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Defining a species-specific K constant in American Bullfrogs (*Lithobates catesbeianus*) through use of computed tomography

Jaime N. Calcagno, BS[1,2]; Nathan C. Nelson, DVM, MS[3]; Elizabeth G. Duke, DVM[2,4]; Tara M. Harrison, DVM, MPVM[2,4]*

[1]College of Veterinary Medicine, North Carolina State University, Raleigh, NC
[2]Exotic Species Cancer Research Alliance, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
[3]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
[4]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

*Corresponding author: Dr. Harrison (tara_harrison@ncsu.edu)

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OBJECTIVE
To use CT measurements to define the body surface area (BSA) formula in American bullfrogs (*Lithobates catesbeianus*) and calculate the species-specific shape constant (K) to suggest chemotherapeutic doses.

ANIMALS
12 American bullfrogs owned by the North Carolina State College of Veterinary Medicine Department of Laboratory Animal Resources underwent CT scans without anesthesia or sedation in November 2022.

METHODS
As part of this prospective study, each American bullfrog underwent a complete physical exam and CT scan. 3-D surface models were created using CT data, and the resulting measurements were used for BSA calculations. Animals were grouped by sex. Nonlinear regression analysis of BSA versus body weight was performed, and a species-specific formula was derived for calculating BSA in American bullfrogs.

RESULTS
The mean body weight of the bullfrogs was 354 grams. The mean CT-derived BSA was 414.92 cm^2. The calculated K constant was 8.28 for the 12 American bullfrogs, and the CT-derived BSA formula was BSA in cm^2 = 8.28 X (body weight in g)$^{2/3}$. The K constant was 8.07 for females and 8.44 for males and was not significantly different between sexes (P = .5).

CLINICAL RELEVANCE
Results indicated that the species-specific K constant for American bullfrogs is 8.28. This is the first calculated K constant that exists for amphibians to our knowledge.

Keywords: body surface area, amphibian, bullfrog, K constant, *Lithobates catesbeianus*

The characteristic call and large size of the American bullfrog (*Lithobates catesbeianus*) make these animals popular in zoological institutions as well as a common pet in many American households. Amphibian populations, including those of the American bullfrog, are vital to our ecosystem, and their numbers are dwindling worldwide due to anthropogenic activities, disease states, climate change, and invasive species.[1,2] Captive populations serve as a safety net for wild populations, and they are known to develop neoplasia. According to a 2020 literature review for the Exotic Species Cancer Research Alliance; 50 cases of amphibian neoplasia have been published between 1954 and 2018. For 69% of these cases, no therapy was pursued.[3] The importance of establishing chemotherapeutic protocols is emphasized when you consider endangered populations. A European retrospective study[4] focusing on the endangered mountain chicken frog (*Leptodactylus fallax*) states that 42 out of 212 necropsies had evidence of neoplasia. Intestinal adenocarcinoma was the known cause of death for 31 of these frogs. Cancer in amphibians can be challenging to treat due to their unique physiological and morphological characteristics that make it difficult to define a species-specific treatment protocol without known published studies.

The majority of chemotherapeutic drugs are administered on the basis of body surface area (BSA) rather than weight.[5–11] BSA measurements are proportional to metabolic rate and provide a more accurate representation of the entire animal.[12] The dosing of chemotherapeutics between different members of the same species is therefore more accurate for each individual. BSA is more accurate than weight-to-dose chemotherapeutics for subjects that differ in size not shape,[13] which is commonly seen in exotics including frogs. The equation used to calculate BSA[6] is BSA = K X (body weight in g)$^{2/3}$. K is a species-specific shape constant and is the key to determining appropriate chemotherapeutic doses. A noninvasive method to evaluate BSA in novel species can be through CT scans. This method has been used successfully in mammals such as ferrets,[14] rabbits,[15] human neonates,[16] and human adults.[17] Most recently, a CT-derived species-specific formula for BSA in a reptile, bearded dragons, has been defined.[18]

The following study uses a similar methodology to the bearded dragon report[18] with alterations made in respect to the American bullfrog. To our knowledge, no K constant for BSA has been published for amphibians or, specifically, American bullfrogs. The objective of the following study is to use CT-derived values to calculate a species-specific shape constant (K) and BSA formula in American bullfrogs. By establishing a K constant for American bullfrogs, we aim to assist in the ability to calculate chemotherapeutic medications for this species. This study does not evaluate the efficacy, toxicity, and pharmacokinetics of chemotherapeutic drugs in American bullfrogs, and further research is indicated in these areas.

