

A PROPOSAL TO THE OHIO UNIVERSITY RESEARCH COUNCIL

TITLE OF PROJECT: **In vivo physiological analysis of vascular thermoregulatory structures using noninvasive infrared thermography on free-ranging birds**

NAME OF APPLICANT: **Lawrence M. Witmer, PhD**

STATUS: ___ Lecturer ___ Asst. Prof. ___ Assoc. Prof. **X** Prof. ___ Administrator

DEPARTMENT: **Biomedical Sciences**

CAMPUS ADDRESS: **Mail: 228 Irvine Hall; Office: 123 Life Sciences Building**

E-MAIL ADDRESS: **witmerL@ohio.edu**

RE-SUBMISSION: ___ YES (Original Submission Date _____)
X NO

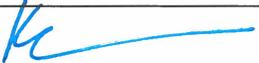
BUDGET: Total Request **\$7980**
 (May not exceed \$8,000)

IRB AND IACUC APPROVAL:

To ensure that the University is in compliance with all federal regulations, complete the checklist below. Note: your proposal can be approved prior to IRB or IACUC approval, but funding will be withheld until notification of approval or exemption.

Yes	No	Office of Research Compliance	Policy #
	X	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
X		Animal Species: multiple bird species, see Appended Materials Institutional Animal Care & Use Committee (IACUC) Approval #: 16-O-028 Expiration Date: 2019-12-22	19.049

SIGNATURES:

Applicant's Signature		Chair/Director's Signature	
Signature		Signature	
Name	Lawrence M. Witmer	Name	Calvin James, PhD
Dept/School	Biomed. Sci., HCOM	Unit	Biomed. Sci., HCOM
Date	2017-01-22	Date	
Dean's Signature			
Name	Kenneth Johnson, DO	Signature	
College	Heritage College	Date	1/25/17

Optional:

If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises.

Signature:  Date: **2017-01-22**

A PROPOSAL TO THE OHIO UNIVERSITY RESEARCH COUNCIL

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NAME OF APPLICANT: **Wm. Ruger Porter, PhD**

STATUS: Lecturer ___ Asst. Prof. ___ Assoc. Prof. ___ Prof. ___ Administrator

DEPARTMENT: **Biomedical Sciences**

CAMPUS ADDRESS: **Mail: 228 Irvine Hall; Office: 113B Life Sciences Building**

E-MAIL ADDRESS: **porterw1@ohio.edu**

RE-SUBMISSION: ___ YES (Original Submission Date _____)
 NO

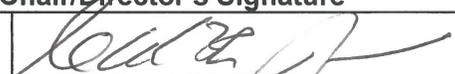
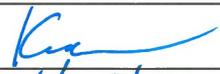
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Applicant's Signature		Chair/Director's Signature	
Signature		Signature	
Name	Wm. Ruger Porter PhD	Name	Calvin James, PhD
Dept/School	Biomed. Sci., HCOM	Unit	Biomed. Sci., HCOM
Date	2017-01-22	Date	
Dean's Signature			
Name	Kenneth Johnson, DO	Signature	
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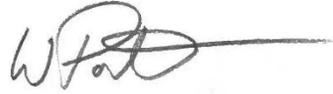
Ohio University Research Council Proposal Checklist

Applicants **must** complete and sign the checklist. The checklist should be included as the second page of the application (following the cover page).

- | | |
|--|---|
| <input checked="" type="checkbox"/> Cover Page | use OURC form |
| <input checked="" type="checkbox"/> Checklist | use OURC form |
| <input checked="" type="checkbox"/> Abstract* | 1 double-spaced page |
| <input type="checkbox"/> Introduction (<i>for resubmissions only</i>)* | 1 double-spaced page |
| <input checked="" type="checkbox"/> New Project Description (<i>for established applicants only</i> [†])* | 1 double-spaced page |
| <input checked="" type="checkbox"/> Discussion | 10 double-spaced pages |
| <input checked="" type="checkbox"/> Glossary/Definition of Terms* (<i>not required</i>) | 2 double-spaced pages |
| <input checked="" type="checkbox"/> Bibliography (<i>not required</i>) | 3 pages |
| <input checked="" type="checkbox"/> Biographical information (<i>applicant(s) and key personnel</i>) | 3 pages per person |
| <input checked="" type="checkbox"/> Other Support (<i>applicant(s) and key personnel</i>) | 1 page per person |
| <input checked="" type="checkbox"/> Budget and Justification | no limit specified |
| <input checked="" type="checkbox"/> Appended Materials | 10 pages; no more than 10 minutes of footage |
| <input checked="" type="checkbox"/> Recommended Reviewers | 5 required |
| <input checked="" type="checkbox"/> Electronic copy of proposal | Single Acrobat file, containing entire proposal and required signatures |

* These sections should be written in language understandable by an informed layperson to assist the committee in its review. Established applicants ([†]) are faculty members who have tenure **and** have been at the university at least three years or administrators who have been at the university at least five years.

****Please note: The committee has the right to return without review any proposals that do not conform to these format requirements.****

Applicant signature: _____  _____  _____

Vertebrates use the blood vascular system to moderate the temperature of specific anatomical regions (e.g., to cool the brain). However, fundamental thermoregulatory mechanisms—i.e., the actual link between physiological function (heating and cooling) and the underlying vascular “plumbing”—have largely remained obscure, which challenges our understanding of issues ranging from organismal responses to climate change to the human health consequences of vascular disease. *In vivo* (live animal) physiological testing involving highly invasive procedures are hard to do and impose confounding artifacts. Fortunately, high-resolution infrared (IR) thermal imaging provides a noninvasive means of *in vivo* physiological measurement of surface temperatures in free-ranging, normally behaving animals. Our previous research has identified vascular devices in birds that may be important for shedding heat as well as for cooling venous blood destined for the brain and eye. We will partner with a thermal physiologist from Canada who has set the standard for IR thermal imaging. Thermal imaging cameras will document IR heat maps of freely ranging birds in wildlife rehabilitation centers, zoos, and parks in South Carolina and Florida. Quantitative thermographic data will be analyzed statistically to assess sites of heat exchange. Thermal data will be compared with existing and new anatomical data based on our well-established techniques (microCT scanning of vascular injections) on legally obtained cadaveric specimens of the study species. This study will be the first to establish the mechanistic links that ultimately allow birds to manage thermal stressors. The intent of this OURC-funded project is (1) to provide publishable pilot research for a full NSF proposal, (2) to promote the professional development of an early-career OU faculty member (Porter), (3) to allow an established OU faculty member (Witmer) to pursue a new direction (*in vivo* experimental physiology) with new funding horizons, (4) to provide training opportunities for OU undergraduates, and (5) to nurture a new collaboration with a world-class ecophysiologicalist.

New Project Description (for established applicants only) — Lawrence M. Witmer, PhD

As a faculty member who has had tenure for at least three years, it is my responsibility to establish that the work in this OURC proposal truly constitutes a new direction that breaks with my past work. The OURC guidelines provide the following detail: “*This new direction can take various forms, such as a technique that is new to the applicant that will offer a new or unique insight; a subject area that is new to the applicant; or...*” These first two criteria both pertain in my case. My past research has been in comparative anatomy and paleontology, using structure to infer function. Although by most measures (e.g., grants, publications) this has been a successful enough research program, it has been incomplete in that my work has *inferred* but not directly *tested* functional hypotheses. The OURC proposal represents an entirely new direction for me in that it will take me into the realm of *in vivo* experimental physiology (the “*new subject area*”) allowing me to work with live animals using quantitative infrared thermography (*the “new technique*”). To help me in this new direction for which I have neither experience nor publications, I’m enlisting a collaborator on the OURC to provide training and mentorship. The OURC grant is intended to set me on this new path to develop the expertise, pilot data, and publications that will allow me to apply to different federal agencies (e.g., USDA, NIH) or different areas of NSF. I currently would have little credibility with these agencies, and so the OURC grant would allow me to build that credibility, diversify my research portfolio, broaden my funding opportunities, and create new training opportunities for OU students. It’s true that this project doesn’t completely “turn its back” on my past research (comparative anatomy), but I regard building that bridge between my past and future research as an essential strategy to build this new direction. Moreover, for the new-faculty co-PI on this OURC proposal (Porter), the comparative anatomical technique of vascular injection constitutes his research identity.

Significance and Background—The goal of this project is to investigate the functional relationship between blood vessels and thermal **ecophysiology** in birds, specifically the heating or cooling of specific parts of the body. Blood vessels fundamentally shuttle nutrients and cellular byproducts around the body, but blood also provides a medium to move thermal energy around the body to either warm or cool specific tissues (Morimoto 1990). Our own experience confirms this phenomenon in that when we are very hot, our skin often “flushes” due to dilation of surface blood vessels to help shed heat. Other well-known examples of thermoregulatory devices in the animal world are the large highly vascular ears of elephants that flap to help dissipate heat (Weissenböck et al. 2010). Likewise, birds use areas of the body as sites of thermal exchange to shed heat, particularly those areas that are located close to the surface, are well vascularized, and are moist to facilitate evaporative cooling (Nolan et al. 1988), such as the oral and nasal cavities (Bernstein et al. 1979) and legs (Johansen & Miller 1973). The anatomy of blood vessels in these putative sites of thermal exchange has been described in a few species of birds such as ducks (Midtgård 1984a), gulls (Midtgård 1984b), and vultures (Arad et al. 1989), and our lab has significantly broadened the sample with our vascular studies of a number of other bird species, such as turkeys, cormorants, ostrich, and loons (Porter & Witmer 2016).

