PAH-INDUCED IMPACTS ON THE BIOENERGETICS AND POPULATION DYNAMICS OF *FUNDULUS HETEROCLITUS*: MODEL APPLICATION FOR ASSESSING LONG-TERM EFFECTS FROM OIL SPILLS[1]

Amy A. Merten[2,3], Eileen B. Beard,[3] and Joel E. Baker[3].

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ABSTRACT

Long-term residual contamination from oil spills and other sources of organic contaminants may exert sublethal baseline toxicity (narcosis) on fish populations. Low level, chronic exposures to narcotic chemicals such as polycyclic aromatic hydrocarbons (PAHs) may impose a metabolic cost on a fish, changing its allocation of energy available for growing, reproducing, foraging for food, and escaping predation. To examine this hypothesis, naïve Fundulus heteroclitus, a ubiquitous estuarine fish species with a short home range, were exposed to a gradient of PAH concentrations in their diets over 120 days. On days 0, 35, 62, 90, and 120, we measured individual fish growth parameters and standard metabolic rates. Standard metabolic rates increased with increasing PAH load (p = 0.087).

Long-term exposure to dietary PAHs altered fish maintenance respiration on individuals. We are developing a bioenergetic model to evaluate whether long-term exposure to PAHs sufficiently impacts individual parameters to the extent that the Fundulus population will be affected. Multiple life stages (e.g., eggs, larvae, juveniles, and adults) and generations will be represented with bioenergetic and bioaccumulative processes altered to mimic impacts measured in the experiment. The narcosis induction submodel subjects cohorts to metabolic alterations by imposing a narcotic gradient from no effect to a level where time to critical body residue shifts, thus changing population mortality probabilities. Each model scenario will be compared and contrasted to quantify PAH effects, and to discuss the importance of cumulative chemical stress to fish. Results from the study and model application will be used to evaluate long-term, population-level consequences of sublethal exposures to narcotic contaminants to improve the basis for risk assessment analyses.

INTRODUCTION

Fish living in contaminated environments, including those affected by long-term residual oil, are continuously challenged with chemicals at low levels that exert baseline toxicity, or narcosis, in higher concentrations. Under sublethal conditions, the fish may combat "sublethal narcosis," inducing a metabolic cost on the bioenergetics of the fish. Accumulation of non-polar narcotic chemicals in organisms alters their metabolic rates and, therefore their energetic demands. Under a long-term exposure scenario from residual oil, exposure to PAHs is primarily through the fish diet. We hypothesized that feeding on contaminated prey decreases metabolic rates caused by accumulation of narcotics which in turn reduces feeding, further decreasing exposure. Thus, dietary exposure to and accumulation of narcotics may cause a negative feedback, reducing net bioaccumulation and increasing time to critical body residue. These shifts in bioenergetics may have consequences at the population level.

The purposes of this paper are to present the results of long-term PAH exposure to fish through dietary uptake and to discuss modeling methods to further explore the cumulative effects of chemical stress on fish bioenergetics at the individual and population levels. We present the results of the laboratory experiment regarding PAH-contaminated food effects on the standard metabolic rate of *Fundulus heteroclitus*, and explore our findings using a population modeling approach. We describe the model development, and propose scenarios for evaluation.

METHODS

Experimental Component: We conducted a long-term (120 days) fish exposure experiment to examine the consequences on the bioenergetics of fish continuously challenged with sublethal levels of polycyclic aromatic hydrocarbons (PAHs). One half of the experiment was run with clean water, while the other half included low level aqueous exposure of MS222 (a model narcotic chemical). MS222 was used as a positive control for narcosis. We measured growth, standard metabolic rates, lipid content, and congener-specific PCB levels in individual fish. Although there were interesting interactions between the MS222 and PAH exposures, the results from the MS222 exposures and PCB tissue residues are published elsewhere (Merten, In Press).

Experimental Methods: The experimental design consisted of a 2x4x3 factorial, random block design. Fish were exposed to two levels of aqueous MS222 (a model narcotic chemical), four levels of food treatments, and sampled over five time points (0, 32, 65, 90, 120 days). Each treatment was run in triplicate for a total of twenty-four tanks. Food exposures consisted of feeding a mixture of clams (*Mercenaria mercenaria*) and fish gel at different levels of contamination. Clean clams were purchased from a local commercial fisher, collected from the Chester River, Maryland, USA.

