Period Covered by the Report: October 1, 2001 – September 30, 2005
Date of Report: December 12, 2005
EPA Agreement Number: R82941091
Title: Developmental Stability in Amphibians as a Biological Indicator of Chemical Contamination and Other Environmental Stressors, SEER project of SIP: Experimental Program To Stimulate Competitive Research (EPSCoR) From The Commonwealth Of Kentucky
Investigators: Whiteman, Howard H., Loganathan, Bommanna G.
Institution: Murray State University
Research Category: EPSCoR
Project Period: October 1, 2001 - September 30, 2005

Objective: We proposed to evaluate the potential of using developmental stability as a biological indicator of anthropogenic and natural stress in amphibians. Amphibians are ideal biological indicators, because their semi-permeable epidermis and complex life cycle expose them to multiple stressors in both aquatic and terrestrial environments. Because of this, amphibians should be among the first vertebrates affected by anthropogenic stressors in either of these environments. Furthermore, some of the same stressors affecting amphibians are known to have negative effects on other species, including humans. Although we proposed to evaluate a wide range of possible stressors, a major thrust of this project is to correlate amphibian developmental stability with contaminant levels accumulated in their tissues. We hypothesized that amphibian developmental stability would decrease with increased levels of anthropogenic (contaminants, land use practices) and natural (population size and density) stressors. Our specific goals were to: (1) correlate the effects of environmental stressors with amphibian developmental stability; (2) evaluate the effect of species, life history stage, trophic level, and habitat type on measures of developmental stability; and (3) develop methods for separating the effects of anthropogenic and natural stressors.

Results Summary: The vast majority of the work involved in this grant is now completed, with some post-grant work still required from two graduate students that will be finishing their work by December 2005. A summary of our results are described below.

Much of our work over the past few years has concentrated on bullfrogs (*Rana catesbeiana*), in part because they are large and thus provide adequate tissue for contaminant analysis, are easy to sample, ubiquitous throughout the region, and provide an excellent model for the dual-life cycle of many amphibians. We sampled bullfrogs of various ages from several different areas in western Kentucky that we predicted would differ in terms of pollutants and thus contaminant within bullfrog tissues (see Map next page). These sites included those near agricultural lands (Terrapin Creek = TC, TD), those on forested recreation areas (Central Land Between the Lakes (LBL--includes EB, GB, TP) and Grand Rivers (GR)), and some of which were downwind of major industrial centers and/or superfund sites (primarily GR). Bullfrogs were captured, digitally photographed, tissue excised for later contaminant analysis, and a toe taken from adults

to analyze for age differences through skeletochronology. At a later date, digital images were evaluated using morphometric software to assess levels of asymmetry at both the individual and population level.

The results of this work suggest that, in contrast to our predictions, there were no significant differences between the three sites in their total PCB and pesticide levels (Figure 1). We suspect that agricultural impacts, including the use of herbicides and pesticides as part of the management of LBL and the Terrapin Creek area, may have influenced these results.