Although these vascular studies, including our own, generated physiological hypotheses about heat exchange, almost none of these studies are linked to *in vivo* physiological data that adequately test these hypotheses. The reverse is also largely true in that almost all experimental studies in thermal physiology have had few to no direct links to the underlying anatomical structures—i.e., specific blood vessels—conferring the physiological function. Moreover, the physiological literature has the major caveat that the birds in many studies experienced unnatural conditions due to invasive experimental procedures, and the experiments did not adequately

control for the stress responses of the bird, especially sympathetically mediated vascular responses like vasodilation or vasoconstriction (Jerem et al. 2015). The animal's thermal response may thus be an artifact of the vascular response to stress, confounding the data gathered in a laboratory setting (Fuller et al. 2003; Balcombe et al. 2004; Lankilde & Shine 2006).

With the advent of non-invasive techniques like thermal imaging, the thermal ecophysiology of free-ranging, naturally behaving animals is now being re-investigated during normal behaviors (Jerem 2015; Tattersall & Cadena 2010; Tattersall 2016), giving us insights into actual thermoregulatory patterns. *A central problem that this study addresses is that, although the literature for both the anatomical and physiological components of thermoregulation are independently rich, few studies have adequately united these disciplines to investigate the physiological processes that are supported by identifiable blood vessels.* For this project we are collaborating with Professor Glenn Tattersall (Brock University, Canada) who is an experienced physiologist and expert in thermal ecophysiology using infrared thermography. *This project seeks to combine Tattersall's expertise in avian thermal ecophysiology and our expertise in avian vascular anatomy to identify physiological sites of thermal exchange in living birds and to identify in existing avian cadavers of the same species those blood vessels directly responsible for the observed heat loss or gain.* The significance of this project is to move our understanding of avian thermoregulatory strategies from one based largely on phenomenology (physiology) or supposition (anatomy) to a more mechanistic and integrated understanding based on demonstrable links between measurable temperature differentials and known vascular "machinery." This project will be a big step forward in our knowledge of how birds use both their physiology and anatomy to adjust body temperatures and interact with the external environment. We regard this OURC-funded project as a pilot study that will enable our team to

be competitive for major federal funding from the National Science Foundation and/or the US Department of Agriculture. Cross-training of each team member will allow Witmer and Porter to acquire expertise with experimental ecophysiology and thermography, as well as enhancing future collaboration by providing Tattersall with experience with anatomical data analysis.

Specific Aim 1 (SA1): Investigate the vascular anatomy of potential physiologically relevant **vascular physiological devices** in species within the study sample.

Introduction—Our previous research (Porter & Witmer 2016) has provided detailed anatomical descriptions of several possible vascular structures (e.g. **retia mirabilia**, **vascular plexuses**) anatomically positioned to exchange heat with either the environment, with other blood vessels (e.g., **counter-current heat exchange**), or a specific organ (e.g., eyeball, brain, spinal cord). We focused on several bird species (e.g., turkeys, loons, cormorants, ostriches), but did not include any *in vivo* physiological analyses. SA1 includes objectives that will lead to a broader understanding of the vascular anatomy of bird species encountered in Specific Aim 2 when infrared thermographic images are collected. These anatomical investigations will include vascular injections, CT-scanning, and gross dissection to test hypotheses of blood vessel anatomy that relate to thermophysiological function.

Hypotheses—(H1) Blood vessels are anatomically positioned to supply sites of thermal exchange in a manner that increases surface area via significant branching and are positioned to exchange heat with both the environment and specific tissues or organs. (H2) Blood vessels within species are more similar than between species, especially in sites of thermal exchange that are part of a unique **thermoregulatory strategy**. (H3) Birds with novel structures, like the pouch of a pelican or the unfeathered head of a turkey vulture, will have a novel vascular arrangement

to support either heat gain or loss (as blood vessels in the legs of ducks are arranged to *reduce* heat loss to cold water; Midtgård 1980, 1981).

Objectives—(1) Use existing cadaveric material in the OU Vertebrate Collection of the birds on the proposed study list: brown pelican, *Pelecanus occidentalis*; double-crested cormorant, *Phalacrocorax auritus*; herring and/or ring-billed gull, *Larus argentatus* or *L. delawarensis*; common loon, *Gavia immer*; turkey, *Meleagris gallopavo*; black and/or turkey vulture, *Coragyps atratus* or *Cathartes aura*; Caribbean flamingos, *Phoenicopterus ruber*; roseate spoonbill, *Platalea ajaja*; heron & egret species, Ardeidae; anhinga, *Anhinga anhinga*; northern gannet, *Morus bassanus*; and others that may be available (e.g., stork or frigatebird). (2) Anatomical analyses will determine their vascular anatomy, with special attention to known sites of thermal exchange, such as the nasal and oral cavities (Bernstein et al. 1979), orbital region (Pinshow et al. 1982), and legs (Johansen & Miller 1973). (3) These vascular patterns will then be analyzed for vascular arrangements, such as a rete or plexus that facilitate heat exchange. (4) Perform a network analyses on the vasculature to quantify the extent of vascularization.

Qualitative methods—(1) A qualitative comparison of anatomical regions will involve well-established methods (see Porter & Witmer 2016), ranging from injecting a radiodense latex solution in the blood vessels (making them visible in μ CT scans using the OU μ CT scanner) to gross dissection with scalpel and forceps. Advanced 3D computer software such as Avizo, Amira, and Maya will be used to analyze and display these blood vessels so that comparisons can be made within and between species, as well as to provide data for network analyses. These methods will identify which species possess potential vascular physiological devices, testing H1, H2, and H3. (2) The anatomical analyses will include recording parameters (e.g., location, size, proximity to other vessels) of any retia mirabilia, plexuses, or specialized vascular networks,

testing H1, H2, and H3. (3) Compare vascular physiological devices among species (e.g., which vessels or retia are enlarged or reduced in different sites of thermal exchange in different species) to gain insight into the diversity of thermoregulatory strategies, testing H2 and H3.

Quantitative methods—A technique new to our lab called Vascular Network Analysis (Wan et al. 1999) will be adapted and refined. Network analyses using Amira’s Filament Editor toolkit will allow us to collect quantitative data relating to branching patterns (e.g., number of vessels, length between branching events, proximity to other vessels) to test for species differences in vascularity (Derkson et al. 2014). Network analyses will allow us to apply metrics and use statistical tests, such as principal components and cluster analyses (Poloavrom et al. 2014) and Kruskal-Wallis tests (Leahy et al. 2015), to assess differences in the vascular networks based on vascular parameters to compare specific vascular physiological devices from each species and to quantify enhanced or reduced vascularity within sites of thermal exchange.

Significance—The significance of SA1 is that it allows us to understand the vascular anatomy of the birds sampled with thermal imaging in Specific Aim 2. The data gathered will be added to the avian datasets already collected in our lab, not only increasing the sample size but also adding more species diversity, vascular diversity (i.e., different arrangements of vessels), and ecophysiological diversity.

Specific Aim 2 (SA2): Use **infrared (IR) thermography** (thermal imaging) to determine the location of physiologically relevant sites of thermal exchange in free-ranging birds.

Introduction—IR thermography has emerged as an important non-invasive tool to investigate the thermal ecophysiology of animals (Jerem 2015; Tattersall et al. 2009; Tattersall 2016). The non-invasive nature of this technique is important because stress placed on the animal during experimentation can cause the release of stress hormones and increased activity of the

sympathetic nervous system, both of which initiate vasomotor responses that will alter the normal relationship between surface temperature and blood flow, thereby confounding the results of the experiment (Balcombe et al. 2004; Lankilde & Shine 2006). This new technique captures infrared light from the animal without them being significantly disturbed. A potential drawback to this technique is that it only detects surface temperatures (i.e. <2mm deep; Tattersall 2016). For our study, however, surface temperatures are, in fact, the parameters we seek to investigate as heat gain and loss usually occurs at the surface (Tattersall 2016) or very near the surface within the nasal and/or oral cavities (Bernstein et al. 1979).

Hypotheses—(H1) Undisturbed and thermoregulating birds will deploy specific regions of the body to shed or gain heat. (H2) IR thermography will detect sites of thermal exchange and highlight regions of the body using heat maps (hot and cold colors). (H3) Heat maps will change as the animal thermoregulates according to different thermal environments.

Objectives—(1) Travel to the Avian Conservation Center (Charleston, SC) and Zoo Miami (Miami, FL) (see permission letters in Appended Materials) and public areas adjacent to these sites to observe free-ranging, normally behaving birds under varied thermal conditions (e.g., in full sun or in shade). Public areas include places like docks, piers, and parks, where wild birds are accustomed to human activity. These settings will allow us access to large-bodied birds that are located in warm environments and often have unfeathered regions thought to have a thermoregulatory role (e.g. the pouch of pelicans or the legs of a gannet). (2) Using infrared cameras, record images of each thermoregulating bird, allowing us to directly observe heat loss or gain from specific areas of the body and inform further anatomical investigations. (3) Analyze IR images to determine which sites of thermal exchange are being deployed to either gain or shed heat, allow us to gauge the location and calculate the magnitude of the heat transfer from

the bird to the environment and rank the significance of sites of thermal exchange in the bird's thermoregulatory strategy.

