PAH-induced sublethal narcosis alters the bioenergetics and bioaccumulation of Fundulus heteroclitus

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Introduction

Accumulation of non-polar narcotic chemicals in organisms alters their metabolic rates and, therefore their energetic demands. Since exposure of these hydrophobic chemicals is primarily through the diet, we hypothesize that decreasing metabolic rates caused by accumulation of narcotics reduces feeding which, in turn, decreases further exposure to the narcotic. Thus, dietary exposure to and accumulation of narcotics may cause a negative feedback, reducing net bioaccumulation. The purpose of this short paper is to discuss the results of a long-term fish contaminant exposure study and to describe a fish bioenergetics model coupled to a PCB-bioaccumulation model. The laboratory component consisted of exposing the estuarine fish *Fundulus heteroclitus* to environmentally-relevant levels of hydrophobic organic contaminants (HOCs) through their diet. We developed the model to further examine and understand the consequences of sublethal narcosis at both the individual and population levels of *Fundulus heteroclitus*.

Fish living in contaminated environments 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. We conducted a long-term (120 days) fish exposure experiment to examine the consequences on the bioenergetics and bioaccumulation rates of fish continuously challenged with sublethal levels of HOCs. We used polycyclic aromatic hydrocarbons (PAHs) as the main HOC stressor in a gradient of contaminated food and PCB congeners at much lower levels to trace uptake dynamics. 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). We measured growth, standard metabolic rates, lipid content, and congener-specific PCB levels in individual fish. Results from these experiments were interpreted using the Wisconsin Fish Bioenergetics Model 3.0[1] as the initial framework for developing a narcosis induction model. The Wisconsin model is an individual-based model, where discrete cohorts are developed to simulate different stage (age) classes of fish to model population level parameters. The model uses the basic energetic equation to balance energy input (consumption) with energy outputs (metabolism, excretion, egestion, and growth) of individual fish.

Our model was run under three main sets of conditions: base case energetics with no HOC exposure (but with the bioaccumulation equations 'turned on'), base case energetics with bioaccumulation (contaminated prey), and cases where the energetics are altered due to HOC accumulation. Comparative model runs were evaluated to quantify contaminant effects. The model was used to evaluate long-term, population-level consequences of sublethal exposures to narcotic contaminants. Results from the scenarios runs include endpoints such as total population growth and the parameters contributing to changes in total population growth.

Methods

Experimental Component: We conducted an experiment using a 2x4x3 factorial, random block design. Fish were exposed to two levels of 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. 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.

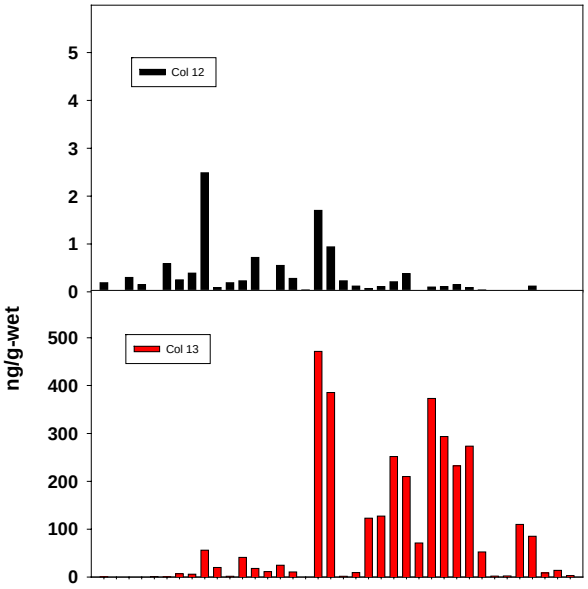

Figure 1. PAH profiles in control and contaminated clams used as food in this experiment. Each bar represents an individual PAH component, arranged by GC retention time.

Fish were sampled on days 0, 35, 62, 90 and 120. Standard metabolic rates were determined by measuring the oxygen consumption rates of three fish from each tank using a respirometer. Afterwards, fish were measured, weighed, sex determined, placed in clean, foil pouches, and frozen until chemical analysis.

Modeling Component: We employed the Wisconsin Fish Bioenergetics Model 3.0[1] as the framework for developing our narcosis induction model. We developed species profiles for three life stages (larval, juvenile and adult) for *Fundulus heteroclitus* using literature values[2,3] and laboratory measurements. The Wisconsin model is an individual-based model where discrete cohorts were used to simulate different stage (age) classes of fish to generate population level

parameters. We modeled several cohorts until the population reached steady-state (r = 0). The number of individuals within a cohort was estimated from literature data and from field experience. The model was initialized using a starting cohort of 1 year old adults which generated multiple cohorts to approximate appropriate life stages: eggs (spawn to hatch) – 6 – 10 day period; larval stage – 28 day period; juvenile stage - < 35 mm; 1 year old adults – (35 – 50 mm); 2 year old adults – (> 50 mm); 3 year old adults – (> 50 mm).