Methods

Animals

Five female and 7 male adult American bullfrogs of the same lineage housed at the North Carolina State College of Veterinary Medicine Department of Laboratory Animal Research (NC State CVM DLAR) participated in this study. The bullfrogs received physical examinations in November 2022, and inclusion criteria were determined based on health status and normal body conformation. Low-stress handling and restraint were utilized so that no sedative or anesthetic medications were required for the American bullfrogs. Researchers wore nitrile, latex-free, exam gloves to handle the frogs. This study was approved by the North Carolina State University Institutional Animal Care and Use Committee (No. 19-614-O).

Examinations

The American bullfrogs underwent thorough and complete physical examinations under the supervision of a licensed veterinarian. The examination focused on assessing normal health and included heart and respiratory rate measurements, abdominal palpation, oral cavity and ocular exam, sex determination, evaluation of normal body conformation, and weight measurements. Sex was determined through evaluation of secondary sex characteristics.[19] Males had larger tympanums than their eyes as well as a defined digit known as the nuptial pad while females had tympanums comparable in size to their eyes.

The American bullfrogs were not sedated or anesthetized for the examinations or CT scans. To minimize movement, animals were placed in individual plastic boxes (Style Selections Storage Container; Belle Storage Solutions; 32.79 cm X 23.14 cm X 13.06 cm) with foam around all four sides and a lid **(Figure 1)**. The foam had a hole carved into it that was the size of the bullfrog. Moisture was removed from the frogs with a towel before being placed in the boxes. A 64-slice helical CT scanner was used to acquire transverse images with a 0.6-mm-slice thickness at 130 kVp and 25 mA. The entire body of the frog was scanned.

Figure 1—American bullfrog being placed in a plastic container lined by foam with a frog-sized hole in the center. A plastic lid was placed for the CT scans.

3-D CT reconstructions

CT surface models were created following the methodology established in a previous study.[18] The CT images were reconstructed in a bone reconstruction algorithm (viewed at window width, 2,000 HU; window level, 400 HU) and analyzed with dedicated CT image analysis software (Mimics Innovation Suite; Materialise NV). A minimum threshold value of −500 HU with no upper limit was defined for each frog to ensure densities outside of the frog were excluded. The gas imaged in each frog was manually removed with a tracing tool (Mimics Innovation Suite; Materialise NV) and filled in with the appropriate density to ensure that these cavities were not included in the frog's surface area. Cavities filled included the lungs, tympanum, and gastrointestinal tract. Water and mucous contamination did not occur due to the drying of the frogs dry before the CT scan. If there was residual water and mucus interference, it would be easily recognized by noticing an abnormal conformation of the frog on the resulting 3-D image. The software generated 3-D models, which were visually assessed for appropriate body contour and completeness. Additionally, the software calculated BSA for each frog, which was then used for K constant determination.

Statistical analysis

BSA determined from the CT-derived measurements and body weight data allowed us to solve for the K constant in our study with the formula BSA = K X (body weight in g)$^{2/3}$. Utilizing nonlinear regression, we plotted BSA against body weight and calculated additional K constants to be used in frogs of varying sizes. The results were finalized for all animals, grouped by sex, and combined.

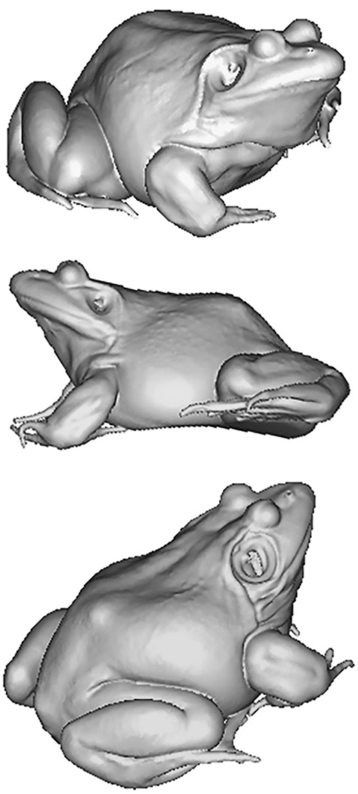

Figure 2—Representative surface models of bullfrogs used to calculate body surface area K values.

