1 **PREDICTING PAH TOXICITY TO GRASS SHRIMP (*P. pugio*)**

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PREDICTING SURVIVAL DURING ETHYLNAPTHALENE, DIMETHYLNAPHTHALENE AND PHENANTHRENE EXPOSURES DIFFERING IN CONCENTRATION AND DURATION

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1 **This paper is contribution XXXX of the Virginia Institute of Marine Science,**

2 **College of William and Mary.**

1 **Abstract-**Both exposure duration and concentration determine lethal consequences
2 of polycyclic aromatic hydrocarbons (PAH) released during oil spills. Many
3 factors, such as weathering, tidal transport and the addition of surfactants, can
4 change the composition of individual dissolved compounds and the duration of
5 exposure over which an individual is exposed. Modern analytical techniques are
6 now capable of quantifying the chemical composition and relative persistence of the
7 aqueous soluble components in oil that lead to toxic effects. However, conventional
8 toxicity testing methods produce effect metrics such as the median lethal
9 concentration (LC50) that are not amenable to predicting toxic effects at all toxicant
10 exposure durations likely to occur during a spill. In the present study, survival time
11 models were used that implicitly include toxicant exposure duration and
12 concentration to predict time-to-death for grass shrimp, *Palaemonetes pugio,*
13 exposed to three PAH (1-ethylnaphthalene, 2,6-dimethylnaphthalene, phenanthrene)
14 commonly found in the water soluble fraction derived from oil. Conventional 48h
15 LC50 values were also calculated for the compounds (ethylnaphthalene, 295 μg/L,
16 dimethylnaphthalene, 500 μg/L, and phenanthrene 360 μg/L). In contrast, survival
17 models and associated response surfaces predicted the proportions dying of shrimp
18 exposed for various times throughout the exposure period.

19

20 **Keywords-** survival analysis, oil spill, PAH, toxicity, grass shrimp

INTRODUCTION

To predict the lethal impact of oil spills, it is important to understand the fate and effects of the individual compounds that make up these complex mixtures. To this end, researchers have studied the composition and relative toxicities of the various aliphatic, aromatic, and heterocyclic compounds released into waters from oil spills [1, 2]. Although an understanding of qualitative and quantitative chemical characteristics of the oil spill is important, the lethal effect that exposure duration has on receptors must also be quantified. Tidal variations, weathering, and the application of surfactants can all alter the relative concentration and length of a toxicant exposure. Better predictive models are needed to assess the potential short-term and long-term effects to biota exposed to oil derived toxicants. Survival models that implicitly include durations as well as concentrations allow managers to make more effective spill response and remediation decisions [3].

Most toxicity tests use a concentration-effect design that produces a lethality metric, such as an LC50 at a set exposure time, e.g., 96 h. A more encompassing approach exists to predict lethal consequences from realistic exposures including pulsed, short term and long-term exposures resulting from oil spills. Occasionally, a few test durations might be used to coarsely predict how mortality changes with exposure time. Gross prediction is inevitable if mortality information was collected for only a few intervals. Temporal information is not included in the calculation of individual LC50 values and the test concentrations are optimized for only one duration.

1 A further compromise arising in the conventional approach, mortality occurring
2 after exposure ends is not included in conventional predictions. In the few studies
3 that quantified post-exposure mortality, it was found to vary widely and could be
4 quite high [4, 5].

5 The conventional approach is limited relative to accurately predicting all
6 mortality from pulsed exposures or for exposure durations other than that used in the
7 test. These weighty shortcomings impede accurate prediction of effects from spilt oil
8 exposures that can vary in both duration and concentration through time, and that
9 require prediction of all mortality resulting from a range of plausible exposure
10 scenarios.

