EPA EPSCoR Progress Report

Title: Developmental Stability in Amphibians as a Biological Indicator of Chemical Contamination and Other Environmental Stressors
Investigators: Whiteman, Howard H., Loganathan, Bommanna G.
Institution: Murray State University
Project Period: October 1 , 2001 through September 30, 2004
Project Amount: $165,775 (this is the EPA total)
Research Category: EPA EPSCoR

Description:

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 are 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.

Progress Summary: In Year 2 of this grant, our research continued in earnest with the recruitment of a quality graduate student (Ms. Beth Kobylarz) concentrating on asymmetry analysis and a postdoctoral student (Jason Neale) working on contaminant analysis. Specifically, we processed two species of amphibians, bullfrogs and spotted salamanders for developmental stability; contaminant analysis is currently being conducted on these animals. We completed statistical analyses of our earlier asymmetry results and should have a manuscript submitted during Fall 2003 (see below). Three other graduate students have become involved in this project: Ms. Christy Meredith has conducted experiments on the effects of nitrate on amphibian development, completed her M.S. during Fall 2003, and will be submitting her work to Ecological Applications during Fall 2004. Ms. Jessica Boynton joined our group in Fall 2003, and with funding from the Kentucky Space Grant Consortium has been analyzing our developmental stability results from a GIS perspective. Most recently, Mr. I-Lun Chien has joined the project to complete the remaining contaminant analysis.

Preliminary Results: Our most recent contaminant results can be seen in the tables below.

Table.1. PCB and pesticide concentrations in normal and deformed amphibians. Numbers in the parenthesis indicate number of specimens pooled for analysis.

Sampling Location	Species	Life Stage	Concentration (ng/g wet wt.)			
			Total PCBs	Total DDTs	HCB	Chlordane
Shuman#2 (Def)	Bullfrog	Larva	22.43	3.36	0.11	2.20
Bishop #1 (2)	Bullfrog	Larva	10.36	1.98	0.75	2.21
M.Morgan#3 (3)	Bullfrog	Larva	8.70	5.05	0.77	2.28
Bishop #4 (6)	Bullfrog	Larva	9.99	4.02	1.40	2.53
Tower LBL (4)	Bullfrog	Larva	11.30	6.43	BDL	1.38
ELK & Bison (1M)	Spotted salamander	Adult Male	24.56	6.88	3.98	33.96
Site 68/80	Spotted salamander	Adult Male	13.61	9.70	3.47	1.13
Star Camp 2 (1M)	Spotted salamander	Adult Male	18.72	BDL	1.80	1.21
LBL-142 (4M)	Eastern Newt	Adult Male	20.73	6.07	0.49	1.20
LBL-220 (6M)	Eastern Newt	Adult Male	33.40	6.80	0.78	3.14

PCB congener composition of the above samples.

Compound			Sampling Locations M-Morgan#3 Concn. ng/G.s.wt.	Shuman#2 Deformed Tadpole Concn. ng/G.s.wt.	Bishop #1 Concn. ng/G.s.wt.	Bishop #4 Concn. ng/G.s.wt.	Blank Concn. ng/G.s.wt.	ELK and Bison Concn. ng/G.s.wt.	Site 68/80 Concn. ng/G.s.wt.	LBL-22 Concn ng/G.s.w
2-4'-D_2CB		8	0.16	1.95	0.33	0.27	0.55	3.79	1.98	1.65
2,2', 5-T_3CB		18	0.25	0.73	0.37	0.22	0.16	0.00	0.00	0.00
2,4, 5-T_3CB		29			0.45			0.00	0.00	2.08
2,4, 4'-T_3CB/2,2', 4,6-T_4CB	28/50		0.27	0.51	0.31	0.46	0.29	0.00	0.00	0.00
2,2',5,5'-T_4CB		52	0.73	3.55	1.11	0.88	0.74	2.06	1.11	3.90
2,2',4,6,6'-P_5CB		104			0.09		0.04	0.00	0.00	0.00
2,2,'3,5'-T_4CB		44	0.41	1.25	0.55	0.56	0.59	1.80	0.85	1.91
2,3',4,4'-T_4CB		66	1.09	3.02	1.20	1.40	1.14	2.28	1.04	2.27
2,2',4,5,5'-P_5CB		101	1.13	3.04	1.41	1.35	1.05	4.14	1.55	3.18
2,2',3,4,5'-P_5CB		87	0.56	1.24	0.61	0.56	0.43	1.82	0.98	1.36
3,3',4,4'-T_4CB		77	1.15	3.68	1.44	1.44	1.32	0.00	0.00	0.00
2,2',4,4',5,6'-H_6CB		154						1.51	0.76	1.53
2,3',4,4',5-P_5CB		118	0.51	1.21	0.55	0.63	0.32	1.47	0.90	1.85
2,2',3,4',5,6,6'-H_7CB		188						0.64	0.00	0.00
2,2',4,4',5,5'-H_6CB		153	0.69	0.94	0.61	0.71	0.30	1.90	1.28	3.93
2,3,3',4,4'-P_5CB		105	0.32	0.39	0.31	0.28		0.55	0.64	1.06
2,2',3,4,4',5'-H_6CB		138	0.68	0.77	0.59	0.55	0.21	1.28	0.83	1.73
3,3',4,4',5-P_5CB		126	0.09		0.06	0.08		0.00	0.00	0.85
2,2',3,4',5,5',6-H_7CB		187	0.16	0.07	0.16	0.14		0.00	0.46	0.94

