PERSISTENT ORGANIC POLLUTANT CONCENTRATIONS AND STRESS PROTEIN LEVELS IN FRESHWATER MUSSELS FROM KENTUCKY LAKE, USA

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Abstract
In this study, selected organochlorine pollutant concentrations and Hsp 70 stress protein levels were determined in freshwater mussels collected from Kentucky Lake and Lake Barkley, USA. Freshwater mussel species representing declining and increasing populations in the lakes were collected and analyzed. Standard analytical procedures were followed to determine pollutant concentrations in freshwater mussels and Hsp 70 levels in gills. Polychlorinated biphenyls, chlorinated pesticides were detected in all samples analyzed. Western blotting of gills revealed that all gills tested contain two kinds of stress proteins, a weak upper band (78kDa) and a strong lower band (72kDa). Hsp 70 were found in freshwater mussel representing both declining and increasing populations with relatively higher incidences in species declining populations To our knowledge, this is the first report examining the association of persistent organic pollutant concentrations and stress protein levels in freshwater mussels.

Introduction
Persistent organic pollutants including polychlorinated biphenyls (PCBs), chlorinated pesticides (DDTs, chlordane, hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB) are well known for their widespread contamination in the global environment, bioaccumulate, biomagnify in the food chain and cause toxic effects on wildlife and human health.[1-5] The stress protein or heat-shock protein (Hsp70) is widespread in plants, bacteria and animals.[6] All organisms, except *Hydra oligactis* and *Trematomus bernacchii* react to stress by increasing the expression of Hsps.[7] Hsp 70 are synthesized intensively in cell under a variety of harmful stimuli, including heat, heavy metals, organic pollutants, injuries, diseases and other stressors.[8] Presence of Hsp represents a general response of cells to various environmental stress situations.[9] Therefore, the induction of stress proteins can be used as an indicator of proteotoxic stress to organisms, thus representing a suitable biomarker for assessment of the toxic impact of various environmental stressors on a variety of organisms.[9] In freshwater ecosystems such as Kentucky Lake, mussels are especially suitable for biomarker studies since these animals live in direct contact with sediments and are widespread, well known bioindicator and exhibit high accumulation rate of most toxicants including heavy metals and persistent organic pollutants.[10] In this study, an attempt has been made to determine whether an association exist between the levels of Hsp 70 protein in gills and persistent organochlorine pollutant concentrations in freshwater mussels collected from Kentucky Lake and Lake Barkley, USA. Kentucky Lake and Lake Barkley are among the major human-constructed lakes in the US.[10] Populations of some mussel species especially, Mapleleafs (*Quadrula quadrula)* in Lake Barkley is declining, on the other hand, in Kentucky Lake, *Plectomerus dombeyanus* is steadily increasing.[11] The objective of the present study was to determine the concentrations of polychlorinated biphenyls, chlorinated pesticides and Hsp 70 in freshwater mussels collected from Kentucky Lake and Lake Barkley and examine any relationship between the pollutants levels and stress protein levels.

Materials and Methods
Freshwater mussels were collected by SCUBA diving. Date of sampling and sample details are presented in Table 1, Table 2 and Table 3. Total PCBs and chlorinated pesticides were analyzed in mussel tissues following the procedure described in Loganathan et al.[10] Hsp 70 analysis was performed as follows: Gill tissue were dissected and were homogenized in 6 volumes of Ca2+-, Mg2+-free buffer (20 mM HEPES (pH 7.3), 12.5 mM KCl, 1% Nonidet P-40). The homogenate were centrifuged and the supernatant were centrifuged again. This supernatant was used for Hsp 70 immunodetection. The protein concentration was measured using the BCA Protein Assay Reagent Kit. Western blot analysis: SDS-polyacrylamide gel electrophoresis of gill samples was performed on 10% acrylamide gels and stained with Coomassie Brilliant Blue R. For immunoblots, proteins on

gels were electrophoretically transferred to 0.45 um nitrocellulose membrane using the Transblot SD. Nitrocellulose strips were probed with primary monoclonal antibody against bovine brain HSP70. The alkaline phosphatase-conjugated goat anti-mouse immunoglobulins were used as the secondary antibody. Immunoreactive bands were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