11 The main goal of the present work was to develop better models to predict the
12 toxic effects of water-soluble polycyclic aromatic hydrocarbons (PAH) derived from
13 spilt oils. We conducted toxicity experiments with the grass shrimp, *Palaemonetes*
14 *pugio,* exposed to three PAH (1-ethylnaphthalene, 2,6-dimethylnaphthalene, and
15 phenanthrene) representative of those found in the water soluble fraction derived
16 from oil [6]. Results from these exposure experiments were modeled with survival
17 time methods [7] to produce models for each compound that predicted proportion of
18 individuals dying during and after an exposure of specified duration and
19 concentration. In addition, dosed shrimp were collected and analyzed for PAH
20 concentrations post mortem to determine if accumulated PAH were consistent with
21 a critical body burden at time of death.
22
23

MATERIALS AND METHODS

Grass shrimp collection and maintenance

Grass shrimp were collected locally from the York River and Back River, and were maintained in the laboratory in filtered York River water for at least 2 weeks prior to being use in the exposures. Shrimp were fed daily with Tetramin® Tropical flake food (Tetra Holding, Blacksburg, VA). Individual shrimp with no outward signs of damage or disease were gently placed into glass tubes and the exposure aquaria one day before exposures began.

PAH survival analysis experiments

Range finding tests were conducted in May-August 2005 to determine the concentration ranges that were used in the survival time experiments. The survival analysis experiments were conducted in August (ethylnaphthalene), October (dimethylnaphthalene), and November (phenanthrene) of 2005. Three replicates of four PAH concentrations and a control were prepared from saturated solutions generated with filtered York River water by techniques described in detail below. To minimize toxicant volatilization, exposure chambers were not aerated during the experiment and solutions were renewed every 12 h. All experiments were conducted under constant fluorescent light. Experimental chambers were constructed from 25 cm x 50 cm x 58 cm glass aquaria with glass lids. A water-tight glass partition was

1 installed down the center of each aquarium to create tandem, 30 L exposure
2 chambers 25 cm x 25 cm x 58 cm tall. This design reduced the surface area to
3 volume ratio to minimize volatile losses of the PAH. One side of each aquarium was
4 used to expose the test organisms and the other was used to prepare the new test
5 solution every 12 h. Individual grass shrimp were placed in 2.8 cm x 10.8 cm, x 40
6 mL glass vials with an open ended screw cap fitted with a stainless steel mesh
7 screen on one end. The vials were suspended in the exposure chambers on
8 aluminum racks to facilitate monitoring the test organism's condition and to allow
9 easy transfer of the test organisms to newly prepared solutions every 12 h. Shrimp
10 were monitored for mortality every 4 h and scored as dead if no appendage
11 movement was apparent. All dead shrimp were removed, weighed, and frozen.
12 Shrimp alive after the exposure period (ethylnaphthalene, 48 h; dimethylnaphthalene
13 and phenanthrene, 60 hours) were transferred to clean, filtered, and aerated York
14 River water. The water was renewed every 12 h and shrimp were routinely
15 monitored for latent mortality for 48 h post exposure, or until no latent mortality
16 was apparent. At the completion of the test, all shrimp were weighed and frozen.
17
18 *Generating PAH solutions*
19
20 A generator column was used to produce the saturated PAH solutions to
21 avoid the use of solvent carriers for the time-to-death exposure experiments.
22 Because up to 350 L of saturated solution was required for each water renewal, a
23 large 7.5 cm x 59.2 cm aluminum generator column was fabricated. It was packed

with 3.8 kg of wet sieved (> 250 um) sand that had been dried at 110° C overnight and ignited at 550° C for 4 hours prior to use. The sand was coated (0.1% to 0.7% w/w) with the analyte dissolved in an organic solvent (pentane or dichloromethane). The solvent was evaporated and the column was then dry packed with the coated sand. The column was fitted with pressure sealed end caps and ¾" NPT fittings and connected to a water pump (Poseidon PS3 "Silent" Titanium, Bayside Aquarium Supply, Anaheim, CA) fed by sand filtered, aerated, 20° C York River water. Column flow was adjusted to 2.0 L/minute by a ¾" gate valve. Prior to the dosing experiments, the solution flowing from the column was sampled and analyzed after flushing ~10 liters to determine the actual concentration of the saturated effluent. Aerated York River water was mixed with the saturated solution directly in the dosing chambers to produce the four dosing concentrations. Contaminated or uncontaminated York River water was transferred from tanks via dedicated water pumps (Poseidon PS3 "Silent" Titanium). The tandem 30 L test aquaria permitted the filling of one side with new exposure water and transferring of the shrimp in racks from old to renewed water with minimal disturbance. Wastewater was pumped from aquaria to a reservoir and then passed at 500 mL/minute over a 20 L activated carbon column to remove any PAH before disposal.

