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RESEARCH REPORT

Susceptibility of Eastern Oyster Early Life Stages to Road Surface Polycyclic Aromatic Hydrocarbons (PAHs)

Richard V. Lacouture
Morgan State University Estuarine Research Center

Randolph K. Larsen
Saint Mary's College of Maryland

Jon T. Anderson
Booz Allen Hamilton, Inc.

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16. Abstract

Polycyclic aromatic hydrocarbons (PAHs) are a class of chemical compounds that are mostly anthropogenic in nature, and they can become persistent organic contaminants in aquatic ecosystems. Runoff from impervious surfaces is one of the many ways contaminants enter the aquatic realm. Three different PAH concentrations were used to investigate the effects on oyster-egg fertilization, embryonic transformation to the D-shell veliger larva, and larvae settlement onto hard substrate. The three different concentrations of PAHs ranged from one that is environmentally relevant to the concentrations found in runoff from road surfaces, another that was tenfold more concentrated, and finally one that was one hundred times more concentrated. The extremely concentrated solution had negative effects on fertilization success after one hour of exposure, while the lesser concentrations showed significant deleterious effects after two hours of exposure. All of the three PAH solutions caused significant numbers of abnormalities in the embryonic transformation of embryos to D-stage larvae. Finally, the two more concentrated PAH solutions significantly inhibited the oyster larvae in successfully setting on the hard substrate. This study's results provide evidence that PAHs entering an aquatic ecosystem from runoff from road surfaces have the potential to inhibit oyster reproduction by negatively impacting three critical processes in the early life cycle of the Eastern oyster.

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Kara Benton and Kevin McEligot preparing samples

EXECUTIVE SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) are a class of chemical compounds that are mostly anthropogenic in nature, and they can become persistent organic contaminants in aquatic ecosystems. Runoff from impervious surfaces is one of the many ways contaminants enter the aquatic realm. This study focuses on runoff from road surfaces. The results of a prior study undertaken at Morgan State University Estuarine Research Center showed that particulate matter from road surfaces was a significant source of PAHs (Anderson et al., 2010). These road-based PAHs can make up as much as 50 percent of the total PAH load.

A number of studies have indicated the deleterious effects of PAHs on a variety of aquatic organisms. Most of these studies have worked with individual compounds in relatively high concentrations to examine the impact of PAHs from oil spills or from the combustion of fossil fuels. The potential impacts of PAHs on aquatic biota range from carcinogenic to mutagenic to lethally toxic.

While studies have focused on the impacts of PAHs on the early life stages of oysters, the majority have used the Pacific oyster (*Crassostrea gigas*) and have analyzed specific PAH compounds. However, this study uses the Eastern oyster (*Crassostrea virginica*) and a ‘cocktail’ of a variety of PAHs that were detected in road surface runoff.

Three different PAH concentrations were used to investigate the effects on oyster-egg fertilization, embryonic transformation to the D-shell veliger larva, and larvae settlement onto hard substrate. The three different concentrations of PAHs ranged from one that is environmentally relevant to the concentrations found in runoff from road surfaces, another that was tenfold more concentrated and finally one that was one hundred times more concentrated.

The extremely concentrated solution had negative effects on fertilization success after one hour of exposure, while the lesser concentrations showed significant deleterious effects after two hours of exposure. All of the three PAH solutions caused significant numbers of abnormalities in the embryonic transformation of embryos to D-stage larvae. Finally, the two more concentrated PAH solutions significantly inhibited the oyster larvae in successfully setting on a hard substrate. This process known as spat settlement is the transformation of the motile larval stage of the oyster to its final sessile stage that we are more familiar with on the bottom of the estuary.

This study’s results provide evidence that PAHs entering an aquatic ecosystem from runoff from road surfaces have the potential to inhibit oyster reproduction by negatively impacting three critical processes in the early life cycle of the Eastern oyster.

INTRODUCTION

Impervious road surfaces have the potential to release chemical pollutants to nearby lakes, streams, and rivers, where these chemicals may adversely affect aquatic organisms and, ultimately, humans (Kennish, 1998). Among some of the most common chemical pollutants in road surfaces are polycyclic aromatic hydrocarbons (PAHs). PAHs are a class of complex organic molecules that can originate from petroleum-based products, including bituminous road surfaces and sealants. Furthermore, road surfaces can accumulate oils and contaminants from vehicle-related spills and tire wear. During precipitation, impervious road surfaces can leach PAHs into runoff and stormwater discharge which ultimately can end up in nearby aquatic systems. Stormwater runoff from roadways has been shown to contribute over 50 percent of the total PAH input to adjacent aquatic systems (Hoffman et al., 1985).

