Atmospheric oxygen level does not alter size-specific femoral cross-sectional geometry in the American alligator (*Alligator mississippiensis*)

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Last updated: 2015-07-23

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## Author information

<http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1932-8494/homepage/ForAuthors.html>

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# Abstract

# Intro Outline

* Oxygen is important for physiology.
* Oxygen levels have varied over geologic time.
* Estimates of pO2 have also varied. So we are not sure of the exact levels.
* The ancestors of alligators arose during substantial hyperoxia (Archosauria), slight hyperoxia (Crocodyliformes, *sensu* Berner 2009), and essentially normoxia (*Alligator*).
* Atmospheric oxygen has the potential to alter tissue-level oxygen availability, which may affect bone morphology (among other things: cardiopulmonary physiology, digestion, muscle physiology).
* Bone morphology influences locomotor performance.
* If we are to successfully infer cross-sectional biomechanics from fossils, we need to know whether bone is plastic in response to the atmosphere.
* Alligators are a good model organism.

# Introduction

Both acute and chronic changes of atmospheric oxygen concentration potentially have profound effect on basic physiological processes and the evolutionary histories of extant organisms (Dudley 1998; Berner et al. 2007; Flück et al. 2007). For example, insects in the Paleozoic are thought to have reached very large body sizes in part due hypothesized high levels of atmospheric oxygen (Dudley 1998). **xxTomasz other good examples? Mammals?**

In part because of the role that organisms played in altering the environment (McAlester 1970; Graham et al. 1995; Berner et al. 2003), the initial populating of Earth's atmosphere with oxygen and its profound effects on organismal evolution have received considerable attention (McAlester 1970; Canfield 2005; Canfield et al. 2013; Crowe et al. 2013). The physiological and evolutionary implications of the comparatively more recent establishment of atmospheric oxygen to present-day atmospheric levels (Berner and Canfield 1989; Berner 1994, 1999, 2001, 2006, 2009; Berner et al. 2003, 2007) during the Phanerozoic are being explored (Graham et al. 1995, 1997; Falkowski et al. 2005; Ward et al. 2006; Whitman 2008; Harrison et al. 2010; VandenBrooks et al. 2011). Too many refs? Cite a review instead?

Although present atmospheric oxygen level (PAL) is approximately 21%, it is thought to have varied considerably across the Phanerozoic (ca. 540 Ma to the Present). The evolutionary history of terrestrial vertebrates has spanned the maximum and minimum estimates for atmospheric O2 during this time span (Figure ). According to some atmospheric models, oxygen level is thought to have reached a peak in the Permo-Carboniferous (~300-275 Ma) at 30-35% and a low in the earliest Jurassic (~15%; Berner 2006, 2009; Glasspool and Scott 2010). Although some estimates placed the lower bound of atmospheric oxygen at ~12-13% (Berner 2006), the long-term feasibility of oxygen levels less than 15-16% has been questioned (Belcher and McElwain 2008; Belcher et al. 2010). Revised estimates have places the minimum at ~15% (Berner 2009), although alternative atmospheric models suggest hyperoxia relative to PAL since the Triassic (Glasspool and Scott 2010). Although these models lack consensus and are subject to revision, variability in atmospheric oxygen across time is without question.

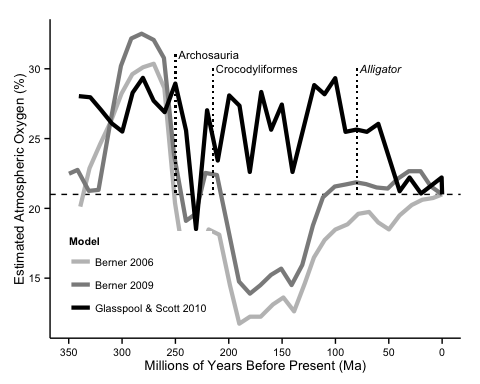
Experimental studies with extant mammals (Hunter and Clegg 1973a,b; Matsumoto et al. 2011) and reptiles (Metcalfe et al. 1981; Williams and Swift 1988; Kam 1993; Warburton et al. 1995; Dzialowski et al. 2002; Chan and Burggren 2005) have shown that acute and chronic alterations of atmospheric oxygen have significant effects both somatic growth and bone morphology. For example, moderate hypoxia in chicken results in decreased growth (Chan and Burggren 2005).