Water chemistry

Old and fresh test solution water chemistries were measured every 12h and included temperature, dissolved oxygen, salinity, and pH. A Hydrolab^{TM} Surveyor 4a (Hydrolab Corp., Austin, TX) was used for these measurements. Unfiltered water samples were also collected and frozen for ammonia analyses (SKALAR SAN plus System continuous flow autoanalyzer using the phenol method).

PAH Analysis of water samples

Exposure waters were analyzed for PAH concentrations using a High Performance Liquid Chromatograph (HPLC) with a fluorescence detector (Waters 600E Controller, Waters 474 Fluorescence Detector, Waters 717 plus Autosampler with a 200 uL loop and a VYDAC C-18 column). The controller was programmed to run a gradient with water/acetonitrile starting with 100% HPLC grade water to 70% acetonitrile at a flow of 1.0 mL/minute over 35 minutes. The fluorescence detector excitation wavelength was 265 nm and the emission wavelength was 370 nm for all PAH analytes. One hundred uL of aqueous sample spiked with an internal standard(1-methyl naphthalene) was injected by the autosampler. Data from the fluorescence detector was collected and analyzed using Hewlett Packard ChemStation Software and then stored on a HP Net Server LC 3. Calibration of the HPLC method was performed for each PAH prior to sample analysis using an internal standard method and a seven point calibration curve. Prior to the start of the shrimp scoping/exposure experiments, calibration standards were made in York River water from stock solutions of internal standard (1-methyl naphthalene @ 0.38

ug/mL) and the PAH analyte of interest. Following calibration, 4.0 mL water samples collected from the generator column, stock tanks, shrimp exposure tanks (time 0 & 12 hours) and waste stream were added to a vial containing 0.15 mL of the internal standard in acetonitrile. The vials were immediately analyzed or frozen to minimize volatility losses. Once the analyses for a particular exposure were completed, the calibration of the HPLC was again verified with fresh standards.

PAH Analysis of tissue samples

Shrimp tissues were analyzed for PAH concentrations by mass spectrometry using selective ion monitoring (MS-SIM). Individual shrimp were weighed, rinsed with DI water and placed into a 50 mL Teflon centrifuge tube containing 2.0 mL of concentrated HCL and 500 ng of deuterated PAH surrogate standards. The shrimp were homogenized with a spatula and ultrasonicated for 10 m. The aqueous homogenate was extracted with two aliquots of hexane (2.0 mL each), centrifuging between extractions to separate the layers. The combined hexane extracts were reduced to 0.1 mL under dry nitrogen and 0.6 ug of p-terphenyl internal standard was added before analysis on a Varian Saturn 4D GC/MS/MS ion trap mass spectrometer operated in electron ionization mode (EI). Analytes and ions monitored were; p-terphenyl I.S. [152+230], naphthalene-d8 [108+135+136], acethylnaphthalenehthene-d10 [160-165], phenanthrene-d10 [187-189], chrysene-d12 [239-241], perylene-d12 [263-266], 1-ethylnaphthalene[141+155+156], 2,6-dimethylnaphthalene[141+155+156] and phenanthrene[176-179]. Six point

