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FRESHWATER BIVALVES

Polycyclic aromatic hydrocarbons in surface waters, sediments, and unionid mussels: relation to road crossings and implications for chronic mussel exposure

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Abstract Transportation infrastructure is a prominent feature across all landscape types, and road crossings over streams are a source of pollutant influx, especially polycyclic aromatic hydrocarbons (PAH) associated with vehicle oils and fuel combustion. Freshwater mussels (Unionoida) are vulnerable to pollutants entering streams because of their sessile benthic lifestyle and their filter- and deposit-feeding exposure routes. We assessed the effect of road crossings on PAH concentrations in mussels, sediment, and water via passive sampling devices (PSDs) at 20 sites in the upper Neuse River basin of North Carolina, and investigated the utility of PSDs for estimating PAH concentrations in mussels. Road crossings significantly increased Total PAH in downstream reaches compared to upstream for all compartments sampled ($P < 0.01$). Total PAH in mussels were correlated with those in PSDs both upstream and downstream of road crossings ($P \leq 0.01$), and PSDs provided a reliable prediction equation for mussel tissue concentrations of Total PAH ($r^2 = 0.90$). Overall, PAH contributions from crossings to streams in a single, relatively rural watershed are substantial and statistically significant, suggesting that the cumulative PAH influx within an entire river basin may be considerable and exert adverse effects over a chronic lifetime exposure for mussels, especially as their contributions may amplify downstream.

Keywords Environmental monitoring · Ecosystem function · Passive sampling device (PSD) · Stormwater runoff · Unionidae

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Introduction

Freshwater mussels (Unionoida) are vulnerable to pollutants entering the aquatic environment because of their sessile benthic lifestyle and their filter- and deposit-feeding exposure routes (Cope et al., 2008). More than 71% of North America's species are endangered, threatened, or of special concern (Williams et al., 1993), and in the southeastern USA where we conducted our study, unionid mussel biodiversity and endemism are highest compared to any other

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region in the world (Haag, 2012). Modern causes implicated in freshwater mussel declines include pollution and water quality degradation, habitat alteration, or a combination of large-scale, chronic stressors (Lydeard et al., 2004; Strayer et al., 2004; Downing et al., 2010). Furthermore, research within the past 15 years has revealed that unionids are among the most sensitive organisms to several classes of contaminants entering surface waters [e.g., copper (Wang et al., 2007a, b), ammonia (Newton et al., 2003; Newton & Bartsch, 2007; US EPA, 2013), and pesticides (Conners & Black, 2004; Bringolf et al., 2007; Archambault et al., 2015)], and mussels are exposed differentially through various routes during development from their parasitic larval (upon a host fish) life stage to their benthic juvenile and adult life stages (Cope et al., 2008).

Pollutants enter streams through multiple point and non-point sources, ranging from industrial discharges to surface runoff. Transportation infrastructure is a prominent feature in urban areas, but also serves as perhaps the most notable infrastructure feature in many rural areas. Road crossings over streams are one direct, but non-point source of pollutants, especially polycyclic aromatic hydrocarbons (PAHs) associated with vehicle fuels and oils leaked onto roads and flushed into nearby waterways after precipitation events. Combustion sources such as those from vehicle engines and other aerial emissions (e.g., fossil fuel power plants) also contribute to the daily load of PAHs settling into surface waters.

PAHs are one class of hydrophobic persistent organic pollutants, which are ubiquitous in the aquatic environment—primarily as a result of anthropogenic activities—and are known to cause adverse human and ecological health effects (Neff, 1979, 2002; Baumard et al., 1998; Oikari et al., 2002; Newton & Cope, 2007; Martins et al., 2013; Bihanic et al., 2014). Several groups of marine and freshwater organisms, including unionid mussels, are sensitive to PAH pollution. In fishes, researchers have documented the induction of cytochrome P450 enzymes in Rainbow Trout (*Oncorhynchus mykiss*) (Oikari et al., 2002), and delayed and abnormal egg development, impaired larval swimming behavior, and DNA damage in Japanese Medaka (*Oryzius latipes*) at environmentally relevant PAH concentrations (Bihanic et al., 2014). Martins et al. (2013) reported genotoxic effects in the gills of the marine bivalve, *Ruditapes decussatus*, in a 28-day laboratory exposure to environmental concentrations of PAHs. Studies with freshwater mussels have reported genotoxic responses in mussel hemolymph exposed to PAHs in vitro (Prochazka et al., 2012) and upregulated biomarker enzymes from mussels in waterways primarily contaminated with PAHs and other organic pollutants (e.g., PCBs; Newton & Cope, 2007). The combination of freshwater mussel sensitivity to contaminants with their sedentary lifestyle renders them vulnerable to perennial and abundant pollutant sources such as PAH influx at road crossings.

Despite the overall imperiled status of unionid mussels, common and abundant species have long been used for environmental biomonitoring (Van Hassel & Farris, 2007). Non-destructive monitoring techniques, including surveys of species richness, relative abundance, or growth, offer qualitative or quantitative estimates of population change over time, but do not provide any insight into potential causative agents of change. Because of their sessile benthic lifestyle, decades-long life spans, and large tissue mass in some species, freshwater mussels are an excellent choice for biomonitoring via tissue collection (albeit a lethal sampling method), and elucidating spatially explicit time-integrated contaminant loads (Van Hassel & Farris, 2007). The wide gap between the rapid uptake and slow depuration rate constants for PAHs and other lipophilic compounds by mussels highlights their limited ability to metabolize them. Thus, freshwater mussels tend to bioaccumulate and bioconcentrate PAHs, with extremely high bioconcentration factors (e.g., 1,000–100,000) (Thorsen et al., 2007). However, removing resident fauna from the ecosystem is an increasingly less desirable option because the sampling is labor intensive and many mussel populations are in decline. Passive sampling devices (PSDs) estimate ecologically relevant exposure to contaminants (Heltsley et al., 2005). In some circumstances, they may offer a more cost- and labor-efficient method of monitoring pollutants in surface waters without the need for removing fauna, and they have been successful in monitoring organic contaminant exposure, including PAHs. PSDs have been used to estimate accumulation in marine and freshwater bivalves (Alvarez et al., 2014; Cope & Jones, 2016) and freshwater fishes (Heltsley et al., 2005), and have been studied extensively over nearly two decades for monitoring PAHs and other organic contaminants (Hofelt & Shea, 1997; Luellen & Shea, 2002, 2003).