Because soft tissues are infrequently preserved for vertebrates, inferences about limb biomechanics and locomotion are usually made based only on skeletal anatomy, shapes, and proportions. With the exception of direct evidence, e.g. such as trackways for locomotion (e.g., Gatesy et al. 1999; Gatesy 2003; Kubo and Benton 2009; McCrea et al. 2014) or nesting or feeding behavior (Horner 1982; Erickson et al. 1996; Clark et al. 1999), skeletal anatomy usually represent the only data than can be applied to such questions. An often acknowledged assumption of functional inferences is that physical and biological processes observed in extinct vertebrates function as they do in extant vertebrates (**REF to Alexander? Vogel?**). An often unstated assumption is that the environment in which extinct vertebrate were living in was similar to present day (**REF to exceptions? Maybe some of the dinosaur respiratory papers? Claessens?**).

Studies of long bone cross-sectional geometry have a long history both in extant (e.g., Demes et al. 1991; Demes and Jungers 1993; Cubo and Casinos 1998; Blob and Biewener 1999, 2001; Demes 2000; Blob 2000; Polk et al. 2000; Ruff 2002, 2003, 2008; Meers 2002; Habib and Ruff 2008; Middleton et al. 2008a,b, 2010; Wallace et al. 2010, 2015; Simons et al. 2011) and extinct vertebrates (Trinkaus 1997; Trinkaus and Ruff 1999; Laurin et al. 2006; Cowgill and Hager 2007; Cowgill 2007; Ruff 2008; Daegling et al. 2014; Dumont et al. 2014). Different aspects of cross-sectional shape and derived properties (area and polar moments of inertia) have been used as a proxy for limb function or locomotor ability.

Thus understanding skeletal plasticity in response to atmospheric oxygen levels is important because limb morphology of fossil taxa are the sole basis for many functional hypotheses and determining the magnitude of response to alterations in oxygen environment is important in understanding limits to locomotion of fossil taxa. Herein we explore the plastic response of femoral cross-sectional biomechanics in American alligators raised for up to two years in atmospheric enviroments that mimic those experienced across the evolutionary history of this clade.

Why alligators??? Specific question: how much do we need to consider what was going on in the ancient oxygen environment when we are doing histology.



Three models for estimated oxygen levels during the past 350 Ma and major events in Alligator evolutionary history. The two gray lines show the results of Berner's 2006 and 2009 and GEOCARBSULF models, which hypothesize that hypoxia relative to PAL predominated through the Mesozoic. The black line shows estimates based on Glasspool and Scott (2010), which hypothesizes hyperoxia throughout most of the Phanerozoic, with a brief exception during the Triassic. Terrestrialization in vertebrates coincided with increased oxygen in the Permo-Carboniferous (375-280 Ma). Most of early the evolutionary history of Crocodyliformes spanned the hypoxic region during beginning ca. 200 Ma. Depending on the model, Alligator evolution has occurred primarily in hypoxia (Berner 2006, 2009) or hyeroxia (Glasspool and Scott 2010) relative to PAL (horizontal dashed line). Oxygen levels from Berner (2006, 2009) and Glasspool and Scott (2010). Divergence dates for Archosauria and Crocodyliformes follow Nesbitt (2011).