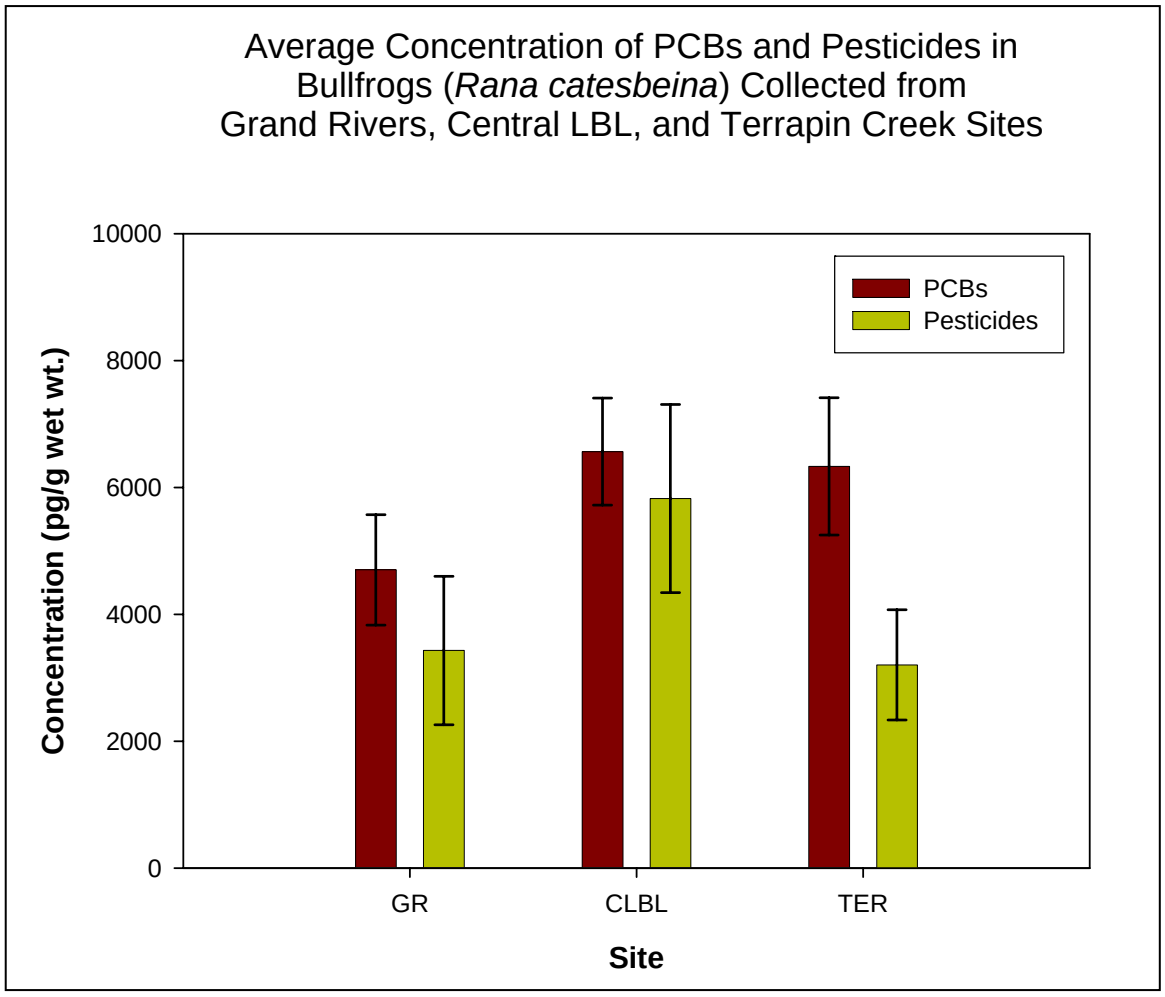