Effects on Aquatic Ecosystems

Many studies have examined how PAHs can affect aquatic organisms (Bender et al., 1988; Harvey, 1997; Varanasi, 1989). PAHs often occur in complex mixtures in aquatic ecosystems; therefore, it is necessary to assess mixture effects to biota (Kennish, 1992). Once in an aquatic environment, the majority of PAHs are quickly adsorbed by sediment particles or biotic material (including living organisms and dead organic material, Kirso et al., 2001), where they can persist for several years depending on their molecular weight (Wild et al., 1991). PAHs are considered toxic to a variety of aquatic organisms, acting as carcinogens, DNA mutagens, and endocrine disruptors (Pittinger et al., 1987; Hellou et al., 2006). For example, in finfish like flounder and trout, PAHs can interfere with estrogen production (Anderson et al., 1996; Rocha et al., 2000). Kim et al. (2007) found that adult Pacific oysters (*Crassostrea gigas*) had marked decreased productivity when exposed to even low concentrations of PAHs. Stream biota, such as insect larvae and crustaceans, are also affected adversely, resulting in reduced species diversity in affected areas (Beasley and Kneale, 2002). Single-celled aquatic plants (algae) can also be adversely affected by PAH concentrations (Anderson et al., 2010; Hjorth et al., 2007; Grotte et al., 2005; Petersen et al., 2008).

Importance of Oysters to the Chesapeake Bay

Oysters are a vital component of the Chesapeake Bay’s economy and ecology. Oysters provide a dominant commercial fishery for the Chesapeake Bay region. Oysters filter particles and algae to improve water clarity, and provide habitat (through oyster reefs) for other important species in the bay. However, in recent decades, the oyster population, as measured by landings, has declined dramatically. For example, statewide harvests, which exceeded 15 million bushels in the late 19th century and sustained an average of 2-3 million bushels through much of the mid-20th century (Kennedy and Breisch, 1981), dropped to 26,495 bushels in 2004. Heavy harvest pressure, land-based pollution, loss of critical habitat, and two parasitic diseases have been identified as reasons for this decline. The decline has had a significant impact on Maryland’s seafood industry and the Chesapeake Bay’s health.

Currently, efforts are underway to restore the bay’s oysters to historical levels and to ensure a sustainable stock. For example, a NOAA grant has allowed Morgan State University’s Estuarine

Research Center to develop a research hatchery for native oysters (*Crassostrea virginica*). The hatchery produces eggs, sperm, larvae, and spat, which the Calvert County Watermen's Association uses to develop its members' remote-setting or grow-out operations. These operations allow the watermen to produce a supply stream of marketable oysters.

As restoration efforts continue, it is important to understand the effects and extent of detrimental factors (e.g., land-based pollution) on oyster survivorship, including oysters' PAH susceptibility during different life stages.

Oyster Susceptibility to PAHs

It is believed that oysters and other bivalves readily accumulate and are susceptible to PAHs since they have a poorly developed metabolic pathway that will not readily allow the breakdown and deactivation of PAHs (Kennish, 1998). For example, Pittinger et al. (1985) and Lee et al. (1978) showed that oysters exposed to crude oil and contaminated sediments accumulated toxic PAH levels in as little as 2 days. Jeong and Cho (2005) found that exposing reproducing adult oysters to PAHs adversely affected egg and sperm viability and ultimately, larval development. Another study directly exposed oyster embryos and larvae to PAH-contaminated sediments, resulting in increased mutations and deleterious deformations (Geffard et al., 2002).

It is important to understand and quantify PAH susceptibility during oysters' early life stages because this is where most of the bottlenecks occur in survivorship and growth of the population (Houde, 1987; see Figure 1). For example, a landmark study by Nelson and Perkins (1931) showed that oyster larvae decreased exponentially from 79,400 at four days after fertilization to 84 viable individuals at day 13.

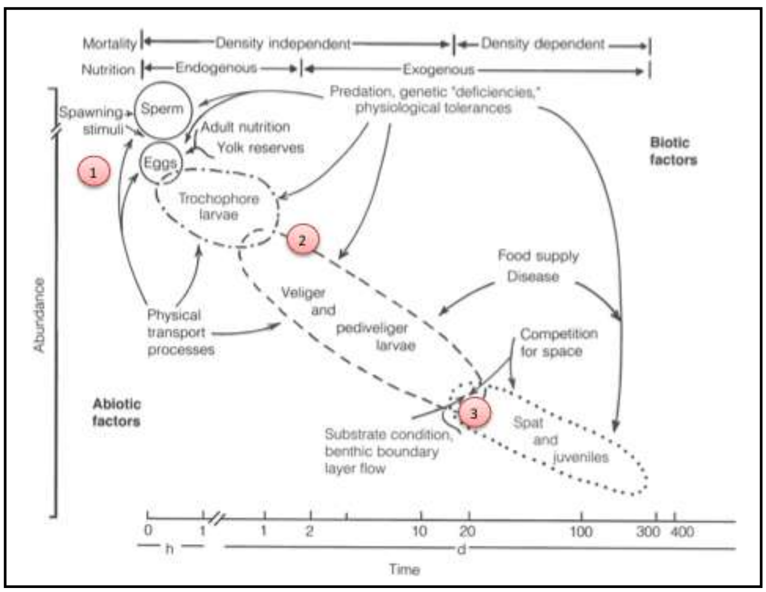

Figure 1: Diagram of the life stages and survivorship of the eastern oyster. Early life stages examined in this study are highlighted with numbers: (1) fertilization success, (2) Veliger ("D-Stage") transformation success, and (3) spat settlement success. Image Source: Kennedy et al. (1996).