Methods—At each study site, we will use a FLIR SC660 and a FLIR T1030 to take thermal images of birds under a variety of conditions (e.g., ambient temperature, light levels, time of day), testing H1, H2, and H3. Thermal imaging analysis software will be used to analyze each image to determine the temperature range, mean temperature, standard deviation, and relative size of the site of thermal exchange, testing H1, H2, and H3. Statistically, a **linear mixed-effect model** will be used to further analyze thermal-exchange-site temperature as a function of ambient temperatures, with the ambient temperature as a fixed effect and the animal as a random effect, testing H3. To further explore the factors influencing thermal-exchange-site temperature, a **phylogenetically informed generalized least squares analysis** will be performed, which will allow us to test for the influence of phylogeny (i.e., relatedness) to understand the effects of factors and interactions that determine the temperature and size of sites of thermal exchange, again testing H3. All statistical tests will be conducted using R (R Core Team, 2016). Dr. Tattersall has written an R package (Thermimage) that converts surface temperatures into estimated heat flux values; we will incorporate these calculations into statistical models comparing species' differences in surface heat exchange.

Significance—The significance of SA2 is that it will allow our lab, for the first time, to collect physiological data of living, thermoregulating birds. These birds will be undisturbed, ensuring that the data collected actually relate to thermoregulation and will show which areas of the body are being used to gain and shed heat. SA2 is crucial to the success of the project as it will inform not only the physiological processes each bird deploys to thermoregulate, but also allow us to correlate these findings with our anatomical studies to identify the blood vessels.

Specific Aim 3 (SA3): Integrate data from SA1 and SA2 to map specific blood vessels onto sites of thermal exchange.

Introduction—The literature for both thermal ecophysiology and vascular anatomy is fairly extensive, but few studies have integrated these two disciplines in a manner involving non-invasive thermal measurements and mapping them onto vascular branching patterns. Several

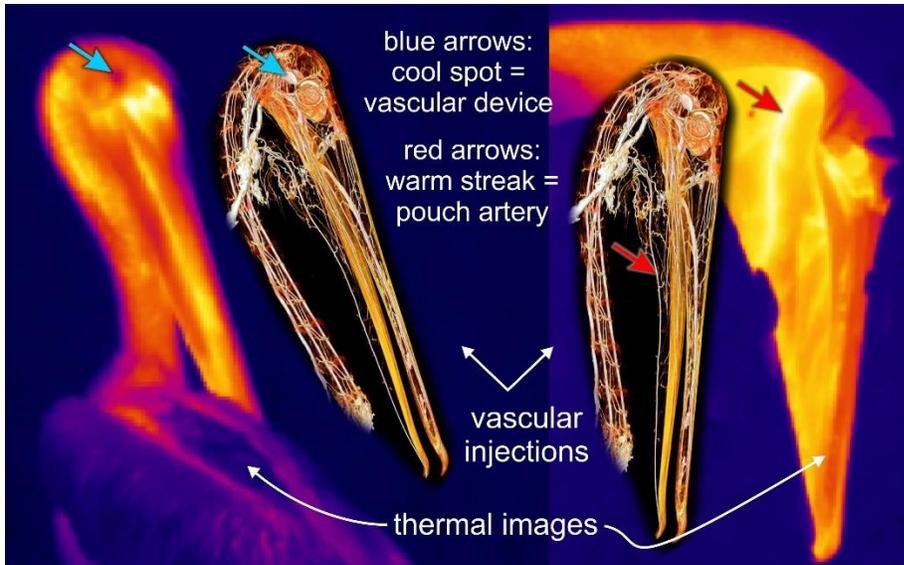


Fig.1. Thermal images of pelicans compared to CT scans of vascular injections of blood vessels. The thermal image “heat maps” show variation in surface temperatures ranging from cooler (bluish) to warmer (reddish).

studies have investigated the anatomy of sites of thermal exchange (Midtgård 1984a, b; Arad et al. 1989) determined by physiological experiments (Bernstein et al. 1979; Pinshow et al. 1982), but the experiments were invasive and this may have introduced stress-related artifacts. The goal of this project is to collect non-invasive thermal images of unstressed individuals of diverse bird species to identify sites of thermal exchange used during thermoregulation and match these sites to known locations of putative vascular devices (Fig. 1). This is a big step forward in the understanding of thermoregulatory strategy in birds.

Hypotheses—(H1) Regions identified by IR thermography will contain blood vessels identified in SA1. (H2) Individual blood vessels that supply or drain these identified sites of thermal exchange will be identifiable as regions of increased or decreased temperature on the IR

image. Blood vessels within sites of thermal exchange will have an anatomical arrangement that supports the physiological functions of regions identified in SA2. (H3) IR thermography will detect sites of thermal exchange previously unidentified by our vascular studies.

Objectives—(1) Compare the results of the anatomical analysis from SA1 to the thermographic images from SA2 to determine which blood vessels are involved in significant thermal exchange (Fig. 1). (2) Investigate the blood vessels associated with any regions that unexpectedly appeared in the thermal images as thermally active, which will allow us to explore previously unknown sites of thermal exchange to refine our understanding of the anatomical arrangement of vascular physiological devices. (3) Compare the vascular anatomy of each site of thermal exchange to identify the vascular arrangement supporting heat gain or loss to understand how this region of the body fits into the bird's overall thermoregulatory strategy.

Methods—The regions of the bird's body that were identified as sites of thermal exchange in the thermographic images will undergo a detailed anatomical analysis using the same methods in Objective 1. The anatomical and IR images will be compared side-by-side, testing H1, H2, and H3. The two images will be superimposed to create a composite image that will co-localize blood vessels and heat transfer sites, testing H1, H2, and H3.

Significance—The significance of SA3 is that it allows us to map blood vessels onto regions that are verified as sites of thermal exchange in free-ranging, undisturbed, normally thermoregulating birds, which allows us to positively identify blood vessels involved in the gain or loss of heat and determine what tissues or organs that these vessels are supporting (e.g. brain and eyes). Each species likely has a unique strategy for thermoregulation, and so assessing the diversity of strategies both qualitatively and quantitatively will allow us to understand the underlying themes of how birds interact with the environment and control body temperatures.

Moreover, by comparing several bird species, we can understand the thermal issues birds face and how each species deploys blood vessels in each site of thermal exchange. With a large enough sample, we can determine the basic anatomical needs for efficient thermoregulation and apply that understanding in the context of climate change.

Personnel and Roles: Porter (OU, PI) will be performing the anatomical methods (vascular injection, etc.), analyzing the vascular data, and performing the quantitative analyses. Witmer (OU, PI) will be assisting Porter in the methods and data analysis and will be coordinating the microCT scanning, as well as coordinating the data collection with our external research partners. Glenn Tattersall, Ph.D. (Brock University, Canada, Collaborator) will be the key player in thermal imaging at our external field study sites, providing training to both Witmer and Porter in the technique and data interpretation. All three of the above will participate in manuscript preparation and ultimately NSF grant writing. Two Ohio University undergraduate students will be invited to participate in the field component as unpaid (but grant supported) assistants. They may also participate during the academic year as research assistants, taking research credits (BIOS 4940) with Witmer.

Timeline: (1) Anatomical preparation (vascular injection, μ CT) and preliminary analysis of anticipated study taxa using existing avian cadavers in the OUVC collection (starting Apr 2017). (2) Drive to Charleston, SC, for field work (Jun 2017). (3) Analysis of SC thermal imaging data and comparison with OU vascular data (Jun–Dec 2017). (4) Fly to Miami for field work (Decr 2017 or Mar [spring break] 2018). 5. Analysis of Miami thermal imaging data and comparison with OU vascular data, manuscript preparation (Dec or Mar 2017 – June 2018).

Glossary

μCT: micro Computed Tomography, an X-ray-based imaging technique that non-invasively digitizes internal and external anatomy and facilitates 3D visualization at sub millimeter resolution.

counter-current heat exchange: blood flow in opposite directions exchanges thermal energy (cool blood is warmed, warm blood is cooled), preventing excessive heat loss or gain to the environment and energy savings.

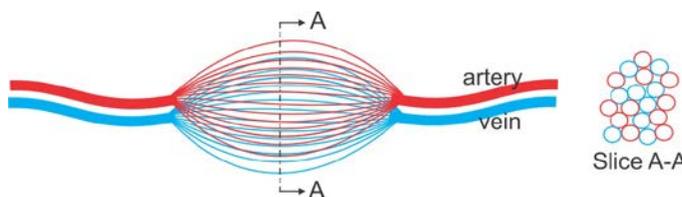
infrared (IR) thermography: a method of detecting infrared energy emitted from an object and converting this information into temperature ranges that are displayed in color values visible to the human eye.

ecophysiology: study of the relationships between an organism's physiological processes and its environment.

linear mixed-effect model: statistical tests that are extensions of linear regressions that describe the relationships of the response and independent variables and allows for the control of sampling or other random variables.

phylogenetically informed generalized least squares analysis: a form of linear regression that tests the relationship of parameters while adjusting the variances to allow for the non-random effects of species relatedness.

retia mirabilia: (plural of rete mirabile) a rapid branching of an artery and vein into numerous fine diameter vessels, which then coalesce into a single vessel. These fine branches of



A rete mirabile in side view (left) and cross section (right) displaying the unique arrangement of arteries and veins.

both the artery and vein are in close contact, allowing the counter-current transfer of thermal energy.

thermoregulatory strategy: the method each species uses to control body temperature by deploying different behaviors or sites of thermal exchange to gain or shed heat.

vascular physiological device: a unique arrangement of blood vessels that has physiological functions beyond shuttling molecules, such as transferring heat (see retia mirabilia).

vascular plexus: a network of arteries or veins that is formed by frequent connections between the blood vessels often found in areas of high metabolic rate or processes requiring high surface areas.