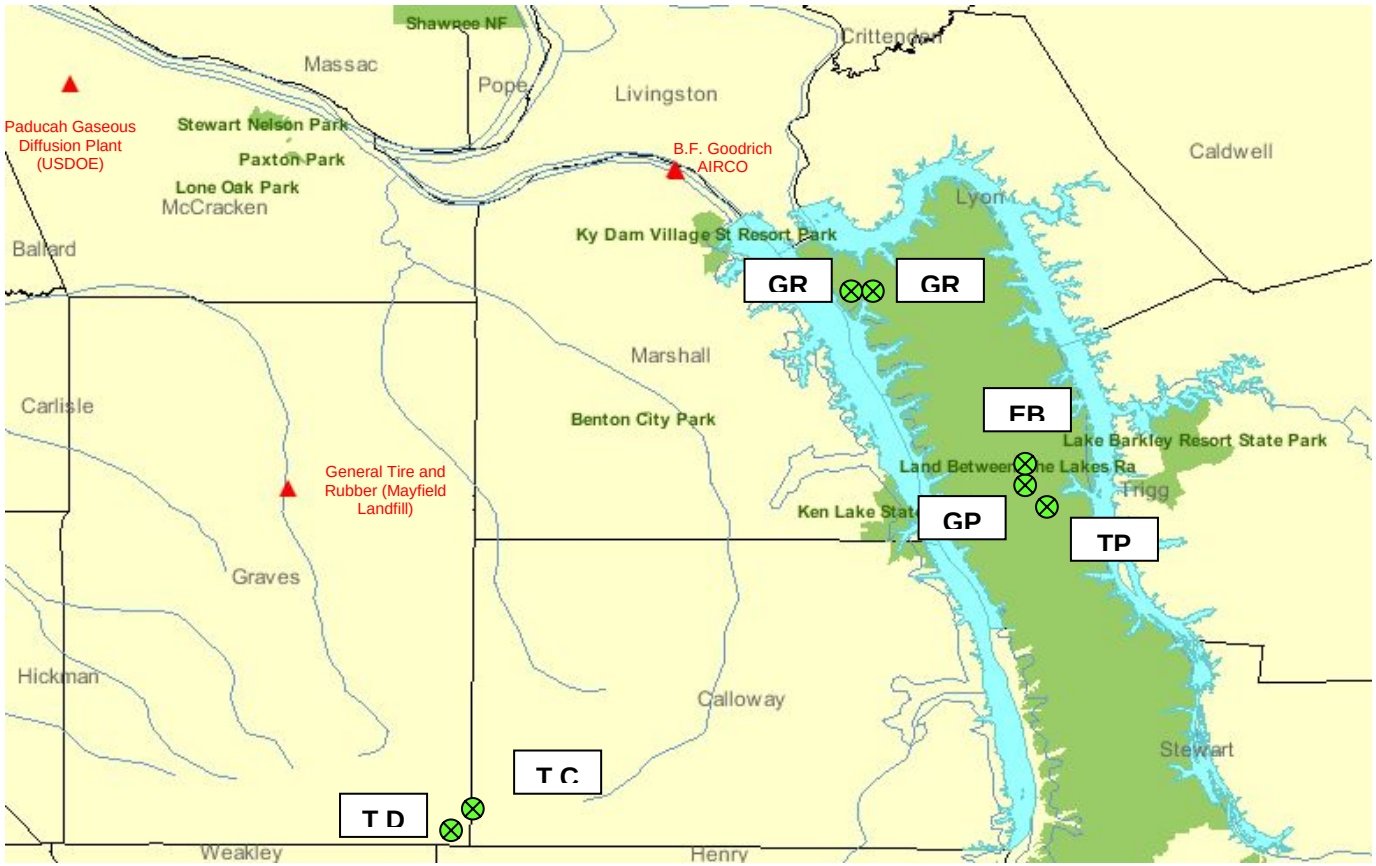