Surprisingly, previous tests of PAH toxicity on oysters have either examined Pacific oyster species or used a small subset of PAH compounds. Furthermore, concentrations were much higher than would typically be experienced in the environment or leached from road surfaces, and adult oysters were used. Several studies have shown that PAH-laden elutriates from sediment and algae raised in contaminated water had negative effects on the Pacific oyster's larval embryogenesis and growth (Geffard et al., 2002) and embryotoxicity (Lyons et al., 2002; Wessel et al., 2007). Therefore, this study is unique in that it looks at the impacts of dissolved PAHs on the early life stages of the Eastern oyster (*C. virginica*).

Objectives

As previously stated, this study examines the effects of realistic concentrations of PAH leachate from road surfaces on the early life stages of oysters. This, in conjunction with results from the 2009 NTC award ("Assessing the Magnitude of Polycyclic Aromatic Hydrocarbon Loading from Road Surfaces and its Effect on Algal Productivity," J. Anderson, R. Larsen, and R. Lacouture), provides necessary data and information on effects of PAHs on aquatic ecosystems. Resource managers and transportation administrators can use the findings to make informed decisions about particular road surfaces, to provide cost-effective transportation infrastructure services, and to minimize the impacts on aquatic ecosystems.

METHODOLOGY

PAH Concoctions (cocktails)

PAH solutions were created based on results from the authors' previous NTC-funded project, "Assessing the Magnitude of Polycyclic Aromatic Hydrocarbon Loading from Road Surfaces and its Effect on Algal Productivity." In brief, the previous study examined four road surface and traffic combinations: low-traffic asphalt, high-traffic asphalt, high-traffic concrete, and new asphalt. Leachate was collected from each sampling site using a modified technique described by a USGS study (Mahler et al., 2005), with the amount and type of 46 PAHs quantified using a GC/MS. A PAH solution was prepared based on the compounds identified in the leachate analyses from the different road surfaces.

PAH Measurements

To ensure that the PAHs were re-created accurately, samples of each solution were taken prior to experimental addition.

The PAH content of the stock solutions was determined by gas chromatograph mass spectrometry (GC/MS). Three hundred microliters of stock was combined with one hundred nanograms of each of the following internal standards immediately prior to GC/MS analysis: d10-Acenaphthalene, d10-Phenanthrene, d12-Benz[a]anthracene, d12-Benzo[a]pyrene, and d12-Benzo[g,h,i]perlyene. The masses of PAHs in the extracts were determined by GC-MS (Agilent 6890/5973N) operating in select-ion mode. A Restek Rxi 5Sil with integra guard fused capillary column was used with helium as the carrier gas. The column was 30 m in length, 0.25 mm in diameter, and 0.25 mm in film thickness. The oven temperature was programmed from 45 to 280°C at a rate of 10°C per minute and held at 310°C for 16.5 minutes. An external standard mixture was used to generate response factors for the mass calculation.

Oyster Bioassays

The Estuarine Research Center's oyster hatchery provided the oysters used for the experiments. The hatchery rears adult brood stock of the native oyster, *C. virginica*, and has the capacity to spawn oysters multiple times throughout the warmer portion of the year. At each spawning, the hatchery can produce an abundant supply of sperm and eggs, and maintain their development through all stages of larvae. Brood stock oysters were dredged from the Patuxent River in Calvert County, Maryland. They began to spawn in June 2010, and continued producing early life stages throughout the summer of 2010.

Three phases of the early oyster development were examined: fertilization, transformation to D-stage larvae, and spat settlement (Figure 1). For each spawning event, multiple subsamples of each life stage were taken and split into one of five treatments: no PAH exposure (CNTRL), no PAH exposure but acetone (CNTRL/ACET), PAH solution at environmentally relevant concentrations in nature (ERC), PAH solution ten-fold greater than ERC (ERCx10), and PAH solution 100 times greater than ERC (ERCx100). The CNTRL/ACET group was tested because the PAH solution contained a small amount of acetone which in itself could have effects on the different oyster life stages. Experiment setup and duration were specific for each life stage and are described in detail below. Fertilization success and embryonic development experiments were conducted during June and July of 2010, while the spat settlement experiment was conducted in July 2011.

Fertilization Success

According to Kennedy et al. (1996), oyster sperm and eggs fertilize in less than 2 hours. The current experiments were conducted in 250-ml Erlenmeyer glass flasks, which were gently mixed on a shaker table under bright fluorescent light conditions (270-389 μE). Fertilized eggs were placed in 100 ml of artificial seawater (15 ppt salinity and dissolved oxygen = 7.0 mg/L). At time 0, 1 hour and 2 hours after exposure, a 10-ml subsample of the fertilized eggs was taken from each flask and incubated for 15 minutes with the fluorescent stain SYTOX blue using the methodology described by LeBaron et al. (1998). SYTOX is a large-molecular weight nucleic acid stain. The molecule's large size inhibits passage through intact cell membranes. Therefore, stained nuclei are indicative of cells with compromised cell membranes, and classify cells as live or dead.