The aims of this study were to assess the effect of road crossings on PAH concentrations in freshwater mussels, stream sediments, and water (via PSDs) and to investigate the utility of PSDs to serve as surrogates for estimating PAH concentrations in mussels. We hypothesized that elevated PAH concentrations would be measured in all compartments sampled immediately downstream of road crossings, compared to those immediately upstream, and made no a priori hypothesis regarding the potential for PAHs in PSDs or other environmental compartments to accurately estimate mussel tissue PAHs.

Materials and methods

Study area and site selection

The study area is a relatively small, 1,686-km^2 watershed within the upper Neuse River basin, which drains into the upper arms of Falls Lake Reservoir in the north-central Piedmont physiographic region of North Carolina, USA (Fig. 1). The main water courses in the study area are the Eno, Little, and Flat Rivers, though several other small streams drain directly into Falls Lake Reservoir. Locations with known populations of federally endangered species were eliminated to avoid sampling protected organisms or disturbing their habitats. Streams with the highest water quality were identified using the Basinwide Water Quality Plan of the Neuse River basin (NC DENR, 1998). Land use and land cover data for the Neuse River basin were obtained from the United States (US) Environmental Protection Agency's (EPA) Neuse River Land Use/Land Cover GIS layer, and the 30-m resolution grid was derived from several Landsat 7 Enhanced Thematic Mapper Plus scenes, ranging in dates from October 1998 to March 1999 (US EPA, 2000). The dominant land uses within the sub-basin were forested (61%), urban (16%), and agriculture (18%) (ArcGIS Desktop v.10.3, ESRI, Redlands, CA, USA). The study area and subsequent sampling sites for this project were a subset of sites concurrently sampled to measure the effect of road crossing structures on freshwater mussel abundance (Levine et al., 2003). The subset consisted of 20 locations—17 bridged, two culverted, and one piped crossing—with 10 sites from the greatest percentage of forest (range 74–98% forested; <1 to 17% agricultural) and 10 sites from the most agriculturally influenced areas (range 16–58% forested; 33– 74% agricultural) (Fig. 1). Each study site was separated

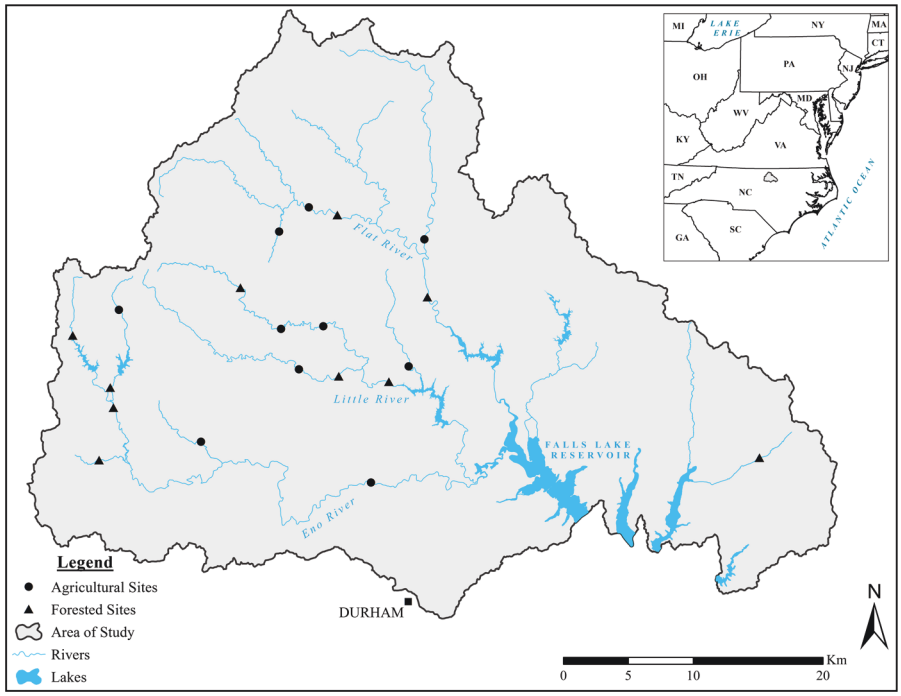

Fig. 1 Study area and the 20 sites in agricultural (*circles*) and forested (*triangles*) landscapes, located in the upper Neuse River Basin, north-central North Carolina, USA

into an upstream reach that included the stream area beginning at the road crossing and continuing 50 m upstream, and a downstream reach that encompassed the area from the crossing to 50 m downstream ($n = 40$ total sample locations).

Sample collection and handling

Eastern elliptio mussels (*Elliptio complanata*) were hand-collected within the 50-m upstream and downstream reaches of each road crossing (May–July 2001). Five mussels were randomly selected among those collected at each sampling reach for chemical analysis, placed in zip-sealable food-grade plastic bags and transported on ice to the laboratory at North Carolina State University (Raleigh, NC, USA). Shortly after arrival to the laboratory (mussels were not held in aquaria or gut cleared), soft tissue was dissected from each shell (mean length 67.9 mm (range 27–100 mm); mean total body wet weight 43.9 g (range 1.7–111.2 g)), and material from the five mussels was composited, freeze dried, and homogenized before aliquots were portioned for extraction and analysis of PAHs.

Surficial (top 2–3 cm) sediment samples were collected with a stainless steel scoop (July 2001); a composite sample of equal portions was obtained by sampling five randomly selected locations at each upstream and downstream reach at each of the study sites. An effort was made to collect only fine-grained sediment inhabited by the mussels. Sediment was transported on ice to the laboratory, frozen at −20°C, and then freeze dried and homogenized before aliquots were portioned for extraction and analysis of PAHs.

Construction and deployment of PSDs

The PSDs were constructed of 12.7-μm-thick low-density polyethylene (LDPE) sheeting containing no plasticizers or additives (ExxonMobil, Baton Rouge, LA, USA), as described by Luellen & Shea (2002). The LDPE sheeting (5 cm × 30 cm, surface area of 300 cm^2) was pre-extracted with hexane for 48 h prior to use and fixed inside a protective polyethylene cage. One cage containing two PSDs was deployed within each of the 50-m zones upstream and downstream of every road crossing in mid-June 2001. They were retrieved approximately 30 days later, allowing time for the PSD to reach equilibrium with aqueous PAHs in the aquatic environment (Thorsen et al., 2004). Following deployment, one PSD per cage was cleaned with de-ionized water and a brush, followed by a brief rinse in acetone to remove material from the surface of the LDPE prior to extraction, while the duplicate was archived at −20°C.