Results and Discussion:

In our experiment, we found no statistically significant differences in weight, length, or condition factors among treatments, indicating that the exposures did not dramatically impact the fish. However, we found significantly different standard metabolic rates and PCB accumulation between the clean group and the group fed the highest levels of PAH-contaminated clams (Figure 2.) Figure 2 plots bioaccumulation factors (concentration of PCBs in fish ng/g w/w normalized to concentration of PCBs in food ng/g w/w). The group exposed to clean clams, or background levels of PAHs, exhibited no net accumulation of PCBs across the course of the experiment. However, the group fed the most PAH-contaminated clams, continuously accumulated PCBs across the course of the experiment, and did not reach steady state even after 120 days. It is important to note that the PCB exposure was almost identical across these treatments, so differences in PCB accumulation is due to other factors, including the PAH exposure gradient, rather than differences in PCB exposure.

These results are counter to our hypothesis that sublethal exposure to narcotics (*i.e.*, PAHs) will depress standard metabolic rates, leading to less consumption and dietary PCB exposure and, therefore, a reduction in PCB accumulation. Our preliminary interpretation of the observed results are: 1) PAH-induced stressed increased the efficiency of absorption of PCBs by changing the specific dynamic action expenditure of the metabolic process, 2) PAH-induced stress reduced depuration efficiencies, and/or 3) other contaminants (*e.g.*, metals) interact with the PAH effect to produce the observed results.

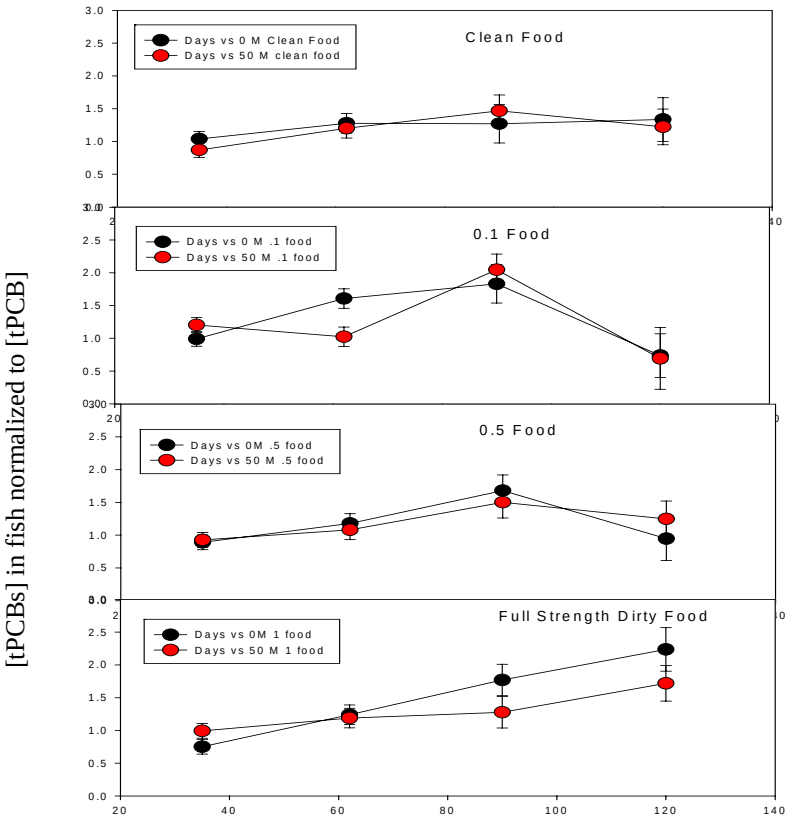

Figure 2. Accumulation of PCBs as tracers of bioaccumulation in Fundulus *versus* dose of narcotic in food (PAHs) and in water (MS-222)

We used the model to further explore the effects of sublethal narcosis on fish bioenergetics and bioaccumulation. We ran several comparative scenarios to evaluate the effects of sublethal contaminant exposures.

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 is also calculated.

2. *Fundulus heteroclitus* PCB accumulation runs: The same methodology was used to develop a baseline bioaccumulation model for PCBs without inducing contaminant effects. PCB uptake rates

measured in our experiment (control groups w/ clean clams, no MS222) are inputs into the model. All of the outputs for this run are the same as for the base case, plus outputs of [t-PCBs] vs. time for each cohort. This provides a distribution of PCB tissue residues across the population. Standard metabolic rate outputs do not vary with PCB accumulation in this run.

3. Sublethal narcosis induction model: A sublethal narcosis model that drives model 2 above is being developed. This model provides a narcotic dose-dependent mechanism for reducing standard metabolic rate. Different levels of exposure concentration of narcotics (*i.e.*, PAH-contaminated food) will induce varying levels of reduction. Results from our laboratory experiment and literature relationships between accumulated narcotic burden and depressed metabolic rates drive this model. These runs explore increasing PCB accumulation via prey concentrations so that portions of cohorts reach critical body residues (CBR). This in turns affects time to CBR, and provides a feedback mechanism for changing mortality probabilities, as well as the growth and reproduction processes.

References:

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