Transformation from Embryos to D-Stage Larvae

Embryos take approximately 24 hours to transform to D-stage larvae (Kennedy et al., 1996). Samples of fresh, unexposed embryos were taken and rinsed of all eggs and sperm. The rinsed embryos (~30-40,000) were incubated in a 250-ml Erlenmeyer glass flask in 100 ml of artificial seawater (15 ppt salinity and 6.5 mg/L dissolved oxygen) with gentle mixing on a shaker table for 24 hours. D-stage transformation success was assessed using the methodology of Geffard et al. (2002). In brief, this involves using light microscopy to examine the proportion of abnormal larvae. Fifty larvae were counted and classified for each sample as normal, indented or incomplete shell, convex hinge, or a protruding mantle.

Spat Settlement Success

Viable larvae take approximately two days to settle on a substrate and form spat (Kennedy et al., 1996). At the appropriate time, fresh larvae were added to 1.5-L Pyrex containers. To facilitate settlement, a gridded PVC plate was primed with a bacterial film and placed on the bottom of each container. An airstone attached to an air pump is placed in each container to assure sufficient aeration during the experiment. Food (*Isochrysis sp*.) is supplied daily for nutrition. Spat settlement was quantified after three days by counting the surface of the entire PVC plate with a dissecting microscope at 50X.

Statistical Analyses

Differences between the control treatments and PAH solution treatments were assessed using analysis of variance (ANOVA) tests.

RESULTS

PAH Solution

The working stock solution contained 24 different PAHs. Relative to concentration, the four major PAHs were napthacene, naphthalene, phenanthrene, and fluoranthene.

PAH	Concentration (ng/mL)
Napthalene	10400.0
1Methylnapthalene	3590.0
Biphenyl	166.0
Acenapthylene	682.0
Acenapthene	1.0
Fluorene	1210.0
1Methylfluorene	0.1
Dibenzothiophene	51.5
Phenanthrene	8800.0
Anthracene	909.0
2Methylanthracene	210.0
9Methylanthracene	13.6
Fluoranthene	5180.0
Pyrene	2180.0
Napthacene	16300.0
Benzo[b]fluoranthene	2040.0
Benzo[k]fluoranthene	2060.0
Benzo[e]pyrene	867.0
Perylene	14.1
Indone[1,2,3-cd]pyrene	1520.0
Dibenz[a,h + a,c]perylene	8.8
Benzo[g,h,i]perylene	4710.0
Anthanthrene	158.0
Corenene	11.1

Table 1: List of PAHs and concentrations in the stock solution.

Fertilization Success

The success of oyster fertilization was assessed by looking at the mean differences the controls and the three PAH treatments at the three different times during the experiment (time0, time1, and time2). The ANOVA test indicated no significant differences in the mean values of percentage of live embryos between the control groups and the PAH treatment groups at time0 at $p < 0.05$ (Figure 2 and Table 2).

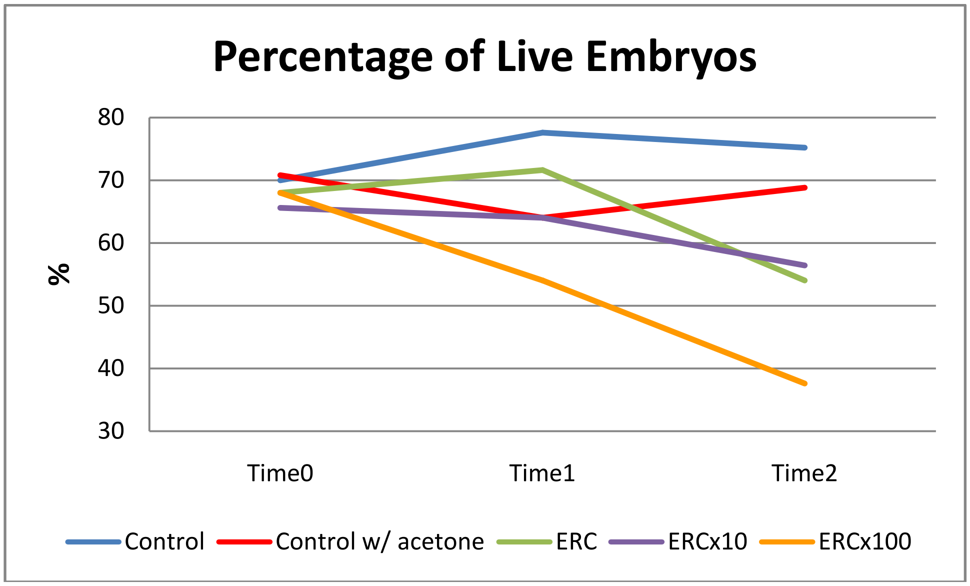

Figure 2: The percentage of live embryos during the experimental time series for control groups and PAH treatment groups. ERC= environmentally relevant concentration; ERCx10 = ten times the environmentally relevant concentration; ERCx100 = one hundred times environmentally relevant concentration.

