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Glucocorticoid Analysis as a Potential Biomarker of Chronic Contaminant Exposure in Wildlife

Hypothesis and Significance: A prevalent concern in the field of ecotoxicology is establishing the impact of chemicals upon individual organisms, populations and communities. Research in this field is used to monitor environmental impacts, conduct hazard assessments, and make intervention and regulatory decisions. The measurement of contaminant concentrations in blood and other tissues is important for confirming exposure but in order to establish impact investigators search for suitable biomarkers. Research that looks at the population, community and ecosystem levels has limited value because the detrimental effects have already occurred at the time of detection (Mayer *et. al.*, 1992). Measurable changes at the suborganismal and organismal levels allow for rapid detection and assessment of environmental effects on organism health, prior to significant impacts at the population level. My primary aim is to evaluate the utility of the glucocorticoid (GC) stress response as a biomarker of chronic contaminant exposure and altered physiological state in wild free-living vertebrates. GCs will be assessed in conjunction with tissue concentrations of contaminants (measured by EPA collaborators) and other parameters of organism health. Proper assessment of the physiological health of subsets of wildlife populations in contaminated sites will ultimately assist in making management decisions to benefit animal and human health.

Background and Methodology: Vertebrates, including humans, respond to noxious stimuli or stressors by evoking the stress response, involving a suite of behavioral and physiological responses. The stress response is a necessary survival mechanism for coping with adverse environmental conditions (e.g. Wingfield and Romero, 2001). However, when the stress response is inappropriately initiated or maintained, there are many detrimental effects on the health of the organism. Stress increases overall energy consumption, reducing energy available for other energetically expensive processes. Several important bodily processes are affected including glucose metabolism, musculoskeletal health, growth, tissue repair; and immune, cardiovascular, reproductive and neurologic function (Sapolsky, 2000; Sapolsky *et. al.*, 2000). Therefore, a measurable indicator of the stress response can provide information about the health of an organism.

One of the main systems activated during the stress response is the hypothalamic-pituitary-adrenal (HPA) axis. Several minutes following exposure to a stressor, steroid stress hormones, called glucocorticoids (GCs) (e.g. cortisol in humans), are secreted into the bloodstream from the adrenal or interrenal glands. GC secretion is regulated by adrenocorticotropin hormone (ACTH) released by the pituitary. GCs are influential in many physiological processes and have multiple effects on the body (Sapolsky *et. al.*, 2000). The metabolites of these hormones are excreted in the urine and feces hours later. GCs are relatively inexpensive and easy to measure. Sampling does not require sacrificing the animals because levels can be determined in blood, urine and feces. Feces can be collected in some species without handling the animals, thereby minimizing interference.

GC measurement allows for analysis of relative stress levels between individual animals and animal populations within a species. Several studies have shown that the GC stress response is altered as a result of anthropogenic environmental perturbation. For example, GC measurement has been used to study the effects of habitat degradation on spotted salamanders (Homan et. *al.*, 2003), tourism on Galapagos marine iguanas (Romero and Wikelski, 2002), snowmobile activity on wolves and elk (Creel *et. al.*, 2000) and logging activities on Northern spotted owls (Wasser *et. al.*, 1997).

There are a limited number of studies in free-living wildlife, primarily in fish, indicating that the GC stress response is altered in wildlife following chronic contaminant exposure. Wikelski *et al.* (2001) found increased GC levels in marine iguanas 7 days following an oil spill. Hopkins *et. al.* (1999; 1997) found elevated GC levels and decreased response to ACTH injection in Southern toads exposed to coal combustion wastes. Similarly, mudpuppies (aquatic salamanders) exposed to chlorinated hydrocarbons (polychlorinated biphenyls and organochlorines) showed lower GC responses to ACTH stimulation (Gendron *et. al.*, 1997). A number of studies in fish exposed to a variety of pollutants (heavy metals and organic compounds) have shown a reduced GC response to ACTH administration (Norris *et. al.*, 1999;

Hontela, 1998; Hontela *et. al.*, 1997; 1995). Clearly, the available evidence suggests that contaminants can affect regulation of GC release, but more research is needed in vertebrates, especially in non-fish species, to determine whether these changes are consistent among different species and contaminants. These comparisons are vital to assess GCs' potential as a biomarker of contaminant exposure and wildlife health.