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Biographical Information — Lawrence M. Witmer, PhD

Education

- 1992 PhD, Johns Hopkins University School of Medicine, Baltimore, Maryland
1987 MA, University of Kansas, Lawrence, Kansas
1982 BA, Biology, Cornell University, Ithaca, New York.

Relevant Professional Experience

- Professor, Department of Biomedical Sciences, Heritage College of Osteopathic Medicine, Ohio University, since July 2005; Assoc. Prof. from 2001–2005; Asst. Prof. from 1995–2001
- Director, Ohio University MicroCT Scanning Facility (OU μ CT): 2006 – present
- Assistant Professor, Department of Anatomy, New York Institute of Technology College of Osteopathic Medicine, Old Westbury, New York: 1992 – 1995

Publications (28 in last five years, only reporting through 2015)

1. Bailleul, A. M., **L. M. Witmer**, and C. M. Holliday. 2017. Cranial joint histology in the mallard duck (*Anas platyrhynchos*): new insights on avian cranial kinesis. *Journal of Anatomy*.
2. Brusatte, S. L., A. Muir, M. T. Young, S. Walsh, L. Steel, and **L. M. Witmer**. 2016. The braincase and neurosensory anatomy of an Early Jurassic marine crocodylomorph: implications for crocodylian sinus evolution and sensory transitions. *Anatomical Record* 299:1511–1530. DOI: 10.1002/ar.2346.
3. Porter, W. R. and **L. M. Witmer**. 2016. Avian cephalic vascular anatomy, sites of thermal exchange, and the rete ophthalmicum. *Anatomical Record* 299:1461–1486. doi:10.1002/ar.23375.
4. Porter, W. R., J. C. Sedlmayr, and **L. M. Witmer**. 2016. Vascular patterns in the heads of crocodylians: blood vessels and sites of thermal exchange. *Journal of Anatomy* 229:1–25. doi: 10.1111/joa.12539
5. Stocker, M. R., S. J. Nesbitt, K. E. Criswell, W. G. Parker, **L. M. Witmer**, T. B. Rowe, R. C. Ridgely, and M. A. Brown. 2016. A dome-headed stem-archosaur exemplifies convergence among dinosaurs and their distant relatives. *Current Biology* 26:2674–2680. <http://dx.doi.org/10.1016/j.cub.2016.07.066>.
6. Bourke, J. M., and **L. M. Witmer**. 2016. Nasal conchae function as aerodynamic baffles: Experimental computational fluid dynamic analysis in a turkey nose (Aves: Galliformes). *Respiratory Physiology & Neurobiology* 234:32–46.
7. Martínez, R. D. F., M. C. Lamanna, F. E. Novas, R. C. Ridgely, G. A. Casal, J. Martínez, J. R. Vita, and **L. M. Witmer**. 2016. A basal lithostrotian titanosaur (Dinosauria: Sauropoda) with a complete skull: Implications for the evolution and paleobiology of Titanosauria. *PLoS ONE* 11(4): e0151661. doi:10.1371/journal.pone.0151661.
8. Gignac, P. M., N. J. Kley, J. A. Clarke, M. W. Colbert, A. C. Morhardt, D. Cerio, I. N. Cost, P. G. Cox, J. D. Daza, C. M. Early, M. S. Echols, R. M. Henkelman, A. N. Herdina, C. M. Holliday, Z. Li, K. Mahlow, S. Merchant, J. Müller, C. P. Orsbon, D. J. Paluh, M. L. Thies, H. P. Tsai, and **L. M. Witmer**. 2016. Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. *Journal of Anatomy* 228(6):889–909. doi: 10.1111/joa.12449.
9. O'Brien, H. D., P. M. Gignac, T. L. Hieronymus, and **L. M. Witmer**. 2016. A comparison of postnatal arterial patterns in a growth series of giraffe (*Artiodactyla: Giraffa camelopardalis*). *PeerJ* 4:e1696; DOI 10.7717/peerj.1696.
10. Porro, L. B., **L. M. Witmer**, and P. M. Barrett. 2015. Digital preparation and osteology of the skull of *Lesothosaurus diagnosticus* (Ornithischia: Dinosauria). *PeerJ* 3:e1494 <https://doi.org/10.7717/peerj.1494>.
11. Leahey, L. G., R. E. Molnar, K. Carpenter, **L. M. Witmer**, and S. W. Salisbury. 2015. Cranial osteology of the ankylosaurian dinosaur formerly known as *Minmi* sp. (Ornithischia: Thyreophora) from the Lower Cretaceous Allaru Mudstone of Richmond, Queensland, Australia. *PeerJ* 3:e1475. doi.org/10.7717/peerj.1475.
12. Porter, W. R. and **L. M. Witmer**. 2015. Vascular patterns in iguanas and other squamates: blood vessels and sites of thermal exchange. *PLOS ONE* 10(10): e0139215. doi:10.1371/journal.pone.0139215.

13. Knoll, F., **L. M. Witmer**, R. C. Ridgely, F. Ortega, and J. L. Sanz. 2015. A new titanosaurian braincase from the Cretaceous “Lo Hueco” locality in Spain sheds light on neuroanatomical evolution within Titanosauria. *PLOS ONE*. 10(10): e0138233. doi:10.1371/journal.pone.0138233.
14. Balanoff, A. M., G. S. Bever, M. Colbert, J. A. Clarke, D. Field, P. M. Gignac, D. T. Ksepka, R. C. Ridgely, N. A. Smith, C. Torres, S. Walsh, and **L. M. Witmer**. 2015. Best practices for digitally constructing endocranial casts: examples from birds and their dinosaurian relatives. *Journal of Anatomy*. doi: 10.1111/joa.12378.
15. Dufeu, D. L., and **L. M. Witmer**. 2015. Ontogeny of the middle-ear air-sinus system in *Alligator mississippiensis* (Archosauria: Crocodylia). *PLOS ONE* 10(9): e0137060. doi:10.1371/journal.pone.0137060E.
16. Sues, H.-D., A. O. Averianov, R. C. Ridgely, and **L. M. Witmer**. 2015. Titanosauria (Dinosauria, Sauropoda) from the Upper Cretaceous Bissekty Formation of Uzbekistan. *Journal of Vertebrate Paleontology* 35(1):e889145-1–e889145-14.

Presentations (58 in last five years, not counting 11 invited lectures, keynotes, etc.; only reporting through 2015)

1. Early, C. M., and **L. M. Witmer**. 2017. Inferring vision-related neuroanatomy and behavior from the brain endocasts of birds. Annual Meeting of the Society of Integrative and Comparative Biology, New Orleans, LA.
2. Bourke, J. M., W. R. Porter, and **L. M. Witmer**. 2016. Complicated noses keep cool heads: the thermoregulatory effects of nasal passage shape in extant birds and reptiles, with implications for dinosaurs. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 205–206*.
3. W. R. Porter, and **L. M. Witmer**. 2016. Vascular anatomy and thermophysiological strategies in the heads of extinct and extant dinosaurs. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 203–204*.
4. Morhardt, A. C., R. C. Ridgely, and **L. M. Witmer**. 2016. Diffusible iodine-based contrast enhancement of large, post-embryonic, intact vertebrates for CT scanning: staining, destaining, and long-term storage. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 89–90*.
5. Holliday, C., H. P. Tsai, I. N. Cost, K. C. Sellers, S. Lautenschlager, and **L. M. Witmer**. 2016. DiceCT and its applications for understanding the reptile musculoskeletal system. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 266*.
6. Nassif, J. P., R. C. Ridgely, and **L. M. Witmer**. 2016. Virtual reconstruction of the skull of a large parrot (Aves: Psittaciformes: *Ara macao*), highlighting the anatomy of the brain endocast, inner ear, rhamphotheca, and kinetic apparatus. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 259*.
7. Holliday, C. M., A. Bailleul, I. N. Cost, K. C. Sellers, **L. M. Witmer**, and M. K. Vickaryous. 2016. The significance of novel palatal joints in the adaptive radiations of archosaurs. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 209*.
8. Cost, I. N., A. Spates, K. C. Sellers, J. L. Davis, K. M. Middleton, **L. M. Witmer**, and C. M. Holliday. Relative kinetic competency in the palatal complexes of birds and other diapsids. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 208–209*.
9. Cerio, D. G., and **L. M. Witmer**. 2016. The visual apparatus of archosaurs: correlates of orbital anatomy, eye size, and behavior. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 232–233*.
10. Degrange, F. J., C. P. Tambussi, R. C. Ridgely, and **L. M. Witmer**. 2016. Neuroanatomy of the extinct terror birds (Aves: Phorusrhacidae): implications for a predatory mode of life. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 224*.