# Methods

## Materials

American alligator (*Alligator mississippiensis*) eggs from eight clutches (*n* = 179 total) were collected from the Rockefeller Wildlife Refuge (Grand Chenier, LA). Incubating, hatchling, and juvenile alligators were raised under chronic conditions of hypoxia (16% O2), hyperoxia (26%, 31%, and 36% O2), or normoxia (21% O2) simulating the range of Phanerozoic atmospheric oxygen levels. Alligators were maintained in their respective treatment groups from the time of collection until they were sacrificed at specified ages (2, 4, 16, 24, 52, and 104 weeks; Table 1). All procedures were approved by the University of California, Irvine Animal Care and Use Committee.

xxTomasz: Other husbandry information? Feeding, tank size, gators/tank, etc. What is the UCI ACUC called officially?

## Histological Preparation

The right femur of each specimen was dissected and prepared for histological analysis by blunt removal of soft tissue, formalin fixation (1 h), passage through a graded ethanol dehydration (1 h each 30%, 50%, 70%, 80%, 90%, 95%, 100% ethanol), and air drying (24 h). Prior to embedding, measurements were taken for each bone using digital calipers (precision = 0.01 mm), including femoral length, width and length of the proximal and distal articular surfaces, and dorsoventral and anteroposterior diameters. Measurements followed Farlow (2005).

Undecalcified ground sections were embedded in epoxy resin (EpoThin; Buehler Ltd.; Lake Bluff, IL) following methods outlined in An and Martin (2003) and Padian and Lamm (2013). Briefly, small femora (<~45 mm length), were placed in a labeled plastic tray lined with a base of cured epoxy resin. All bones were positioned in the same anatomical orientation and glued in place with a small amount of epoxy. After the epoxy had cured, bones were completely covered with epoxy resin and allowed to cure for 48 hours. For large femora (more than 45 mm in length), the epiphyses were removed with a band saw, leaving ~15 mm of the diaphysis to be embedded. Before cutting, the dorsal surface, mid-diaphysis and proximal end of the bone was marked in pencil to ensure correct anatomical orientation, and then labeled and embedded. During the polymerization process, the femora were simultaneously impregnated with resin and embedded in a hardened block which supported the bone and allowed for sections to be evenly cut. The anatomical orientation of each bone (indicating dorsal and medial surfaces) was marked by labeling the corresponding sides of the block with different colors of permanent ink, and three serial transverse thick sections (about 1 mm each) were removed from the mid-diaphysis of each specimen with a low-speed saw (IsoMet; Buehler, Ltd.; Lake Bluff, IL).

The proximal surface of each section was marked to maintain orientation. This mark, together with the colored labels from the block now visible along the dorsal and medial edges of the section, allowed the original anatomical orientation to be preserved as the sections were mounted onto a labeled slide with additional epoxy resin. After a second round of curing, sections were ground to a thickness of approximately 100-125 using a graded series (280 to 600 grit) of abrasive paper on a grinder-polisher (MetaServ; Buehler, Ltd.; Lake Bluff, IL). Slides were then cover-slipped using Permount (Fisher Scientific) mounting medium thinned with xylene.

## Biomechanical Parameters

Each section was viewed and photographed using a polarizing microscope (Nikon Eclipse V100 or Zeiss Discovery) fitted with a digital camera and image capture software (Diagnostic Instruments, Inc.; Sterling Heights, MI). Most images were taken of the entire cross section at 40X magnification, and larger femoral cross-sections were imaged at 15X. Images were then analyzed using NIH ImageJ (version 1.49o; <http://rsbweb.nih.gov/ij/>). The internal (medullary) and external dorsoventral and anteroposterior diameters as well as cortical thickness in each orthogonal quadrant were measured from images of the entire cross-section using the measure tool in ImageJ.

The images were converted to binary silhouettes of the cortex (Figure ), following Matsuda et al. (1986), and analyzed using the ImageJ plug-in MomentMacro (<http://www.hopkinsmedicine.org/fae/mmacro.htm>). Conversion of cortices to binary silhouettes necessarily results in the loss of information about bone microstructure, including osteocyte density and porosity. We defined orthogonal anteroposterior and dorsoventral axes of the femur based on a plane passing through the femur lying parallel to the midline plane of the animal's body, with the long axis of the femur directly perpendicular. Although femoral oriantation is more anteromedial and movements during the stride cycle are more complex (Gatesy 1991), we use this standard position to allow future comparisons with other archosaurs.

