.07	9.38	10.21	9.43	8.44
lipid (%)	2.30	2.22	2.05	2.17	2.08	1.98	2.31	1.90	2.05	1.90
naphthalene	6.68	1.65	1.99	3.07	3.93	0.80	0.86	0.46	2.19	2.57
2-methylnaphthalene	10.13	1.44	2.77	5.33	3.36	0.92	2.51	0.31	3.55	3.21
1-methylnaphthalene	4.84	0.42	0.78	2.26	2.40	0.54	1.05	0.18	2.07	1.56
biphenyl	2.63	1.28	0.82	1.74	1.37	0.37	0.53	0.51	1.54	1.45
2,6-dimethylnaphthalene	4.64	0.84	0.95	1.84	2.32	0.42	0.87	0.00	2.06	1.10
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	2.81	0.00	0.40	1.86	1.47	0.25	0.43	0.00	0.00	1.24
dibenzofuran	3.32	0.34	0.68	1.48	1.71	0.29	0.63	0.00	2.18	1.06
2,3,5-trimethylnaphthalene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	17.25	3.15	3.90	9.21	6.37	1.36	2.68	0.68	6.68	3.29
C2-naphthalenes	25.33	4.30	5.22	5.92	8.61	1.26	3.63	1.13	6.31	5.67
C3-naphthalenes	14.28	9.00	2.69	5.40	5.73	0.58	3.29	1.90	0.00	3.84
C4-naphthalenes	3.30	1.36	1.05	0.89	1.00	0.35	0.92	0.34	0.00	0.78
fluorene	2.84	0.64	0.59	1.01	1.67	0.28	0.59	0.33	1.37	1.40
1-methylfluorene	3.68	1.90	0.83	1.26	1.78	0.40	0.77	0.46	2.43	1.23
C1-fluorenes	7.63	5.15	2.60	2.08	3.40	0.55	2.26	1.51	4.15	3.56
C2-fluorenes	9.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.46
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	0.63	0.40	0.00	0.00	0.00	0.00	0.32	0.18	0.00	0.11
C1-dibenzothiophenes	2.83	4.01	0.00	0.00	0.00	0.00	2.40	0.00	0.00	0.00
C2-dibenzothiophenes	0.00	9.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-dibenzothiophenes	0.00	8.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
phenanthrene	17.14	4.68	1.37	2.74	5.58	1.45	1.62	1.86	5.43	5.49
anthracene	0.51	0.36	0.23	0.08	0.00	0.00	0.29	0.58	0.12	0.21
1-methylphenanthrene	5.76	2.00	0.00	0.45	1.14	0.26	0.00	0.00	0.81	1.23
C1-phenanthrenes/anthracenes	35.58	11.26	0.00	3.04	7.54	1.83	0.00	0.00	7.08	5.97
C2-phenanthrenes/anthracenes	24.61	10.52	0.00	3.61	3.79	1.15	21.97	0.00	6.27	4.14
C3-phenanthrenes/anthracenes	9.72	3.40	0.00	2.91	3.66	1.25	0.00	0.00	2.84	2.29
C4-phenanthrenes/anthracenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fluoranthene	9.36	4.05	1.53	2.57	5.20	0.76	1.91	1.67	3.62	3.04
pyrene	14.75	7.53	1.81	3.62	4.54	1.28	3.16	2.09	5.53	6.64
C1-fluoranthenes/pyrenes	3.04	1.40	0.78	1.16	1.06	0.40	0.83	0.52	1.17	1.31
retene	2.13	1.78	4.37	2.33	0.71	0.26	1.63	1.56	2.31	0.83
benz[a]anthracene	0.41	0.18	0.08	0.17	0.13	0.00	0.10	0.02	0.68	0.24
chrysene	1.54	0.55	0.45	0.60	0.85	0.09	0.47	0.30	1.27	1.11
C1-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	0.89	0.35	0.00	0.00	0.22	0.00	0.26	0.12	0.00	0.49
benzo[k]fluoranthene	0.58	0.14	0.00	0.00	0.12	0.00	0.09	0.07	0.00	0.16
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	6.01	0.00	0.00	5.69	0.00	0.00	2.38	2.26	2.83	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	1.60	0.94	0.20	0.00	0.45	0.00	0.34	0.12	0.58	0.72
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	256	102	36	72	80	17	59	19	75	67
Sum of 16 PP PAH	65	21	9	21	24	5	12	10	23	23
Petrogenic PAH	172	76	20	40	50	11	41	8	41	39
Pyrogenic PAH	41	16	5	10	16	3	8	6	15	17
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table II-5. PAHs in Downstream Mussels (ng/g lipid).										
Bridge Number	5	173	200	53	54	57	36	38	80	151
dry wt (%)	9.59	10.16	9.67	9.52	9.01	8.82	8.39	10.51	10.03	6.85
lipid (%)	2.09	2.27	2.66	1.80	1.98	2.02	1.94	2.49	2.55	1.87
naphthalene	286	176	203	305	155	259	314	121	131	319
2-methylnaphthalene	445	158	198	388	138	283	322	90	121	314
1-methylnaphthalene	208	82	105	182	73	133	155	52	63	158
biphenyl	166	109	127	123	133	118	135	82	96	155
2,6-dimethylnaphthalene	189	0	117	140	107	121	125	0	0	136
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	125	0	63	104	0	115	93	0	0	115
dibenzofuran	170	0	93	137	106	147	129	0	0	125
2,3,5-trimethylnaphthalene	0	0	162	0	0	0	0	0	0	0
C1-naphthalenes	770	283	345	579	286	432	517	202	231	484
C2-naphthalenes	812	424	554	589	469	540	562	372	365	618
C3-naphthalenes	696	893	1338	374	554	366	402	849	416	0
C4-naphthalenes	175	161	1539	104	158	110	115	409	141	72
fluorene	142	89	186	114	105	122	107	109	80	89
1-methylfluorene	115	114	881	186	95	212	157	234	91	162
C1-fluorenes	515	351	1767	296	311	358	256	652	289	248
C2-fluorenes	0	0	1948	188	0	331	0	0	0	0
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	0	0	267	26	43	27	0	110	0	0
C1-dibenzothiophenes	375	0	2594	161	0	154	0	806	0	0
C2-dibenzothiophenes	0	0	4017	154	0	0	0	1500	0	0
C3-dibenzothiophenes	0	0	3278	0	0	0	0	1272	0	0
phenanthrene	504	450	1633	596	430	510	621	591	419	393
anthracene	58	40	196	13	0	22	0	76	74	0
1-methylphenanthrene	138	146	1645	115	0	137	107	200	0	76
C1-phenanthrenes/anthracenes	610	467	7058	644	0	728	578	1076	0	492
C2-phenanthrenes/anthracenes	311	503	6038	486	0	679	408	957	0	397
C3-phenanthrenes/anthracenes	103	260	4834	334	0	500	267	509	0	253
C4-phenanthrenes/anthracenes	0	0	655	0	0	0	0	120	0	0
fluoranthene	500	470	4467	346	484	644	342	431	567	267
pyrene	746	801	1002	590	523	930	508	442	718	541
C1-fluoranthenes/pyrenes	259	286	1348	105	202	267	111	380	169	90
retene	402	344	5949	203	446	326	237	1490	168	81
benz[a]anthracene	34	25	63	21	101	69	0	30	11	11
chrysene	184	123	247	83	181	236	95	154	131	70
C1-chrysenes	0	0	504	0	354	0	0	633	175	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	93	40	80	39	96	88	0	45	41	0
benzo[k]fluoranthene	38	21	26	23	46	53	0	13	21	0
benzo[e]pyrene	0	0	0	0	0	120	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	0	0	0	452	757	388	245	265	0	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	112	58	448	58	0	106	42	114	57	0
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	9281	6874	55974	8257	6353	9630	6950	14387	4576	5666
Sum of 16 PP PAH	2788	2269	8551	2723	2777	3471	2368	2363	2239	1795
Petrogenic PAH	5307	4119	39288	4756	2852	5128	3948	10373	2208	3258
Pyrogenic PAH	2142	1803	7409	1574	1646	2637	1391	1602	1830	1201
Values listed as 0 are below detection limit (~10 ng/g lipid)										