Figure 1: Total PCBs and pesticides in bullfrogs from the three sample areas. Although we initially expected contaminant levels would be higher in the Grand Rivers (GR) locality due to downwind exposure, levels at Terrapin Creek (TER) and Central Land Between the Lakes (CLBL) were not significantly different from GR or each other.


Shawnee NF
Massac
Pope
Livingston
Crittenden
Caldwell
Paducah Gaseous Diffusion Plant (USDOE)
Stewart Nelson Park
Paxton Park
Lone Oak Park
McCracken
B.F. Goodrich AIRCO
Lyon
Ky Dam Village St Resort Park
Ballard
GR
GR
Marshall
Carlisle
Benton City Park
FR
Lake Barkley Resort State Park
General Tire and Rubber (Mayfield Landfill)
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Hickman
Calloway
Stewart
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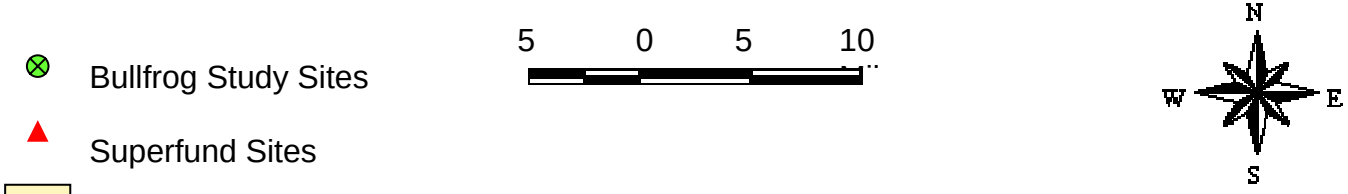
Bullfrog Study Sites
Superfund Sites
County Boundaries
Parks/Natural Recreation Areas
Lakes
Rivers and Streams
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Although adult bullfrogs captured from these sites also did not vary in total asymmetry, focusing on the more vulnerable larval stages revealed that the Grand Rivers site had higher levels of PCBs, pesticides, and total asymmetry than the other two sites, as predicted (Figure 2). These results also correspond to the presence of phenodeviants (grossly deformed tadpoles) collected in the Grand River site several years ago, an observation which served as the basis for the current research.