To estimate the biomechanical strength of the bone in resistance to stresses due to compression, tension, bending and shear forces that might be encountered during locomotion, we measured several cross-sectional geometric properties on each specimen (see Lieberman et al. 2004 for a review). Cross-sectional area of the cortical bone (CSA) estimates resistance of the bone to axial compression. Orthogonally measured area moments of inertia and estimate bending resistance about the dorsoventral and anteroposterior axes, respectively. Similarly orthogonal section moduli ( and ), calculated as , perhaps better approximate how a given cross-sectional geometry resists bending in about an axis. () approximates resistance to multidirectional bending (Schaffler et al. 1985). Because longer beams (bones) are more easily loaded in torsion than short beams, we standardized by dividing by femoral length to allow comparisons between bones of different lengths ().



Example cross sectional image

## Statistical Analysis

All statistical analyses were carried out using the statistical computing language R (version 3.2.1; R Core Team 2014). Preliminary statistical analyses showed no differences among the three hyperoxic treatment groups (26%, 31%, and 36% O2), so we pooled these treatments into one "hyperoxic" group, resulting in three treatments to be compared: normoxia (16%), hypoxia (21%), and hyperoxia (combined 26%, 31%, and 36% O2 treatment groups).

### Growth Rate Equality Among Treatments

In all analyses, femur length was to be used as a covariate to account for differences in size between different age classes. However, we first needed to determine whether femur length varied across age classes with respect to treatment group. To this end, we compared growth trajectories among the three treatment groups (normoxia, hypoxia, and hyperoxia, the latter group combining the different levels of hyperoxia treatments).

Growth was not linear in the sample of alligators studied here (Fig. 2), so traditional linear regression methods were not appropriate for this sample (linear models had extremely poor support relative to other models and were not explored further). The appropriate model for reptilian growth has been long debated, with some studies using a determinate model, e.g., von Bertalanffy or logistic growth (Erickson and Tumanova 2000; Erickson et al. 2001, 2004; Lee and Werning 2008; Saalfeld et al. 2008; Lee and O’Connor 2013) and others favoring an indeterminate growth model, e.g., linear, polynomial, or power law (Jacobsen and Kushlan 1989; Myhrvold 2013). So-called "determinate" and "indeterminate" growth do not encompass a single pair of growth patterns but rather a range of strategies (Sebens 1987; also reviewed in Myhrvold 2013).

The goal of this portion of the analysis was not to evaluate the appropriate growth model within *Alligator* or more broadly across Archosauria (for thorough comparison of models and discussion see Myhrvold 2013) but rather to determine whether the rate of increase in femoral length differed among treatment groups. We tested two different growth models in two different parameterizations: (1) with separate curves fit for each of three treatment groups: normoxic, hypoxic, and hyperoxic and (2) with all samples pooled. Comparisons between these different parameterizations determine if growth rates differed between treatment groups, which, if true, would complicate use of femoral length as a covariate in later analyses.

For a determinate growth model, we used a three parameter von Bertalanffy growth curve (Bertalanffy 1960; Chen et al. 1992; Pardo et al. 2013). The von Bertalanffy growth curve (VGBC) is defined as

where is interpreted as the asymptotic femoral length at infinite age. To model indeterminate growth, we used the second-order polynomial model

which has been suggested as a preferred alternative to a von Bertalanffy growth model (Roff 1980; Chen et al. 1992).

These models can be used to fit one or more growth curves simultaneously using ordinary least squares with a polynomial term for the polynomial function and non-linear least squares for the VBGC. These growth curves can then be compared statistically via -tests and by comparison of information criteria (Chen et al. 1992; Roff 2006). We used Akaike Information Criterion (AIC; Akaike 1974) to assess model fit and considered models with AIC values of units as equally well supported given the data (Burnham and Anderson 2002).