Treatment	Treatment	Mean Difference	p-value
Control	Control w/acetone	-0.8	0.861
	ERC	2	0.662
	ERCx10	4.4	0.341
	ERCx100	2	0.662

Table 2: Results of analysis of variance testing the difference between the mean percentage of live embryos in different treatments at time0.

After approximately one hour (time1), the mean percentage of live embryos diverged (Figure 2). At this time, there was a significant difference between the control and the ERCx100 treatment (Table 3).

Treatment	Treatment	Mean Difference	p-value
Control	Control w/acetone	13.6	0.065
	ERC	6	0.4
	ERCx10	13.6	0.065
	ERCx100	23.6	0.003

Table 3: Results of analysis of variance testing the difference between the mean percentage of live embryos in different treatments at time1.

After approximately two hours, the divergence in the percentage of live embryos increased between the control group and all PAH treatments (Figure 2). By this time, there was a significant difference ($p < 0.05$) between the control mean percentage of live embryos and the means for the ERC, ERCx10, and ERCx100 treatments, indicating a significant PAH impact on the oyster embryos' survival (Table 4).

Treatment	Treatment	Mean Difference	p-value
Control	Control w/acetone	6.4	0.222
	ERC	21.2	0
	ERCx10	18.8	0.001
	ERCx100	37.6	0

Table 4: Results of analysis of variance testing the difference between the mean percentage of live embryos in different treatments at time2.

<u>Transformation from Embryos to D-Stage Larvae</u>

The transformation of the embryos to healthy D-stage larva occurred in varying degrees (Figure 3). The variety of abnormalities was relatively equal in the control group, while convex hinge and incomplete shell abnormalities appeared in the other groups to varying degrees. The vast majority of the abnormalities in the ERCx100 treatment were incomplete shells.

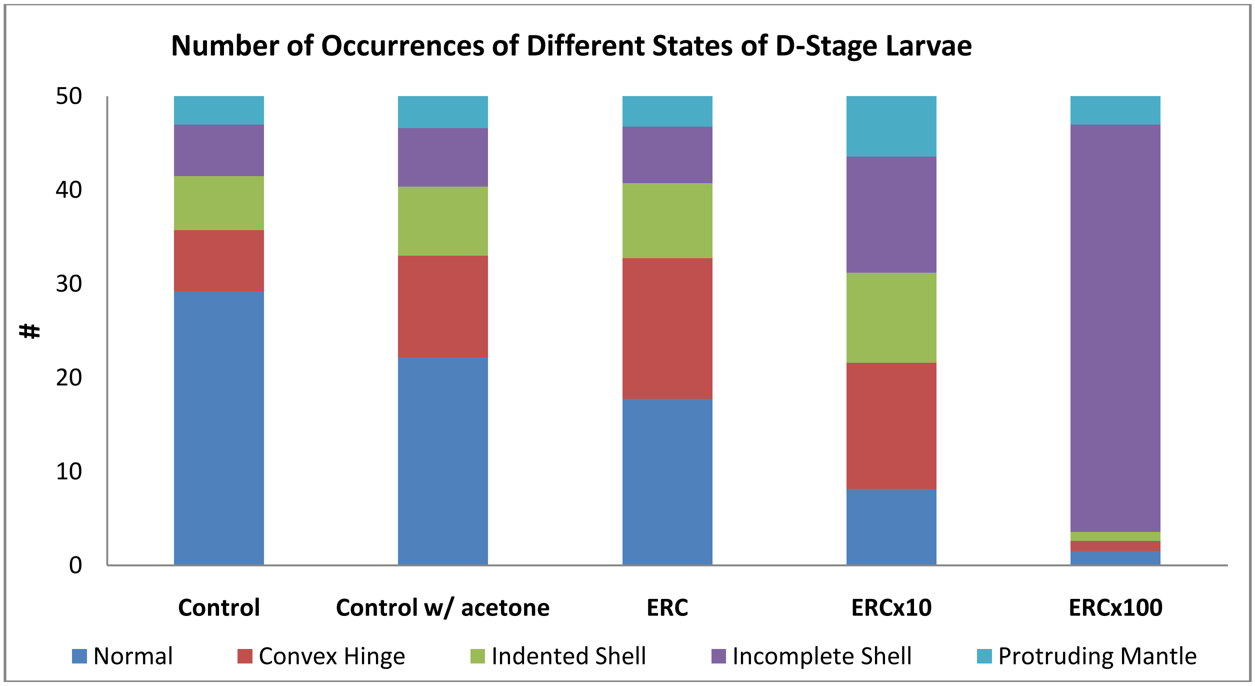

Figure 3: The number and types of abnormalities occurring during the transformation from oyster embryos to D-Stage larvae in the control groups and different PAH treatments.