1 calibration curves were generated for each analyte and identifications were based on
2 retention time and matches to library spectra.
3
4 *Calculating LC50 values*
5
6 The measured PAH exposure concentrations, number of dead shrimp and
7 total exposed shrimp were fitted by maximum likelihood estimation to a log normal
8 model with the PROBIT procedure in the SAS software (SAS Corp., Cary, NC).
9 Spontaneous mortality was included in the log normal model because low levels of
10 mortality (≤ 6%) occurred in the control aquarium shrimp during exposures. The 48
11 h LC50 values and associated 95% fiducial limits were estimated with these models.
12 Initially, log logistic and log normal models with spontaneous mortality were
13 explored but, based on the associated X^2 statistics, the log normal was selected as the
14 best fitting model.
15
16 *Survival models*
17
18 Survival time was modeled as a function of PAH concentration using mean
19 concentrations in each exposure tank and the SAS LIFEREG procedure (SAS Corp.,
20 Cary, NC). The general approach was that described in detail in previous
21 publications e.g. [7]. Initially, models predicting time-to-death for shrimp as a
22 function of ln of the PAH concentrations were produced with three candidate
23 models: log normal, log logistic, and Weibull. Akaike's information criteria (AIC)

were then used to select the generally best among these candidate models (see Newman [7] for details). The log logistic model was chosen to predict survival in an exposed population based on exposure concentration,

where TTD = the predicted time-to-death for a specified proportion of the exposed shrimp, μ = the MLE-estimated intercept, β = the estimated coefficient for the influence of ln of PAH concentration on time-to-death, σ = the MLE-estimated scale parameter, and W = the response metameter for the model distribution associated with the proportion dying (P) of the exposed shrimp for which prediction is being made. The W can be generated by special functions within most statistical or spreadsheet software, or taken from tables such as Appendix Table 7 in Newman [7]. By changing W, the various combinations of exposure concentration and duration can easily be found that result in P of the exposed shrimp dying. However, prediction is only recommended within the range of concentrations and durations used in the tests from which the data were generated.

RESULTS

1 *Water chemistry*

2

3 Table 1 summarizes the temperature, salinity, dissolved oxygen, pH and
4 ammonia for the three exposure experiments. Measurements were taken for both
5 freshly prepared solutions and 12 h-exposed test solutions. Measured parameters
6 were within a narrow range for all exposure experiments.

7

8 *Toxicant concentrations*

9

10 The measured PAH concentrations are summarized in Table 2. Saturated
11 solutions prepared by the generator column technique had the following mean
12 concentrations: ethylnaphthalene 6510 ug/L, dimethylnaphthalene 630 ug/L and
13 phenanthrene 500 ug/L and proved to be the practical upper limit for dosing
14 concentrations for dimethylnaphthalene and phenanthrene. Variation was present in
15 PAH concentrations because they were measured in both the newly prepared and the
16 12 h old solutions. Average loses during the 12 h were: ethylnaphthalene 9%;
17 dimethylnaphthalene 17%; phenanthrene 15%. The PAH concentrations in all
18 control samples were below detection limits (1 ug/L) during the exposures.
19 Problems with incomplete mixing in some of the initial ethylnaphthalene replicates
20 produced an unacceptable range in concentrations and increased variance in some
21 replicate treatments. These outlier replicates were not included in the survival
22 analysis. Careful mixing in subsequent experiments alleviated this problem.

23

Survival analysis

There was no apparent latent mortality for the ethylnaphthalene and dimethylnaphthalene experimental shrimp. Once shrimp were placed in clean water they rapidly revived and returned to what appeared to be normal behavior. During the phenanthrene test, six shrimp died in the first 24 h post exposure. This represented < 5% of the total mortality that occurred during the exposures. Conventional LC50 values and 95% fiducial limits were calculated and were: ethylnaphthalene 295 µg/L (162-331), dimethylnaphthalene 500 µg/L (463-535) and phenanthrene 360 µg/L (333-402). Survival data were fitted to accelerated failure time models with the candidate survival time distributions of log normal, log logistic, and Weibull. For the three compounds tested, the log logistic model was selected as the best choice based on the results of the Minimum AIC Estimation (MAICE). Contours of predicted mortality for exposed shrimp were developed from the models for each compound (Figure 1A-1C). Conventional LC50 values and 95% fiducial limits are also included in Figure 1.