PAH extraction, preparation, and analysis

Mussel, sediment, and PSD samples were extracted and analyzed for 42 PAHs, as described by Thorsen et al. (2004) and Luellen & Shea (2002). Briefly, samples were shaker-extracted (200 rpm) for 24 h using dichloromethane (DCM) for both mussels and PSDs, and 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 column, collecting the lipid fraction, evaporating and weighing. The purified extracts were analyzed for PAHs on two separate chromatographic runs using an Agilent 6890 gas chromatograph connected to an Agilent 5973 N MSD utilizing a Restek 30 m × 0.25 mm Rtx-5 (film thickness 0.25 μm) mass spectrometer with Integra-Guard column. The mass fraction of total organic carbon (TOC) in sediment was determined by Carbon, Hydrogen, Nitrogen analysis with an elemental analyzer according to standard methods (APHA et al., 1995).

Quality assurance protocols were followed for all PAH analyses. Briefly, the accuracy of all determinations was assessed by analyzing procedural blanks and polyethylene blanks (with the PSD samples), replicate samples, spiked samples, and surrogate internal standards. All quality control measures were within acceptable ranges (i.e., all blanks were clean (<1 ng/g of several PAHs observed); recoveries of surrogate internal standards and matrix spike recoveries ranged from 40 to 120%; the difference between matrix spike and spike duplicates and duplicate sample analysis was usually <10% and always <30%). Data were not corrected for surrogate recoveries and the limit of detection was 1 ng/g.

Characterizing Toxicity of PAHs

The potential toxicity of PAHs was evaluated with the US EPA methods for evaluating toxicity in water and sediments, based on a mechanistic approach that incorporates the additivity of PAH toxicity and, in sediments, also the reduction in PAH bioavailability caused by TOC content (US EPA, 2003, 2010). Individual PAH concentrations in water were estimated by converting the concentrations in PSDs with their respective PAH octanol–water partition coefficients (Neff & Burns, 1996; Luellen & Shea, 2002). Raw individual PAH concentrations in each sediment sample were normalized to the TOC fraction. Individual PAH concentrations in water and sediment were then divided by the respective acute or chronic US EPA Potency Divisors (available for 34 PAHs) (US EPA, 2003, 2010) to calculate the water benchmark toxic unit (WBTU) for water and the equilibrium-partitioning sediment benchmark toxic unit (ESBTU) for sediment. The individual PAH WBTUs and ESBTUs were totaled; the resulting sum ($\sum$WBTU and $\sum$ESBTU) is a measure of Total PAH toxic units. Values $\geq$1.0 indicate the potential for toxicity to aquatic life.

Statistical analyses

We evaluated the effect of road crossings on stream PAH concentrations with paired t-tests of Total PAH (sum of the 42 PAHs analyzed) measured upstream and downstream of the crossings in mussel tissue, bed sediments, and PSDs (PROC TTEST, SAS v.9.4, SAS Institute, Cary, NC, USA). We also assessed the effect of roads on the total lipid content (percent of soft tissue body weight) of mussels upstream versus downstream of crossings as a measure of effect on mussel health. Before each paired t-test was performed, a Shapiro–Wilk test was used to test the assumption that paired differences were normally distributed. Mussel lipid paired differences were normally distributed ($P = 0.49$). The assumption was violated for each set of paired differences of PAH concentrations (P values $\leq$0.01), except for sediment dry weight ($P = 0.08$); therefore, all the non-normal datasets were log-transformed to satisfy the normality assumption. Shapiro–Wilk tests of the log-transformed paired differences confirmed that the assumption was satisfied in all cases (P values $\geq$0.42).

Correlation analyses were performed to determine whether the Total PAH concentration in PSDs offered a comparable estimate for mussel tissue and lipid-normalized Total PAHs (PROC CORR, SAS v.9.4). Spearman rank-order correlation coefficients (ρ_s) were used because datasets had extreme values, a condition that renders the parametric Pearson product-moment correlations less appropriate. Additionally, we correlated mussel PAH concentrations with those in sediment to investigate whether sediment PAH concentrations were related to the mussel tissue. Finally, we used correlation to assess the relations between annual average daily traffic count with the environmental compartment changes in PAH concentrations (i.e., the difference in PAHs downstream vs. upstream of road crossings). Annual average daily traffic count ranged from 50 to 16,000 vehicles among the study sites (mean = 1994, median = 955, standard deviation = 3471) (NC DOT, 2014).

The effect of landscape cover (agricultural or forested) at the sites on PAH concentrations was evaluated by an analysis of variance (ANOVA) for each given proximity and compartment (e.g., mussel dry weight upstream of crossings). This comparison was assessed separately for each proximity (i.e., upstream and downstream) within each ecological compartment (i.e., mussel tissues, bed sediments, and surface water (PSDs); PROC GLM, SAS v.9.4) because the aforementioned paired t-tests showed a significant effect of proximity to the crossings (see results). Diagnostic plots of residuals and Levene's test were used to determine whether residuals were independent and normally distributed. If violations were observed, a Welch's test was used (robust to such violations) and/or data were log-transformed and re-analyzed.

Following other analyses, we used simple linear regression (PROC GLM, SAS v.9.4) to predict mussel tissue PAH concentrations from those in PSDs, using upstream and downstream data at road crossings in both land types (i.e., data were pooled based on results of other analyses).

Results

Measured PAHs and toxicity

The mean mussel tissue Total PAH concentrations in upstream reaches were 115 ng/g dry weight (Fig. 2;

range 14–818 ng/g) and 5380 ng/g lipid (range 720–39,718 ng/g); in downstream reaches, mean mussel tissue concentrations were 250 ng/g dry weight (Fig. 2; range 70–1403 ng/g) and 11,407 ng/g lipid (range 4,300–52,774 ng/g) (Supplemental Data, Tables S1 & S2). Mean sediment concentrations were 161 ng/g dry weight (Fig. 2; range 38–488 ng/g) and 7513 ng/g OC (range 1153–43,151 ng/g) upstream, and 352 ng/g dry weight (Fig. 2; range 161–834 ng/g) and 16,298 ng/g OC (range 5561–73,824 ng/g) downstream of road crossings (Supplemental Data, Tables S3 & S4). Mean Total PAH concentrations in PSDs were 2309 ng/g sampler (Fig. 2; range 284–9400 ng/g) in upstream reaches and 3542 ng/g sampler (Fig. 2; range 548–20,585 ng/g) downstream of road crossings (Supplemental Data, Tables S5, S6).