In addition to GCs, I will be investigating other variables including heterophil/lymphocyte (H/L) ratios (a measure of immune response to stress in birds), body condition and fecal parasite load as indicators of relative health status because of the established links between stress and disease. H/L ratios are frequently elevated in chronically stressed birds and elevations are generally linked with an increased adrenal stress response (Maxwell *et. al*., 1992; Puvalpirod and Thaxton, 2000; Siegel, 1995; Vleck, 2001). Increased parasite load may be indicative of a weakened immune system and decreased ability to fight disease. Loss of body condition may ensue during chronic stress because of increased energetic expenditure and is likely to be positively correlated with parasite load because parasites utilize the host's resources. Hormonal changes may be early indicators of contaminant exposure whereas changes in relative health assessment parameters are more indicative of subsequent effects.

Approaches and Objectives: I will compare wildlife between heavily contaminated areas (Superfund sites) and relatively pollutant free reference sites in New England, in cooperation with collaborators from the EPA, USFWS and USGS. A variety of contaminants and sites are being included in this investigation including the Woonasquatucket and Housatonic Rivers Superfund Sites (primarily organic pollutants) and the Nyanza Superfund Site in Sudbury (primarily mercury). Different contaminants may have a variety of effects on different species depending on several factors related to taxonomic classification (e.g. birds vs. mammals), physiology and natural history. For this reason, my investigation is being conducted in representative species from different vertebrate classes (mammals, birds, reptiles and amphibians) with diverse natural histories (e.g. position in the food chain; aquatic vs. terrestrial).

Following capture, I will take initial blood samples collected within 3 minutes to reflect pre-capture GC levels, fecal samples to measure GC secretion from a number of hours before capture, body weights and measurements, and conduct basic health exams. Animals will be held for 30 minutes at which time another blood sample will be collected prior to release. This sample measures GC secretion resulting from the stress of capture. Following sample processing and extraction, plasma and fecal GC concentrations will be determined by radioimmunoassay (RIA). I will also count leukocyte numbers to assess immune status and screen fecal samples for gastrointestinal parasite presence (direct smear and fecal flotation) and parasite egg numbers (McMaster Technique).

Data Analysis and Anticipated Results: Data from contaminated sites will be compared to reference sites by ANOVA. Steroid stress hormone findings will be compared with the results of H/L ratios, body condition and parasite load. I expect that initial plasma and fecal GC levels will be elevated and the difference between initial and capture-stress-induced plasma GC levels reduced in animals living in highly contaminated sites. I also expect to find elevated avian H/L ratios, decreased body condition and increased parasite load in wildlife from contaminated sites. If findings prove significant, the results would indicate that an animal's stress response, and specifically GC concentrations, can be used to evaluate the relative health of exposed wildlife populations, identify populations and ecosystems at risk, justify intervention and be used as predictors of impending hazards.

Educational and Career Goals: This project is the basis of my Ph.D. thesis research and unites several of my career interests including wildlife conservation, stress physiology, hormone measurement as an environmental tool, animal and human health, and anthropogenic impact on ecosystem health. My educational and career goals are to combine my clinical knowledge and understanding of health and disease processes learned in veterinary school with the research skills from my Ph.D. training and to apply them to environmental problems. While I enjoy research as a discipline in itself, I see its greater value as an environmental tool. I do not know whether I will end up in a faculty position in an academic institution, working for an environmental organization or both. I will follow whichever direction allows me to have the greatest positive impact on the environment. I view this thesis project as a major step along the way.

Literature Cited

-Creel, S. Fox, J.E., Hardy, A., Sands, J., Garrot, B. and R.O. Peterson (2002). Snowmobile activity and glucocorticoid stress responses in wolves and elk. *Conservation Biology* 16(3): 809-814.

-Gendron, A.D., Bishop, C.A., Fortin, R and A. Hontela (1997). In vivo testing of the functional integrity of the corticosterone-producing axis in mudpuppy (amphibia) exposed to chlorinated hydrocarbons in the wild. *Environmental Toxicology and chemistry* 16(8): 1694-1706.