Tissue concentrations

PAH concentrations (wet weight) were measured in select whole shrimp that died during the exposure experiments. Ethylnaphthalene concentrations spanned a wide range from 14 µg/g in the 300 µg/L treatment to over 100 µg/g in the 525 µg/L treatment. Tissue concentrations measured in shrimp from the 300 µg/L and 450 µg/L ethylnaphthalene treatments are presented in Figure 2 for comparison. Tissue

concentrations measured in dimethylnaphthalene and phenanthrene exposed shrimp also showed dose dependence and a corresponding wide range of values. Shrimp from the dimethylnaphthalene experiment ranged from 18 µg/g to 98 µg/g and phenanthrene exposed shrimp ranged from 30 µg/g to 500 µg/g at time of death.

All three PAH were eliminated rapidly from the shrimp post exposure. Body burdens measured in phenanthrene exposed shrimp that died during the depuration phase showed an exponential decrease in concentration with time. Elimination rate constants were calculated for each PAH from the slope of the log transformed concentration data (Figure 3). Corresponding half-lives ranged from 5.3-7.8 hours.

DISCUSSION

These apparent solubility values for ethylnaphthalene, dimethylnaphthalene and phenanthrene measured in our experiments were 36-63% lower than those reported previously for these compounds in freshwater [8]. This discrepancy likely resulted in part from reduced solubility in the higher salinity of the 20 $^{o}/_{oo}$ estuarine water used to make the test solutions. This trend has been documented for hydrophobic organic compounds in saline water [9] and should be considered when conducting or evaluating toxicity experiments for estuarine species with hydrophobic organic compounds near solubility limits. Although salinity has a significant effect on PAH solubility it has been shown to have little effect on the toxicity or bioaccumulation of PAH to larval *P. pugio* [10].

Conventional LC50 values calculated for *P. pugio* were: ethylnaphthalene 295 µg/L (162-331), dimethylnaphthalene 500 µg/L (463-535) and phenanthrene 360 µg/L (333-402). Surprisingly, there is little information in the literature on the acute toxicity of these individual PAH to *P. pugio* for comparison. Tatem and Anderson [11] did report a 48 h LC50 concentration of 700 µg/L for *P. pugio* exposed to mixed dimethylnaphthalenes that is in reasonable agreement with our results for 2,6 dimethylnaphthalene (500 µg/L). Looking at other aquatic crustaceans, 48h LC50 toxicities of 617-780 µg/L were reported for dimethylnaphthalenes to the copepods *Eurytemora affinis* [12] and *Oithona davisae* [13]. Similar to the trend we found for *P. pugio*, the 48 h LC50 value for phenanthrene (522 µg/L) was lower than the dimethylnaphthalene LC50 (617 µg/L) for *O. davisae* [13].

The PAH concentrations measured in whole shrimp at time of death varied by a factor of 5 and showed an increasing trend with dose (Figure 2). Our acute exposure experiments were designed to develop time to death models and not to address toxicokinetics so samples for tissue analysis were limited. However, these results suggest that body burdens from acutely toxic exposures are dose dependent and are not good predictors of time of death for the compounds and durations used in our experiments. There was a similar range in body burden concentrations for shrimp from the ethylnaphthalene (14 µg/g to 100 µg/g) and dimethylnaphthalene (18 µg/g to 98 µg/g) exposures but a higher range for the phenanthrene (30 µg/g to 500 µg/g) exposed shrimp. Previous work has shown that critical body residues of various PAH are similar on a molar basis (7.5 ± 2.6 µmol g^{-1}) when amphipods

(*Diporia sp.*) were exposed for 28 days [14]. We saw a similar trend of increasing body burden concentrations with increasing molecular weight but the range of body burdens at time of death for each compound was greater than the compound-to-compound differences. Further work is needed on a wide range of PAH to evaluate the utility of the critical body burden concept for predicting toxicity of short-term acute exposures like those occurring during oil spills.