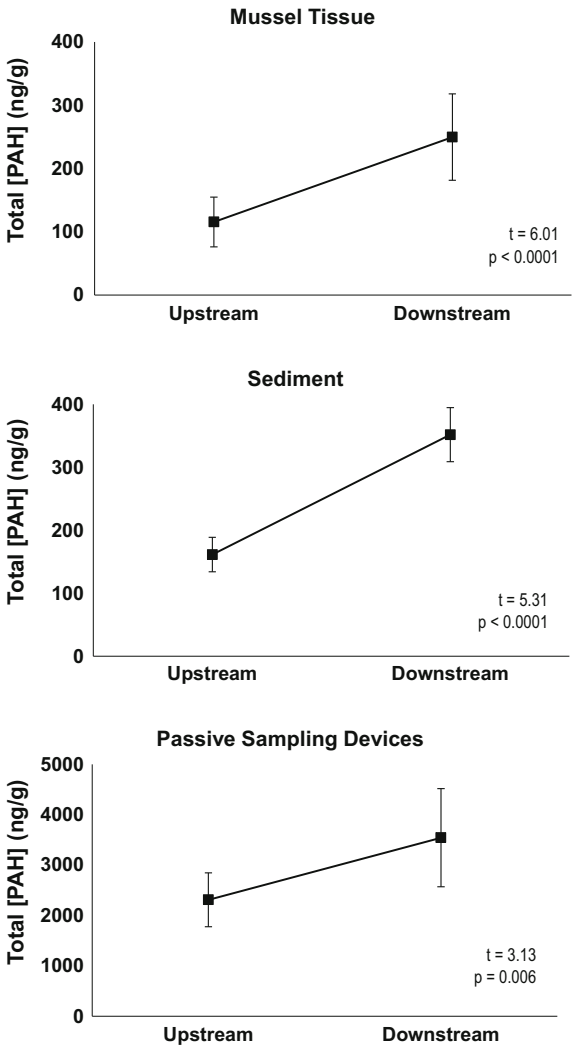

Fig. 2 Change in mean (±SE) PAH concentrations from upstream ($n = 20$) to downstream ($n = 20$) of road crossings in mussel tissue, sediment, and passive sampling devices

The acute $\sum$WBTUs for PAH concentrations in water estimated from the PSDs were all <0.01 upstream and downstream of road crossings, with chronic values ranging 0–0.01 upstream and 0.01–0.02 downstream of road crossings. The mean increase of acute and chronic $\sum$WBTUs for water concentrations downstream of crossings, compared to upstream, was negligible (max = 0.01). The acute $\sum$ESBTUs for sediments ranged 0–0.01 upstream of road crossings and 0–0.02 downstream, with chronic values ranging 0–0.05 upstream and 0.01–0.09 downstream of road crossings. The mean increase of acute $\sum$ESBTUs downstream of crossings, compared to upstream, was 0 (max = 0.01), and the mean increase of chronic $\sum$ESBTUs was 0.01 (max = 0.06). These low $\sum$WBTU and $\sum$ESBTU values indicate that the water and sediments did not contain PAH concentrations that would cause potential toxicity to aquatic organisms, and the minimal increases in the acute and chronic $\sum$ESBTUs suggest that individual roads contributed minimally to increasing toxicity of the sediments immediately downstream of a given crossing.

Effect of road crossings on mussel lipids and PAH concentrations

Road crossings had a highly significant impact on Total PAH concentrations in downstream reaches compared to upstream. Downstream concentrations were higher than those measured upstream of the crossings in every compartment ($t_{(19)} = 5.31$–6.63 and $P < 0.0001$ for mussel tissue dry weight and lipid-normalized PAH concentrations and sediment dry weight and OC-normalized concentrations; $t_{(19)} = 3.13$ and $P = 0.006$ for surface water PSDs) (Fig. 2). Mean increases in downstream samples, compared to upstream, were 134 ng/g mussel dry weight, 6027 ng/g mussel lipid, 190 ng/g sediment dry weight, 8786 ng/g sediment OC, and 1234 ng/g sampler in PSDs. These differences translate to an average 3 × higher PAH concentration in downstream mussel tissue and sediment and 2× higher PAHs in downstream PSDs. Lipid content of mussels was not different ($t_{(19)} = 1.09$, $P = 0.29$) between samples upstream and downstream of a crossing.

Correlation of PAH concentrations

Total PAH concentrations in mussel tissue (dry weight and lipid-normalized) were correlated with those in PSDs both upstream and downstream of road crossings (ρ_s ranged from 0.55 to 0.82 and corresponding P values ranged from <0.0001 to 0.0116; Table 1). The linear relationships of mussel PAH concentrations with PSDs upstream of road crossings were strong ($r^2 = 0.77$ for dry weight and 0.73 for lipid), and those between mussels and PSDs downstream of crossings were even stronger ($r^2 = 0.95$ for dry weight and 0.90 for lipid) (Fig. 3, dry weight shown). Mussel tissue concentrations were also correlated with PAHs in the sediment ($\rho_s = 0.82$–0.96 and P values all ≤ 0.0001; Table 1). The relationships between sediment dry weight PAHs and mussel tissue concentrations supported the correlations ($r^2 = 0.68$–0.91; Fig. 4), as did those between OC-normalized sediment PAH concentrations and mussel tissue concentrations ($r^2 = 0.96$–0.97).

Average annual daily traffic count was correlated with Total PAH concentration increases downstream of crossings for sediment dry weight ($\rho_s = 0.71$, $P = 0.0004$) and sediment OC ($\rho_s = 0.72$, $P = 0.0004$), mussel dry weight ($\rho_s = 0.64$, $P = 0.0025$) and lipid-normalized tissue concentrations ($\rho_s = 0.73$, $P = 0.0003$), but not for PSDs ($\rho_s = 0.22$, $P = 0.3522$). Despite the apparent correlations with traffic count, the relationships with increased PAH concentration downstream of the crossings were not particularly strong ($r^2 = 0.44$–0.58), except possibly for mussel lipid ($r^2 = 0.81$), and an extreme traffic count value (16,000) had a substantial effect on the relationships, such that when it was removed, r^2 values were substantially reduced (range 0.08–0.17, and mussel lipids = 0.40).