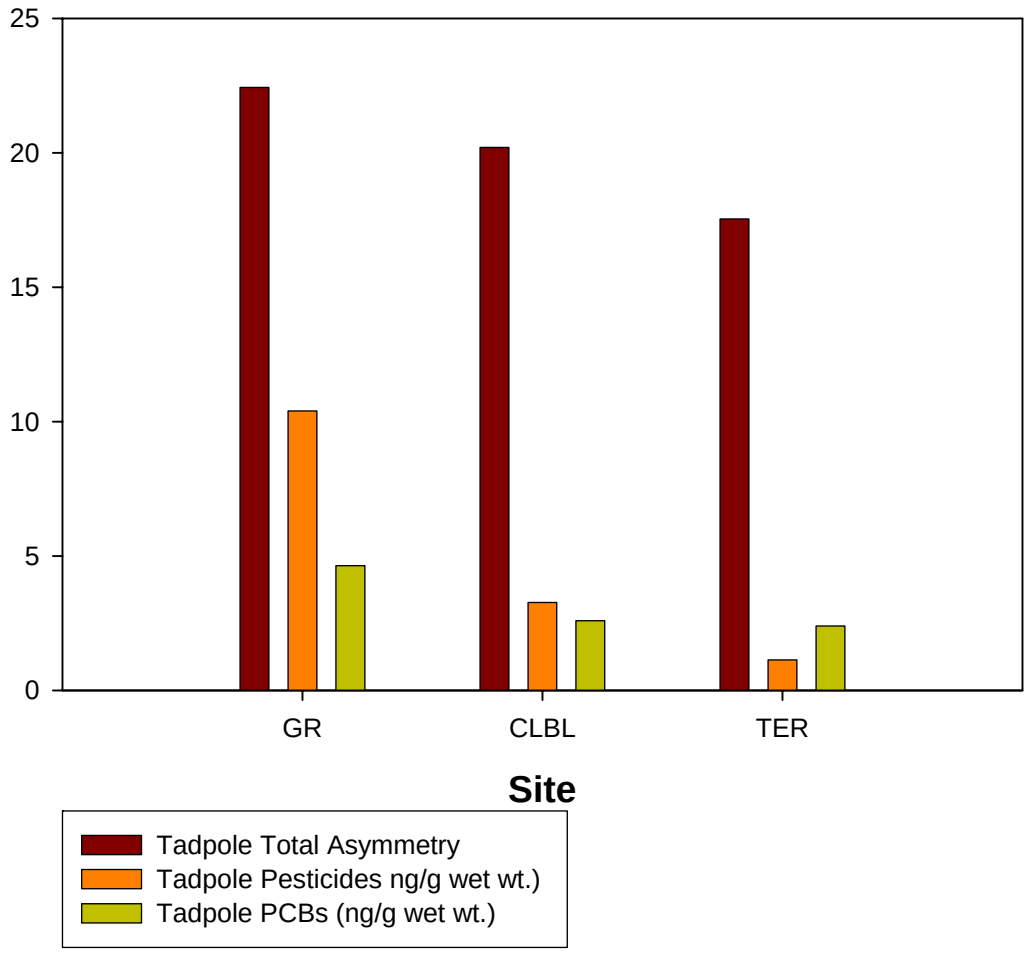
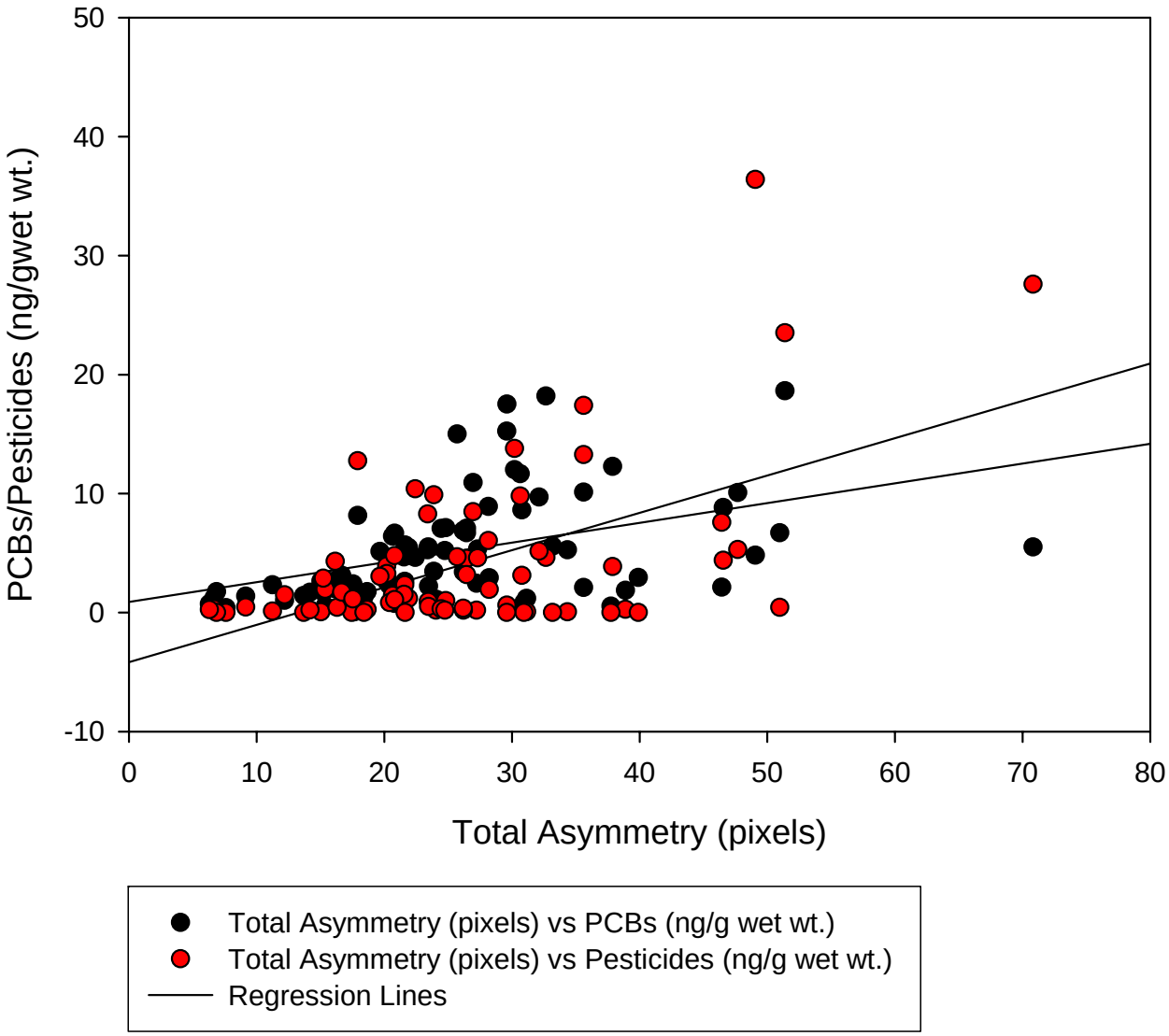