The percentage of abnormalities ranged from 41.5% in the control group, 55.6% in the control group with acetone, 63.2% in the ERC treatment, 83.6% in the ERCx10 treatment, and 96.8% in the ERCx100 treatment. There were significant differences ($p < 0.05$) in this percentage between the control group and all of the other treatments (Table 5).

Treatment	Treatment	Mean Difference	p-value
Control	Control w/acetone	-14.1	0
	ERC	-21.7	0
	ERCx10	-42.1	0
	ERCx100	-55.3	0

Table 5: Results of analysis of variance testing the mean difference between the percentage of abnormalities occurring in oysters during the transformation of embryos to D-stage larva.

<u>Spat Settlement Success</u>

The mean number of spat that set on the PVC plate was highest in the control group (132) and decreased steadily in the control with acetone group (115), the ERC PAH treatment (89), the ERCx10 PAH treatment (52), and the ERCx100 PAH treatment (28) (Figure 4).

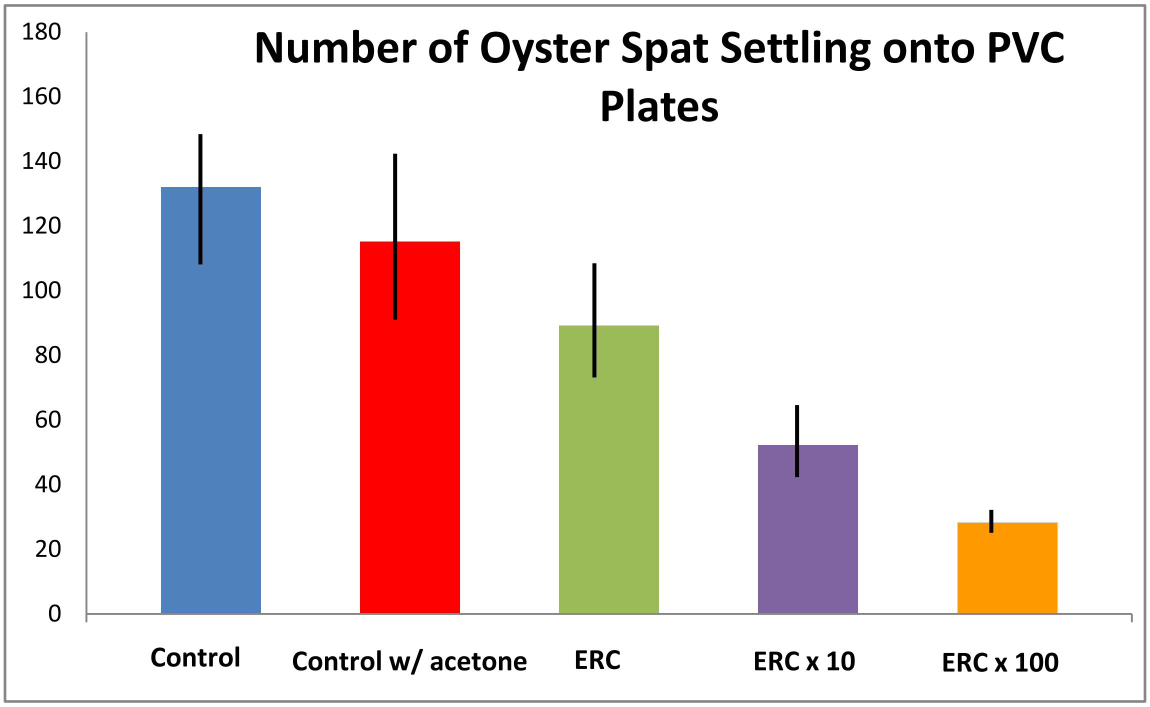

Figure 4: The number of oyster spat settling onto PVC plates in the control groups and different PAH treatments. Black vertical lines indicate the standard deviation of each group.

The ANOVA statistical test indicated significant differences ($p < 0.05$) for the mean number of oysters setting for the control group and the ERCx10 and ERCx100 treatments (Table 6). There was very nearly a significant difference between the mean of the control and the ERC treatment ($p = 0.083$).

Treatment	Treatment	Mean Difference	p-value
Control	Control w/acetone	16.8	0.483
	ERC	42.8	0.083
	ERCx10	79.8	0.003
	ERCx100	103.82	0

Table 6: Results of analysis of variance testing the mean difference between spat set in the control groups and treatment groups.

CONCLUSIONS

The results of the three experiments indicate significant negative impacts of a PAH cocktail on various early life stages of *C. virginica*. Especially noteworthy is the fact that a concentration of this cocktail that is similar to that found in road surface runoff had a deleterious effect on fertilization success, larval transformation, and spat settlement success. However, several issues should be discussed regarding the relevance and real-world application of the laboratory results.