2,2',3,3',4,4'-H_6CB	128	0.10	0.09	0.09	0.08	0.02	0.78	0.00	0.60
2,2',3,3',4,5,5',6'-O_8CB	200						0.00	0.00	0.00
2,2',3,4,4',5,5'-H_7CB	180	0.26			0.19		0.54	1.23	1.66
2,2',3,3',4,4',5-H_7CB	170	0.09		0.10	0.06		0.00	0.00	0.98
2,2',3,3',4,4',5,6-O_8CB	195				0.06		0.00	0.00	0.00
PCB-194	194						0.00	0.00	0.00
2,2',3,3',4,4',5,5',6-N_9CB	206						0.00	0.00	0.55
decachlorobiphenyl	209	0.05			0.06		0.00	0.00	1.38
Totals:		**8.70**	**22.43**	**10.36**	**9.99**	**7.15**	**24.56**	**13.61**	**33.40**
HCB		0.77	0.11	0.72	1.13	0.05			
2,4'-DDE		0.28	0.72	0.33	0.30	0.28			
Trans-Nona		0.42	0.33	0.48	0.31	0.03			
4,4'-DDE		1.66	0.60	0.56	0.91				
Totals:		3.13	1.77	2.09	2.65	0.36			
PCB-103									

Future Activities: We are currently collecting larger numbers of bullfrog adults in an effort to correlate asymmetry with age, contamination level, and the presence of corticosterone, a known stress hormone. We are also currently collecting tissue samples of other species for contaminant processing, and completing the contaminant analysis.

Publications:

Manuscript in preparation:

1. Loganathan, B.G. and H. H. Whiteman. PCB congeners and chlorinated pesticide concentrations in amphibians collected from western Kentucky. In preparation for Int. J. Anal. Chem.

2. 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.

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

Presentations:

1. Whiteman, H. and Loganathan, B.G. 2002. EPA-EPSCoR Project Status. Presented at 8th Annual Kentucky EPSCoR Conference. October 20-21, 2002.

2. Loganathan, B.G. and Whiteman, H. 2002. PCB congeners and chlorinated pesticide concentrations in amphibians collected from western Kentucky. Poster presented at 8th Annual Kentucky EPSCoR Conference. October 20-21, 2002. Poster #: 14.

3. Whalen, M.M. and Loganathan, B.G. 2002. Immunomodulation of human natural killer cell cytotoxic function by triazine and carbamate pesticides. Poster presented at 8th Annual Kentucky EPSCoR Conference. October 20-21, 2002. Poster #: 15.

4. Boynton, J. and H. Whiteman. 2003. Utilization of remote sensing to model current and future threats to amphibian populations in western Kentucky. 10th Symposium on thee Natural History of Lower Tennessee and Cumberland River Valleys, Land Between the Lakes, March 21-22.

5. Boynton, J. and H. Whiteman. 2003. Using remote sensing and GIS to model habitat change and fragmentation in western Kentucky. Joint Meeting of Ichthyologists and Herpetologists, Manaus, Brazil, June 26-July 1.

6. Meredith, C. and H. Whiteman. 2003. Response of amphibian embryos and larvae to increasing nitrate concentrations: sublethal and lethal effects at levels found in agricultural run-off. Society for Conservation Biology, University of Minnesota-Duluth, June 29-July 2.

Students Supported:

Graduate theses:

2003- I-Lun Chien: "Contaminant analysis of amphibians from Western Kentucky". M.S.
2002- Jessica Boynton: "Utilization of remote sensing to model current and future threats to amphibian populations". M.S.
2002- Beth Kobylarz: "Effects of age on stress bioindicators and chemical contamination in bullfrogs". M.S.
2001-02 Christy Meredith: "Effect of nitrates on embryonic and larval development in amphibians". M.S. Currently a water quality specialist with the Western Pennsylvania Conservancy.
1999-02 Amy Benson, MSU: "Developmental stability as an indicator of stress in amphibians". M.S. Currently a research technician at the RMBL.

Other graduate RAs:

Chris Eden

Undergraduate RAs:

Catherine Aubee,
Trace Hardin
Joshua Kitchens
Sarah Viernum,

Supplemental Keywords: amphibians, bioindicators, contaminants

Scientific Discipline: conservation biology, toxicology