-test comparisons of polynomial models with and without O2 treatment levels is straightforward using built-in functions in R (the test = 'F' option in the anova() function). Comparisons between VBGC models was more challenging. To compare these models, we generalized the three parameter VBGC model above into a nine parameter model, which allowed for fitting separate curves to each of the three treatment groups using a pair of dummy variables to code for two additional treatment groups. Residual sums of squares were used to construct an -test to compare between the two different model parameterizations following Roff's (2006) model with degrees of freedom adjustments for additional parameter estimates (also see Chen et al. 1992 for further discussion):

where is the residual sum of squares of the 3 parameter model, is the residual sum of squares of the 9 parameter model, and is the total number of observations.

### Clutch Effects

Because eggs were taken from each of eight clutches (Table 1), different genetic background and/or maternal effects (e.g., egg provisioning) may have resulted in differing growth trajectories between clutches. We used non-linear mixed models to test for differences in the covariate femoral length across ages within clutch and with clutch nested within treatment group. We first fit the second-order polynomial function with pooled treatment groups as above, and then fit the a mixed-effects model (Pinheiro and Bates 2000) with the addition of clutch as a random effect using the nlme function in the nlme package (Pinheiro and Bates 2000; Pinheiro et al. 2014). These models were compared via Wald tests (Pinheiro and Bates 2000) and comparison of AIC. As for growth models above, models with AIC vlues <4 were considered equally supported (Burnham and Anderson 2002).

### Biomechanical Measures

Biomechanical measures, including cross-sectional area (CSA), anteroposterior and dorsoventral diameters, orthogonal dorsoventral and anteroposterior moments of inertia ( and ) and section moduli ( and ), and polar moment of inertia standardized to femoral length () were analyzed using ANCOVA, with femoral length as covariate and oxic treatment group as the main effect.

Models were first fit with an interaction term between treatment and femoral length to allow each treatment group to have a separate scaling relationship with femoral length. In all cases, the interaction term was not significant (), and so the interaction term was dropped for subsequent analyses. This effectively fit a model were the scaling relationship (i.e., slope) between the treatment groups and femoral length was force to be equal across groups, while the intercept term was allow to vary for each group. Slopes were compared to isometric scaling predictions using reduced major axis (RMA) regression implemented in the R package smatr (Warton et al. 2006, 2011).

### Multiple comparisons

Write something post hoc about multiple comparisons here after we do the post-hoc multiple comparisons procedures.

# Results

## Growth Rate Equality Among Treatments

Femoral length increased rapidly in this sample of hatching alligators and gradually slowed (Fig. 2), which is a typical pattern in alligators (Wilkinson and Rhodes 1997), although at 104 weeks of age, femoral length was still increasing.

### Determinate Growth Model

We fit two models to the von Bertalanffy growth equation, one in which all treatments were combined and one in which the three oxic treatment groups were separated. The latter nine parameter, separate treatment groups, model was not significantly improved over the poodled-samples model ( = 1.99; = 0.07; = 0.2). The growth equation for the pooled-samples data was

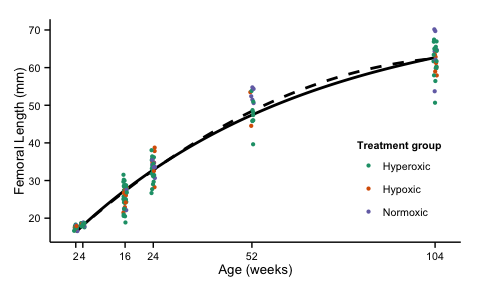
which predicts an asymptotic femoral length of 75.87 mm in this sample. This asymptotic femoral length is significantly less than what would be found in adult wild alligators [Farlow et al. (2005). The size of the alligators in this study (~1 m maximum total length **xxTomasz: is this correct?**) is a function of the 104 week duration of the experiment and captive conditions. The maximum femoral length at this age qualitatively agrees with Farlow's (2005) predictions from total length. Although the -value for the overall -test of equality among models approaches significance ( = 0.07), the value is less than 1. Thus we conclude that there is equal support for both models and choose the simpler and more parsimonious three-parameter model (Figure , solid line). These models show that in the determinate growth model, osygen treatment groups does not have a significant effect on femoral length growth rate.