11. Early, C. M., R. C. Ridgely, W. R. Porter, D. G. Cerio, and **L. M. Witmer**. 2016. The skull and endocranial anatomy of the extinct giant moa *Dinornis robustus* (Aves: Palaeognathae) and implications for the behavioral role of vision in moa. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 223–224*.
12. Morhardt, A. C., R. C. Ridgely, and **L. M. Witmer**. 2016. Gross Anatomical Brain Region Approximation (GABRA): a new landmark-based approach for estimating brain regions in archosaurs. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 223*.
13. Bailleul, A. M., J. R. Horner, **L. M. Witmer**, and C. M. Holliday. 2016. Functional cranial joint histology in reptiles and birds and its significance for avian cranial kinesis. Program & Abstracts of the 11th International Congress of Vertebrate Morphology, Washington, D.C. 2016. *Anatomical Record, Volume 299, Special Feature: 72*.
14. Holliday, C. M., K. C. Sellers, H. P. Tsai, M. K. Vickaryous, C. F. Ross, L. Porro, J. Davis, and **L. M. Witmer**. 2015. PMJs and TMJs: convergence in the craniomandibular joints of crocodyliforms and mammals. 75th Annual Meeting of the Society of Vertebrate Paleontology, Dallas, TX. *Journal of Vertebrate Paleontology Supplement—Meeting Program and Abstracts: 145*.
15. Tsuihiji, T., M. Watabe, K. Tsogtbaatar, and **L. M. Witmer**. 2015. A new specimen of *Shartegosuchus* (Archosauria: Crocodyliformes) from the Upper Jurassic in Shar Teg, western Gobi Desert, Mongolia. 75th Annual Meeting of the Society of Vertebrate Paleontology, Dallas, TX. *Journal of Vertebrate Paleontology Supplement—Meeting Program and Abstracts: 227*.
16. Early, C. M. and **L. M. Witmer**. 2015. Neuroanatomy, endocasts, and the evolution of brains and behavior in birds. 75th Annual Meeting of the Society of Vertebrate Paleontology, Dallas, TX. *Journal of Vertebrate Paleontology Supplement—Meeting Program and Abstracts: 119*.
17. Knoll, F., R. C. Ridgely, and **L. M. Witmer**. 2015. Comparative paleoneurology of the basal dicraeosaurid sauropod *Suuwassea emilieae*. 75th Annual Meeting of the Society of Vertebrate Paleontology, Dallas, TX. *Journal of Vertebrate Paleontology Supplement—Meeting Program and Abstracts: 156*.
18. Cost, I. N., A. Spates, K. C. Sellers, J. L. Davis, K. M. Middleton, **L. M. Witmer**, and C. M. Holliday. 2015. Biomechanics of the avian feeding apparatus. 75th Annual Meeting of the Society of Vertebrate Paleontology, Dallas, TX. *Journal of Vertebrate Paleontology Supplement—Meeting Program and Abstracts: 110*.
19. Cerio, D. G., R. C. Ridgely, and **L. M. Witmer**. 2015. The eyes have it: bounding estimates of eye size in dinosaurs with soft-tissue reconstruction and the extant phylogenetic bracket approach. 75th Annual Meeting of the Society of Vertebrate Paleontology, Dallas, TX. *Journal of Vertebrate Paleontology Supplement—Meeting Program and Abstracts: 104*.
20. Morhardt, A. C., R. C. Ridgely, and **L. M. Witmer**. 2015. Iodine-based CT Contrast-Enhancement of Vertebrates: best practices for staining, de-staining, and long-term storage of large, post-embryonic, intact specimens. The Austin Working Group: advancing contrast-enhanced CT imaging in the Biological Sciences. The University of Texas at Austin & the High-Resolution X-Ray CT Facility. Austin, Texas.
21. Porter, W. R. and **L. M. Witmer**. 2015. Evidence for blood vessels and physiological heat exchange in the heads of dinosaurs. 127th Annual Meeting of the American Association of Anatomists, Experimental Biology Conference, Boston, MA.
22. Holliday, C. M., K. C. Sellers, L. B. Porro, C. F. Ross, **L. M. Witmer**, and M. K. Vickaryous. 2015. PMJs and TMJs: Convergence in the craniomandibular joints of crocodylians and mammals. 127th Annual Meeting of the American Association of Anatomists, Experimental Biology Conference, Boston, MA.
23. Bourke, J. M., W. R. Porter, R. C. Ridgely, and **L. M. Witmer**. 2015. Using airflow patterns to aid inferences of nasal soft-tissue reconstructions in dinosaurs. 127th Annual Meeting of the American Association of Anatomists, Experimental Biology Conference, Boston, MA.
24. Holliday, C. M., K. C. Sellers, M. K. Vickaryous, C. F. Ross, L. B. Porro, **L. M. Witmer**, and J. L. Davis. 2015. The functional and evolutionary significance of the crocodyliform pterygomandibular joint. Annual Meeting of the Society of Integrative and Comparative Biology, West Palm Beach, FL.

Biographical Information — William Ruger Porter, Ph.D.

Education

2015 PhD, Ohio University, Athens, Ohio

2006 BS, Indiana University, South Bend, Indiana

Relevant Professional Experience

- Lecturer of Human Anatomy, Ohio University Heritage College of Medicine
Human Medical Gross Anatomy, July 2014

Publications (last five years)

1. **Porter, W. R.** and L. M. Witmer. 2016. Avian cephalic vascular anatomy, sites of thermal exchange, and the rete ophthalmicum. *Anatomical Record* 299:1461–1486.
doi:10.1002/ar.23375.
2. **Porter, W. R.**, J. C. Sedlmayr, and L. M. Witmer. 2016. Vascular patterns in the heads of crocodylians: blood vessels and sites of thermal exchange. *Journal of Anatomy* 229:1–25. doi: 10.1111/joa.12539
3. **Porter, W. R.** and L. M. Witmer. 2015. Vascular patterns in iguanas and other squamates: blood vessels and sites of thermal exchange. *PLOS ONE* 10(10): e0139215.
doi:10.1371/journal.pone.0139215.
4. Bourke J.M., **Porter W.R.**, Ridgely R.C., Lyson T.R., Schachner E.R., Bell P.R., and L.M. Witmer. 2014. Breathing Life into Dinosaurs: Tackling Challenges of Soft-Tissue Restoration and Nasal Airflow in Extinct Species. *Anatomical Record* 297:2148–2186.

Presentations (last five years)

1. Holliday CM, **Porter W**, Owerkowicz T, Vliet K, Witmer LM. 2016. The soft tissues of the dorsotemporal fenestra of archosaurs and its significance for dinosaur biology. Society of Vertebrate Paleontology Annual Meeting, Salt Lake City, Utah.
2. Bourke J, Witmer LM, Ridgely RC, **Porter W**, Zanno LE. 2016. Airflow simulations in the antorbital sinus of *Acrocanthosaurus*. Testing the potential for theropod paranasal sinuses to function as accessory cooling structures. Society of Vertebrate Paleontology Annual Meeting, Salt Lake City, Utah.
3. Nassif JP, Bourke J, Cerio D, Dufeu D, Early CM, Morhardt AC, **Porter W**, Ridgely R, Spaw A, Witmer LM. 2016. A digital menagerie: building the Witmerlab's visible Interactive anatomy library as an open-access resource for research and education. Society of Vertebrate Paleontology Annual Meeting, Salt Lake City, Utah.
4. **Porter WR** and Witmer LM. 2016. Vascular anatomy and thermophysiological strategies in the heads of extinct and extant dinosaurs. 11th International Congress of Vertebrate Morphology, Washington, D.C., USA.

5. **Porter WR** and Tattersall G. 2016. Co-Organizers of the ICVM Symposium entitled New insights into the functional relationship between anatomy and physiology of extinct and extant vertebrates. 11th International Congress of Vertebrate Morphology, Washington, D.C., USA.
6. **Porter WR** and Witmer LM. 2015. Evidence for blood vessels and physiological heat Exchange in the heads of dinosaurs. American Association of Anatomists Annual Meeting, Boston, MA. (Poster)
7. Bourke JM, **Porter WR**, Ridgely, RC, and Witmer, LM. 2015. Using airflow patterns to aid inference of nasal soft-tissue reconstructions in dinosaurs. American Association of Anatomists Annual Meeting, Boston, MA.
8. Bourke JM, **Porter WR**, Ridgely RC, Witmer LM. 2014. Airflow reconstruction and heat-transfer potential in the elongate nasal passages of the ankylosaurs *Panoplosaurus mirus* and *Euoplocephalus tutus*. Society of Vertebrate Paleontology Annual Meeting, Berlin, Germany.
9. **Porter WR** and Witmer LM. 2013. Evidence for sites of physiological heat exchange in the heads of dinosaurs. Society of Vertebrate Paleontology Annual Meeting, Los Angeles, CA.
10. Bourke JM, **Porter WR**, Lyson T, Schachner E., Bell P. 2013 Nasal turbinates in Pachycephalosaurids (Dinosauria:Ornithiscia): Reconstructing nasal anatomy and airflow, with implications for physiology. Society of Vertebrate Paleontology Annual Meeting, Los Angeles, CA.
11. **Porter WR** and Witmer LM. 2013. Dinosaur vascular anatomy and its physiological implications. 10th International Congress of Vertebrate Morphology, Barcelona, Spain.
12. Witmer LM, Ridgely, RC, Snively, E, Holliday, C, **Porter WR**, Bourke JB, and Morhardt A. 2013. Analytical and modeling approaches for the digital restoration of dinosaur head functional anatomy within the visible interactive dinosaur project. 10th International Congress of Vertebrate Morphology, Barcelona, Spain.
13. Bourke JB, Ridgeley RC, **Porter WR**, Lyson TR, Schachner ER, Bell PR, and Witmer LM. 2013. Breathing life into dinosaurs: tackling challenges of soft-tissue reconstruction and nasal airflow in extinct species. 10th International Congress of Vertebrate Morphology, Barcelona, Spain.
14. **Porter WR** and Witmer LM. 2012. Dinosaur cephalic vascular anatomy and its physiological implications: evidence from the fossils. Society of Vertebrate Paleontology Annual Meeting, Raleigh, NC.
15. Bourke JB, **Porter WR**, and Witmer LM. 2012. Dorsal or rostral nostrils? Testing fleshy nostril position and airflow in sauropods using computational fluid dynamics. Society of Vertebrate Paleontology Annual Meeting, Raleigh, NC.