Table 1 Spearman rho (ρ_s) correlation coefficients and corresponding P values (shown below ρ_s) between mussel tissue and lipid-normalized Total PAH concentrations and those in passive sampling devices (PSDs) and sediments (dry weight and organic carbon (OC)-normalized)

	Upstream		Downstream	
	Mussel dry weight	Mussel lipid	Mussel dry weight	Mussel lipid
PSD	0.7790	0.8196	0.5518	0.6346
	<0.0001	<0.0001	0.0116	0.0027
Sediment dry weight	0.9158	0.9098	0.8466	0.8496
	<0.0001	<0.0001	<0.0001	<0.0001
Sediment	0.9519	0.9609	0.8150	0.8436
OC	<0.0001	<0.0001	<0.0001	<0.0001

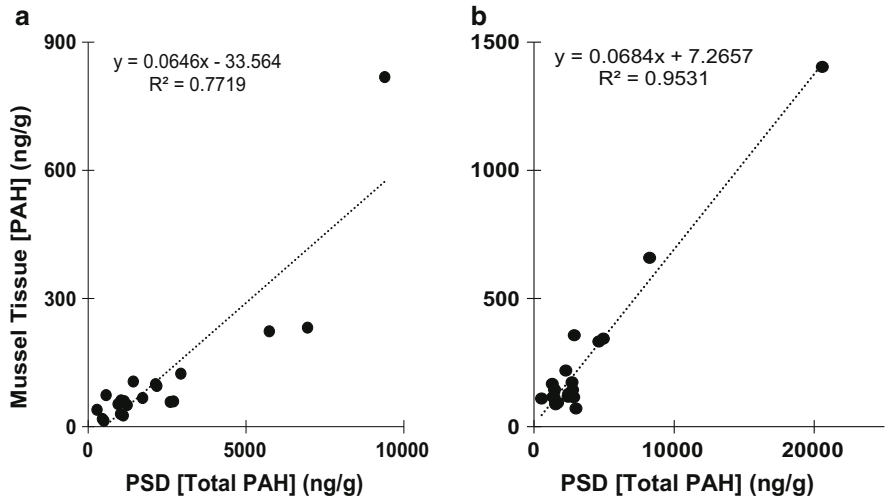

Fig. 3 Relationships between Total PAH concentrations in passive sampling devices (PSDs) and mussel tissue dry weight upstream (**a**) and downstream (**b**) of road crossings

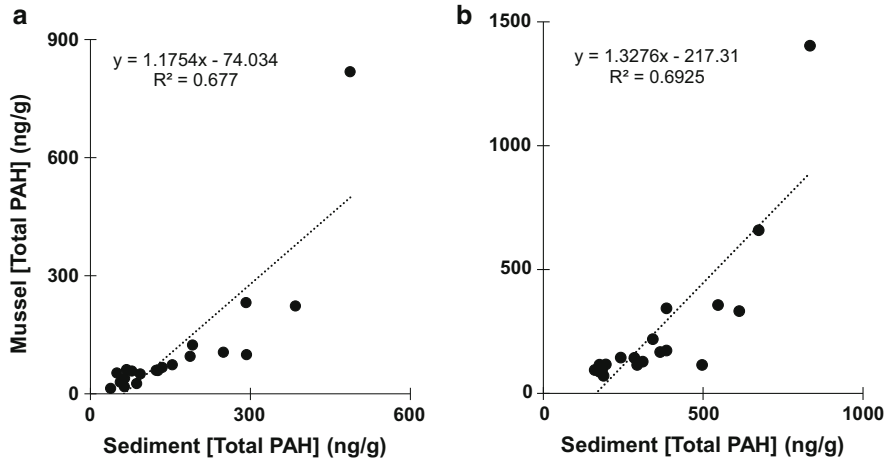

Fig. 4 Relationships between Total PAH concentrations in sediment dry weight and mussel tissue dry weight upstream (**a**) and downstream (**b**) of road crossings

Landscape effect on PAH concentrations

Land cover type had no effect on the PAH concentrations upstream or downstream of road crossings for any compartment. Comparisons of upstream PAH concentrations between agricultural and forested sites for each compartment resulted in ANOVA P values ranging 0.27–0.33, and comparisons of those downstream had similar results (ANOVA P values = 0.17–0.46). Because land cover type had no effect on PAH concentrations, data from both land cover types were pooled for a more robust dataset to predict mussel tissue concentrations from the PSDs.

Predicting mussel PAH concentrations

The concentrations of Total PAH in PSDs provided a reliable prediction equation for mussel tissue concentrations of Total PAH. The combined data of mussel dry weight and PSD concentrations from upstream and downstream samples ($n = 40$) resulted in a highly significant relationship ($F_{(1,38)} = 359.09$, $P < 0.0001$, $r^2 = 0.90$) (Fig. 5) and yielded the equation:

$$[\text{Mussel dry weight Total PAH}] = -18.766 + 0.0688 \times [\text{PSD Total PAH}]$$

Despite the correlations between mussel tissue and sediment Total PAH concentrations in their respective locations, prediction equations with pooled sediment data from upstream and downstream sites were not attainable without violating the residual assumptions for simple linear regression.

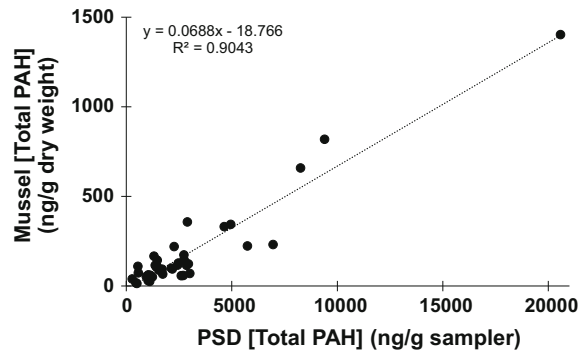

Fig. 5 Linear regression of the relationship between passive sampling device (PSD) concentrations of Total PAHs sampled over 30 days compared to that accumulated by freshwater mussels collected from the 20 study sites and combined from all stream locations ($n = 40$; 20 upstream and 20 downstream), indicating that PSDs may be a viable predictor of PAH concentrations in mussels, thus reducing the need for mussel tissue collection via destructive sampling