Table II-5 (continued). PAHs in Downstream Mussels (ng/g lipid).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	8.52	9.58	8.54	11.81	5.63	10.03	13.58	10.89	8.11	7.27
lipid (%)	1.84	2.45	2.17	1.47	1.78	2.08	1.70	2.02	1.82	2.12
naphthalene	754	258	320	316	515	284	304	144	276	1416
2-methylnaphthalene	996	236	460	403	488	348	473	84	331	1651
1-methylnaphthalene	462	127	210	192	234	163	214	49	163	727
biphenyl	370	154	157	130	233	103	184	122	129	594
2,6-dimethylnaphthalene	387	138	185	141	228	125	195	0	147	600
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	248	0	119	118	198	88	129	0	0	525
dibenzofuran	343	102	171	116	252	99	161	0	153	643
2,3,5-trimethylnaphthalene	0	0	0	0	0	0	0	0	0	0
C1-naphthalenes	1514	451	791	612	724	528	789	235	498	2569
C2-naphthalenes	1785	576	697	604	959	539	806	377	655	2792
C3-naphthalenes	1086	1065	574	339	659	230	973	742	0	1670
C4-naphthalenes	321	229	214	71	151	88	206	126	0	498
fluorene	274	111	139	77	196	73	139	97	113	571
1-methylfluorene	644	231	123	110	180	104	198	124	193	988
C1-fluorenes	939	568	452	161	312	204	544	436	274	1431
C2-fluorenes	649	0	0	0	0	0	0	0	0	852
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	54	76	0	0	0	0	67	56	0	82
C1-dibenzothiophenes	270	503	0	0	0	0	505	0	0	0
C2-dibenzothiophenes	0	1205	0	0	0	0	0	0	0	0
C3-dibenzothiophenes	0	980	0	0	0	0	0	0	0	0
phenanthrene	1795	848	341	190	599	384	735	527	351	3066
anthracene	43	58	41	7	0	0	67	184	10	79
1-methylphenanthrene	438	348	0	45	125	78	0	0	75	558
C1-phenanthrenes/anthracenes	2490	1687	0	236	726	408	0	0	434	3081
C2-phenanthrenes/anthracenes	1993	1551	0	272	589	364	4507	0	427	2165
C3-phenanthrenes/anthracenes	1028	666	0	270	509	342	0	0	318	1425
C4-phenanthrenes/anthracenes	0	0	0	0	0	0	0	0	0	0
fluoranthene	788	611	264	207	478	218	466	463	338	1874
pyrene	1512	1189	315	374	704	411	941	818	682	3464
C1-fluoranthenes/pyrenes	312	206	182	83	185	124	256	169	106	658
retene	293	229	661	171	116	77	389	384	182	476
benz[a]anthracene	46	23	17	17	22	0	27	11	60	88
chrysene	138	95	80	66	116	48	100	140	71	652
C1-chrysenes	0	0	0	0	0	0	0	0	0	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	81	58	0	0	36	0	61	54	0	257
benzo[k]fluoranthene	47	24	0	0	16	0	22	20	0	180
benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	398	0	0	502	0	0	557	566	223	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	178	152	41	0	54	0	141	36	69	551
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	22680	14757	6553	5829	9603	5431	14155	5962	6278	36184
Sum of 16 PP PAH	6258	3403	1660	1857	2911	1507	3661	3049	2134	12636
Petrogenic PAH	14369	10557	3539	3135	5822	3376	9464	2645	3276	20743
Pyrogenic PAH	3979	2633	1047	884	1924	958	2321	1989	1406	9203
Values listed as 0 are below detection limit (~10 ng/g lipid)										

Table II-6. PAHs in Upstream Mussels (ng/g lipid).										
Bridge Number	5	17	200	53	54	57	36	38	80	151
dry wt (%)	10.38	10.36	9.06	9.65	10.62	9.92	9.51	9.44	11.15	10.60
lipid (%)	2.39	1.97	2.06	2.14	1.87	2.19	2.20	2.40	2.35	2.24
naphthalene	42	120	168	107	46	87	84	101	120	221
2-methylnaphthalene	78	190	183	146	48	172	65	53	110	269
1-methylnaphthalene	63	55	61	69	15	58	37	40	47	212
biphenyl	55	95	69	43	36	50	41	62	94	204
2,6-dimethylnaphthalene	54	0	101	64	22	75	40	0	0	217
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	35	0	57	42	0	64	32	0	0	137
dibenzofuran	57	0	78	58	25	55	36	0	0	144
2,3,5-trimethylnaphthalene	0	0	127	0	0	0	0	0	0	0
C1-naphthalenes	264	252	277	272	55	307	186	111	190	508
C2-naphthalenes	249	423	430	124	109	339	207	261	231	700
C3-naphthalenes	236	573	899	154	85	209	103	727	228	0
C4-naphthalenes	33	110	1008	38	36	66	20	262	125	51
fluorene	32	89	184	33	18	52	25	87	72	107
1-methylfluorene	24	104	588	81	25	154	57	172	71	190
C1-fluorenes	145	295	1382	115	73	265	85	433	131	286
C2-fluorenes	0	0	1143	59	0	195	0	0	0	0
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	0	0	314	8	12	16	0	72	0	0
C1-dibenzothiophenes	102	0	1855	58	0	81	0	443	0	0
C2-dibenzothiophenes	0	0	3360	68	0	0	0	1466	0	0
C3-dibenzothiophenes	0	0	2432	0	0	0	0	1016	0	0
phenanthrene	113	433	1273	279	121	361	140	468	257	540
anthracene	18	48	144	6	0	11	0	52	59	0
1-methylphenanthrene	41	95	996	45	0	68	36	102	0	110
C1-phenanthrenes/anthracenes	190	391	4621	242	0	357	198	781	0	783
C2-phenanthrenes/anthracenes	71	436	5065	250	0	428	110	543	0	507
C3-phenanthrenes/anthracenes	21	206	4160	158	0	172	75	514	0	369
C4-phenanthrenes/anthracenes	0	0	551	0	0	0	0	77	0	0
fluoranthene	103	339	3708	139	149	256	106	192	407	313
pyrene	176	682	731	218	144	416	180	289	666	481
C1-fluoranthenes/pyrenes	50	309	1035	38	60	144	32	274	108	110
retene	73	315	3750	59	128	122	63	741	129	119
benz[a]anthracene	6	21	46	5	21	38	0	22	10	10
chrysene	51	122	181	27	54	115	20	85	80	84
C1-chrysenes	0	0	360	0	78	0	0	331	158	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	23	35	70	15	32	43	0	31	29	0
benzo[k]fluoranthene	11	15	24	9	9	27	0	11	14	0
benzo[e]pyrene	0	0	0	0	0	64	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	0	0	0	189	142	200	75	152	0	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	29	48	421	17	0	59	14	61	47	0
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	2445	5801	41854	3234	1542	5126	2066	10030	3384	6672
Sum of 16 PP PAH	632	1930	6961	1081	714	1692	675	1529	1751	1884
Petrogenic PAH	1491	3422	29880	1863	632	2898	1195	7733	1492	3912
Pyrogenic PAH	508	1526	6020	618	469	1274	422	976	1440	1295
Values listed as 0 are below detection limit (~10 ng/g lipid)										

Table II-6 (continued). PAHs in Upstream Mussels (ng/g lipid).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	10.18	11.24	9.70	9.25	9.10	9.07	9.38	10.21	9.43	8.44
lipid (%)	2.30	2.22	2.05	2.17	2.08	1.98	2.31	1.90	2.05	1.90
naphthalene	290	74	97	141	189	41	37	24	107	135
2-methylnaphthalene	440	65	135	246	162	47	109	16	173	169
1-methylnaphthalene	210	19	38	104	115	27	45	10	101	82
biphenyl	114	58	40	80	66	19	23	27	75	76
2,6-dimethylnaphthalene	202	38	46	85	112	21	38	0	100	58
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	122	0	19	86	71	12	18	0	0	65
dibenzofuran	144	15	33	68	82	15	27	0	106	56
2,3,5-trimethylnaphthalene	0	0	0	0	0	0	0	0	0	0
C1-naphthalenes	750	142	190	425	306	69	116	36	326	173
C2-naphthalenes	1101	194	254	273	414	64	157	60	308	298
C3-naphthalenes	621	406	131	249	275	29	142	100	0	202
C4-naphthalenes	143	61	51	41	48	17	40	18	0	41
fluorene	123	29	29	47	80	14	26	17	67	74
1-methylfluorene	160	85	41	58	85	20	33	24	119	65
C1-fluorenes	332	232	127	96	163	28	98	79	202	187
C2-fluorenes	409	0	0	0	0	0	0	0	0	77
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	27	18	0	0	0	0	14	9	0	6
C1-dibenzothiophenes	123	181	0	0	0	0	104	0	0	0
C2-dibenzothiophenes	0	422	0	0	0	0	0	0	0	0
C3-dibenzothiophenes	0	362	0	0	0	0	0	0	0	0
phenanthrene	745	211	67	126	268	73	70	98	265	289
anthracene	22	16	11	4	0	0	13	31	6	11
1-methylphenanthrene	250	90	0	21	55	13	0	0	40	65
C1-phenanthrenes/anthracenes	1547	507	0	140	362	92	0	0	346	314
C2-phenanthrenes/anthracenes	1070	474	0	166	182	58	951	0	306	218
C3-phenanthrenes/anthracenes	423	153	0	134	176	63	0	0	139	120
C4-phenanthrenes/anthracenes	0	0	0	0	0	0	0	0	0	0
fluoranthene	407	182	75	119	250	39	82	88	177	160
pyrene	641	339	88	167	218	65	137	110	270	350
C1-fluoranthenes/pyrenes	132	63	38	53	51	20	36	27	57	69
retene	93	80	213	107	34	13	70	82	113	44
benz[a]anthracene	18	8	4	8	6	0	4	1	33	13
chrysene	67	25	22	28	41	4	20	16	62	58
C1-chrysenes	0	0	0	0	0	0	0	0	0	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	39	16	0	0	10	0	11	6	0	26
benzo[k]fluoranthene	25	6	0	0	6	0	4	4	0	8
benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	261	0	0	262	0	0	103	119	138	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	69	42	10	0	22	0	15	6	28	38
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	11123	4614	1760	3333	3851	864	2544	1008	3663	3547
Sum of 16 PP PAH	2813	941	418	979	1155	248	536	519	1120	1213
Petrogenic PAH	7464	3423	952	1828	2382	532	1756	419	1989	2060
Pyrogenic PAH	1783	740	263	473	758	157	340	310	709	873
Values listed as 0 are below detection limit (~10 ng/g lipid)										