### Indeterminate Growth Model

We similarly fit two different models to the second-order polynomial growth curve (Figure , dashed line), one in which all treatments were combined and one in which the three oxic treatment groups were separated by the inclusion of a three-level categorical variable for treatment. The separate treatment groups model was not significantly improved over the model in which all treatment groups were pooled ( = 2.08, = 0.128; = 0.24). The polynomial growth equation for the pooled samples was

### Model comparison

Within both types of growth models (determinate and indeterminate), simpler models that do not include a separate parameter for treatment group are preferred. More complex models that include treatment group do not differ significantly from ones that do and only show small gains in AIC (ca. 0.2). Thus, by either metric (-value or ), we found that femoral length increase does not differ between groups. Comparing between indeterminate and determinate models, we find that an indeterminate growth model is preferred over a determinate model (AIC = 7.53). Based on these results showing no effects of treatment group on growth rates, we therefore proceeded to use femoral length as a covariate proxy for body size in all remaining analyses.



Femoral length (mm) vs. Age (weeks) at six time points (2, 4, 16, 24, 52, and 104 weeks) with non-linear least squares regression of the three parameter von Bertalanffy growth curve (determinate growth; solid line) and second order polynomial regression (indeterminate growth; dashed line) through all groups combined. Points have been staggered slightly along the Age axis to avoid overplotting.

## Clutch effects

Non-linear mixed models revealed no significant effects (1) of clutch membership on growth or (2) of clutch on growth when nested within treatment group. The model including clutch as a random effect did not fit the observed pattern of femoral growth significantly better than the second-order polynomial model without clutch ( = 0.051; = 1.8). Similarly, inclusion of oxygen treatment group did not significantly improve the model fit ( = 0.354; = 1.92). Based on these results, we did not consider clutch membership as a factor in any subsequent analyses.

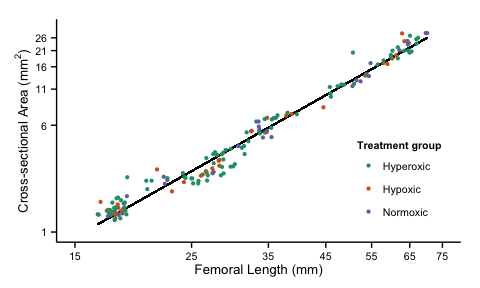
## Biomechanical Parameters

Results of analysis of covariance with oxic treatment group as the main effect and femoral length as the covariate (with and without the oxic X femoral length interaction term) revealed no significant differences between normoxic, hypoxic, and hyperoxic groups in any measured biomechanical parameters. Consequently, we pooled all experimental groups and used reduced major axis regression (LaBarbera 1989) to assess scaling relationships between biomechanical measures of bone robusticity and femoral length. Results of these analyses are summarized in Table 2.