Discussion

Road crossings significantly increased stream PAH concentrations, and those changes were observed in sediment, water, and freshwater mussels. Road crossings appear to provide a consistent source of PAHs that likely gets renewed in streams with each precipitation and runoff event, contributing to accumulation in the sediment and chronic exposures to mussels and other aquatic biota. Furthermore, there was consistency between upstream and downstream PAH signatures (Supplemental Data, Tables S1–S6), indicating that the PAHs in the upstream reaches we sampled may be influenced by road runoff farther upstream in

the watershed. Our 20 sampling sites in this study represented only a small portion of the 326 NC DOT inventoried road crossings and approximately 1.4% of the more than 1400 road/stream intersections identified in our study area (ArcGIS Desktop, v.10.3, ESRI). While an individual road crossing had a minimal effect on the overall toxicity ranking of PAHs in water and sediments immediately downstream at a given site, as evidenced by the negligible changes in $\sum$WBTUs and $\sum$ESBTUs compared to those in upstream samples, the similarity in PAH signatures on either side of the crossings suggests that PAH pollution from traffic sources may increase exposure and cumulative toxicity as streams proceed downstream with more crossings and distance covered in a watershed. To illustrate, multiplying the average increase in Total PAH concentrations downstream of a crossing in the rural headwater sub-basin that we studied here by its 1400 road crossings could result in extensive cumulative input from this one relatively small watershed. While this cumulative input from multiple crossings would certainly be mitigated, in part, by PAH degradation or reduction in bioavailability, the relatively large number of road crossing inputs to streams create the potential for increased toxicity downstream and is a topic worthy of future study. The toxic effects of PAH parent compounds and those of their associated metabolites are already known to alter mussel physiology and biochemistry at sub-cellular scales (Newton & Cope, 2007; Prochazka et al., 2012) and may adversely impact immunity, reproduction, or other processes that can lead to long-term population-level declines. Any potential effects may be exacerbated by other factors such as thermal pollution or stress from the same road runoff (Herb et al., 2008). Moreover, road residues and runoff contain metals related to vehicle wear and emissions (e.g., Cd, Pd, and Pt (Haus et al., 2007; McKenzie et al., 2009)), and a recent review of the co-toxic effects of metal-PAH mixtures concluded that synergistic impacts were more likely than additive effects (Gautier et al., 2014).

We demonstrated that Total PAH concentrations in PSDs were strongly related to those in tissue samples of *E. complanata* both upstream and downstream of road crossings. As such, the future use of PSDs in stream monitoring seems to offer a reliable method to estimate PAH concentrations in mussels, thereby reducing or eliminating the need to destructively sample the often imperiled mussels to assess their exposure. This relation yielded a linear regression model that explained more than 90% of the variation in mussel tissue PAH concentrations, and indicates promise for PSDs as a predictive tool for estimating PAHs and other contaminants in mussels, as has been demonstrated recently for other freshwater fauna [e.g., crayfish (Paulik et al., 2016)]. The analysis of PAHs in PSDs rather than collecting and analyzing mussels, as stated above is a beneficial non-destructive alternative, but they also have the advantage of use in places where mussel beds are not easily accessible (e.g., deep rivers, backwaters) or are absent (e.g., due to previous extirpation). Moreover, some freshwater mussel species like *E. complanata* may be widespread and abundant throughout their range, but most species are in decline, leaving few choices for collection and use in biomonitoring applications. Finally, PSDs may require less time and effort to deploy and retrieve than collecting resident mussels and are a less cost intensive and logistically challenging option compared to deploying caged mussels (Patnode et al., 2015; Cope & Jones, 2016) or other organisms for biomonitoring.

While the correlation of sediment PAH concentrations with those accumulated in mussels suggests that sediment also may offer a surrogate to directly sampling mussel PAH concentrations, we were unable to demonstrate a predictive relationship model with the concentrations generated from our samples. However, the apparent trend may be worthy of future investigation in a larger scale study or with a larger dataset of combined studies. We also found no quantitative evidence that land use cover type affected the PAHs introduced in runoff from road crossings in our study. However, other land use types not investigated here (e.g., urban, suburban), compared to lower intensity uses like forest and agriculture, would be appropriate in future studies of PAH contributions from such crossings, especially because other high-intensity land uses sourcing PAHs (e.g., fossil fuel mining) have been implicated in detrimental effects to stream communities (Diamond et al., 2002; Locke et al., 2006).

The PAH contributions to streams from individual road crossings studied here were substantial, and the potential for accumulation of PAHs and other traffic-related contaminants from such crossings within watersheds highlights the opportunity for transportation and environmental regulatory agencies to implement best management practices or to improve upon

existing infrastructure designs that reduce road runoff into streams. If contaminated runoff were anticipated when designing new roads that cross surface waters and accounted for at existing crossings with retrofitted design features, then runoff could be reduced (Luell et al., 2011). For example, runoff can be sequestered and minimized with engineered stormwater control measures such as bioretention cells, constructed stormwater wetlands, vegetated filter strips, or other innovative and emerging best management practices, such as the use of pervious concrete pavements (Winston et al., 2015; Chandrappa & Biligiri, 2016). Even in locations where space is not optimal for adding a stormwater control measure retroactively, undersized bioretention cells have been shown to improve runoff water quality (Luell et al., 2011).

Conclusion

We found that the PAH contribution of road crossings to streams in a single, relatively rural watershed is substantial and statistically significant, suggesting that the cumulative PAH influx within an entire river basin may be considerable and exert adverse effects over a chronic lifetime exposure for mussels, especially as their contributions may amplify downstream. Additionally, PAHs entering streams in urban areas with a higher density of roads and greater abundance of traffic may be many times greater than our results here (Van Metre et al., 2000). Understanding the risks of PAHs in road runoff is especially important because freshwater mussels are not only highly imperiled, but also critically important to the functional ecology of freshwater ecosystems (e.g., filtration and bioturbation (Vaughn & Hakencamp, 2001); biodeposition of nutrients and organic matter (Spooner & Vaughn, 2006); stream community biodiversity (Allen et al., 2012)). Because freshwater mussels are filter- and deposit-feeding organisms and each may filter up to 11 L water/day, depending on species and body size (Naimo, 1995), they offer critical ecosystem services (Vaughn & Hakencamp, 2001), including sequestering environmental contaminants like PAHs.

Contaminant removal by mussels is a benefit for other aquatic species that rely on healthy surface waters for suitable habitat, for terrestrial wildlife that forage and drink from streams, and for humans, who rely on surface waters for food, recreation, and most importantly, a primary drinking water supply in many regions. However, this important ecosystem service is often a detriment to the mussels themselves; as previously discussed, they have limited ability to metabolize and eliminate PAHs (Thorsen et al., 2007), and may suffer toxic responses resulting from long-term exposure (Newton & Cope, 2007; Prochazka et al., 2012; Martins et al., 2013). Such effects may impact immune function, reproduction, or other processes that can lead to long-term population-level declines like those reported in North America and much of the world (Lydeard et al., 2004; Haag, 2012), thus reducing the capacity of freshwater mussels to continue providing this and other vital ecosystem services. Minimizing the contaminant loads entering our surface waters (e.g., engineering road crossings with runoff treatment or retention) is imperative for maintaining mussel populations and the valuable ecosystem services they provide.