Table II-7. PAHs in Downstream Sediment (ng/g dry weight).										
Bridge Number	5	173	200	53	54	57	36	38	80	151
dry wt (%)	71.23	64.50	59.38	55.08	60.79	56.30	60.92	59.94	60.87	47.86
TOC (%)	3.24	3.03	1.13	2.97	2.95	2.71	2.55	2.70	1.99	2.52
naphthalene	7.35	3.15	1.60	5.33	1.89	4.90	4.21	1.20	3.02	4.16
2-methylnaphthalene	6.99	1.94	2.60	10.15	3.94	4.68	4.77	1.12	1.92	5.23
1-methylnaphthalene	6.44	3.58	0.69	2.80	1.34	1.94	3.20	0.93	0.88	2.88
biphenyl	7.70	3.27	2.14	3.94	4.16	2.77	789.26	2.56	3.12	7.17
2,6-dimethylnaphthalene	5.09	0.00	1.13	4.74	2.08	3.43	2.00	0.00	0.00	2.19
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	6.86	0.00	2.18	5.87	0.00	5.67	4.79	0.00	0.00	6.18
dibenzofuran	6.65	0.00	1.61	7.79	6.61	4.40	4.37	0.00	0.00	3.45
2,3,5-trimethylnaphthalene	0.00	0.00	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	12.41	3.53	1.53	8.41	4.28	5.55	12.22	2.97	2.93	6.43
C2-naphthalenes	15.18	8.43	3.61	16.65	9.38	7.31	7.27	7.82	4.90	11.44
C3-naphthalenes	14.16	15.52	8.44	5.33	9.80	9.28	6.53	17.90	6.55	0.00
C4-naphthalenes	4.40	3.82	7.33	2.41	4.25	1.65	2.64	4.58	2.06	1.20
fluorene	3.03	1.43	1.34	1.77	3.05	1.30	1.14	1.18	0.80	1.10
1-methylfluorene	1.81	1.34	5.54	4.01	2.70	2.28	1.93	3.29	1.38	2.79
C1-fluorenes	8.74	11.46	-234.24	5.87	5.95	4.23	6.34	11.72	4.49	2.92
C2-fluorenes	0.00	0.00	9.98	3.61	0.00	4.05	0.00	0.00	0.00	0.00
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	0.00	0.00	1.15	0.40	0.65	0.40	0.00	1.95	0.00	0.00
C1-dibenzothiophenes	7.15	0.00	11.27	5.61	0.00	1.78	0.00	17.67	0.00	0.00
C2-dibenzothiophenes	0.00	0.00	23.10	2.00	0.00	0.00	0.00	28.96	0.00	0.00
C3-dibenzothiophenes	0.00	0.00	26.29	0.00	0.00	0.00	0.00	20.06	0.00	0.00
phenanthrene	38.35	27.27	74.56	22.01	18.29	50.21	20.09	20.50	13.94	12.49
anthracene	5.15	3.63	5.02	1.98	0.00	1.62	0.00	6.85	3.18	0.00
1-methylphenanthrene	2.47	4.19	15.12	2.31	0.00	2.59	1.76	8.33	0.00	1.23
C1-phenanthrenes/anthracenes	20.23	9.26	49.00	21.85	0.00	9.71	6.64	20.35	0.00	7.58
C2-phenanthrenes/anthracenes	4.38	8.23	55.68	7.84	0.00	13.91	6.61	15.50	0.00	10.25
C3-phenanthrenes/anthracenes	2.80	4.69	61.30	9.16	0.00	7.24	3.82	21.23	0.00	3.64
C4-phenanthrenes/anthracenes	0.00	0.00	4.20	0.00	0.00	0.00	0.00	1.51	0.00	0.00
fluoranthene	89.98	34.56	130.67	84.92	65.09	63.50	19.18	27.52	50.69	56.15
pyrene	71.19	76.65	23.98	36.35	30.73	111.88	34.21	29.92	64.43	37.75
C1-fluoranthenes/pyrenes	8.77	5.02	13.83	1.96	5.20	4.93	1.44	6.75	2.09	1.64
retene	9.83	6.59	59.74	5.87	10.32	8.77	3.42	54.29	2.96	1.94
benz[a]anthracene	1.83	1.09	1.26	1.06	10.56	5.19	0.00	1.09	0.40	1.15
chrysene	4.47	3.01	1.91	2.54	2.74	5.38	2.31	4.67	2.81	2.33
C1-chrysenes	0.00	0.00	2.87	0.00	9.91	0.00	0.00	11.17	2.19	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	6.53	2.80	4.28	3.34	7.11	5.94	0.00	1.85	1.70	0.00
benzo[k]fluoranthene	4.02	2.27	0.88	2.04	2.47	5.15	0.00	0.68	0.86	0.00
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	9.26	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	36.16	87.88	26.68	19.44	32.14	0.00	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	10.76	6.79	10.86	7.09	0.00	6.89	5.44	10.97	4.65	0.00
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	395	254	393	343	310	404	975	399	182	193
Sum of 16 PP PAH	248	162	257	209	219	289	111	137	146	120
Petrogenic PAH	128	88	86	109	64	101	69	203	36	57
Pyrogenic PAH	220	144	218	156	128	246	76	94	136	110
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table II-7 (continued). PAHs in Downstream Sediment (ng/g dry weight).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	60.36	54.55	56.23	59.20	63.93	65.41	63.68	66.37	62.87	61.94
TOC (%)	2.39	3.46	3.09	2.90	2.26	3.30	1.78	3.27	1.47	1.53
naphthalene	10.63	6.01	4.53	5.16	7.06	6.76	3.76	2.56	2.97	15.76
2-methylnaphthalene	20.67	5.68	7.45	7.58	6.66	9.86	4.37	1.40	3.26	20.85
1-methylnaphthalene	6.25	1.76	4.03	3.13	3.72	3.53	2.34	0.82	1.60	6.90
biphenyl	11.02	12.57	5.26	3.66	12.04	4.01	4.74	3.60	3.07	14.31
2,6-dimethylnaphthalene	4.97	3.08	3.65	3.69	4.43	5.54	3.93	0.00	1.03	4.49
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	41.99	0.00	9.02	7.10	9.60	6.78	4.92	0.00	0.00	12.61
dibenzofuran	18.64	4.77	8.53	6.25	13.68	5.23	4.28	0.00	4.56	28.52
2,3,5-trimethylnaphthalene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	19.21	6.05	17.32	9.21	16.76	8.68	9.87	4.07	4.44	20.04
C2-naphthalenes	23.40	20.22	11.51	17.63	11.63	14.97	8.04	7.18	12.71	31.55
C3-naphthalenes	12.99	29.59	9.95	6.04	8.18	8.38	8.61	18.67	0.00	19.76
C4-naphthalenes	3.62	4.24	16.01	1.03	1.97	3.51	1.47	2.09	0.00	7.02
fluorene	2.69	1.91	1.82	1.73	2.32	1.46	3.35	1.36	0.71	5.84
1-methylfluorene	9.63	4.88	2.32	1.20	2.25	1.92	1.40	3.17	2.71	7.26
C1-fluorenes	15.33	11.05	7.97	3.02	12.73	5.60	7.68	12.11	2.33	13.99
C2-fluorenes	11.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.46
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	0.62	1.19	0.00	0.00	0.00	0.00	0.47	0.94	0.00	0.70
C1-dibenzothiophenes	3.83	15.01	0.00	0.00	0.00	0.00	6.11	0.00	0.00	0.00
C2-dibenzothiophenes	0.00	22.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-dibenzothiophenes	0.00	26.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
phenanthrene	69.56	54.00	21.51	8.02	16.93	23.83	26.08	30.99	10.08	94.55
anthracene	2.91	6.67	2.71	0.44	0.00	0.00	2.28	14.95	0.42	5.87
1-methylphenanthrene	9.69	11.26	0.00	0.43	1.27	1.26	0.00	0.00	0.45	5.28
C1-phenanthrenes/anthracenes	57.68	30.81	0.00	3.34	7.36	13.27	0.00	0.00	3.69	23.55
C2-phenanthrenes/anthracenes	28.76	32.94	0.00	6.26	8.06	7.48	60.86	0.00	2.66	87.20
C3-phenanthrenes/anthracenes	15.63	9.36	0.00	4.91	11.89	6.86	0.00	0.00	2.34	19.30
C4-phenanthrenes/anthracenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fluoranthene	31.74	147.96	30.79	20.52	45.32	25.68	27.57	123.85	9.73	76.03
pyrene	99.63	101.31	14.96	30.11	98.33	39.76	109.13	85.23	79.64	154.13
C1-fluoranthenes/pyrenes	9.47	14.49	5.09	3.67	3.55	3.59	4.89	3.00	1.85	10.41
retene	5.47	7.12	29.95	3.37	1.66	2.01	5.94	11.53	2.62	4.70
benz[a]anthracene	4.97	1.46	1.09	0.97	1.44	0.00	1.12	0.65	1.94	2.08
chrysene	4.97	2.98	2.57	2.03	2.09	1.09	1.91	4.35	1.90	12.22
C1-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	11.21	6.75	0.00	0.00	1.83	0.00	3.08	3.57	0.00	11.51
benzo[k]fluoranthene	3.01	1.59	0.00	0.00	0.91	0.00	0.74	1.16	0.00	4.91
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	29.46	0.00	0.00	50.25	0.00	0.00	32.20	161.12	14.37	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	15.02	39.51	3.10	0.00	3.54	0.00	8.12	3.59	3.45	19.71
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	617	645	221	211	317	211	359	502	175	748
Sum of 16 PP PAH	323	369	91	125	188	105	223	433	123	413
Petrogenic PAH	251	259	85	66	100	92	128	67	39	309
Pyrogenic PAH	250	335	75	65	172	85	172	253	102	346
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table II-8. PAHs in Upstream Sediment (ng/g dry weight).										