Results

Our study group consisted of 12 healthy adult American bullfrogs (5 females and 7 males). The mean body weight of the bullfrogs was 354 grams (range, 324 to 419 grams; median, 347.5 grams). All animals were identified by previously placed microchips, healthy, and owned by NC State CVM. No frogs had abnormal body contour conformation. The average heart rate was 60 beats/min (all frogs had the same heart rate) and the average respiratory rate was 64 breaths/min (range, 30 to 80 breaths/min; median, 70 breaths/min).

The mean BSA was 414.92 cm^2 (range, 382.76 to 445.42 cm^2; median, 412.87 cm^2) **(Figure 2)**. Results were displayed as BSA plotted against body weight for each American bullfrog. Results were grouped by sex and as an overall group **(Figure 3)**. The derived K constant for the female bullfrogs was 8.07, and the derived K constant for male bullfrogs was 8.44. There was no statistical difference between the K constant for male or female bullfrogs (P = 0.5). The results for all 12 bullfrogs were considered and the K constant derived was 8.28. Based on our results, the CT-derived BSA formula for American bullfrogs is BSA = 8.28 X (body weight in grams)$^{2/3}$. Results are summarized **(Table 1)**.

Table 1—Body weight and body surface area (BSA) of American bullfrogs (*Lithobates catesbeianus*) for overall measurements and those specifically for male and female frogs.

Sex	All animals (n = 12)	Male (n = 7)	Female (n = 5)
Body weight (g)	Mean: 354 Median: 347.5 Range: 324–419	Mean: 246.14 Median: 334 Range: 324–388	Mean: 362.2 Median: 354 Range: 340-419
CT-derived BSA (cm^2)	Mean: 414.92 Median: 412.87 Range: 382.76–445.42	Mean: 418.44 Median: 416.33 Range: 410.32–437.68	Mean: 410 Median: 403.79 Range: 383.76–445.72

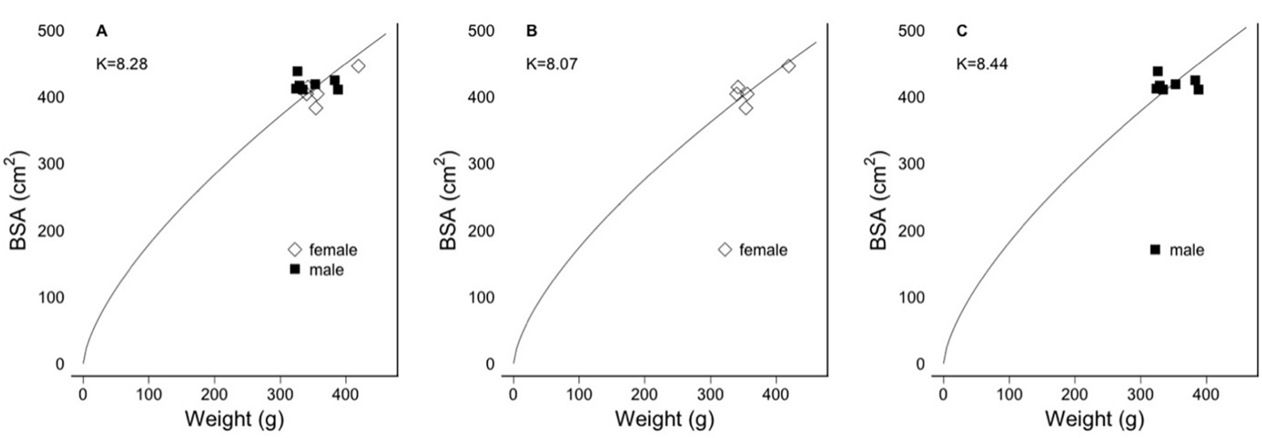

Figure 3—K values in American bullfrogs in relation to body weight (g) and body surface area (BSA; cm^2) grouped by sex. A—Male and female. B—Female. C—Male.