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References

Allen, D. C., C. C. Vaughn, J. F. Kelly, J. T. Cooper & M. H. Engel, 2012. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. Ecology 93: 2165–2174.

Alvarez, D. A., K. A. Maruya, N. G. Dodder, W. Lao, E. T. Furlong & K. L. Smalling, 2014. Occurrence of contaminants of emerging concern along the California coast (2009–2010) using passive sampling devices. Marine Pollution 81: 347–354.

APHA et al. (American Public Health Association, American Water Works Association, and Water Environment Federation), 1995. Standard Methods for the Examination of Water and Wastewater. 19th edition. APHA, AWWA, WEF. Washington, DC.

Archambault, J. M., C. M. Bergeron, W. G. Cope, R. J. Richardson, M. A. Heilman, J. E. Corey III, M. D. Netherland & R. J. Heise, 2015. Sensitivity of freshwater molluscs to hydrilla-targeting herbicides: providing context for invasive aquatic weed control in diverse ecosystems. Journal of Freshwater Ecology 30: 335–348.

Baumard, P., H. Budzinski & P. Garrigues, 1998. Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean sea. Environmental Toxicology and Chemistry 17: 765–776.

Bihanic, F. L., C. Clérandeau, K. L. Menach, B. Morin, H. Budzinski, X. Cousin & J. Cachot, 2014. Developmental toxicity of PAH mixtures in fish early life stages. Part II: adverse effects in Japanese medaka. Environmental Science and Pollution Research 21: 13732–13743.

Bringolf, R. B., W. G. Cope, S. Mosher, M. C. Barnhart & D. Shea, 2007. Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae). Environmental Toxicology and Chemistry 26: 2094–2100.

Chandrappa, A. K. & K. P. Biligiri, 2016. Pervious concrete as a sustainable pavement material – research findings and future prospects: a state-of-the-art review. Construction and Building Materials 111: 262–274.

Conners, D. E. & M. C. Black, 2004. Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Archives of Environmental Contamination and Toxicology 46: 362–371.

Cope, W. G. & J. Jones, 2016. Recent precipitous declines of endangered freshwater mussels in the Clinch River: an in situ assessment of water quality stressors related to energy development and other land-use. Final Completion Report, submitted to the US Fish and Wildlife Service Regions 4 and 5 offices, Cookeville, Tennessee and Abingdon, Virginia.

Cope, W. G., R. B. Bringolf, D. B. Buchwalter, T. J. Newton, C. G. Ingersoll, N. Wang, T. Augspurger, F. J. Dwyer, M. C. Barnhart, R. J. Neves & E. Hammer, 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. Journal of the North American Benthological Society 27: 451–462.

Diamond, J. M., D. W. Bressler & V. B. Serveiss, 2002. Assessing relationships between human land uses and the decline of native mussels, fish, and macroinvertebrates in the Clinch and Powell River watershed, USA. Environmental Toxicology and Chemistry 21: 1147–1155.

Downing, J. A., P. Van Meter & D. A. Woolnough, 2010. Suspects and evidence: a review of the causes of extirpation and decline in freshwater mussels. Animal Biodiversity and Conservation 33: 151–185.

Gautier, P. T., W. P. Norwood, E. E. Prepas & G. G. Pyle, 2014. Metal-PAH mixtures in the aquatic environment: a review of co-toxic mechanisms leading to more-than-additive outcomes. Aquatic Toxicology 154: 253–269.

Haag, W. R., 2012. North American Freshwater Mussels: Natural History, Ecology, and Conservation. Cambridge University Press, New York.

Haus, N., S. Zimmerman, J. Wiegand & B. Sures, 2007. Occurrence of platinum and additional traffic related heavy metals in sediments and biota. Chemosphere 66: 619–629.

Heltsley, R. M., W. G. Cope, D. Shea, R. B. Bringolf, T. J. Kwak & E. G. Malindzak, 2005. Assessing organic contaminants in fish: comparison of a non-lethal tissue sampling technique to mobile and stationary passive sampling devices. Environmental Science & Technology 39: 7601–7608.

Herb, W. R., B. Janke, O. Mehseni & H. G. Stefan, 2008. Thermal pollution of streams by runoff from paved surfaces. Hydrological Processes 22: 987–999.

Hofelt, C. S. & D. Shea, 1997. Accumulation of organochlorine pesticides and PCBs by semipermeable membrane devices and *Mytilus edulis* in New Bedford Harbor, MA. Environmental Science & Technology 31: 154–159.

Levine, J. F., A. E. Bogan, P. A. Russell, E. F. Andersen & C. B. Eads, 2003. Distribution of freshwater mussel populations in relationship to crossing structures. Final report submitted to the North Carolina Department of Transportation (HWY-2003-02), Raleigh, NC. 182 pp.

Locke, B. A., D. S. Cherry, C. E. Zipper & R. J. Currie, 2006. Land use influences and ecotoxicological ratings for the Upper Clinch River tributaries in Virginia. Archives of Environmental Contamination and Toxicology 51: 197–205.

Luell, S. K., W. F. Hunt & R. J. Winston, 2011. Evaluation of undersized bioretention stormwater control measures for treatment of highway bridge deck runoff. Water Science & Technology 64: 974–979.

Luellen, D. R. & D. Shea, 2002. Calibration and field verification of semipermeable membrane devices for measuring polycyclic aromatic hydrocarbons in water. Environmental Science & Technology 36: 1791–1797.

Luellen, D. R. & D. Shea, 2003. Semipermeable membrane devices accumulate conserved ratios of sterane and hopane petroleum biomarkers. Chemosphere 53: 705–713.

Lydeard, C., R. H. Cowie, W. F. Ponder, A. E. Bogan, P. Bouchet, S. A. Clark, K. S. Cummings, T. J. Frest, O. Gargominy, D. G. Herbert, R. Herschler, K. E. Perez, B. Roth, M. Seddon, E. E. Strong & F. G. Thompson, 2004. The global decline of nonmarine molluscs. BioScience 54: 321–330.

Martins, M., P. M. Costa, A. M. Ferreira & M. H. Costa, 2013. Comparative DNA damage and oxidative effects of carcinogenic and non-carcinogenic sediment-bound PAHs in the gills of a bivalve. Aquatic Toxicology 142–143: 85–95.