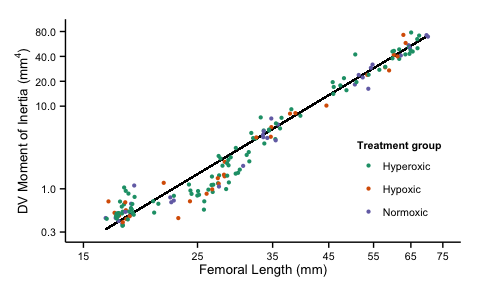
Overall we observed scaling patterns that reflected the underlying changes in cortical morphology with age. As alligators increased in size, bone was added periosteally with relatively little apparent endosteal resportion. The net effect of this pattern was an increase in cortical thickness and only small increases in the size of the medullary cavity (Figure ). Although both anteropesterior and dorsoventral mid-diaphyseal diameters scaled with negative allometry (), we found that cross-sectional area of the femoral cortical bones was positively allometric (Figure ; ; ). Thus, although the bone is narrower than would be predicted, it's resistance to axial loading is increased (although the degree to which alligator femora, or any vertebrate femoral for that matter, are axially loaded is questionable **REF???**). Scaling patterns with respect to bending loads revealed that moments of inertia ( and ) scale with significant negative allometry (; Table 2). Section moduli ( and ), calculated as divided by the radius of the region being measured, and which approximates the bending rigidity of a particular section, scaled isometrically relative to femoral length (Figure ; Table 2). We interpret this finding to result from dividing a negatively allometric trait by another negatively allometric trait.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Trait |  | 95% CI |  |  |
| AP Diameter | 0.85 |  | 1 | < 0.001 |
| DV Diameter | 0.88 |  | 1 | < 0.001 |
| Cross-sectional Area | 2.17 |  | 2 | < 0.001 |
| AP Moment of Inertia () | 3.77 |  | 4 | < 0.001 |
| DV Moment of Inertia () | 3.76 |  | 4 | < 0.001 |
| AP Section Modulus () | 2.93 |  | 3 | 0.11 |
| DV Section Modulus () | 2.93 |  | 3 | 0.17 |
| Standardized Polar Moment of Inertia () | 2.78 |  | 3 | < 0.001 |

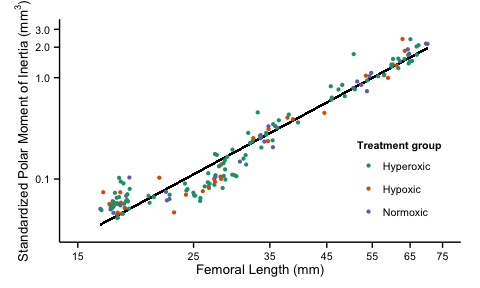
Summary of analysis of covariance results. Estimates of slope () with 95% confidence intervals, null-hypothesized (isometric) slope (), and the -value for a test of the estimated slope vs. the isometric slope () are given. Diameters, moments of inertia, and section moduli were all measured with respect to orthogonal anteroposterior (AP) and dorsoventral (DV) axes. Cross-sectional area and standardized polar moment of inertia are measured without respect to anatomical orientation.



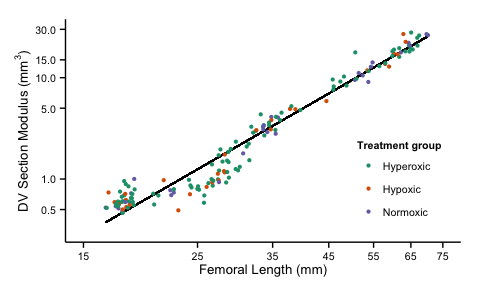
Femoral mid-diaphseal cross sectional area.



**SI only???** Dorsoventral Moment of Inertia



**SI only???** Standardized Polar Moment of Inertia



Dorsoventral Section Modulus

# Discussion

## Main Conclusions

1. No effect of O2 treatment on growth rates
   * Both determinate and indeterminate show the same pattern where in models that include teatment groups are not significantly improved over models that do not include separate groups. Although the models must by definition fit better, this improvement is not significant.
   * Studies of dinosaur growth & estimates of dinosaur growth curves (e.g., Erickson and Tumanova 2000; Erickson et al. 2001, 2004; Lee and Werning 2008; Myhrvold 2013) do not need to consider the prevailing atmospheric oxygen level
2. No effect of O2 treatment of XS biomechanical parameters
   * Not terribly surprising given that there are no differences in growth
   * How does this compare with other animals? CSA in particular

## Physiological Effects of Hypoxia and Hyperoxia

Studies of the effects of altered atmospheric oxygen---hypoxia (low [O2] relative to PAL) and hyperoxia (high [O2] relative to PAL)---have traditionally focused on rodents and birds, although some studies have addressed other species. For example, alligators, the subject of this study, as well as turtles have emerged as model organisms for the study of "oxia" (**REF to Eme, Hicks, Crossley, Warren/Jackson papers**).