1 The information in this paper reflects the views of the author, and does not necessarily reflect the official positions or policies of NOAA or the Department of Commerce.

2 National Oceanic and Atmospheric Administration, Office of Response and Restoration, 7600 Sandpoint Way, NE, Seattle, WA, 98115.

3 University of Maryland, Chesapeake Biological Laboratory, 1 Williams Street, Solomons, MD, 20688.

Clams were split into two groups. One group was frozen until the experiment. One group was kept alive, transported, and caged in the PAH-contaminated Elizabeth River, Virginia, USA to accumulate contaminants for exposure experiments. Clams were caged for approximately 30 days, transported to the laboratory, and frozen until use.

Fish were sampled on days 0, 35, 62, 90 and 120. On each sampling day, standard metabolic rates were determined by measuring individual oxygen consumption rates of three fish per tank using a MicroOxymax™ respirometer system (Columbus Instruments). Afterwards, fish were measured, weighed, sex determined, placed in clean, foil pouches, and frozen until chemical analysis.

Modeling Components: We developed a *Fundulus heteroclitus* bioenergetics model to examine whether long term, dietary exposure to PAHs can predict long term impacts on population level parameters. We employ the basic bioenergetics equations described in by Hanson et al., 1997 to build our individual stage class and population models. The model contains discrete cohorts to simulate different stage (age) classes of fish to model population level parameters. The model allows one to calculate population vital rates (e.g., growth, reproductive value) and to compare different exposure scenarios.

A bioenergetics approach provides a basis for developing a narcosis induction model. We developed species profiles for three life stages (larval, juvenile and adult) for *Fundulus heteroclitus* using literature values (Munns et al., 1997, and Madon et al., 2001) and our laboratory measurements. The model follows an assembly of discrete cohorts to simulate different stage (age) classes of fish to generate population level parameters. The number of individuals within a cohort was estimated from literature data on *Fundulus* populations and from field experience. The model was initialized with field-based stage distributions to approximate appropriate life stages: larval stage (eggs — larval ~ 28 days) juvenile stage—< 35 mm; 1 year old adults — (35 – 50 mm); 2 year old adults — (> 50 mm); 3 year old adults — (> 50 mm) and run until stable distributions in the populations were reached.

The model uses species-specific physiological parameters to describe the flow of energy into and out of a fish. Outputs to our model include total population biomass, and energy budgets of individuals and summary populations.

RESULTS AND DISCUSSION

Characterization of Food for PAH compounds: The experimental design developed a gradient of PAH-contaminated food fed to different groups over the time course of the experiment. All batches of food were characterized for individual PAH analytes to verify and quantify dietary exposures to fish, and correlate narcotic dose to sublethal responses in *Fundulus heteroclitus*. Figure 1 depicts the PAH profiles of forty-two PAH analytes measured in the food batches and the established food gradient. The clean food treatment contained an average Σ-PAH concentration of 600 ng/g w/w (± 20, n = 4). For the contaminated treatments, the average Σ-PAH concentrations were 840 (± 270, n = 4), 2400 (± 1300, n = 4), and 2800 (± 2200, n = 6) for the 10% contaminated, 50% contaminated, and 100% contaminated treatments, respectively. The contaminated treatments represent a environmentally relevant mixture and the bioavailable fraction of PAHs accumulated from a contaminated estuarine system (Elizabeth River, sub-estuary of the Chesapeake Bay) where PAHs are the dominant chemical class of concern. The variability in the full strength batches is due to two different caging periods, and thus reflects different environmental conditions of the Elizabeth River at times of clam deployments.

Results of experiment: All endpoints were analyzed using an ANOVA, general linear model, to examine treatment effects. No