Bridge Number	5	17	200	53	54	57	36	38	80	151
dry wt (%)	10.38	10.36	9.06	9.65	10.62	9.92	9.51	9.44	11.15	10.60
TOC (%)	1.66	0.95	2.80	1.44	1.25	2.70	1.30	0.77	1.52	2.39
naphthalene	0.57	3.85	1.04	1.15	0.62	1.29	1.21	1.70	1.23	4.73
2-methylnaphthalene	1.61	1.78	1.85	1.87	0.88	1.65	0.67	0.72	3.18	2.21
1-methylnaphthalene	0.99	0.84	0.32	0.70	0.20	1.11	0.54	0.65	0.53	4.18
biphenyl	2.91	2.39	0.72	1.39	1.91	1.38	1.47	2.54	3.23	3.91
2,6-dimethylnaphthalene	1.28	0.00	0.79	1.32	0.46	1.11	1.60	0.00	0.00	2.76
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	7.03	0.00	3.39	7.41	0.00	5.45	2.83	0.00	0.00	11.36
dibenzofuran	14.90	0.00	7.18	15.82	4.75	15.87	20.83	0.00	0.00	90.61
2,3,5-trimethylnaphthalene	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	4.12	3.36	1.38	3.66	0.60	8.20	2.51	1.95	2.85	8.68
C2-naphthalenes	4.32	6.02	1.81	1.65	1.37	6.08	2.75	3.38	3.80	7.37
C3-naphthalenes	4.79	8.81	5.53	1.77	1.75	2.50	2.31	11.67	2.05	0.00
C4-naphthalenes	0.47	2.19	4.53	1.13	0.75	1.29	0.33	4.13	1.17	0.60
fluorene	1.96	1.71	0.94	0.57	0.33	1.13	0.29	2.22	1.10	2.01
1-methylfluorene	0.56	2.49	3.17	1.65	0.64	4.36	0.84	2.92	0.87	2.90
C1-fluorenes	2.36	5.18	6.38	1.15	0.87	3.56	1.46	6.79	0.94	2.65
C2-fluorenes	0.00	0.00	9.73	2.07	0.00	2.39	0.00	0.00	0.00	0.00
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	0.00	0.00	1.83	0.27	0.26	0.32	0.00	1.16	0.00	0.00
C1-dibenzothiophenes	2.05	0.00	11.92	1.09	0.00	2.22	0.00	6.73	0.00	0.00
C2-dibenzothiophenes	0.00	0.00	17.35	0.79	0.00	0.00	0.00	15.96	0.00	0.00
C3-dibenzothiophenes	0.00	0.00	15.80	0.00	0.00	0.00	0.00	19.61	0.00	0.00
phenanthrene	9.98	70.63	69.25	19.34	12.99	29.37	11.49	71.92	24.51	44.03
anthracene	2.71	6.39	18.03	1.27	0.00	1.83	0.00	9.80	6.05	0.00
1-methylphenanthrene	0.51	1.23	9.39	0.59	0.00	1.12	0.42	1.41	0.00	1.58
C1-phenanthrenes/anthracenes	4.39	5.79	24.29	4.63	0.00	4.12	2.50	13.30	0.00	11.36
C2-phenanthrenes/anthracenes	2.95	10.13	35.36	5.54	0.00	12.32	3.36	8.47	0.00	8.95
C3-phenanthrenes/anthracenes	0.30	3.67	28.98	4.73	0.00	4.75	1.58	8.29	0.00	4.20
C4-phenanthrenes/anthracenes	0.00	0.00	5.31	0.00	0.00	0.00	0.00	2.56	0.00	0.00
fluoranthene	14.67	37.07	125.36	16.86	22.18	86.94	7.17	23.59	52.07	27.87
pyrene	13.04	46.32	16.10	21.09	12.34	38.34	11.93	19.86	35.65	46.55
C1-fluoranthenes/pyrenes	1.22	5.29	14.38	0.93	1.45	5.44	0.48	5.19	2.72	3.18
retene	1.51	6.43	32.25	0.93	2.48	2.27	1.43	11.45	2.38	2.10
benz[a]anthracene	1.39	1.95	1.60	0.80	2.58	3.54	0.00	1.54	0.66	0.79
chrysene	1.13	2.86	1.58	0.70	1.18	4.06	0.42	3.32	1.51	1.46
C1-chrysenes	0.00	0.00	3.08	0.00	1.72	0.00	0.00	5.89	3.52	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	4.67	7.75	4.93	2.60	6.74	8.85	0.00	7.93	3.61	0.00
benzo[k]fluoranthene	1.46	3.22	0.66	0.87	0.80	3.40	0.00	1.68	0.64	0.00
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	5.34	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	20.29	14.19	35.47	7.58	14.46	0.00	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	3.91	8.52	24.10	2.05	0.00	11.13	1.47	5.19	4.33	0.00
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	114	256	511	149	94	318	89	298	159	296
Sum of 16 PP PAH	61	188	265	94	71	227	44	162	131	138
Petrogenic PAH	34	91	224	41	16	70	25	155	32	76
Pyrogenic PAH	55	149	230	63	52	184	30	109	117	110
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table II-8 (continued). PAHs in Upstream Sediment (ng/g dry weight).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	10.18	11.24	9.70	9.25	9.10	9.07	9.38	10.21	9.43	8.44
TOC (%)	2.10	1.80	2.60	1.04	2.28	2.60	1.70	1.30	2.15	1.45
naphthalene	8.98	1.34	2.09	2.49	1.82	0.86	0.27	0.33	1.10	0.80
2-methylnaphthalene	11.34	1.17	5.23	3.93	1.67	0.50	0.71	0.34	1.84	1.40
1-methylnaphthalene	2.78	0.54	0.76	2.35	1.09	0.26	0.89	0.16	1.20	0.55
biphenyl	2.89	2.81	1.51	2.43	1.55	0.63	0.36	1.18	1.05	0.97
2,6-dimethylnaphthalene	2.55	0.65	0.54	1.94	1.60	0.66	0.45	0.00	1.29	0.49
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	12.60	0.00	2.00	15.42	9.33	1.95	1.45	0.00	0.00	4.22
dibenzofuran	17.82	5.22	9.55	23.47	18.92	5.12	3.67	0.00	17.26	9.42
2,3,5-trimethylnaphthalene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-naphthalenes	6.72	1.96	2.98	5.64	4.04	0.88	1.20	0.70	4.07	1.53
C2-naphthalenes	10.20	3.93	7.20	4.81	4.48	1.20	1.37	1.45	1.94	2.07
C3-naphthalenes	10.83	7.49	2.00	3.01	4.02	0.43	1.28	1.47	0.00	1.39
C4-naphthalenes	2.71	0.91	0.66	0.88	0.75	0.38	0.57	0.47	0.00	0.32
fluorene	1.88	0.47	1.29	1.05	1.32	0.26	0.27	0.32	0.74	1.41
1-methylfluorene	1.67	2.06	0.76	0.77	1.59	0.53	0.39	0.42	0.90	1.27
C1-fluorenes	3.88	5.32	1.08	2.58	3.87	0.54	0.81	1.25	1.18	1.93
C2-fluorenes	3.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
C3-fluorenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	0.38	0.50	0.00	0.00	0.00	0.00	0.10	0.14	0.00	0.06
C1-dibenzothiophenes	3.46	3.05	0.00	0.00	0.00	0.00	1.56	0.00	0.00	0.00
C2-dibenzothiophenes	0.00	5.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-dibenzothiophenes	0.00	7.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
phenanthrene	54.11	31.03	13.00	15.60	40.12	5.52	4.20	10.47	12.87	13.77
anthracene	3.43	4.39	2.70	0.77	0.00	0.00	1.03	6.39	0.47	1.14
1-methylphenanthrene	2.62	2.65	0.00	0.33	0.72	0.39	0.00	0.00	0.24	0.39
C1-phenanthrenes/anthracenes	41.77	17.92	0.00	1.94	4.62	1.50	0.00	0.00	3.79	3.81
C2-phenanthrenes/anthracenes	22.74	8.50	0.00	6.84	12.49	1.14	9.54	0.00	2.55	3.38
C3-phenanthrenes/anthracenes	6.22	4.64	0.00	2.58	2.45	1.91	0.00	0.00	1.69	1.21
C4-phenanthrenes/anthracenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fluoranthene	55.83	24.86	7.25	12.46	20.55	12.41	4.20	8.90	6.64	11.11
pyrene	81.08	35.02	4.99	10.17	15.41	6.19	6.77	10.22	16.14	18.48
C1-fluoranthenes/pyrenes	3.37	2.54	0.96	1.33	1.26	1.93	0.76	0.83	0.73	0.90
retene	2.88	3.59	3.62	2.01	1.16	0.31	0.97	2.20	1.04	0.39
benz[a]anthracene	1.17	0.68	0.58	0.56	0.49	0.00	0.25	0.11	1.61	0.68
chrysene	1.11	0.84	0.63	0.64	0.52	0.10	0.34	0.43	0.75	0.77
C1-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[b]fluoranthene	8.76	3.06	0.00	0.00	1.91	0.00	1.21	1.46	0.00	3.12
benzo[k]fluoranthene	2.20	2.49	0.00	0.00	0.41	0.00	0.25	0.71	0.00	0.67
benzo[e]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[a]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	22.67	0.00	0.00	30.48	0.00	0.00	9.14	12.71	7.82	0.00
indeno[1,2,3-c,d]pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenz[a,h]anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo[g,h,i]perylene	9.32	6.47	1.85	0.00	2.24	0.00	1.92	2.16	2.09	3.04
coronene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sum of PAH	424	200	73	156	160	46	56	65	91	92
Sum of 16 PP PAH	262	110	36	89	94	27	31	54	49	59
Petrogenic PAH	154	88	25	41	61	14	20	12	24	27
Pyrogenic PAH	203	93	27	48	71	23	20	36	34	50
Values listed as 0.00 are below detection limit (~0.1 ng/g)										