### Evolutionary considerations

Regardless of the lower limit of atmospheric oxygen, most major extant clades of vertebrates or their ancestors were already established by the early Jurassic, approximately 200 Ma (Romer 1966; Carroll 1988). The transition from high to low oxygen levels relative to PAL spanned the Triassic (Berner 2006, 2009; Berner et al. 2007), during which major clades of terrestrial vertebrates originated. Thus, crocodylians, dinosaurs, turtles, and mammalian ancestors may have all originated in hypoxia relative to PAL and, not only survived, but continued to diversity in an oxygen deficient environment (Figure ).

### Mammals

* (Gordon et al. 1943) - Aviation medicine. Endocrine testes, ovaries, thyroid, anterior putuitary
* (Moore and Price 1948)
* (Timiras et al. 1957)
* (Frisancho and Baker 1970; Frisancho 1970) - High altitude human populations
* (Hunter and Clegg 1973a,b) - Changes in rat and mouse skeletons
* (Frappell and Mortola 1994) - Hamsters vs. rats: metabolic and ventilatory response to development in chronic hypoxia
* (Widmer et al. 1997) Respiratory adaptations in blind mole rats
* (Hochachka et al. 1999) - Human hypoxia tolerance
* (Mortola 2004) - Ventilatory response in chick embryos
* (Ramirez et al. 2007) - Review mammals and birds
* (Matsumoto et al. 2011) - Three-Dimensional Cortical Bone Microstructure in a Rat Model of Hypoxia-Induced Growth Retardation

### Reptilia

* (Metcalfe et al. 1981)
* (Williams and Swift 1988)
* (Deeming and Ferguson 1990) Growth only, nothing about atmosphere or physiology
* (Richards et al. 1991) Trace elements only
* (Burton and Palmer 1992) Only concerns chorioallantoic membrane
* (Kam 1993)
* (Warburton et al. 1995)
* (Jackson et al. 2000a,b; Jackson 2000) Lactic acid buffering
* (Andrews 2002) O2 as a constraint on viviparity in reptiles using *Sceloporous* as a model
* (Dzialowski et al. 2002)
* (Chan and Burggren 2005)
* (Bickler and Buck 2007)

### Lots of cardiovascular changes

Are these relevant here?

* (Eme et al. 2013) What are the mechanisms at work in the response to hypoxia in the cardiovascular system? Gene expression changes are linked to plasticity in cardiovascular morphology and function. Alterations in autonomics (changes in adrinergic receptors).
* (Kam 1993) Florida red-bellied turtle. In hypoxia (10%), slower growth, lower metabolism, reduced mass. But equal hatching time.
* (Crossley and Altimiras 2005; Crossley and Burggren 2009)
* Other Crossley papers
* (Eme et al. 2011a,b,c)
* (Burggren et al. 2014)
* (Harrison et al. 2010)

## Growth rates in controlled conditions

* Differences from previous results.
* Come back to Berner et al 2007: Animals that evolved under hypoxia might have had a selective advantage.

"Many of these new body plans may have supported more efficient respiratory systems, which may have been selected for under low-O2 regimes that coincide with postextinction time periods."

## No growth in the medullary cavity

* CA

## Scaling

## Lack of Results

## Alligators as a model organism

* (Porteus et al. 2011) - HVR
* Physiology
* Biomechanics
* Evolution

## Future Directions

* Mineral apposition rates to test whether there is only periosteal deposition

# Acknowledgments

We gratefully acknowledge the assistance of Dr. Ruth Elsey at the Rockefeller Wildlife Refuge (Grand Chenier, LA). This project was supported by the National Science Foundation grant IOS 0922576 (TO, JWH, and KMM), the California State University Department of Biology (SLL, KMM), and the University of Missouri Department of Pathology & Anatomical Sciences (KMM).

xxTomasz Anyone else to add?

This manuscript was written using rmarkdown (RStudio Inc 2014), knitr (Xie 2013), and pandoc (<http://johnmacfarlane.net/pandoc/>).

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