The first issue relates to the fact that the PAH cocktail was an acetone-based solution. Acetone is a toxic chemical in itself and may have had impacts on the experimental results that would not be part of a 'real-world' PAH situation. With this in mind, the researchers ran a separate set of controls that contained the highest volume of acetone of any of the PAH treatments (same as the ERCx100). In order to assess the effects of the acetone alone, the researchers ran an ANOVA comparing the control data to the control plus acetone data. There were two instances when the control data was significantly different than the control plus acetone data. In the fertilization test at time1, the percentage of live embryos in the control plus acetone group decreased, while that of the control group increased from time0 ($p = 0.065$). This separation in the data of the two groups narrowed considerably at the time2 sampling ($p = 0.22$). At time1, the control plus acetone group was not significantly different from the PAH treatment groups; however, at time2 there were significant differences between the control plus acetone group and all of the PAH treatments. This may indicate one of two things: 1) an initial effect of the acetone on the fertilization success of the oyster, followed by a compounded effect of the PAH cocktail; or 2) a problem with two of the counts from the time1 control plus acetone group, as the other three counts were very comparable from time0 to time2. The control plus acetone group was also significantly different from the control group in the D-stage transformation experiment. The control group in this experiment was significantly different than the control plus acetone group as well as all of the PAH treatments. In addition, the control plus acetone group was significantly different than all of the PAH treatments. These results certainly indicate a negative effect of the acetone relative to the normal transformation into D-stage larva, but also that the PAH cocktail had a significant negative impact beyond that of the acetone.

The second issue involves the interaction of PAHs and UV-radiation. A number of studies have demonstrated that PAH toxicity increases with UV-light exposure (Pelletier et al., 1997; Lyons et al., 2002). In our study, the first two experiments were run under bright fluorescent light conditions, while the spat settlement experiment had to be conducted in the dark. It would be expected that both light conditions would result in less severe consequences for the particular oyster life stages examined in this study. Nonetheless, significant negative impacts of the PAHs were documented in each of the three experiments. The PAH/UV interaction may be lessened in an environment such as the Chesapeake Bay, due to the estuary's turbidity and poor light attenuation.

In a study conducted in Rhode Island, rainwater flowing primarily off an interstate highway contained 14 different PAHs, with phenanthrene and fluoranthene dominating the concentration (Hoffmann et al., 1984). The study analyzed stormwater from four primary land use categories: residential, commercial, highway, and industrial. The highway and industrial sites showed significantly higher loads of PAHs than the other two categories. The total urban runoff accounted for approximately 71% of the higher molecular weight PAHs and 36% of the total PAHs. The largest PAH load in the stormwater was associated with particulate matter rather than in a soluble state (79-93%). A second study by this group focused more rigorously on the highway contribution and reported that highways contribute >50% of the total PAH load entering the Pawtuxet River (Hoffman et al., 1985). These results are pertinent to our study in the documentation of the significance of runoff from highways as a large source of PAHs to waterbodies and indicate that subjecting the oyster life stages to a soluble form of PAHs is more simplistic than what actually happens in nature since a majority of the PAHs are associated with particulate matter.

The final concern in the experimental design is related to the PAH concentration of the treatments. The researchers intended to create an environmentally relevant concentration (ERC), a concentration comparable to runoff water or receiving water bodies after a rain event. In addition to this ERC treatment, several higher concentrations were used to test the effects of the elevated PAHs. Table 7 compares the various PAH concentrations used in this study and those found in several other studies.

PAH	**Stock**	**Working Sltn**	**100X**	**10X**	**ERC**	**Road Runoff**	**Dissolved Runoff**	**Dissolved PAHs**
						Maryland, USA	Beijing, China	Chesapeake Bay
	Conc.	**(7:100 Dil.)**	**Treatment**	**Treatment**	**Treatment**	(Anderson, Larsen & Lacouture, 2010)	(Zhang et al., 2008)	(Bamford et al., 1999)
	(ng/mL)	(ng/mL)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)
Napthalene	10400.0	728.0	1092.0	109.2	10.9	132.0	38.5	
1Methylnapthalene	3590.0	251.3	377.0	37.7	3.8	35.0		
Biphenyl	166.0	11.6	17.4	1.7	0.2	8.0		
Acenapthylene	682.0	47.7	71.6	7.2	0.7	4.0	4.5	0.8
Acenapthene	1.0	0.1	0.1	0.0	0.0	3.0	10.2	5.5
Fluorene	1210.0	84.7	127.1	12.7	1.3	9.0	25.8	5.0
1Methylfluorene	0.1	0.0	0.0	0.0	0.0	1.0		
Dibenzothiophene	51.5	3.6	5.4	0.5	0.1	0.0		
Phenanthrene	8800.0	616.0	924.0	92.4	9.2	68.0	112.7	7.4
Anthracene	909.0	63.6	95.4	9.5	1.0	5.0	24.3	1.6
2Methylanthracene	210.0	14.7	22.1	2.2	0.2	1.0		
9Methylanthracene	13.6	1.0	1.4	0.1	0.0	2.0		
Fluoranthene	5180.0	362.6	543.9	54.4	5.4	30.0	136.9	8.9
Pyrene	2180.0	152.6	228.9	22.9	2.3	17.0	86.2	6.6
Napthacene	16300.0	1141.0	1711.5	171.2	17.1	4.0		
Benzo[b]fluoranthene	2040.0	142.8	214.2	21.4	2.1	27.0	15.1	
Benzo[k]fluoranthene	2060.0	144.2	216.3	21.6	2.2	23.0	10.0	
Benzo[e]pyrene	867.0	60.7	91.0	9.1	0.9	18.0		
Perylene	14.1	1.0	1.5	0.1	0.0	4.0		
Indone[1,2,3-cd]pyrene	1520.0	106.4	159.6	16.0	1.6	9.0	2.0	
Dibenz[a,h + a,c]perylene	8.8	0.6	0.9	0.1	0.0	1.0		
Benzo[g,h,i]perylene	4710.0	329.7	494.6	49.5	4.9	17.0	1.7	
Anthanthrene	158.0	11.1	16.6	1.7	0.2	2.0		
Corenene	11.1	0.8	1.2	0.1	0.0	11.0		