For all three compounds, prediction modeling was expedited because there was very little latent mortality. This is consistent with the results of Zhao et al [5] and Newman and McCloskey [4] where minimal post exposure mortality was detected for pentachlorophenol-exposed *Hyallea azteca* and *Gambusia holbrooki* respectively.

Unlike the single LC50 value, the response surfaces generated with survival time models (Figure 1) predict what proportion of an exposed population would be killed for a given combination of exposure concentration and duration. This type of information can then be used to predict the consequences to resident populations based on different exposure scenarios generated from field observation of computer simulations. An example would be to determine the consequence from adding surfactant to an oil spill on an incoming tide. Although surfactants enhance oil dissipation and protect on-shore species, their use will also increase the dissolved PAH concentrations in waters because of diminished droplet size and increased solubilization. The duration of the increase will be a function of physical factors such as dilution from mixing and volatility. If the increase and duration in dissolved PAH concentrations can be predicted based on known physical parameters, the

manager can use survival models to estimate the increased risk to aquatic species and make an informed decision. Survival models can be expanded to include the combined effects from toxicant mixtures as well as other parameters that can influence toxicity such as temperature, salinity, and light intensity. Combined with appropriate physical modeling, survival models can become valuable management tools. It is the intention of the authors to explore these effects in the near future.

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3

1 Table 1. Water quality measurements in exposure aquaria.

	Exposure Experiment		
	Ethylnaphthalene	Dimethylnaphthalene	Phenanthrene
Temperature (°C)	20.6 ± 0.3, n = 38	20.6 ± 0.4, n = 50	20.5 ± 0.4, n = 50
Salinity (g/kg)	19.6 ± 0.1, n = 38	21.0 ± 0.1, n = 50	20.9 ± 0.2, n = 50
D.O. (mg/L)	7.0 ± 0.4, n = 38	7.0 ± 0.5, n = 50	7.0 ± 0.3, n = 50
pH*	7.78 (6.91-8.01), n = 38	8.05 (7.99-8.09), n = 50	7.89 (7.42-8.00), n = 50
Ammonia (mg/L)	0.05 ± 0.03, n = 38	0.19 ± 0.06, n = 50	0.16 ± 0.06, n = 50

2 *All values are mean ± standard deviation except pH values which are reported as

3 medians and ranges.

4

1 Table 2. Measured toxicant concentrations in exposure aquaria.

Toxicant concentration (µg/L) (mean ± standard deviation, number of samples)			
Treatment Replicate	Ethylnaphthalene	Dimethylnaphthalene	Phenenanthrene
Conc. 1A	*	300 ± 30, n = 10	150 ± 20, n = 10
Conc. 1B	310 ± 40, n = 8	300 ± 40, n = 10	150 ± 20, n = 10
Conc. 1C	310 ± 40, n = 8	290 ± 70, n = 10	160 ± 20, n = 10
Conc. 2A	400 ± 40, n = 6	380 ± 70, n = 10	220 ± 40, n = 10
Conc. 2B	*	380 ± 60, n = 10	230 ± 30, n = 10
Conc. 2C	370 ± 40, n = 8	390 ± 60, n = 10	230 ± 30, n = 10
Conc. 3A	490 ± 70, n = 8	470 ± 100, n = 10	300 ± 30, n = 10
Conc. 3B	440 ± 40, n = 8	470 ± 70, n = 10	300 ± 30, n = 10
Conc. 3C	480 ± 40, n = 6	480 ± 70, n = 10	300 ± 40, n = 10
Conc. 4A	*	570 ± 80, n = 10	380 ± 40, n = 10
Conc. 4B	490 ± 50, n = 6	590 ± 50, n = 10	390 ± 40, n = 10
Conc. 4C	530 ± 40, n = 6	570 ± 90, n = 10	400 ± 60, n = 10