McKenzie, E. R., J. E. Money, P. G. Green & T. M. Young, 2009. Metals associated with stormwater-relevant brake and tire samples. Science of the Total Environment 407: 5855–5860.

Naimo, T. J., 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology 4: 341–362.

[NC DOT] North Carolina Department of Transportation, 2014. Traffic Survey Group Transportation Planning Branch, AADT Stations Shapefile. Raleigh, NC. [Accessed 10 June 2016] Available from: https://www.ncdot.gov/projects/trafficsurvey/.

Neff, J. M., 1979. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. Applied Science, London, UK.

Neff, J. M. & W. A. Burns, 1996. Estimation of polycyclic aromatic hydrocarbon concentrations in the water column based on tissue residues in mussels and salmon: an equilibrium partitioning approach. Environmental Toxicology and Chemistry 15: 2240–2253.

Neff, J. M., 2002. Bioaccumulation in Marine Organisms – Effects of Contaminants from Oil Well Produced Water. Elsevier, Amsterdam.

Newton, T. J., J. W. Allran, J. A. O'Donnell, M. R. Bartsch & W. B. Richardson, 2003. Effects of ammonia on juvenile unionid mussels (*Lampsilis cardium*) in laboratory sediment toxicity tests. Environmental Toxicology and Chemistry 22: 2554–2560.

Newton, T. J. & M. R. Bartsch, 2007. Lethal and sublethal effects of ammonia to juvenile Lampsilis mussels (Unionidae) in sediment and water-only exposures. Environmental Toxicology and Chemistry 26: 2057–2065.

Newton, T. J. & W. G. Cope, 2007. Biomarker responses of unionid mussels to environmental contaminants. In Van Hassel, J. H. & J. L. Farris (eds), Freshwater Bivalve Ecotoxicology. CRC Press, Boca Raton, FL: 257–284.

[NC DENR] North Carolina Department of Environment and Natural Resources, 1998. Basinwide Assessment Report, Neuse River basin. Division of Water Quality, Raleigh, NC.

Oikari, A., N. Fragoso, H. Leppanen, T. Chan & P. V. Hodson, 2002. Bioavailability to juvenile Rainbow Trout (*Oncorhynchus mykiss*) of retene and other mixed-function oxygenase-active compounds from sediments. Environmental Toxicology and Chemistry 21: 121–128.

Patnode, K. A., E. Hittle, R. M. Anderson, L. Zimmerman & J. W. Fulton, 2015. Effects of high salinity wastewater discharges on unionid mussels in the Allegheny River, Pennsylvania. Journal of Fish and Wildlife Management 6: 55–70.

Paulik, L. B., B. W. Smith, A. G. Bergmann, G. J. Sower, N. D. Forsberg, J. G. Teeguarden & K. A. Anderson, 2016. Passive samplers accurately predict PAH levels in resident crayfish. Science of the Total Environment 544: 782–791.

Prochazka, S. T., W. G. Cope & L. Recio, 2012. Genotoxic response of unionid mussel hemolymph to hydrogen peroxide and polycyclic aromatic hydrocarbons. Walkerana: The Journal of the Freshwater Mollusk Conservation Society 15: 113–125.

Spooner, D. E. & C. C. Vaughn, 2006. Context-dependent effects of freshwater mussels on stream benthic communities. Freshwater Biology 51: 1016–1024.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton & S. J. Nichols, 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54: 429–439.

Thorsen, W. A., W. G. Cope & D. Shea, 2004. Bioavailability of PAHs: effects of Soot Carbon and PAH Source. Environmental Science & Technology 38: 2029–2037.

Thorsen, W. A., W. G. Cope & D. Shea, 2007. Toxicokinetics of environmental contaminants in freshwater bivalves. In Van Hassel, J. H. & J. L. Farris (eds), Freshwater Bivalve Ecotoxicology. CRC Press, Boca Raton, FL: 169–207.

[US EPA] United States Environmental Protection Agency, 2000. Neuse River basin land cover land use metadata. US EPA Landscape Characterization Branch. Research Triangle Park, NC. [Accessed 10 June 2016] Available from: http://gisdata.lib.ncsu.edu/fedgov/epa/epalulc/nrb_lclu.htm.

[US EPA] US Environmental Protection Agency, 2003. Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: PAH mixtures (EPA-600-R-02-013). Office of Research and Development. Washington, DC.

[US EPA] US Environmental Protection Agency, 2010. Water quality benchmarks for aquatic life. Washington, DC. Available from: http://www.epa.gov/bpspill/waterbenchmarks.

[US EPA] US Environmental Protection Agency, 2013. Aquatic life ambient water quality criteria for ammonia –freshwater (EPA-822-R-13-001). Office of Water. Office of Science and Technology, Washington, DC.

Van Hassel, J. H. & J. L. Farris, 2007. A review of the use of unionid mussels as biological indicators of ecosystem health. In Van Hassel, J. H. & J. L. Farris (eds), Freshwater Bivalve Ecotoxicology. CRC Press, Boca Raton, FL: 19–49.

Van Metre, P. C., B. J. Mahler & E. T. Furlong, 2000. Urban sprawl leaves its PAH signature. Environmental Science & Technology 34: 4064–4070.

Vaughn, C. C. & C. C. Hakencamp, 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46: 1431–1446.

Wang, N., C. G. Ingersoll, D. K. Hardesty, C. D. Ivey, J. L. Kunz, T. W. May, F. J. Dwyer, A. D. Roberts, T. Augspurger, C. M. Kane, R. J. Neves & M. C. Barnhart, 2007a. Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26: 2036–2047.

Wang, N., C. G. Ingersoll, I. E. Greer, D. K. Hardesty, C. D. Ivey, J. L. Kunz, W. G. Brumbaugh, F. J. Dwyer, A. D. Roberts, T. Augspurger, C. M. Kane, R. J. Neves & M. C. Barnhart, 2007b. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26: 2048–2056.

Williams, J. D., M. L. Warren Jr., K. S. Cummings, J. L. Harris & R. J. Neves, 1993. Conservation status of the freshwater mussels of the United States and Canada. Fisheries 18(9): 6–22.

Winston, R. J., M. S. Lauffer, K. Narayanaswamy, A. H. McDaniel, B. S. Lipscomb, A. J. Nice & W. F. Hunt, 2015. Comparing bridge deck runoff and stormwater control measure quality in North Carolina. Journal of Environmental Engineering 141: 04014045.