Table A-9. PAHs in Downstream Sediment (ng/g organic carbon).

Bridge Number	5	173	200	53	54	57	36	38	80	151
dry wt (%)	71.23	64.50	59.38	55.08	60.79	56.30	60.92	59.94	60.87	47.86
TOC (%)	3.24	3.03	1.13	2.97	2.95	2.71	2.55	2.70	1.99	2.52
naphthalene	227	104	142	180	64	181	165	44	152	165
2-methylnaphthalene	216	64	230	342	134	173	187	41	97	208
1-methylnaphthalene	199	118	61	94	45	71	126	34	44	114
biphenyl	238	108	189	133	141	102	30978	95	157	285
2,6-dimethylnaphthalene	157	0	100	160	71	127	79	0	0	87
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	212	0	193	198	0	209	188	0	0	245
dibenzofuran	205	0	143	262	224	162	172	0	0	137
2,3,5-trimethylnaphthalene	0	0	70	0	0	0	0	0	0	0
C1-naphthalenes	383	116	135	283	145	205	480	110	147	255
C2-naphthalenes	469	278	319	561	318	270	285	289	246	454
C3-naphthalenes	437	512	747	180	333	342	256	663	329	0
C4-naphthalenes	136	126	648	81	144	61	104	170	103	48
fluorene	93	47	119	60	103	48	45	44	40	44
1-methylfluorene	56	44	490	135	92	84	76	122	70	111
C1-fluorenes	270	378	-20729	198	202	156	249	434	226	116
C2-fluorenes	0	0	883	122	0	149	0	0	0	0
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	0	0	101	13	22	15	0	72	0	0
C1-dibenzothiophenes	221	0	998	189	0	66	0	654	0	0
C2-dibenzothiophenes	0	0	2044	68	0	0	0	1073	0	0
C3-dibenzothiophenes	0	0	2326	0	0	0	0	743	0	0
phenanthrene	1184	900	6598	742	621	1851	788	759	701	496
anthracene	159	120	444	67	0	60	0	254	160	0
1-methylphenanthrene	76	138	1338	78	0	95	69	309	0	49
C1-phenanthrenes/anthracenes	625	306	4336	736	0	358	261	754	0	301
C2-phenanthrenes/anthracenes	135	272	4928	264	0	513	260	574	0	407
C3-phenanthrenes/anthracenes	87	155	5425	309	0	267	150	786	0	144
C4-phenanthrenes/anthracenes	0	0	371	0	0	0	0	56	0	0
fluoranthene	2779	1141	11564	2862	2208	2341	753	1019	2547	2228
pyrene	2199	2530	2122	1225	1043	4125	1343	1108	3238	1498
C1-fluoranthenes/pyrenes	271	166	1224	66	176	182	57	250	105	65
retene	303	217	5287	198	350	323	134	2011	149	77
benz[a]anthracene	57	36	111	36	358	191	0	40	20	46
chrysene	138	99	169	86	93	198	91	173	141	92
C1-chrysenes	0	0	254	0	336	0	0	414	110	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	202	92	379	113	241	219	0	68	86	0
benzo[k]fluoranthene	124	75	78	69	84	190	0	25	43	0
benzo[e]pyrene	0	0	0	0	0	341	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	0	0	0	1219	2982	984	763	1191	0	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	332	224	961	239	0	254	214	406	233	0
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	12190	8368	34799	11565	10532	14911	38270	14786	9143	7670
Sum of 16 PP PAH	7649	5332	22769	7058	7439	10660	4349	5092	7341	4768
Petrogenic PAH	3946	2911	7571	3680	2155	3736	2705	7509	1809	2246
Pyrogenic PAH	6793	4767	19321	5265	4338	9054	2982	3474	6819	4357

Values listed as 0.00 are below detection limit (~5 ng/g)

Table II-9 (continued). PAHs in Downstream Sediment (ng/g organic carbon).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	60.36	54.55	56.23	59.20	63.93	65.41	63.68	66.37	62.87	61.94
TOC (%)	2.39	3.46	3.09	2.90	2.26	3.30	1.78	3.27	1.47	1.53
naphthalene	445	174	147	178	312	205	211	78	202	1032
2-methylnaphthalene	865	164	241	261	295	298	245	43	221	1365
1-methylnaphthalene	261	51	131	108	164	107	131	25	109	452
biphenyl	461	364	170	126	533	121	266	110	209	937
2,6-dimethylnaphthalene	208	89	118	127	196	168	220	0	70	294
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	1757	0	292	245	425	205	276	0	0	826
dibenzofuran	780	138	276	216	605	158	240	0	310	1867
2,3,5-trimethylnaphthalene	0	0	0	0	0	0	0	0	0	0
C1-naphthalenes	804	175	561	318	741	263	554	124	302	1312
C2-naphthalenes	979	585	373	608	515	453	451	220	864	2065
C3-naphthalenes	543	856	322	208	362	254	483	571	0	1293
C4-naphthalenes	152	123	519	36	87	106	82	64	0	460
fluorene	112	55	59	60	103	44	188	42	48	382
1-methylfluorene	403	141	75	41	100	58	79	97	184	475
C1-fluorenes	641	320	258	104	563	170	431	370	158	916
C2-fluorenes	496	0	0	0	0	0	0	0	0	423
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	26	34	0	0	0	0	27	29	0	46
C1-dibenzothiophenes	160	434	0	0	0	0	343	0	0	0
C2-dibenzothiophenes	0	647	0	0	0	0	0	0	0	0
C3-dibenzothiophenes	0	761	0	0	0	0	0	0	0	0
phenanthrene	2911	1562	697	276	749	721	1463	948	685	6189
anthracene	122	193	88	15	0	0	128	457	28	384
1-methylphenanthrene	406	326	0	15	56	38	0	0	30	346
C1-phenanthrenes/anthracenes	2414	891	0	115	326	402	0	0	251	1542
C2-phenanthrenes/anthracenes	1203	953	0	216	357	226	3414	0	180	5708
C3-phenanthrenes/anthracenes	654	271	0	169	526	208	0	0	159	1263
C4-phenanthrenes/anthracenes	0	0	0	0	0	0	0	0	0	0
fluoranthene	1328	4281	997	707	2006	777	1547	3789	661	4977
pyrene	4168	2931	485	1038	4351	1203	6123	2607	5413	10089
C1-fluoranthenes/pyrenes	396	419	165	127	157	109	274	92	126	681
retene	229	206	970	116	73	61	334	353	178	308
benz[a]anthracene	208	42	35	33	64	0	63	20	132	136
chrysene	208	86	83	70	93	33	107	133	129	800
C1-chrysenes	0	0	0	0	0	0	0	0	0	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	469	195	0	0	81	0	173	109	0	754
benzo[k]fluoranthene	126	46	0	0	40	0	42	35	0	321
benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	1233	0	0	1732	0	0	1807	4929	977	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	628	1143	100	0	156	0	455	110	235	1290
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	25796	18656	7162	7264	14037	6387	20157	15355	11862	48931
Sum of 16 PP PAH	13507	10666	2948	4321	8316	3188	12520	13238	8378	27043
Petrogenic PAH	10481	7479	2752	2275	4424	2798	7190	2063	2632	20217
Pyrogenic PAH	10470	9698	2429	2246	7590	2579	9646	7735	6941	22672
Values listed as 0.00 are below detection limit (~5 ng/g)										

Table II-10. PAHs in Upstream Sediment (ng/g organic carbon).										
Bridge Number	5	173	200	53	54	57	36	38	80	151
dry wt (%)	71.23	64.50	59.38	55.08	60.79	56.30	60.92	59.94	60.87	47.86
TOC (%)	3.24	3.03	1.13	2.97	2.95	2.71	2.55	2.70	1.99	2.52
naphthalene	17	127	92	39	21	48	48	63	62	188
2-methylnaphthalene	50	59	164	63	30	61	26	27	160	88
1-methylnaphthalene	31	28	28	24	7	41	21	24	27	166
biphenyl	90	79	63	47	65	51	58	94	163	155
2,6-dimethylnaphthalene	40	0	70	45	16	41	63	0	0	110
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	217	0	300	250	0	201	111	0	0	451
dibenzofuran	460	0	636	533	161	585	817	0	0	3596
2,3,5-trimethylnaphthalene	0	0	62	0	0	0	0	0	0	0
C1-naphthalenes	127	111	122	123	20	302	99	72	143	345
C2-naphthalenes	134	199	160	56	47	224	108	125	191	292
C3-naphthalenes	148	291	490	60	59	92	91	432	103	0
C4-naphthalenes	14	72	401	38	25	47	13	153	59	24
fluorene	60	56	83	19	11	42	12	82	55	80
1-methylfluorene	17	82	281	56	22	161	33	108	43	115
C1-fluorenes	73	171	564	39	30	131	57	252	47	105
C2-fluorenes	0	0	861	70	0	88	0	0	0	0
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	0	0	162	9	9	12	0	43	0	0
C1-dibenzothiophenes	63	0	1055	37	0	82	0	249	0	0
C2-dibenzothiophenes	0	0	1535	27	0	0	0	591	0	0
C3-dibenzothiophenes	0	0	1398	0	0	0	0	726	0	0
phenanthrene	308	2331	6128	652	441	1083	451	2664	1231	1747
anthracene	84	211	1596	43	0	67	0	363	304	0
1-methylphenanthrene	16	40	831	20	0	41	17	52	0	63
C1-phenanthrenes/anthracenes	136	191	2150	156	0	152	98	493	0	451
C2-phenanthrenes/anthracenes	91	334	3129	187	0	454	132	314	0	355
C3-phenanthrenes/anthracenes	9	121	2565	159	0	175	62	307	0	167
C4-phenanthrenes/anthracenes	0	0	470	0	0	0	0	95	0	0
fluoranthene	453	1223	11094	568	753	3205	281	874	2617	1106
pyrene	403	1529	1425	711	419	1413	468	735	1791	1847
C1-fluoranthenes/pyrenes	38	175	1272	31	49	201	19	192	137	126
retene	47	212	2854	31	84	84	56	424	120	83
benz[a]anthracene	43	64	142	27	87	130	0	57	33	31
chrysene	35	94	140	24	40	150	17	123	76	58
C1-chrysenes	0	0	272	0	58	0	0	218	177	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	144	256	437	88	229	326	0	294	181	0
benzo[k]fluoranthene	45	106	58	29	27	125	0	62	32	0
benzo[e]pyrene	0	0	0	0	0	197	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	0	0	0	684	482	1308	298	536	0	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	121	281	2133	69	0	410	58	192	218	0
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	3513	8444	45222	5011	3191	11731	3510	11036	7971	11747
Sum of 16 PP PAH	1887	6215	23486	3174	2422	8379	1742	5987	6568	5476
Petrogenic PAH	1065	3014	19846	1375	550	2592	963	5740	1590	3005
Pyrogenic PAH	1698	4931	20388	2134	1775	6768	1160	4032	5868	4366
Values listed as 0.00 are below detection limit (~5 ng/g)										