-Homan, R.N., Regosin, J.V., Rodrigues, D.M., Reed, J.M., Windmiller, B.S. and L.M. Romero (2003). Impacts of varying habitat quality on the physiological stress of spotted salamanders (*Ambyostoma maculatum*). *Animal Conservation* 6: 1-8.

-Hontela, A. (1998). Interrenal dysfunction in fish from contaminated sites: in vivo and in vitro assessment. *Environ. Toxicol. Chem*. 17(1): 44-48.

-Hontela, A., Daniel, C. and J.B. Rasmussen (1997). Structural and functional impairment of the hypothalamo-pituitary-interrenal axis in fish exposed to bleached kraft mill effluent in the St. Maurice River, Quebec. *Ecotoxicology* 6: 1-12.

-Hontela, A., Dumont, P., Duclos, D. and R. Fortin (1995). Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. *Environ. Toxicol. Chem*. 14(4): 725-731.

-Hopkins, W.A., Mendonca, M.T. and J.D. Congdon (1999). Responsiveness of the hypothalamo-pituitary-interrenal axis in an amphibian (*Bufo terrestris*) exposed to coal combustion wastes. *Comp. Biochem. Phys. Part C* 122: 191-196.

-Hopkins, W.A., Mendonca, M.T. and J.D. Congdon (1997). Increased circulating levels of testosterone and corticosterone in Southern toads, *Bufo terrestris*, exposed to coal combustion waste. *Gen. Comp. Endoc*. 108:237-246.

-Maxwell, M.H., Hocking, P.M. and G.W. Robertson (1992). Differential leucocyte responses to various degrees of food restriction in broilers, turkeys and ducks. *British Poultry Science* 33: 177-187.

-Mayer, F.L., Versteeg, D.J., McKee, M.J., Folmar, L.C., Graney, R.L., McCume, D.C. and B.A. Rattner (1992). Physiological and nonspecific biomarkers. In: *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress.* Eds. Huggett, R.J., Kimerle, R.A., Mehrle Jr., P.M. and H.L. Bergman. Lewis Publishers, U.S.A. pp. 5-86.

-Norris, D.O., Donahue, S., Dores, R.M., Lee, J.K., Maldonado, T.A., Ruth, T. and J.D. Woodling (1999). Impaired adrenocortical response to stress by brown trout, *Salmo trutta*, living in metal-contaminated waters of the Eagle River, Colorado. *Gen. Comp. Endocrinol.* 113: 1-8.

-Puvadolpirod, S. and J.P. Thaxton (2000). Model of physiological stress in chickens 1. Response parameters. *Poultry Science* 79: 363-369.

-Romero, L.M. and M. Wikelski (2002). Exposure to tourism reduces stress-induced corticosterone levels in Galapagos marine iguanas. *Biological Conservation* 108: 371-374.

-Sapolsky, R.M. (2000). Immunity, Stress, and Disease. In: *Why Zebras Don't Get Ulcers.* W.H. Freeman and Co., New York, pp. 126-158.

-Sapolsky, R.M., Romero, L.M. and A.U. Munck (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21(1): 55-89.

-Siegel, H.S. (1995). Stress, strains and resistance. *British Poultry Sciences* 36: 3-22.

-Vleck, C.M. (2001). Comparison of corticosterone and heterophil to lymphocyte ratios as indicators of stress in free-living birds. In: *Avian Endocrinology*. Eds. Dawson, A. and C.M. Chaturvedi. Narosa Publishing House, New Delhi, India, pp. 401-411.

-Wasser, S.K., Bevis, K., King, G. and E. Hanson (1997). Noninvasive physiological measures of disturbance in the Northern spotted owl. *Conservation Biology* 11(4): 1019-1022.

-Wikelski, M., Romero, L.M. and H.L. Snell (2001). Marine iguanas oiled in the Galapagos. *Science* 292: 437-438.

-Wingfield, J.C. and L.M. Romero (2001). Adrenocortical responses to stress and their modulation in free-living vertebrates. In: *Handbook of Physiology; Section 7: The Endocrine System. Coping with the Environment: Neural and Endocrine Mechanisms, vol. IV*. Oxford University Press, New York, pp. 211-234.