PROPOSAL-

Validation of Quantitative Magnetic Resonance (QMR) imaging to measure body condition in reptiles

Introduction– The energetic resources acquired by an organism are allocated to meet the demands of maintenance, growth, and reproduction. Though these needs may compete when resources are limited, life-history theory predicts that whenever possible, an individual should allocate consumed energy so that its fitness is maximized (Stearns 1992). Because the quantity and quality of available environmental energy can vary spatially and temporally, the ability to endogenously store excess food energy during periods of abundance is a critical component to dealing with environmental stochasticity (Bonnet et al. 1998). Therefore, understanding the interplay between fluctuating environmental resources with the energetic investment reproduction is essential to addressing questions concerning life-history tradeoffs (Zera and Harshman 2001).

Endogenous fat stores compensate for the competing demands of maintenance, growth, and reproduction by serving as a form of energetic "capital" that can be used to fund reproduction during periods when usable resources are scarce. For ectothermic animals, such as reptiles, the ability to up- or down-regulate their metabolism is conducive to accumulating large amounts of capital energy stores (which contrasts with organisms that rely on available energy that is present in the environment, or "income" energy; Jönsson 1997). Traditional hypotheses concerning "capital" versus "income" breeding suggest that reptiles rely heavily on capital energy stores when food is abundant in order to fund reproduction at a later period (Bonnet et al. 1998), though recent studies on reptile reproductive energetics (e.g. Warne et al. 2012, Warner et al. 2008) have revealed that the factors driving variation in energy allocation are more subtle, thus underscoring the need for further research into factors that affect reptile reproductive ecology.

To predict which breeding strategy an organism uses, an individual's body condition (i.e. fat stores, muscle mass, etc.) is assessed prior to, during, and after reproduction, usually by using body condition indices, or via lipid extraction from homogenized tissue. These techniques yield valuable body condition information; however, issues regarding accuracy, completeness, or animal preservation limit the scope of data that can typically be gathered. A new technology that has recently emerged in the field of body condition analysis is Quantitative Magnetic Resonance (QMR) imaging, which allows for quick and noninvasive body composition analysis for small animals. While the reliability of this technique has been validated for endothermic taxa (McGuire and Guglielmo 2010), to date only one validation study has been conducted on ectotherms (arthropods; O'Regan et al. 2012) and none have been conducted on reptiles.

Objectives– Recently, our lab group has acquired QMR machine to conduct studies on the energetic ecology of small vertebrates in the southwest, and while it has proven it be useful for assessing small endotherms, preliminary scans of reptiles have proven to be sensitive to variation in individual body temperature. I intend to conduct a validation study to assess the reliability and utility of this machine on reptiles. The goal of this study is to compare body composition values of lizards that have been scanned to values gained from lipid extraction. This data will be written as a techniques manuscript and submitted for publication. It will also be essential to my PhD dissertation, as it will allow me to assess the accuracy of readings from lizards scanned in the future.

Methods– I will use the QMR to scan lizards that are representative of body size ranges seen in New Mexico. I will collect 10 individuals of three size classes; 3 – 5 g (tree lizards, *Urosaurus oranatus*), 9 – 12 g (fence lizards, *Sceloporus undulatus*), and 28 – 32 g (collared lizards, *Crotaphytus collaris*). Each individual will be live-scanned at body temperatures that are likely to be

observed in nature: 20°C, 30°C, and 35°C. Body temperatures will be adjusted in an environmental chamber (Percival Intellus Ultra C8). Following scanning, lizards will be humanely euthanized with a 50:50 mix of water and MS-222 injected into the coelomic cavity (IACUC protocol # 13-100983-MC). Lizards will then be oven-dried to determine body water content. Following drying, lizards will be homogenized in a blender, and then placed into a soxhlet extraction unit, where lipids will be drawn from the homogenate via distillation of petroleum ether. Lipid content from the extract will be compared to the QMR readings of lipids for each set of body temperatures. I will use a linear regression with prediction intervals to determine the likelihood of accuracy for each set of scans.

Implications– There has long been an interest in understanding how shifts in environmental resources affect the dynamics of life-history tradeoffs. In recent years, there has been greater focus given to the subtleties that drive variation in energy allocation strategies; however, technological limitations have precluded the ability of researchers to conduct complex field studies that investigate these ideas. Because of my access to the QMR, I will be able to collect data that will provide valuable baseline information on energy allocation strategies of reptiles in nature. The type of data collected will be the first of its kind in the field of reptile physiological ecology, and will be the first stage in formulating testable hypotheses that will be integral to my dissertation.

While this particular study requires euthanasia for lipid extraction, it will be foundational for future studies that wish to use non-invasive techniques such as the QMR. When an animal is scanned by the QMR, data on fat mass, lean muscle mass, and body water, are quickly generated without causing harm to the animal scanned. Our ability to make rapid and repeatable measurements on individual animals, coupled with the ability to transport the machine to multiple field sites is unprecedented, thus making data of this kind very novel among the species being studied.

Literature Cited

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PREVIOUS USE OF GRAC FUNDS

I received $400 from the GRAC committee in the spring of 2015 to conduct surveys for lizards. Funds were spent on gas to travel to and from the San Mateo and Magdalena mountains, near Socorro, New Mexico. I took 6 field trips, which collectively accumulated to over $400 in cost of gas. Several lizard populations were found, and about 30 lizards were collected, scanned in the QMR, and added to the Museum of Southwestern Biology. The results from the scans were erratic and inconsistent, thereby necessitating a validation study.

BUDGET

Expenditures		Cost per Unit	Quantity	Estimated Total Cost	Funding Source	
Item	**Description**				**GRAC**	Other Source
Lab equipment	Whatman Standard Cellulose Extraction thimbles	$118.28/pack of 25	2	$236.56	$236.56	--
	Petroleum ether	$64.73/4 L	1	$64.73	$64.73	--
	PFA tubing	$6.72/ft.	6	$40.32	$40.32	--
Travel	Two field trips, estimated 250 miles per trip	$0.56/mi.	750	$420	$58.39	$361.61*
Field Supplies	Noose poles, for capture of lizards	$29.46	3	$88.38	--	$88.38*
Total Cost				$849.99	**$400**	$449.99

Total funds requested from GRAC: $400

Total funds (to be) requested from other sources: $449.99

Estimated total cost of project: $849.99

ADDITIONAL FUNDING

*GPSA Student Research Grant, provided by the UNM Graduate and Professional Student Association. I will request up to $500 by deadline Oct. 9, 2015.