2

3 *Replicate not used in survival analysis

4

1 **Figure legends**

2

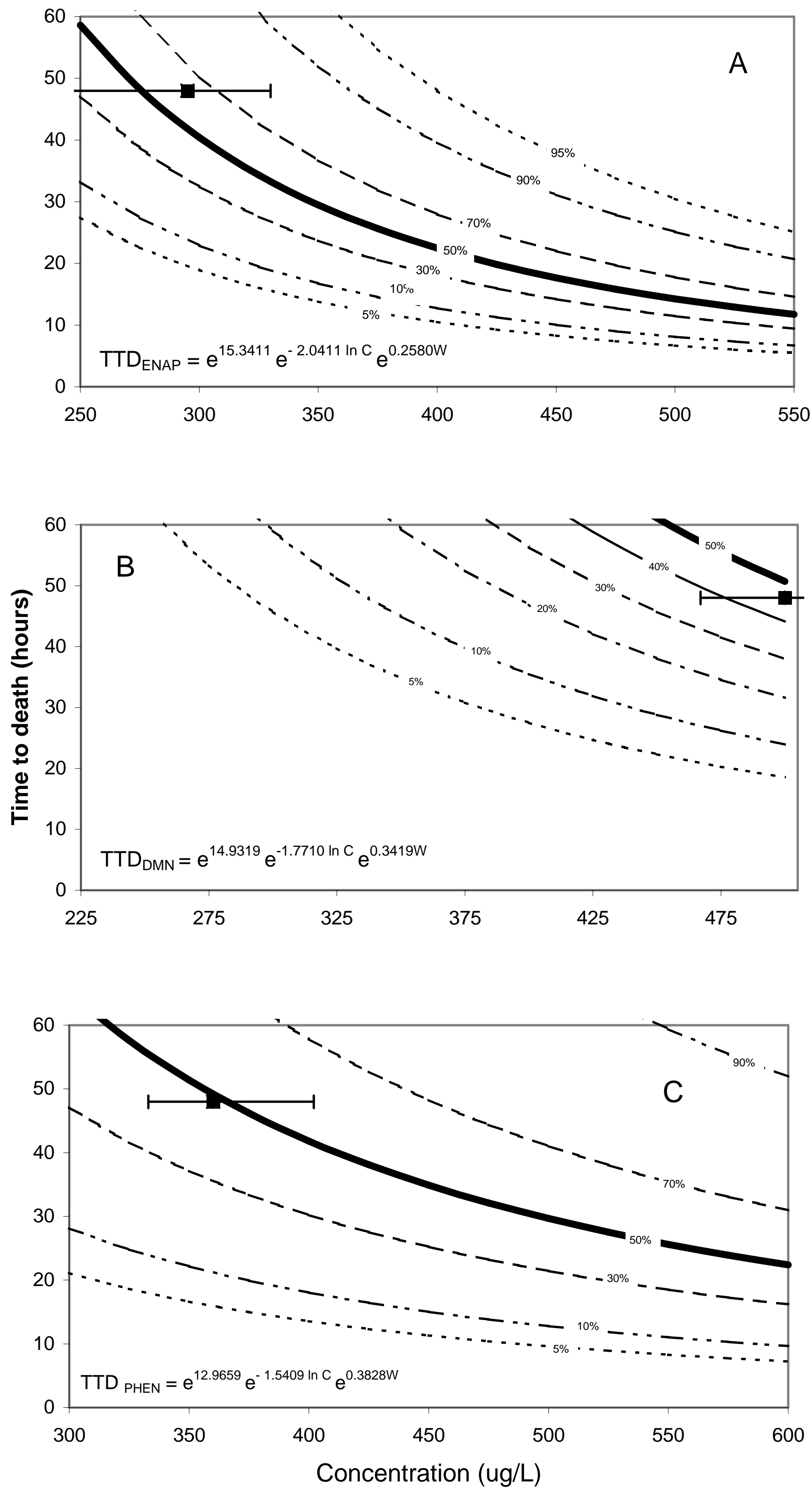
3 Figure 1. Response surfaces predicting mortality levels for *P. pugio* exposed to three
4 PAH, A) 1-ethylnaphthalene, B) 2,6-dimethylnaphthalene and C) phenanthrene.
5 The survival models from which predictions are made are also given in the figure.
6 Lines indicate different proportions dying predicted with the models for different
7 combinations of exposure concentration and duration. The 48-h median lethal
8 concentrations (LC50) and the 95% fiducial limits are shown for comparison.