16. **Porter WR** and Witmer LM. 2012. Vascular patterns in iguanas: blood vessels and cephalic sites of thermal exchange. Society of Integrative and Comparative Anatomy Annual Meeting, Charleston, SC. (Poster)
17. **Porter WR** and Witmer LM. 2011. Vascular anatomy and its physiological implications in extant and extinct dinosaurs and other diapsids. Society of Vertebrate Paleontology Annual Meeting, Las Vegas, NV.
18. **Porter WR** and Witmer LM. 2011. Vascular anatomy and physiological implications in extant and extinct theropod dinosaurs. Geological Society of America Northeast/Northcentral regional meeting, Pittsburgh, PA.
19. **Porter WR** and Witmer LM. 2011. Vascular patterns in galliform birds: regions of thermal exchange. Society of Integrative and Comparative Anatomy Annual Meeting, Salt Lake City, UT. (Poster)
20. **Porter WR**, Witmer LM. 2010. Vasculature and dinosaur physiology: vascular patterns in extant diapsids. Society of Vertebrate Paleontology Annual Meeting, Pittsburgh, PA. (Poster)
21. Martiny AR, Ridgely RC, Dufeu DL, **Porter WR**, Bourke JM, Morhardt AC, Snively ED, and Witmer LM. 2010. Promoting a culture of outreach within an active university research lab setting: Witmerlab at Ohio University. Society of Vertebrate Paleontology Annual Meeting, Pittsburgh, PA. (Poster)
22. Witmer LM, Ridgely RC, Snively ED, Holliday CM, Hieronymus T, Dufeu DL, **Porter WR**, Bourke JM, Martiny AC. 2010. Toward the visible dinosaur: integrating anatomical systems to test inferences of function, physiology, and behavior. 9th International Congress of Vertebrate Morphology, Punta del Este, Uruguay.
23. **Porter WR** and Witmer LM. 2010. Vasculature and dinosaur physiology: patterns in the extant realm. Society of Integrative and Comparative Anatomy Annual Meeting, Seattle, WA. (Poster)

Other Support — Lawrence M. Witmer, PhD

A. Previous University Funding (last three years):

- 1804 Award

Title: “Enhancing 3D imaging research at Ohio University: acquisition of a microCT scanner to replace and upgrade current capabilities” with L. Liu and S. Hooper (Witmer senior PI).

Award dates: 2013–2015

Award amount: \$75,000

Outcomes: Purchase of the OU μ CT scanner, with support of several other OU units and external grants. The OU μ CT is a shared core facility.

Relationship to current OURC proposal: bird specimens will be scanned on this device

B. External Funding (last three years):

- National Science Foundation grant (current)

Title: “Collaborative Research: Dinosaur jaw muscle evolution and the origins of avian cranial kinesis” with R. C. Ridgley (Witmer senior PI on OU side). Collaborative project with University of Missouri (lead institution) & University of Southern Indiana.

Award dates: 2015–2018

Award amount: OU budget \$265,481 (Total project budget for all institutions: \$965,031)

Outcomes: two published articles, several conference presentations and abstracts, research is on-going

Relationship to current OURC proposal: none, completely different project with no overlap of goals, techniques, specimens, or data.

- National Science Foundation grant (expired)

Title: “Toward the Visible Dinosaur: Integrating anatomical systems to test inferences of function, physiology, and behavior, with special emphasis on broader impacts and outreach” with R. C. Ridgley (Witmer senior PI)

Award dates: 2011–2016

Award amount: \$350,500

Outcomes: over two dozen published articles, dozens of conference presentations and abstracts, several OU doctoral & undergraduate students trained, wide dissemination

Relationship to current OURC proposal: Funded some work that comprises anatomical pilot data for the OURC proposal

C. Sustainability: It is important to emphasize that this OURC-funded project is intended to be a sustained research effort over the next several years. Once we have the OURC-collected data, we will publish at least one or two articles and present at conferences to get demonstrated credibility for our team, at which point we will submit external funding proposals to NSF (to programs different from the above awards), as well as to USDA and potentially to NIH later.

D. Statement on the criterion for OURC of funding "for projects where no other funding is available": Again, no current funds are available for this project, and it is our explicit goal to turn the investment of the OURC grant into a sustained, externally funded project.

Other Support — Wm. Ruger Porter, PhD

A. Previous University Funding (last three years): none

B. External Funding (last three years):

- American Association of Anatomists Research Outreach Grant

Title: “New insights into the functional relationship between anatomy and physiology of extinct and extant vertebrates”

Award Date: 2015

Award Amount: \$2500

Outcome: Supported travel for international researchers to present research at a symposium at the 2016 International Congress of Vertebrate Morphology in Washington, D.C.

Relationship to current OURC proposal: Porter co-organized this symposium with Glenn Tattersall, fostering the personal and professional relationship that has led to the collaboration proposed in this proposal.

C. Sustainability: see statement under Witmer’s information on previous page

D. Statement on the criterion for OURC of funding "for projects where no other funding is available": see statement under Witmer’s information on previous page

Budget and Justification

Budget

A. Travel	Detail	Amount
1. Travel to South Carolina sites (four days)		
a. OU motor pool vehicle (Honda Pilot), round trip	4 days @ \$20, 1200 mi @ 46¢	\$632
b. Airfare for external collaborator (Tattersall)	Buffalo – Charleston	\$400
c. Two hotel rooms (men, women)	3 nights @ \$135 x 2	\$810
d. Meals	5 people @ \$30/day x 4	\$600
2. Travel to Miami sites (four days)		
a. Airfare for OU people (2 PIs, 2 undergrads)	Columbus – Miami, \$400 x 4	\$1600
b. Airfare for external collaborator (Tattersall)	Buffalo – Miami	\$380
c. Two hotel rooms (men, women)	3 nights @ \$143 x 2	\$858
d. Meals	5 people @ \$30/day x 4	\$600
e. Car rental	Four-day package	\$300
	Total travel	\$6180
B. OU μ CT scanning: 12 specimens each scanned twice	24 scans @ \$75	\$1800
	Total Budget	\$7980

Justification

A. *Travel costs.*—The success of this project depends on traveling to field sites to gather quantitative infrared thermographic images of free-ranging birds in a variety of thermal settings. The species listed under Objective 1 (page 4 of the Discussion section) are available at the sites we have identified in South Carolina and Florida. The sites are the Avian Conservation Center near Charleston, SC, and Zoo Miami in Miami, Florida. Permission letters for both sites are included in Appended Materials. Additionally, many of the species are observable in locations nearby these facilities, such as public parks and waterfront piers, where birds are wild but highly accustomed to humans. (Note: Our approved IACUC protocol covers all of these observational studies.)

The plan is to have each trip consist of four days, two research days flanked on each side by a travel day. Data collection is rapid since it is basically photographic (but in the infrared spectrum), and thus we should be able to collect enough data in two days at each site to run legitimate statistical analyses. The two sites will be traveled to in different manners. The South Carolina site is an 8.5-hour drive from Athens, and a van will be rented from the Ohio University Motor Pool to reduce travel costs (rates are quoted from OU Parking Services personnel). This vehicle will accommodate the Athens-based Co-Pi's and two undergraduate student workers. The grant will fly our collaborator (Glenn Tattersall) from the nearest airport (Buffalo, NY) to Charleston, where we will pick him up. Travel to the Miami site will be by air, with the OU team flying in from Columbus and Tattersall again flying in from Buffalo; a vehicle will be rented in Miami so that we can transport our personnel and gear. At each site we will stay in an extended-stay hotel with a kitchen, allowing us to moderate food costs by preparing some meals in the hotel. We are planning on booking two rooms at each site, one for the men and one for the women.

We are budgeting for two undergraduate students to join us on these trips to assist with logistics and data collection, as well as to provide training and experience. These likely will be

students who are already working in Witmer's lab, taking research credits during the academic year. We will cover the students' travel costs but not pay them a stipend.

B. *MicroCT scanning costs.*—The anatomical component of the project will require access to the Ohio University MicroCT Scanning facility (OU μ CT). As noted in the methods sections of the Discussion, the vascular systems of existing cadaveric specimens in the Ohio University Vertebrate Collection will be studied via PI Porter's well-honed vascular injection technique. Adequate supplies are already in hand. Twelve specimens comprising the study sample will each require two separate scans on the OU μ CT, one prior to injection and one after injection. Each scan will take one hour, and the hourly scan rate for OU researchers is \$75. PI Witmer has been Director of the OU μ CT since 2006, and thus we have the experience to know that these numbers and costs are correct.

Note: No thermal imaging gear is being requested in this proposal, because two infrared thermography equipment sets are being provided by our collaborator (Glenn Tattersall). His gear is of very high quality and purchasing equivalent gear for OU would exceed the OURC budget limits. Dedicated gear for OU researchers would be part of future federal grant proposals.

Appended Materials

1. Approved IACUC protocol
2. Letter from HCOM Biomedical Sciences Chair affirming Porter's research expectation
3. Letter from HCOM Executive Dean affirming Witmer's new research direction
4. Collaborator support letter from Glenn Tattersall (Brock University, ON, Canada)
5. Support letter from research site in Charleston, South Carolina
6. Support letter from research site in Miami, Florida
7. Zoo Miami Animal Care and Use Committee Observation Only Exemption Form

IACUC REVIEW



Protocol Information

Protocol Number: 16-O-028
Submission Date: 12/19/2016
Expiration Date: 12/22/2019
Approved By: hayhow
Approved Date: 12/23/2016 07:41:42 AM
Form Type: ORIGINAL
Form Status: APPROVED
Waiver: There are no waivers with this approval.