Table 7: List of PAHs used in the study and their concentrations in the treatments compared to several other studies.

The concentrations of the PAHs in the ERC treatment used in the current study were similar to the concentrations found in water from the Patapsco River, a highly urbanized subestuary of the upper Chesapeake Bay (Bamford et al., 1999). The PAH concentrations in the ERCx10 treatments were closest to the PAH concentrations in road runoff in several other studies (Zhang et al., 2008; Anderson et al., 2010). These elevated concentrations were measured in the water running off of road surfaces prior to being diluted in the catchment water of the specific waterbody. The researchers are, thereby, able to conclude that the PAH concentrations used in this study accurately reflect concentrations dissolved in a local, urbanized subestuary and concentrations typical of road runoff. The results attained in this study for the ERC and ERCx10 treatments are, therefore, reflective of what might be expected in real-world situations. It is, therefore, possible to state that various early life stages of the Eastern oyster can be negatively impacted by rainwater running off road surfaces. These results call for the proper design of roadways neighboring aquatic ecosystems. The design and implementation of road runoff control systems and buffering techniques are vital in limiting the pollutants' path to aquatic systems.

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GLOSSARY of TERMS
(All definitions come from Wikipedia or Wiktionary.)

Acetone - is the organic compound with the formula $(CH_3)_2CO$, a colorless, mobile, flammable liquid, the simplest example of the ketones. **Acetone** is miscible with water and serves as an important solvent in its own right, typically as the solvent of choice for cleaning purposes in the laboratory.

ANOVA test - in statistics, analysis of variance (**ANOVA**) is a collection of statistical models, and their associated procedures, in which the observed variance in a particular variable is partitioned into components attributable to different sources of variation. **ANOVA**s are useful in comparing two, three, or more means.

Anthropogenic - having its origin in the influence of human activity on nature.

Biota - are the total collection of organisms of a geographic region or a time period.

Biotic - **biotic** describes a living component of a community; for example organisms, such as plants and animals.

Bituminous - of or relating to **bituminous** coal.

Brood stock - are a group of mature individuals used in aquaculture for breeding purposes.

Carcinogenic - a **carcinogen** is any substance, radionuclide, or radiation that is an agent directly involved in causing cancer.

Embryo - an **embryo** is a multicellular diploid eukaryote in its earliest stage of development, from the time of first cell division until birth, hatching, or germination.

Embryogenesis - **embryogenesis** is the process by which the embryo is formed and develops.

Embryotoxicity - any toxicity that affects an embryo.

Endocrine disruptors - endocrine disruptors are substances that “interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for development, behavior, fertility, and maintenance of homeostasis (normal cell metabolism).”

Estuary - is a partly enclosed coastal body of water with one or more rivers or streams flowing into it, and with a free connection to the open sea.

Larva - a **larva** (plural larvae) is a distinct juvenile form many animals undergo before metamorphosis into adults.

Leachate - is a widely used term in the environmental sciences where it has the specific meaning of a liquid that has dissolved or entrained environmentally harmful substances which may then enter the environment.

Motile - capable of movement or demonstrating movement by independent means.

Mutagenic - a **mutagen** is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level.

Particulate - also known as **particulate matter** (**PM**), **suspended particulate matter** (**SPM**), fine particles – are tiny subdivisions of solid matter suspended in a gas or liquid.

Polycyclic Aromatic Hydrocarbons (PAHs) - **Polycyclic aromatic hydrocarbons** (**PAH**s), also known as **poly-aromatic hydrocarbons** or **polynuclear aromatic hydrocarbons**, are potent atmospheric pollutants that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. Naphthalene is the simplest example of a PAH. PAHs occur in oil, coal, and tar deposits, and are produced as byproducts of fuel burning (whether fossil fuel or biomass). As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic.

Spat - settled larvae of shellfish such as oysters and scallops.

Turbidity - is the cloudiness or haziness of a fluid caused by individual particles (suspended solids) that are generally invisible to the naked eye, similar to smoke in air.

UV-radiation - is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than X-rays, in the range 10 nm to 400 nm, and energies from 3 eV to 124 eV. UV light is found in sunlight and is emitted by electric arcs and specialized lights such as black lights.