9

10 Figure 2. Whole body concentrations (µg/g wet weight) of 1-ethylnaphthalene
11 measured in *P. pugio* at time of death. Shrimp from two exposure concentrations
12 (300 µg/L and 450 µg/L) ranged from 15-50 µg/g. A critical body burden was not
13 evident for the acute exposures of 1-ethylnaphthalene used in these experiments.
14 Similar trends were seen for 2,6-dimethylnaphthalene and phenanthrene.

15

16 Figure 3. Elimination rate constants (k) calculated for *P. pugio* exposed to 1-
17 ethylnaphthalene (n = 6), 2,6-dimethylnaphthalene (n = 6) and phenanthrene (n = 9).
18 Individual rate constants are shown on the figure. All three PAH were eliminated
19 quickly by the shrimp with corresponding half-lives of 7.8, 5.3 and 5.7 hours.


A
60
50
40
30
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10
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250
300
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400
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500
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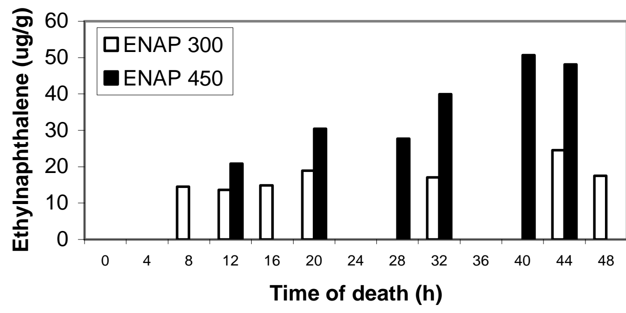
95%
90%
70%
50%
30%
10%
5%
TTD_ENAP = e^15.3411 e^-2.0411 ln C e^0.2580W
B
Time to death (hours)
225
275
325
375
425
475
50%
40%
30%
20%
10%
5%
TTD_DMN = e^14.9319 e^-1.7710 ln C e^0.3419W
C
300
350
400
450
500
550
600
90%
70%
50%
30%
10%
5%
TTD PHEN = e^12.9659 e^- 1.5409 ln C e^0.3828W
Concentration (ug/L)


ENAP 300
ENAP 450
Ethylnaphthalene (ug/g)
60
50
40
30
20
10
0
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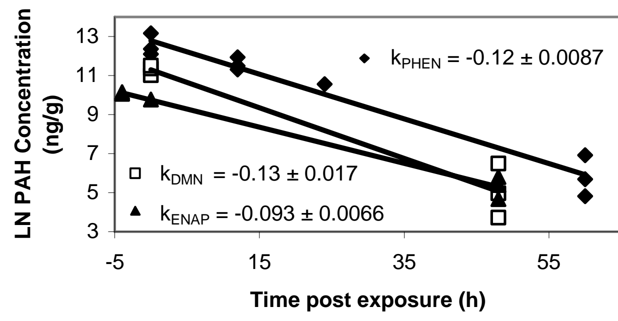
44
48
Time of death (h)


LN PAH Concentration (ng/g)
13
11
9
7
5
3
-5
15
35
55
Time post exposure (h)
k_{PHEN} = -0.12 ± 0.0087
k_{DMN} = -0.13 ± 0.017
k_{ENAP} = -0.093 ± 0.0066