Table II-10 (continued). PAHs in Upstream Sediment (ng/g organic carbon).										
Bridge Number	56	64	25	11	12	242	30	67	33	50
dry wt (%)	60.36	54.55	56.23	59.20	63.93	65.41	63.68	66.37	62.87	61.94
TOC (%)	2.39	3.46	3.09	2.90	2.26	3.30	1.78	3.27	1.47	1.53
naphthalene	376	39	68	86	81	26	15	10	75	53
2-methylnaphthalene	474	34	169	135	74	15	40	10	125	92
1-methylnaphthalene	116	16	25	81	48	8	50	5	82	36
biphenyl	121	81	49	84	69	19	20	36	71	64
2,6-dimethylnaphthalene	107	19	17	67	71	20	25	0	88	32
acenaphthylene	0	0	0	0	0	0	0	0	0	0
acenaphthene	527	0	65	531	413	59	81	0	0	276
dibenzofuran	746	151	309	809	837	155	206	0	1173	617
2,3,5-trimethylnaphthalene	0	0	0	0	0	0	0	0	0	0
C1-naphthalenes	281	57	97	194	179	27	67	21	277	100
C2-naphthalenes	427	114	233	166	198	36	77	44	132	135
C3-naphthalenes	453	217	65	104	178	13	72	45	0	91
C4-naphthalenes	114	26	21	30	33	12	32	14	0	21
fluorene	79	13	42	36	59	8	15	10	50	93
1-methylfluorene	70	60	25	27	70	16	22	13	61	83
C1-fluorenes	162	154	35	89	171	16	46	38	80	126
C2-fluorenes	149	0	0	0	0	0	0	0	0	59
C3-fluorenes	0	0	0	0	0	0	0	0	0	0
dibenzothiophene	16	15	0	0	0	0	6	4	0	4
C1-dibenzothiophenes	145	88	0	0	0	0	87	0	0	0
C2-dibenzothiophenes	0	173	0	0	0	0	0	0	0	0
C3-dibenzothiophenes	0	216	0	0	0	0	0	0	0	0
phenanthrene	2264	898	421	538	1775	167	236	320	875	901
anthracene	143	127	88	26	0	0	58	195	32	75
1-methylphenanthrene	110	77	0	11	32	12	0	0	16	25
C1-phenanthrenes/anthracenes	1748	518	0	67	205	45	0	0	257	249
C2-phenanthrenes/anthracenes	952	246	0	236	553	34	535	0	173	221
C3-phenanthrenes/anthracenes	260	134	0	89	108	58	0	0	115	79
C4-phenanthrenes/anthracenes	0	0	0	0	0	0	0	0	0	0
fluoranthene	2336	719	235	429	909	376	235	272	452	727
pyrene	3393	1013	162	351	682	187	380	313	1097	1210
C1-fluoranthenes/pyrenes	141	73	31	46	56	58	43	25	50	59
retene	121	104	117	69	51	9	54	67	71	26
benz[a]anthracene	49	20	19	19	22	0	14	3	109	44
chrysene	47	24	20	22	23	3	19	13	51	51
C1-chrysenes	0	0	0	0	0	0	0	0	0	0
C2-chrysenes	0	0	0	0	0	0	0	0	0	0
C3-chrysenes	0	0	0	0	0	0	0	0	0	0
C4-chrysenes	0	0	0	0	0	0	0	0	0	0
benzo[b]fluoranthene	366	88	0	0	85	0	68	45	0	205
benzo[k]fluoranthene	92	72	0	0	18	0	14	22	0	44
benzo[e]pyrene	0	0	0	0	0	0	0	0	0	0
benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0
perylene	949	0	0	1050	0	0	513	389	532	0
indeno[1,2,3-c,d]pyrene	0	0	0	0	0	0	0	0	0	0
dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0
benzo[g,h,i]perylene	390	187	60	0	99	0	108	66	142	199
coronene	0	0	0	0	0	0	0	0	0	0
Sum of PAH	17721	5772	2372	5393	7097	1380	3138	1983	6186	5996
Sum of 16 PP PAH	10961	3181	1160	3070	4143	826	1742	1655	3305	3832
Petrogenic PAH	6433	2532	802	1411	2707	418	1113	373	1646	1741
Pyrogenic PAH	8475	2700	859	1648	3138	709	1095	1090	2320	3281
Values listed as 0.00 are below detection limit (~5 ng/g)										

Table II-11. PAHs in Downstream PSDs (ng/g).

Bridge Number	5	173	200	53	54	57	36	38	80	151
naphthalene	8.3	14.1	167.6	13.6	18.4	21.5	7.9	21.1	2.4	8.5
2-methylnaphthalene	0.9	5.5	77.9	4.3	2.8	8.2	5.8	3.3	1.2	3.0
1-methylnaphthalene	2.7	3.8	27.5	6.0	2.4	5.9	1.0	4.1	1.4	2.3
biphenyl	3.0	3.3	45.1	3.6	4.5	3.7	4.0	10.3	0.8	3.9
2,6-dimethylnaphthalene	5.3	10.3	130.0	9.5	8.7	15.1	8.7	22.3	2.7	9.0
acenaphthylene	0.6	0.7	15.8	2.3	0.6	1.7	1.1	2.0	0.4	0.5
acenaphthene	5.5	7.0	114.9	17.3	7.1	16.8	9.8	40.3	2.3	7.1
dibenzofuran	4.1	2.2	69.6	5.0	4.4	5.3	2.5	10.0	1.1	3.6
2,3,5-trimethylnaphthalene	2.3	13.8	115.4	21.7	11.8	14.9	8.5	35.3	5.9	19.9
C1-naphthalenes	6.7	11.0	124.4	9.9	9.5	19.1	4.3	13.6	2.9	8.6
C2-naphthalenes	9.2	15.0	302.7	43.0	27.5	27.9	17.7	59.0	8.2	12.0
C3-naphthalenes	56.9	29.1	675.0	35.6	31.2	89.8	27.3	104.2	21.2	43.6
C4-naphthalenes	58.3	91.2	982.3	55.3	64.6	84.7	83.4	195.2	37.3	115.9
fluorene	7.8	5.6	150.7	8.9	13.1	15.6	4.5	26.6	5.1	7.6
1-methylfluorene	6.3	7.7	127.4	13.5	4.9	1.7	8.7	17.3	5.0	5.8
C1-fluorenes	17.4	13.7	263.0	27.8	12.3	27.6	7.9	64.0	5.7	23.2
C2-fluorenes	52.9	89.9	1266.7	64.6	72.1	166.4	75.7	319.8	35.5	71.2
C3-fluorenes	93.8	89.7	1908.9	448.4	98.0	223.9	157.8	581.8	16.3	105.5
dibenzothiophene	5.7	8.5	94.9	14.8	8.7	22.8	7.4	21.7	2.7	0.4
C1-dibenzothiophenes	26.6	44.3	454.3	46.4	24.5	43.0	43.7	101.0	12.1	11.6
C2-dibenzothiophenes	28.0	32.5	69.9	79.6	43.1	144.7	36.4	170.8	11.2	20.7
C3-dibenzothiophenes	26.6	46.9	566.0	45.3	38.4	80.7	27.8	113.0	19.3	46.9
phenanthrene	70.6	25.2	662.1	149.7	59.7	34.8	76.7	177.0	31.4	35.2
anthracene	6.7	6.2	176.3	19.3	10.1	15.5	15.9	26.0	3.9	11.6
1-methylphenanthrene	17.2	25.9	151.4	29.6	6.2	47.1	21.7	78.2	9.0	13.2
C1-phenanthrenes/anthracenes	96.3	52.7	2561.7	181.8	97.7	225.3	107.7	408.5	57.9	179.2
C2-phenanthrenes/anthracenes	109.0	111.8	1413.8	124.6	64.2	65.4	24.5	355.9	33.5	104.5
C3-phenanthrenes/anthracenes	62.8	93.4	758.8	94.7	52.3	50.0	69.5	140.0	23.6	48.3
C4-phenanthrenes/anthracenes	4.7	12.1	108.3	17.8	4.7	18.3	6.4	24.9	1.6	1.5
fluoranthene	191.4	163.3	1503.7	230.2	95.9	268.4	119.5	429.7	52.9	173.7
pyrene	59.1	136.3	1332.9	180.8	68.3	91.3	67.1	294.6	21.4	86.2
C1-fluoranthenes/pyrenes	47.1	52.3	1101.7	129.0	42.4	127.8	85.3	343.7	14.6	67.8
retene	42.5	30.9	291.5	48.5	46.2	48.4	15.7	84.1	11.6	24.3
benz[a]anthracene	14.2	16.7	305.6	50.4	18.9	48.9	7.3	71.4	7.3	31.8
chrysene	92.9	88.5	400.2	109.3	156.3	319.8	114.4	269.1	41.8	138.5
C1-chrysenes	9.7	22.3	305.2	43.9	22.8	38.0	11.5	82.2	9.4	15.0
C2-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
benzo[b]fluoranthene	27.8	34.0	303.7	51.2	57.3	143.0	29.4	53.2	12.4	33.3
benzo[k]fluoranthene	21.6	22.6	324.5	17.3	26.6	19.8	17.3	43.5	7.2	15.2
benzo[e]pyrene	17.3	41.2	724.0	26.8	72.4	113.9	43.6	142.9	12.1	60.6
benzo[a]pyrene	7.0	8.5	180.1	12.0	9.7	21.7	7.8	38.3	4.8	1.6
perylene	14.8	32.7	494.7	32.3	32.9	37.7	12.2	53.2	8.0	17.0
indeno[1,2,3-c,d]pyrene	7.5	4.0	194.2	6.8	9.6	25.7	5.4	13.6	4.4	9.5
dibenz[a,h]anthracene	3.0	1.3	31.1	3.7	2.5	3.9	2.0	12.3	1.1	2.5
benzo[g,h,i]perylene	0.0	12.6	182.9	26.2	16.2	21.2	14.8	32.0	3.0	12.6
coronene	1.4	1.3	25.4	4.3	2.6	4.5	2.8	11.8	1.1	2.0
Sum of PAH	1353	1542	21284	2567	1485	2831	1431	5123	574	1616
Sum of 16 PP PAH	524	563	6235	881	584	1058	506	1532	202	561
Petrogenic PAH	763	849	13607	1560	775	1510	845	3236	336	910
Pyrogenic PAH	491	557	6146	833	584	1133	497	1569	192	604

Values listed as 0.0 are below detection limit (~1 ng/g)

Table II-11 (continued). PAHs in Downstream PSDs (ng/g).