Discussion

Neoplasia affects all fields of veterinary medicine, and it does not discriminate between small animals, large animals, zoo animals, or exotic species. While published K constants exist for dogs and cats,[5] 10.1 and 10.0, respectively, the determination of K constants in nondomestic animals in a relatively novel endeavor. Previous studies have utilized CT-derived K constants in ferrets (9.94),[14] rabbits (9.9),[15] and bearded dragons (11.6).[18] Interestingly, before the publication of the bearded dragon BSA formula, the K constant for reptiles was estimated as K = 10 on the basis of metabolic energy requirements.[20] These results of the bearded dragon paper[18] are clinically important because they suggest that higher doses of chemotherapeutic agents may be needed than previously thought. When treating mammals such as dogs with chemotherapeutic medications, BSA is typically used[5]; however, the use of BSA has been reported to lead to a greater risk of overdose in smaller breeds, which is why dogs less than 15 kg are dosed on the basis of body weight instead.[5] However, there are some studies[21] suggesting these differing K constants in dogs are more based on height and weight than weight alone. Additionally, there are other publications in cats suggesting that BSA was more accurate for animals that differ in size and not shape,[13] which may be further reason to use species-specific K constant and BSA calculations rather than milligram per kilogram calculations.

The American bullfrogs in this study successfully underwent CT without the use of sedatives or anesthetics. Our hands-off approach and plastic box technique limited stress in the species and allowed us to produce clear CT images for reconstruction. Heart rate and respiratory rate tend to increase in times of stress, and in our bullfrogs the average heart rate was 60 beats/min and the average respiratory rate was 64 breaths/min, which is well within normal resting rates for American bullfrogs.[22] The resulting CT images were clear and 3-D reconstruction required no alterations other than manually removing gas from body cavities. The frogs were required to be partially dried as in our pilot study of these animals we found that when the animals were wet, water would soak into the surrounding foam increasing its effective Hounsfield units. This interfered with the automatic segmentation of the frog from the surrounding foam by the reconstruction software. Ensuring that the frog was dry allowed us to get an accurate reconstruction.

Our CT-derived BSA for American bullfrogs is BSA = 8.28 X (body weight in grams)$^{2/3}$. The K constant derived for females (8.07) and males (8.28) was not significantly different. On average, female American bullfrogs are typically larger; however, the growth rate of wild American bullfrogs does not differ between sex.[19] The males and females in our study group were all adults, suggesting that they could be of similar body size. Our largest frog was a female weighing 419 grams, the smallest frog was a male weighing 324 grams, and the average frog size was 347.5 grams.

Evaluating American bullfrogs of the same age and same lineage was a limitation of the present study as we cannot determine if other age stages would have a similar BSA; however, adults tend to develop more cancer than juveniles, so a surface area based on the adult BSA may be more clinically important. The average lifespan of an American bullfrog is 7 to 9 years in the wild, and they can live up to 16 years of age under human care.[23] Future research could be to evaluate frog postmetamorphosis to the nature of their growth rates as described above.

The usage of BSA for chemotherapeutic medication dosing is reliant on the assumption that glomerular filtration rate and metabolic rate are proportional to BSA.[17,24] Amphibians have lower metabolic rates compared to mammalian species, and further research is indicated to determine if the glomerular filtration rate is proportional to BSA. While this study produced a BSA formula specific to American bullfrogs, which can be applied to chemotherapeutic dosing, it did not evaluate the pharmacokinetics or pharmacodynamics of chemotherapeutic agents in American bullfrogs. Veterinarians should be cautious when dispensing potentially toxic chemotherapeutic agents and should consider the size of the patient when determining chemotherapeutic dosages in all animals, including amphibians.

Our study defined a formula for determining BSA in American bullfrogs that is both minimally invasive and species specific. The information provided in this study could be used to calculate chemotherapeutic doses for American bullfrogs and is the first of its kind to define a K constant for an amphibian. Further research is indicated to study pharmacokinetic data, toxicity possibilities, and species-specific BSA formulas in other amphibians.

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Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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