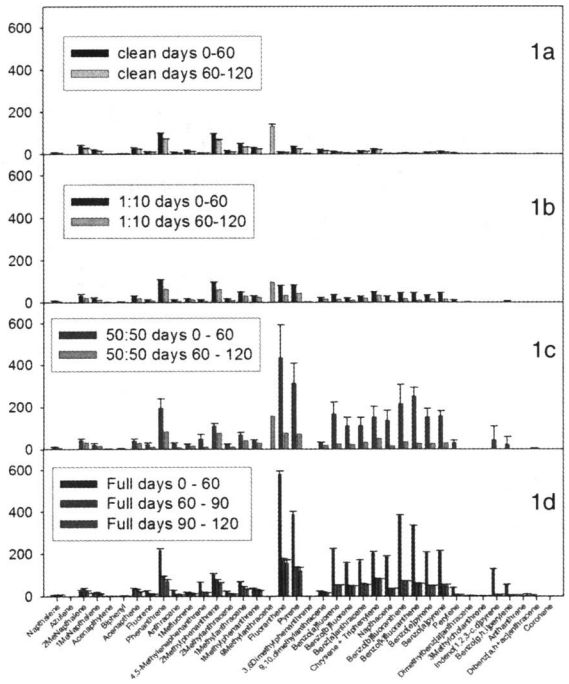

FIGURE 1. MEAN CONCENTRATIONS OF PAH ANALYTES IN FOOD (CLAMS HOMOGENIZED WITH FISH GEL) BATCHES FED TO *FUNDULUS HETEROCLITUS*. STANDARD DEVIATIONS (SD) ARE +/- 1 STANDARD DEVIATION OF THE MEANS.

significant effects in Weight, Length, Condition Factors measured in individual fish were detected from the treatments.

Figure 2 depicts least square means measured for individual fish. Standard metabolic rate (SMR) increases significantly as t-

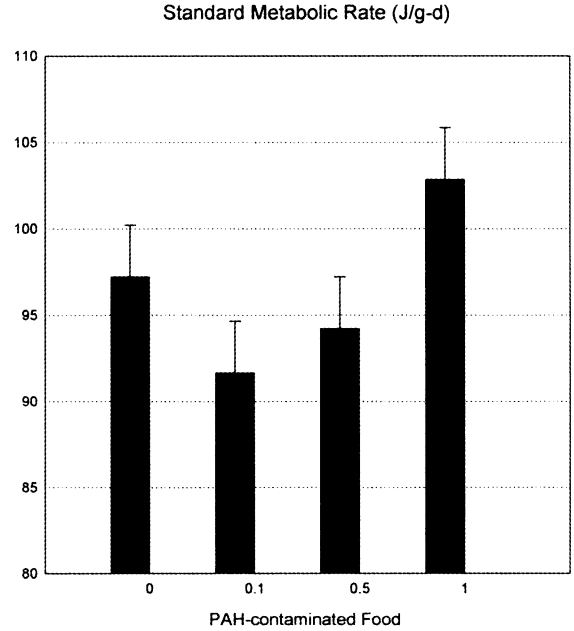

FIGURE 2. LEAST SQUARE MEANS FOR STANDARD METABOLIC RATE (J/G-D) MEASURED IN *FUNDULUS HETEROCLITUS*. ERROR BARS REPRESENT SE OF THE MEAN. FOOD EFFECT, P = 0.02, MS222*FOOD, P = 0.004)

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Table 1. Mean concentrations of total PAHs in food (clams homogenized with fish gel) batches fed to *Fundulus heteroclitus*. Standard deviations (SD) are +/- 1 standard deviation of the means.

Days		% Clean Clams	% PAH-Contaminated Clams	t-PAH avg	SD
0-60	Clean Food	100	0	600	20
	1/10 Food	10	90	1010	250
	50/50 Food	50	50	3050	920
	Full Strength	0	100	3900	1200
60-90	Clean Food	100	0	585	5
	1/10 Food	10	90	660	0
	50/50 Food	50	50	1030	0
	Full Strength	0	100	1330	10
90-120	Clean Food	100	0	585	5
	1/10 Food	10	90	660	0
	50/50 Food	50	50	1030	0
	Full Strength	0	100	1130	100

PAH in the food increases ($p = 0.087$). Consistent with narcosis theory, standard metabolic rates are depressed. However, after PAH levels get above 900 ng/g w/w Σ-PAH, metabolic rates increase with the food gradient. The results indicated that total chemical burden in the contaminated food exposures overcame the narcotic effect at low levels, causing a net increase in the standard metabolic rates of *Fundulus heteroclitus*. The results of the study suggest that sublethal narcosis (as defined by a reduction in standard metabolic rate) can be chemically induced, however, there is a threshold where other modes of action may interact with and mask the sublethal narcosis effect.

The non-linear SMRs (J/g-d) responses are used as explicit inputs to the bioenergetic equations in our model. In theory, the increased energy expenditure exerted at higher levels reduces the energy available to allocate to production. We used these measured differences in SMR as a starting position for generating a wider range of dose-response relationships into the model.