Toggle Comments

Research Members

Name	Role	Anesthesia	Surgery	Euthanasia	CITI Training
Witmer, Lawrence Title: Professor College: Osteopathic Medicine Department: COM-Biomedical Sciences	PI				Expires: 12/16/2019
Porter, William Title: Lecturer, Human Anatomist College: Osteopathic Medicine Department: COM-Biomedical Sciences	CO-I				Expires: 12/16/2019

Project Title: In vivo physiological analysis of vascular thermoregulatory structures based on observations using noninvasive infrared thermography on free-ranging birds

Project Summary: Virtually all vertebrate animals use the blood vascular system to physiologically moderate the thermal environment of specific anatomical regions and/or the whole body, for example, allowing warming of inspired air or cooling of the brain and delicate sensory tissues. Our previous research has identified key vascular devices in the head and neck regions of several bird species (e.g., turkey, ostrich, cormorant, pelican) that we hypothesize are important for shedding heat as well as for cooling venous blood destined for the brain and eye. In vivo physiological testing has been very difficult due to the necessity of highly invasive procedures that are complicated to execute and which also impose artifacts (e.g., sympathetically mediated vascular changes due to the stress of the procedures). Fortunately, the advent of high-resolution infrared thermal imaging using thermography cameras provides a completely noninvasive means of in vivo physiological measurement of surface temperatures in free-ranging, normally behaving birds. Infrared thermography has the shortcoming of recording only temperatures of tissues near the body surface, but, in reality, virtually all of the vascular heat exchangers in our study are located superficially. For this study, we will be partnering with Glenn Tattersall (Brock University) who is an accomplished comparative thermal physiologist and is highly experienced with and has set the standard for infrared thermal imaging. We will use thermal imaging cameras to document infrared heat maps of freely ranging birds in offsite naturalistic settings in wildlife rehabilitation centers, zoos, and public areas where wild birds tend to congregate (e.g., waterfront piers). No birds will be handled or otherwise bothered by us or by others on our behalf. Thermographic data will be compared with existing and new data on the vascular system of the study species based on our well-documented anatomical techniques (microCT scanning of radio-opaque vascular injections) on legally obtained cadaveric specimens of the study species. This IACUC application is in support of an OURC proposal. The resulting data will be written up for journal submission to provide published pilot research for an upcoming NSF proposal.

Associated College: College of Osteopathic Medicine

YES NO



Questions:

Observation Only: This project will only observe animals in their natural environments with no contact. This can include projects such as counting animals seen during a migration or observation and recording of natural behaviors. If any contact, including mist netting, is used it cannot qualify as observation only.

Description of animals being observed.

IACUC REVIEW

1. brown pelican, *Pelecanus occidentalis*
2. double-crested cormorant, *Phalacrocorax auritus*
3. herring and/or ring-billed gull, *Larus argentatus* or *L. delawarensis*
4. common loon, gavia immer
5. turkey, *Meleagris gallopavo*
6. black and/or turkey vulture, *Coragyps atratus* or *Cathartes aura*
7. Caribbean flamingos, *Phoenicopterus ruber*
8. roseate spoonbill, *Platalea ajaja*
9. wood stork, *Mycteria Americana*
10. heron & egret species, *Ardeidae*
11. frigatebird, *Fregata magnificens*
12. anhinga, *Anhinga anhinga*
13. northern gannet, *Morus bassanus*



Purchased Antibody Use Only NO LIVE ANIMALS WILL BE USED: This project will only use commercially produced antibodies purchased from a vendor with a current USDA license.

Filming Animals: This project will only use animals in a film, theater or art.

Adverse Outcomes

YES NO



QUESTION:

Are there any EXPECTED adverse outcomes for animal health and/or well being from the experimental procedures?

ROUTING

User	Status	Emails Sent	Next Email Send	Comments
Witmer, Lawrence	APPROVED	0		12/19/2016 09:16:13 AM approved the IACUC protocol.
Porter, William	APPROVED	1		12/19/2016 09:43:06 AM approved the IACUC protocol.
IACUC	APPROVED			On 12/23/2016 07:41:42 AM hayhow approved the IACUC protocol.

New Expiration Date:

Enter why you are changing the expiration date:

Change Expiration Date

New Protocol Number:

Enter why you are changing the protocol number:

Change Protocol Number

Please provide a reason as to why you are denying this IACUC submission.

DENY PROTOCOL

Please provide a reason as to why you are requesting a revision for this IACUC submission.

REQUEST REVISION

Type your comment below.

ADD COMMENT

Enter revision comments below.

REQUEST REVISION

Please provide a reason as to why you are suspending this IACUC protocol.

SUSPEND PROTOCOL

UNSUSPEND PROTOCOL

Review By Date:

Agenda Review Date:

Submit To Committee

Review By Date:

Agenda Review Date:

IACUC REVIEW

Submit To DMR
Insert any waivers here.

APPROVE PROTOCOL

Date: January 20th, 2017

To: Ohio University Research Committee

From: Calvin James, PhD
Chair, Department of Biomedical Sciences
Heritage College of Osteopathic Medicine

Subject: Support for Wm. Ruger Porter's participation in an OURC proposal

This letter is intended to support the participation of Wm. Ruger Porter, PhD, in the OURC proposal entitled "In vivo physiological analysis of vascular thermoregulatory structures using noninvasive infrared thermography on free-ranging birds," submitted by Lawrence Witmer and Dr. Porter, who are both faculty members in the Department of Biomedical Sciences in the Heritage College.

The OURC guidelines provide the following eligibility criterion: "OURC awards are primarily for Group I and Group II faculty and benefits-eligible, permanent administrative staff." Dr. Porter qualifies as a Group II faculty member, but I would like to further endorse his participation in this project by stating that his research efforts are a permissible part of his 20% service expectation (i.e., service in support of advancing research in our department). Dr. Porter has been able to easily manage meeting his teaching obligations (even winning teaching awards) while remaining active and productive in his research in Prof. Witmer's lab. I can confirm that Dr. Porter will be here and available for the research project during the 12-month term of the OURC grant.

Sincerely,



Calvin B. L. James, Ph.D., Chair



OHIO
UNIVERSITY

Heritage College of
Osteopathic Medicine

Office of the Executive Dean
Grosvenor Hall 201
Athens OH 45701-2979

T: 740.593.9350
F: 740.593.1990

January 24, 2017

Subject: Support for the OURC proposal submitted by Lawrence Witmer & Wm. Ruger Porter

This letter is intended to support the OURC proposal entitled "In vivo physiological analysis of vascular thermoregulatory structures using noninvasive infrared thermography on free-ranging birds," submitted by Lawrence Witmer and Ruger Porter, who are faculty members in the Department of Biomedical Sciences in the Heritage College.

More in line with the guidelines of the funding mechanism, I affirm that this project indeed represents a new research direction for Professor Witmer. The OURC guidelines mandate that "established faculty...must argue that the proposed project is a break with the applicant's past work, rather than continuous with his or her previous scholarship." Prof. Witmer, an established investigator and faculty member in the Department of Biomedical Sciences, has been focused on comparative anatomy and paleontology. While the current OURC proposal establishes an appropriate link to his scholarship, it seeks to explore an entirely new area of experimental physiology. He has no publications in this area, and the OURC project would provide him with a unique opportunity to receive a special mentorship in this field from a leading physiologist from Canada. This collaborative effort is also in line with the College's vision to promote the visibility of our scientists and place them on the research map of their fields.

The Heritage College strongly supports Prof. Witmer's quest to enter this new research venue. We believe that this scientific direction into pathophysiology will broaden his horizon and open entirely new areas of external research funding that go beyond NSF to include other federal agencies, such as the USDA and NIH. We are very enthusiastic about this novel path for the group and strongly support his effort to work closely with Dr. Porter in diversifying his research portfolio in that it will benefit not only his research team, but also the Heritage College and Ohio University.

With a great enthusiasm, we look forward to hearing from you soon.

Sincerely,

Kenneth H. Johnson, D.O., FAAO

Executive Dean

johnsok9@ohio.edu

Brock University
Depart. Biol. Sciences
500 Glenridge Avenue
St. Catharines, ON
L2S 3A1 Canada
T 905-688-5550 ext.4815
F 905-688-1855
brocku.ca

Monday, January 23, 2017

Re: Agreement to collaborate on the thermal biology project

Dear Drs. Porter and Witmer,

I have read a draft of the OURC proposal, and I am pleased to provide my support and acknowledge participation in the project entitled “In vivo physiological analysis of vascular thermoregulatory structures based on observations using noninvasive infrared thermography on free-ranging birds”. My laboratory is well equipped for conducting field and lab research in the realm of thermal biology, we routinely use infrared thermal imaging to assess heat flux in birds, and I have provided support to a wide array of researchers. At present, I am involved in projects with colleagues in Australia investigating insulation in fairy wrens, colleagues in Los Angeles on torpor in nesting hummingbirds, and colleagues in London, Ontario investigating hibernating ground squirrels. I am also lead on a National Geographic Society funded project (2013-2015) on thermal imaging of Darwin’s finches, where I developed and honed the skills mentioned in your proposed research.