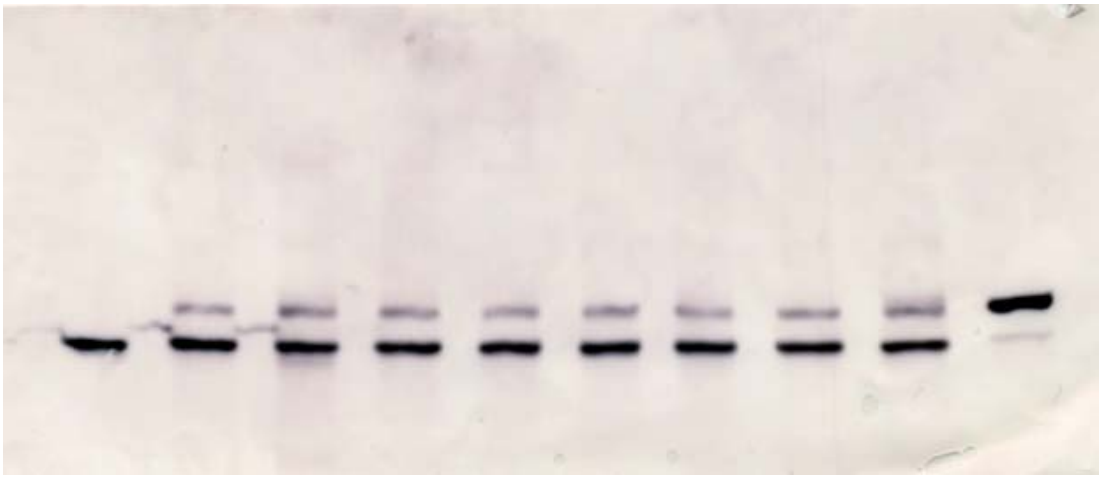
Results and Discussion

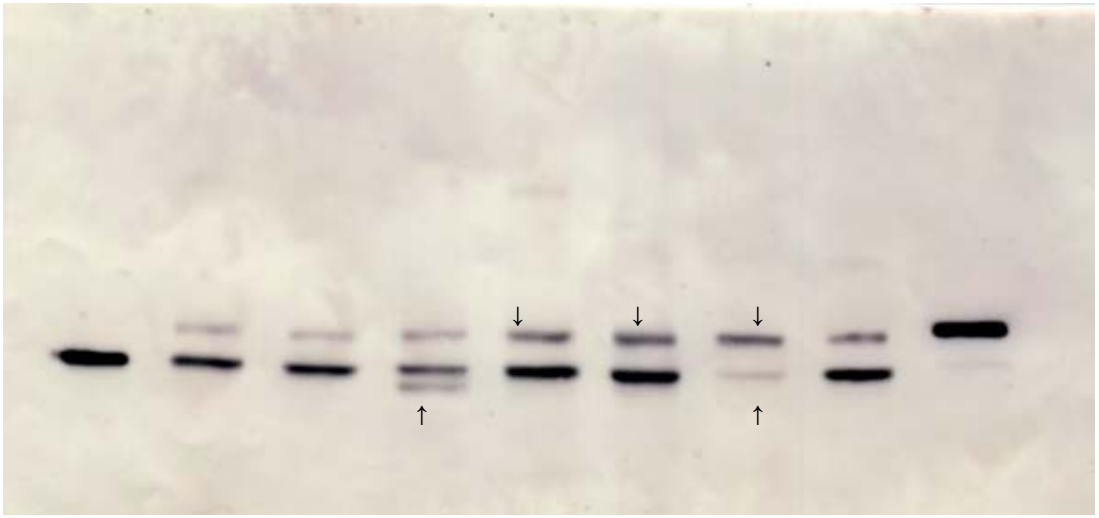
Tables 1-4 and figure 1 shows the sample details, organochlorine concentrations and stress protein levels in Kentucky Lake and Lake Barkley freshwater mussels. Table 1. Details of freshwater mussels collected from Kentucky Lake. Latitude: N36º 44' 634"; Longitude: W 88°06' 306". Species collected: *Plectomerus dombeyanus*. Sampling date: February 28, 2006.

ID	LENGTH (MM)	HEIGHT (MM)	WIDTH (MM)	AGE (YRS)
3PD1	104.7	67.9	38.5	14
3PD2	99.4	61.34	37.8	15
3PD3	104.5	61.7	35	14
3PD4	95	56.7	32	12
3PD5	92.1	56.2	29.8	12
3PD6	93.4	56.7	28.9	14
3PD7	93.7	60.8	31.7	14

Figure 1. Western blotting of freshwater mussel gills with anti-bovine brain Hsp 70.

Protein conc.: 60µg/lane. BB: bovine brain, MG: *Mytilus galloprovincialis* (sea mussel). HSP 70 (70kDa)