The model uses the basic energetic equation to balance energy input (consumption) with energy outputs (metabolism, excretion, egestion, and growth) of individual fish.

(1) Consumption = Metabolism + Growth + Excretion + Egestion

Metabolism losses include standard metabolic rate (R), active rate (A), and specific dynamic action (S), or the cost of digesting food. Growth includes both somatic (B) and reproductive (G) growth. Egestion (F) losses occur through fecal excretions. Excretion (U) losses occur through nitrogeneous waste products.

(2) $C = (R + A + S) + (B + G) + (F + U)$

Specific growth (dB/Bd t) is modeled as

(3) $dB/Bdt = C - (R + F + U)$

and predicts fish growth on a daily basis. All of the core processes are dependent on the size of the fish and water temperature.

We used the bioenergetic equations to develop steady-state populations of *Fundulus* under two sets of conditions: base case energetics with no PAH exposure and cases where the energetics are altered due to PAH exposures. Comparative model runs are evaluated to quantify contaminant effects and to evaluate long-term, population-level consequences of sublethal exposures to narcotic contaminants. Results from the runs include endpoints such as total population growth and the parameters contributing to changes in total population growth.

1. Base-case runs (no contaminant or narcosis effect imposed): The base-case model was developed for each life stage as described above. Outputs include specific rates for an energy mass balance plotted against time for individual fish growth (somatic and reproductive) and metabolism expenditures. Population structure and net population production at steady state without contaminant stress are also calculated.

2. Sublethal narcosis induction model: A sublethal narcosis model was developed. This model provides a narcotic dose-dependent mechanism for changing standard metabolic rate. Different levels of exposure concentration of narcotics (i.e., PAH-contaminated food) will induce dose dependent changes in standard metabolic rates. Results from our laboratory experiment and literature relationships between accumulated narcotic burden and changes in metabolic rates drive this model. These runs explore increasing PAH exposure via prey concentrations so that portions of cohorts reach critical body residues (CBR) using LC50s (water exposures) defined in the literature (DiToro et al., 2000, French-McCay, 2002). This in turns affects time to CBR, and provides a feedback mechanism for changing mortality probabilities, as well as the growth and reproduction processes.

CONCLUSIONS

We found that long-term (120 day) exposure to PAH-contaminated food greater than 900 ng/g Σ-PAHs significantly stimulated standard metabolic rates of *Fundulus heteroclitus*. At the low level exposure, we measured a typical narcotic response (i.e., a depression in biological activity). The elevated standard metabolic rates induced by higher levels of PAH-contaminated food imply that multiple modes of toxicity associatd with PAHs are interacting. We employ a modeling approach to examine the laboratory findings on the total production on the population level. The emphasis on the bioenergetics modeling described above is focused on parameterizing the feedbacks between contaminant-induced changes in metabolic rates, and then imposing that effect across generations. Comparisons of the model outputs (e.g., total production) between baseline populations and impacted populations will be presented. Once the interpretive aspect of the model is completed, it will provide a framework for linking to other studies. The food exposures were well characterized in the laboratory experiment. In that sense, it provides a unique data set that will allow connectivity to other work.

The design of the experiment was not set up to cause deleterious effects in growth, condition factors, or mortality, however, it would be useful to link results from this experiment to other work where other sublethal and lethal endpoints occurred due to exposure to PAHs and other narcotics. Thus, to model upper end effects, we use critical body residue data for fish species (DiToro et al., 2000, French-McCay 2002). DiToro et al., synthesized acute toxicity data, specifically on narcotic exposures to vertebrates where they derived species-specific theoretical CBRs from LC50s (water exposures) due to PAHs, and other narcotics. This approach was extended to our study where we calculated the food concentrations needed in order for a fish to approach its CBR level. This is an exploratory, theoretical use of the modeling application, and can be applied to spill sites impacted by long-term residual oil.

BIOGRAPHY

Amy Merten is a marine scientist with the NOAA Office of Response and Restoration (ORR), Seattle, WA. She specializes in biological assessment preparedness and response activities. Ms. Merten is a Ph.D. Candidate at the University of Maryland, Chesapeake Biological Laboratory, pursuing a doctorate in Marine Estuarine and Environmental Sciences with a specialization in Environmental Chemistry.

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