Bridge Number	56	64	25	11	12	242	30	67	33	50
naphthalene	14.1	27.2	19.6	28.2	12.3	14.2	9.6	20.9	6.9	47.1
2-methylnaphthalene	7.7	6.5	5.8	4.5	5.0	4.8	7.4	3.8	4.7	28.0
1-methylnaphthalene	6.4	11.6	6.7	6.5	4.6	6.9	7.2	4.0	2.9	21.4
biphenyl	5.6	11.3	4.6	5.9	4.6	2.4	6.0	5.8	3.1	26.8
2,6-dimethylnaphthalene	21.4	22.9	15.0	17.8	5.6	5.7	5.3	9.7	8.4	63.9
acenaphthylene	0.5	2.2	1.4	1.0	1.3	0.9	0.9	2.0	0.7	2.3
acenaphthene	14.5	17.8	10.8	13.0	5.4	7.2	18.1	13.3	9.9	23.0
dibenzofuran	10.2	12.2	3.4	9.3	4.1	7.0	6.0	8.0	4.2	21.4
2,3,5-trimethylnaphthalene	27.6	38.8	18.6	23.7	10.3	27.2	17.8	20.8	13.2	90.4
C1-naphthalenes	22.6	18.8	14.0	11.4	7.8	7.2	13.6	11.0	6.4	31.6
C2-naphthalenes	38.0	80.3	33.0	41.9	24.9	19.1	21.4	44.3	20.2	128.1
C3-naphthalenes	101.8	188.2	63.7	103.6	79.6	62.9	38.5	76.2	39.4	414.9
C4-naphthalenes	183.4	237.7	119.7	69.1	160.4	40.1	145.7	135.0	41.0	203.6
fluorene	14.6	32.1	10.6	18.1	15.5	11.8	13.5	13.3	6.8	49.8
1-methylfluorene	30.0	25.5	16.1	23.2	12.2	9.9	7.4	11.7	6.2	33.1
C1-fluorenes	32.9	44.3	50.1	53.9	38.6	38.0	18.9	26.9	26.1	130.8
C2-fluorenes	141.0	294.2	178.0	183.6	174.5	167.8	197.1	333.2	170.0	502.1
C3-fluorenes	209.1	214.9	359.4	349.8	181.1	141.1	144.7	254.0	92.3	562.9
dibenzothiophene	14.0	32.7	8.6	13.3	1.0	8.7	10.5	16.5	4.8	36.5
C1-dibenzothiophenes	56.7	74.1	16.8	38.5	56.9	22.6	45.1	46.1	29.6	184.1
C2-dibenzothiophenes	62.3	92.6	49.1	64.0	86.1	84.4	52.4	120.2	31.0	97.4
C3-dibenzothiophenes	69.3	84.5	36.6	98.7	76.7	44.0	47.9	72.0	45.3	142.0
phenanthrene	140.7	221.8	75.8	55.3	124.4	40.7	78.5	117.5	67.1	423.2
anthracene	19.3	27.4	14.8	15.3	13.3	20.4	11.3	30.6	9.5	26.4
1-methylphenanthrene	43.8	64.2	35.3	24.2	16.8	28.1	35.1	32.4	12.1	156.6
C1-phenanthrenes/anthracenes	156.3	313.8	246.1	305.1	126.8	43.0	195.4	115.7	154.4	775.0
C2-phenanthrenes/anthracenes	47.3	294.3	114.5	97.9	132.1	132.1	114.0	259.1	67.5	821.4
C3-phenanthrenes/anthracenes	146.4	240.6	130.5	102.6	60.6	63.4	90.7	54.6	37.0	152.9
C4-phenanthrenes/anthracenes	20.4	14.3	8.0	13.9	12.1	4.5	16.0	13.9	9.2	28.6
fluoranthene	176.6	426.6	159.8	240.0	328.5	190.4	169.7	124.1	186.6	945.6
pyrene	360.5	615.5	126.9	176.0	273.3	77.4	200.4	206.8	137.6	495.7
C1-fluoranthenes/pyrenes	156.0	106.1	77.9	136.7	80.5	45.7	103.3	76.2	47.7	290.8
retene	53.0	55.4	33.7	40.5	49.4	36.6	43.6	103.3	16.3	133.3
benz[a]anthracene	47.7	82.6	27.4	28.0	42.2	34.1	25.2	34.7	11.2	82.7
chrysene	243.9	312.8	191.6	296.5	273.7	153.8	145.1	224.4	91.2	693.6
C1-chrysenes	41.2	78.0	23.6	22.0	33.9	32.9	29.3	40.2	22.5	108.5
C2-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
benzo[b]fluoranthene	29.5	90.5	77.0	58.9	88.1	40.0	49.6	36.3	52.6	189.3
benzo[k]fluoranthene	54.6	43.1	17.1	59.9	33.7	21.1	17.5	36.9	21.5	74.4
benzo[e]pyrene	84.4	90.0	45.9	122.4	71.3	32.9	90.6	72.3	37.2	154.1
benzo[a]pyrene	20.1	30.0	9.0	23.5	13.8	7.7	9.2	26.0	10.3	36.0
perylene	69.4	87.0	37.7	70.8	29.9	20.7	49.3	42.9	27.3	70.8
indeno[1,2,3-c,d]pyrene	11.4	15.7	11.4	9.6	11.1	14.6	9.6	12.2	8.4	89.0
dibenz[a,h]anthracene	5.8	6.2	2.9	2.6	7.8	2.9	3.8	5.0	1.4	12.6
benzo[g,h,i]perylene	23.8	26.9	21.9	34.2	19.8	11.5	34.5	27.2	13.2	70.4
coronene	5.7	7.3	3.8	5.3	1.2	2.2	2.8	3.6	1.9	7.9
Sum of PAH	3042	4820	2534	3120	2813	1790	2359	2945	1617	8680
Sum of 16 PP PAH	1199	1983	788	1103	1252	635	821	939	651	3249
Petrogenic PAH	1598	2580	1598	1780	1424	1004	1347	1788	892	4920
Pyrogenic PAH	1169	1905	760	1114	1247	637	827	914	627	3114

Values listed as 0.0 are below detection limit (~1 ng/g)

Table II-12. PAHs in Upstream PSDs (ng/g).