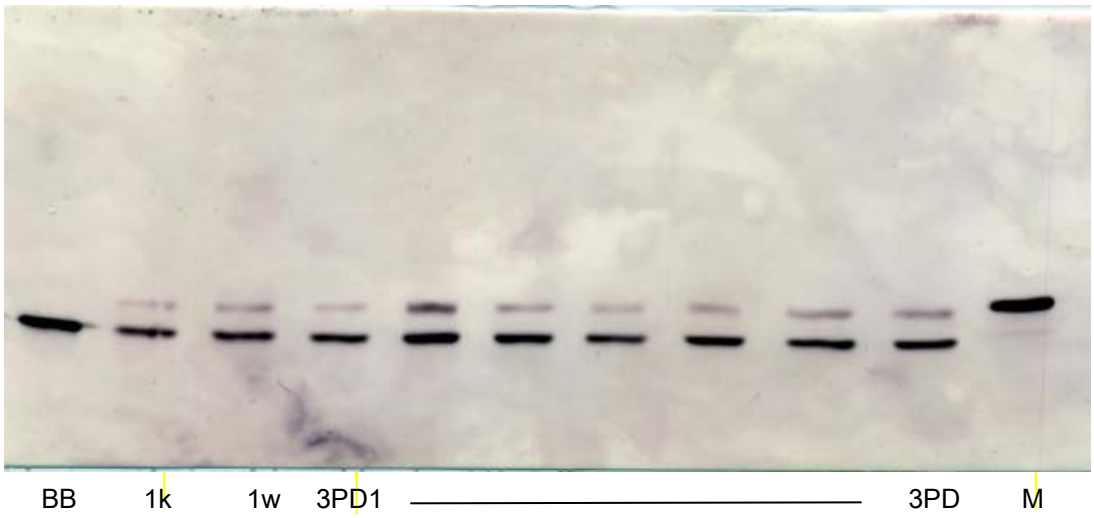


Table 2. Details of freshwater mussels collected from Lake Barkley. Latitude: N 36° 47' 001" and Longitude: W87° 59' 214". Species collected: Mapleleaf (*Qudadrula quadrula)*. This species population in this lake is declining in the recent years. Sampling date: February 27, 2006.

ID	LENGTH (MM)	HEIGHT (MM)	WIDTH (MM)	AGE (YRS)
1a	81.25	80.16	44.53	15
1b	82.47	76.74	47.81	16
1c	85.7	81.74	43.38	18
1d	87.8	82.4	49.5	18
1e	86.2	80.71	43.9	16
1f	86.2	78.2	45.3	15
1g	78.4	71.7	40.6	10
1h	78.9	75.3	41.8	15
1i	77.1	70.9	39.1	12
1j	73.2	67.8	41.5	12
1k	67.6	66.4	37.2	10
1w*	168.5	119.3	65.6	20

Table 3. Details of freshwater mussels collected from Lake Barkely. Latitude: N36° 46' 924"; Latitude: W87°58' 698". Date of sampling: June 27, 2006.

ID	LENGTH (MM)	HEIGHT (MM)	WIDTH (MM)	AGE (YRS)
2a	80.1	66.4	45.49	8
2b	80.1	65.9	44.8	20
2c	126	94.06	57	16
2d	153	123	51.5	12
2e	50.9	42.4	36.8	8

2a and 2b: *Quadrula quadrula*, 2c: *Amblema plicata*, 2d: *Anadonta suborbiculata*, 2e: *Obliquara reflexa*

Table 4. Total PCBs and chlorinated pesticide concentrations in selected freshwater mussel samples from Kentucky Lake and Lake Barkley. (* Hsp 70 analysis was not done in this sample)

ID	SAMPLING LOCATION	SPECIES	TOTAL PCBS (NG/G DRY WT.)	TOTALCHLORINATED PESTICIDES (NG/G DRY WT.)
3PD4	Kentucky Lake	*Plectomerus dombeyanus*	11.95	5.06
3PD5	Kentucky Lake	*Plectomerus dombeyanus*	27.99	10.4
*3PD11& 3PD12 (pooled)	Kentucky Lake	*Plectomerus dombeyanus*	8.06	4.69
*3PD13 & PD14 (pooled)	Kentucky Lake	*Plectomerus dombeyanus*	6.77	6.97
1c	Lake Barkley	*Quadrula quadrula*	13.86	9.15
1d	Lake Barkley	*Quadrula quadrula*	17.1	7.59
1f	Lake Barkley	*Quadrula quadrula*	5.64	6.59
1h	Lake Barkley	*Quadrula quadrula*	13.6	6.38

Figure 1 shows stress protein Hsp 70 levels in various samples. Western blotting results revealed that all mussels have two types of stress proteins, a weak upper band (78 kDa) and strong lower band (72 kDa). It appears that sample numbers 2a-2d and 3PD2 were stressed. Particularly 2b, 2c and 3PD2 were more stressed than other mussels, since these samples expressed both bands of Hsp 70s . We do not have total PCBs and chlorinated pesticides data for these samples at this time to examine the relationship between stress protein levels and persistent organochlorine concentrations in freshwater mussels. Although Hsp 70 was detected in all of the samples analyzed, we need data for more number of samples from contaminated and relatively less contaminated sites. In particular, to elucidate whether mussel sample numbers 1a through 1w are stressed or less stressed could be explained with additional data. This preliminary data provide evidence that freshwater mussels from Kentucky Lake and Lake Barkley expressed Hsp 70s stress proteins. However, it is not clear whether persistent organochlorine concentrations have had any effect on expression of Hsp 70s. Further studies dealing with Hsp 70 stress proteins and organochlorine concentrations are warranted to make clear the effects of organochlorine accumulation in freshwater mussels and expression of Hsp 70.

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