In terms of this proposal, I am able and willing to provide use of my current thermal imaging camera (SC660) or a new camera I will be receiving soon, the T1030. The latter camera will be higher resolution (1024x768) and have telephoto capacity, essential for our proposed research in the field. I should be able to conduct the thermal imaging along with you and be involved in the data analysis, most of which will be computational and performed off site.

Finally, I will gladly provide you with training on using thermal image analysis software, as well as assist with the physical and statistical modelling required to confer heat flux analysis to our data. Recently, I wrote a package for use in R that will make the physical modelling of heat exchange relatively painless. Likewise, I can assist with the statistical modelling in R, as I have considerable experience with linear mixed modelling, commonly used in physiology where we conduct repeated measurements on subjects.

In short, I am enthusiastic to play a part in this project and look forward to building our collaboration.

Sincerely yours,



Glenn Tattersall, PhD, Professor, Biological Sciences

AVIAN CONSERVATION CENTER

The Center for Birds of Prey



January 12, 2017

To Whom it May Concern:

The Avian Conservation Center is a 501c 3 non-profit organization located near Charleston, SC. The organization is multi-faceted, with a focus on providing professional medical care to injured birds of prey and shorebirds, and educational presentations conveying the importance of the crucial roles of wild birds in our ecosystem. The Center regularly participates in research projects with both NGOs and government agencies across the country, and is able to provide invaluable data in support of conservation efforts.

We are keenly interested in participating in a study with Dr. Larry Witmer, Ohio University, and Dr. Glenn Tattersall, Brock University, involving non-invasive thermal imaging of captive birds. The Center hosts nearly 50 species of birds from around the world as permanent residents utilized for educational purposes. In addition, the Avian Medical Clinic admits over 600 birds of prey and shorebirds for treatment annually. We will provide appropriate access to a diverse number of species that will serve as excellent subjects for this research project.

Please do not hesitate to contact us as necessary for any clarification or additional information required.

Jim Elliott, Founder / Executive Director
Debbie Mauney, Avian Medical Clinic Director
Avian Conservation Center & Center for Birds of Prey
Charleston, SC
843-971-7474

thecenterforbirdsofprey.org



Jan. 11, 2017

To Whom It May Concern,

*OTHER OBSERVATIONS:
To encourage an
appreciation for
the world's wildlife
and to help
conserve it for
future generations.*

The purpose of this letter is to convey our support for the project entitled, "In vivo physiological analysis of vascular thermoregulatory structures based on observations using noninvasive infrared thermography on free-ranging birds" that is being proposed by Drs. Lawrence Witmer, Ruger Porter, and Glenn Tattersall.

We have agreed to participate in this study by allowing the PIs access to the Zoo Miami grounds to utilize their imaging equipment on some of our avian collection in their habitats.

We have contributed to Dr. Witmer's imaging research in the past and look forward to collaborating with him again.

Carol Kruse

1/11/17

Zoo Miami Director, Carol Kruse

Date



**One Zoo Boulevard
12400 SW 152 Street
Miami, FL 33177-1499
Tel: 305-251-0400
Fax: 305-378-6381**

www.zoomiami.org





Zoo Miami Animal Care and Use Committee OBSERVATION ONLY EXEMPTION FORM

PROJECT TITLE: In vivo physiological analysis of vascular thermoregulatory structures based on observations using noninvasive infrared thermography on free-ranging birds		
PRINCIPAL INVESTIGATOR: Lawrence Witmer, PhD, & Ruger Porter, PhD	TELEPHONE: 740-591-7712	EMAIL: witmerL@ohio.edu

Guidance

The Animal Welfare Act Regulations (§2.31, d, 1) state that studies "...that are conducted on free-living wild animals in their natural habitat and do not involve an invasive procedure, any harm to the animal, or any material alteration of the behavior of an animal under study" do not require an IACUC approved protocol. PHS Policy II requires compliance with the Animal Welfare Act" (Silverman, et al. Pg. 233). Therefore, an IACUC approved protocol is not required if the study involves unobtrusive observation of free-living wild animals in their natural habitat. All other studies involving the use and care of vertebrate animals require an IACUC approved protocol.

EXEMPTION CHECKLIST:

COULD YOUR STUDY:

1. AFFECT THE BEHAVIOR OF AN ANIMAL UNDER STUDY? YES~ SUBMIT ZMACUC PROJECT SUBMISSION FORM NO
2. INVOLVE AN INVASIVE PROCEDURE? YES ~ SUBMIT ZMACUC PROJECT SUBMISSION FORM NO
3. CAUSE ANY HARM TO THE ANIMAL? YES ~ SUBMIT ZMACUC PROJECT SUBMISSION FORM NO

Examples of behavior effects or invasive procedures include: supplemental feeding; nest checks; playback of recorded vocalizations that might result in territory desertion or infanticide in some cases/species, etc. Passive observation can easily transition into disruption; the PI must recognize when they reach this point and need to stop their work and apply for approval.

IF YOU CHECKED 'NO' TO ALL THREE QUESTIONS AND YOUR STUDY WILL ONLY INVOLVE OBSERVATION OF ANIMALS IN THEIR HABITAT, AN ZMACUC APPROVED PROTOCOL IS NOT REQUIRED. IN ORDER TO KEEP THE ZMACUC INFORMED OF STUDIES INVOLVING VERTEBRATE ANIMALS, WHETHER OBSERVATION OR OTHER, PLEASE READ AND CHECK THE INVESTIGATOR ASSURANCE. SIGN BELOW AND OBTAIN THE CONSERVATION AND RESEARCH MANAGER'S SIGNATURE.

SUBMIT THIS FORM TO FRID@MIAMIDADE.GOV OR FAX TO 305-254-1483.

Investigator Assurance	
<input checked="" type="checkbox"/>	I agree to not make any changes to this observation only study without prior notification and approval of the Zoo Miami Animal Care and Use Committee.
<input checked="" type="checkbox"/>	I agree to stop this study and submit a ZMACUC PROJECT SUBMISSION FORM should observation result in contact with a free-living wild animal.
<input checked="" type="checkbox"/>	I agree to comply with the requirements of the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and provisions of the USDA Animal Welfare Act and Regulations.
SIGNATURES	
PROJECT DIRECTOR OR PRINCIPAL INVESTIGATOR: L. M. WITMER & WM. R. PORTER	DATE: 2017-01-09
CONSERVATION AND RESEARCH MANAGER:	DATE:

Recommended Reviewers

Raymond Danner, PhD

Phone: (910) 962-7895

Email: dannerr@uncw.edu

Address: University of North Carolina, Wilmington
Dobo Hall 120A
601 South College Road, Wilmington, NC 28403

Reviewer's expertise: Dr. Danner is an ecological physiologist investigating bird thermal ecology and habitat selection. His expertise will allow him to evaluate aspects of the proposal that deal with bird thermal ecology, and evaluate the ability to measure heat transfer wild birds.

Relationship to applicant/application: None. Neither PI has met Dr. Danner.

Colleen Farmer, PhD

Phone: (801) 581-4236

Email: cg.fmr@gmail.com

Address: University of Utah
257 South 1400 East, Rm. 201
Salt Lake City, UT 84112-0840 USA

Reviewer's expertise: Dr. Farmer is an expert in the anatomy and physiology of living and extinct reptiles. Her research has an extensive *in vivo* research program investigating the pulmonary physiology of birds and crocodylians, including metabolic rates and air-flow patterns. She has also published on the vascular anatomy of reptiles. Dr. Farmer's expertise in reptile vascular anatomy and physiology would allow her to comment directly on the protocol of this *in vivo* research, but she can also evaluate the hypotheses and objectives.

Relationship to applicant/application: None. We know each other and have a cordial professional relationship.

Tomasz Owerkowicz, PhD

Phone: (909) 537-5312

Email: towerkow@csusb.edu

Address: California State University, San Bernardino
5500 University Parkway, San Bernardino CA 92407

Reviewer's expertise: Dr. Owerkowicz is an expert in the cardiopulmonary system of birds and crocodylians. He has an extensive research program relating to the physiology of the upper respiratory tract and the physiology of heat transfer using IR thermography. Dr. Owerkowicz's expertise in reptile cardiopulmonary physiology would allow him to evaluate the physiological aspects of this project and how they relate to hypothesis testing and *in vivo* protocols. His experience with IR thermography will allow him to evaluate the protocols involving the capture of thermal images.

Relationship to applicant/application: The PIs have not coauthored with Dr. Owerkowicz, but do know him, and are discussing a collaboration with him, although no work has commenced.

Emma Schachner, PhD

Phone: ??

Email: eschachner@gmail.com

Address: Louisiana State University Health Sciences Center,
New Orleans, LA 70112

Reviewer's expertise: Dr. Schachner is an expert in veterinary anatomy and comparative physiology, including birds and crocodylians. She has a research program that relates to the anatomy and evolution of reptiles, both living and extinct. Dr. Schachner's extensive knowledge on reptile anatomy and physiology will allow her to evaluate the proposal.

Relationship to application: The PIs and Dr. Schachner were coauthors on a 2014 article on dinosaur breathing.

Jeanette Wyneken, PhD

Phone: (561) 297-0146

Email: jwyneken@fau.edu

Address: Florida Atlantic University
777 Glades Road
Boca Raton, FL 33431

Reviewer's expertise: Dr. Wyneken is an expert in the anatomy and physiology of reptiles. She has an extensive research program on the behavioral and physiological ecology of sea turtles. Dr. Wyneken's expertise would allow her to evaluate the physiological aspects of the OURC grant proposal.

Relationship to application: None. We know each other and have a cordial professional relationship.