Bridge Number	5	17	200	53	54	57	36	38	80	151
naphthalene	12.0	9.3	65.8	17.3	5.4	18.5	1.8	63.5	3.3	18.5
2-methylnaphthalene	3.5	4.2	24.6	7.2	5.0	4.8	0.6	31.8	1.5	7.2
1-methylnaphthalene	3.8	2.4	18.3	8.5	1.4	4.4	0.5	16.3	1.5	2.9
biphenyl	3.8	3.0	25.2	6.4	2.4	4.4	0.8	14.0	1.1	7.6
2,6-dimethylnaphthalene	11.1	10.0	76.6	13.7	2.6	8.8	0.9	34.1	4.5	8.5
acenaphthylene	0.6	1.0	7.9	1.6	0.5	0.6	0.1	3.6	0.4	1.0
acenaphthene	2.9	8.1	65.4	19.1	4.1	9.7	1.5	22.9	3.4	11.5
dibenzofuran	2.2	3.7	10.2	4.8	2.9	5.8	0.9	8.9	1.2	4.1
2,3,5-trimethylnaphthalene	12.6	17.3	69.3	16.6	6.1	15.2	2.2	66.8	4.6	22.4
C1-naphthalenes	4.0	8.2	50.8	14.9	2.0	6.3	1.1	42.4	3.7	17.8
C2-naphthalenes	20.1	18.1	97.9	26.3	14.9	19.3	7.2	84.3	15.6	26.1
C3-naphthalenes	43.9	33.9	239.3	57.7	42.7	44.5	8.1	234.8	11.1	91.7
C4-naphthalenes	50.3	51.2	557.0	94.0	62.2	76.7	14.6	313.1	24.2	99.9
fluorene	7.1	13.8	49.6	16.8	5.6	16.0	1.3	27.0	2.4	18.1
1-methylfluorene	1.6	7.9	27.7	15.2	5.5	8.2	1.5	42.3	3.7	8.7
C1-fluorenes	21.5	16.7	145.7	40.6	13.8	26.1	3.5	107.0	13.2	43.1
C2-fluorenes	78.6	141.4	492.4	113.8	63.4	189.3	11.5	426.9	37.9	241.9
C3-fluorenes	109.9	90.0	1034.1	212.8	111.2	179.9	22.9	572.9	62.5	154.4
dibenzothiophene	8.7	7.8	53.8	9.5	3.8	13.6	2.1	35.0	3.0	16.8
C1-dibenzothiophenes	16.2	31.3	190.0	39.2	18.2	41.1	5.5	81.9	7.6	63.8
C2-dibenzothiophenes	28.8	26.5	220.3	65.8	32.6	45.4	5.4	147.4	17.7	67.3
C3-dibenzothiophenes	29.2	52.6	279.4	41.8	30.0	32.0	6.1	192.8	13.9	67.1
phenanthrene	66.2	46.8	372.6	74.5	46.2	65.2	11.7	263.5	33.5	132.5
anthracene	7.5	8.6	84.0	22.4	12.4	10.0	1.8	48.6	1.8	19.2
1-methylphenanthrene	16.9	18.3	65.9	33.3	12.7	25.6	4.5	59.3	3.4	34.7
C1-phenanthrenes/anthracenes	95.5	82.6	979.3	238.5	52.6	132.6	19.8	655.2	50.7	216.8
C2-phenanthrenes/anthracenes	96.6	91.4	527.2	246.5	52.2	104.3	16.1	434.3	35.2	281.1
C3-phenanthrenes/anthracenes	30.1	63.9	207.7	121.5	31.6	116.5	12.0	315.5	11.1	114.6
C4-phenanthrenes/anthracenes	8.0	6.0	69.4	13.7	5.0	15.0	1.0	28.7	2.4	10.5
fluoranthene	60.8	143.2	751.5	252.1	78.8	96.8	28.2	446.3	46.5	172.4
pyrene	61.1	66.9	313.2	278.0	62.8	246.1	26.6	467.0	26.8	239.7
C1-fluoranthenes/pyrenes	89.8	74.4	259.2	148.4	51.2	81.8	9.8	448.1	26.2	135.2
retene	20.5	32.4	213.2	52.5	22.7	44.2	4.1	172.0	9.4	37.2
benz[a]anthracene	20.0	20.3	143.1	44.8	15.6	38.4	4.4	122.2	6.2	32.5
chrysene	109.5	116.6	786.8	135.6	118.9	204.7	24.9	477.9	38.3	301.8
C1-chrysenes	21.9	15.0	182.9	22.8	20.3	17.4	3.5	91.3	7.4	27.2
C2-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
benzo[b]fluoranthene	25.4	38.8	203.0	45.0	30.1	61.0	10.6	205.3	10.1	83.1
benzo[k]fluoranthene	12.5	4.1	140.4	27.2	12.4	8.2	4.8	31.0	3.7	15.4
benzo[e]pyrene	37.0	51.2	302.7	105.6	40.1	83.4	2.3	193.1	18.3	79.2
benzo[a]pyrene	4.7	10.6	44.5	15.4	7.6	24.6	0.6	43.8	4.2	18.2
perylene	16.9	23.9	121.7	38.8	19.6	38.6	3.5	71.9	7.8	29.5
indeno[1,2,3-c,d]pyrene	7.6	12.2	61.3	8.7	7.7	9.5	1.1	29.0	3.2	17.3
dibenz[a,h]anthracene	2.0	1.4	8.4	4.9	2.0	2.6	0.3	6.0	0.9	2.3
benzo[g,h,i]perylene	7.4	5.6	43.3	29.4	12.1	10.2	2.6	32.5	4.0	24.0
coronene	1.3	1.2	10.3	4.5	1.5	3.3	0.5	6.2	1.2	4.2
Sum of PAH	1291	1494	9693	2807	1153	2211	295	7218	590	3029
Sum of 16 PP PAH	404	511	3120	987	426	822	121	2240	190	1105
Petrogenic PAH	805	858	5888	1579	642	1209	159	4434	366	1778
Pyrogenic PAH	393	513	3152	1031	430	842	116	2267	186	1088

Values listed as 0.0 are below detection limit (~1 ng/g)

Table II-12 (continued). PAHs in Upstream PSDs (ng/g).

Bridge Number	56	64	25	11	12	242	30	67	33	50
naphthalene	27.0	18.1	3.6	9.4	13.8	4.0	4.8	2.8	4.5	18.4
2-methylnaphthalene	22.3	4.4	2.7	2.5	4.8	2.1	1.1	1.6	2.1	9.4
1-methylnaphthalene	14.9	4.6	2.8	2.5	1.4	1.0	1.2	0.9	2.1	7.5
biphenyl	12.2	2.4	2.2	2.5	3.0	1.2	1.6	0.7	2.5	6.1
2,6-dimethylnaphthalene	23.6	9.2	4.7	7.4	12.0	4.3	5.5	3.2	4.7	17.2
acenaphthylene	3.2	1.3	0.9	0.3	1.0	0.3	0.7	0.2	0.5	2.3
acenaphthene	29.1	17.6	6.2	6.1	12.9	2.1	7.1	2.9	3.1	16.6
dibenzofuran	13.9	3.7	3.0	1.9	3.2	1.0	1.9	0.9	2.0	5.8
2,3,5-trimethylnaphthalene	50.2	25.3	11.0	3.8	12.1	3.8	12.3	3.7	8.0	41.8
C1-naphthalenes	42.5	7.6	4.0	4.2	7.9	1.9	4.1	2.0	6.5	10.6
C2-naphthalenes	118.4	17.7	22.5	15.8	13.1	4.2	8.4	9.0	17.8	23.6
C3-naphthalenes	158.1	57.2	29.9	30.2	58.4	9.7	25.3	12.5	39.5	52.4
C4-naphthalenes	279.2	163.3	58.7	56.5	47.9	19.2	44.9	14.6	51.0	149.5
fluorene	14.4	13.5	4.3	7.1	13.5	1.8	4.0	3.2	4.0	12.0
1-methylfluorene	21.9	16.0	6.9	4.2	15.9	2.7	3.1	2.5	3.8	7.5
C1-fluorenes	77.5	16.5	9.1	12.1	23.8	5.2	8.9	5.1	13.5	23.5
C2-fluorenes	297.0	122.3	42.4	61.4	85.7	25.4	56.3	43.5	56.6	269.5
C3-fluorenes	269.6	127.1	103.1	78.7	160.4	48.5	106.9	31.4	70.8	203.6
dibenzothiophene	43.1	4.1	5.0	8.5	12.6	3.6	6.3	1.5	6.0	15.6
C1-dibenzothiophenes	125.5	64.8	13.6	11.8	32.0	7.5	21.5	10.2	16.6	52.9
C2-dibenzothiophenes	293.1	56.1	41.6	21.9	40.9	12.1	24.3	10.1	18.2	47.3
C3-dibenzothiophenes	96.4	37.3	16.6	17.1	26.9	8.7	24.0	13.8	22.5	58.5
phenanthrene	199.1	91.2	42.1	33.7	74.0	20.0	35.3	12.4	29.7	71.8
anthracene	29.4	8.4	9.5	12.5	10.3	2.7	4.5	3.1	7.9	13.6
1-methylphenanthrene	97.5	22.4	15.0	10.0	17.0	5.3	10.0	4.5	13.8	15.3
C1-phenanthrenes/anthracenes	417.4	252.4	84.7	97.7	148.5	37.2	74.8	54.2	86.0	186.8
C2-phenanthrenes/anthracenes	430.9	102.5	67.4	75.4	72.5	13.4	103.1	24.6	75.3	116.8
C3-phenanthrenes/anthracenes	203.9	49.9	49.9	42.6	70.5	25.7	27.1	17.7	36.0	75.7
C4-phenanthrenes/anthracenes	45.1	10.6	2.1	3.5	7.4	2.4	5.7	2.9	5.5	18.4
fluoranthene	621.4	171.0	39.8	80.1	114.5	58.6	52.3	39.2	72.7	121.5
pyrene	560.8	143.4	75.9	81.3	110.2	34.0	65.0	19.3	95.7	311.2
C1-fluoranthenes/pyrenes	221.6	153.7	40.5	33.0	75.0	24.0	37.7	16.5	35.7	136.2
retene	115.0	43.5	22.2	23.4	27.3	8.8	17.3	8.3	18.2	82.2
benz[a]anthracene	115.2	37.9	21.1	11.9	44.5	5.5	15.2	6.4	16.8	39.7
chrysene	279.5	168.9	84.1	135.9	157.5	59.1	62.7	46.1	100.3	142.0
C1-chrysenes	60.2	20.8	15.1	17.3	19.6	3.2	10.5	4.7	15.1	34.8
C2-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4-chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
benzo[b]fluoranthene	102.4	39.4	12.4	51.9	69.6	14.0	11.4	5.5	42.2	74.6
benzo[k]fluoranthene	73.2	16.5	12.7	19.2	21.2	4.5	13.3	7.7	15.2	33.2
benzo[e]pyrene	182.5	55.1	38.5	33.1	100.8	14.7	32.9	8.9	15.5	88.8
benzo[a]pyrene	30.5	12.6	6.2	7.5	11.1	2.4	9.4	2.0	5.6	11.3
perylene	93.6	44.6	24.0	11.8	13.1	10.8	12.7	7.8	16.0	39.0
indeno[1,2,3-c,d]pyrene	25.5	13.8	8.3	8.5	11.4	3.0	3.0	3.4	7.2	22.0
dibenz[a,h]anthracene	9.2	4.7	1.3	1.7	2.3	0.7	1.3	1.0	1.9	1.6
benzo[g,h,i]perylene	34.5	10.7	8.9	16.5	10.9	4.3	5.6	2.8	10.3	17.9
coronene	5.8	2.7	1.6	2.1	3.1	0.7	1.2	0.7	0.8	2.4
Sum of PAH	5987	2267	1078	1176	1796	525	986	476	1080	2708
Sum of 16 PP PAH	2133	776	340	484	647	222	293	160	417	909
Petrogenic PAH	3320	1341	635	621	967	268	616	286	596	1542
Pyrogenic PAH	2202	750	348	485	718	217	303	156	411	934

Values listed as 0.0 are below detection limit (~1 ng/g)