Figure 2: Effect of bullfrog locality on total asymmetry, pesticides, and PCB levels in tadpoles.

In addition, across all bullfrogs and localities, total asymmetry was found to be a significant indicator of both PCB and pesticide contamination, although this relationship was stronger for pesticides (Figure 3). Bullfrogs with higher asymmetry had the highest levels of both contaminants, suggesting that asymmetry can provide a useful indicator of contaminant levels in bullfrogs, particularly at the highest levels.



PCBs: $R^2 = 0.19$, $p < 0.0001$ Pesticides: $R^2 = 0.32$, $p < 0.0001$

Figure 3: PCB and pesticide levels as a function of total asymmetry of bullfrogs from all localities and age classes combined.

Future Activities:

Our graduate students are completing their theses this fall. Additionally, a new graduate student (Julia Earl) has begun experiments aimed at determining the effects of nitrate and phosphorus pollution on the health and asymmetry of frog populations. Recently, this project has also helped initiate genetic analyses of amphibian populations, in order to better understand how metapopulation dynamics and life history variation may influence population fluctuations, so that we may better distinguish those caused by anthropogenic inputs from those occurring naturally.

Publications:
Manuscript in preparation:

Loganathan,B.G., Chien, I-L., Kobylarz, B. and Whiteman, H. Polychlorinated biphenyls (PCBs), Chlorinated Hydrocarbon Pesticides in Amphibian Samples Collected from Western Kentucky. Intended for publication in Environmental Toxicology and Chemistry. SETAC Press.

Benson, A. R., Whiteman, H. H., J. B. Boynton, M. Dotson, and R. Cates. Developmental stability as an indicator of amphibian population health. In preparation for Conservation Biology.

Meredith, C. S. and H. H. Whiteman. Lethal and sublethal effects of nitrate on amphibian embryos and larvae. In preparation for Ecological Applications.

Kobylarz, B., H. H. Whiteman, B. G. Loganathan and L. Duobinis-Gray. Contaminants and asymmetry: effects of locality, gender, and age. In preparation for Ecological Applications.

Presentations:

Whiteman, H. and Loganathan, B.G. 2004. EPA-EPSCoR Project Status. Presented at 9th Annual Kentucky EPSCoR Conference.

Whiteman, H. H. 2004. Developmental Stability in Amphibians as a Biological Indicator of Chemical Contamination and Other Environmental Stressors. EPA Environmental Research Seminar, Atlanta, GA, Sept 28-29.

Loganathan, B.G., Metzger, C., Kobylarz, B. and Whiteman, H. 2004. PCB Congeners and Chlorinated Pesticides Concentrations in Amphibian Samples Collected from Western Kentucky. A poster presented at the Fourth World Congress and 25th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Portland, OR. Nov. 14-18, 2004.

Kobylarz, B., H. H. Whiteman, B. G. Loganathan and L. Duobinis-Gray. 2005. Contaminants and asymmetry: effects of locality, gender, and age. Ninth Annual

Symposium on the Natural History of the Tennessee and Cumberland River Valleys, Brandon Springs Group Camp, LBL.

Kobylarz, B.A., Chien, I-L., Mruetusatorn, P. and Dunlap, K. 2005. The impact of age and sex on chemical exposure and developmental stability in North American Bullfrogs. Southeastern Ecology and Evolution Conference, University of Georgia, Athens, GA.

Dunlap, K. Brown, A. and Loganathan, B.G. 2005. Preliminary studies on atrazine levels in selected ponds from westernmost Kentucky. MSU Sigma Xi Poster Competition, April 18-22, 2005.

Kobylarz, B.A., Chien, I-L., Mruetusatorn, P. and Dunlap, K. 2005. The impact of age and sex on chemical exposure and developmental stability in North American Bullfrogs. MSU Sigma Xi Poster Competition, April 18-22, 2005.

Students Supported:

Graduate theses:

2002-04 Jessica Boynton: "Utilization of remote sensing to model current and future threats to amphibian populations". M.S.

2003-05 I-Lun Chien: "Polychlorinated biphenyls (PCBs), Chlorinated Hydrocarbon Pesticides in Amphibian Samples Collected from Western Kentucky. M.S.

2002-05 Beth Kobylarz: "Effects of age on stress bioindicators and chemical contamination in bullfrogs". M.S.

Other graduate RAs:
Chris Eden, Prachya Mruetusatorn, Julia Earl, Christy Meredith

Undergraduate RAs:
Catherine Aubee, Trace Hardin, Joshua Kitchens, Clinton Metzer, Michael O'Brien, Justin Kane, Rachael Brown, Anna Brown, Kim Dunlap

Supplemental Keywords: amphibians, bioindicators, contaminants

Scientific Discipline: conservation biology, toxicology