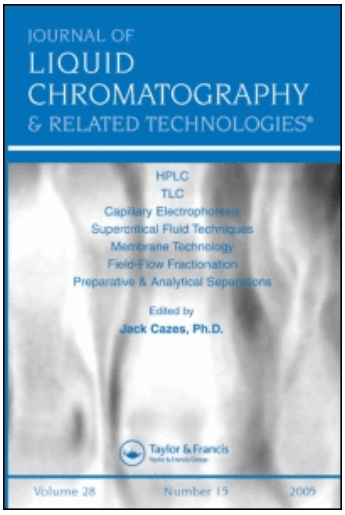
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Characterization and Quantification of the Neutral Lipids in the Lizard *Uta stansburiana stansburiana* by HPTLC-Densitometry

Peter A. Zani[a]; Jessica L. Counihan[a]; James D. Vasta[a]; Bernard Fried[a]; Joseph Sherma[b]
[a] Department of Biology, Lafayette College, Easton, Pennsylvania, USA [b] Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA

To cite this Article Zani, Peter A. , Counihan, Jessica L. , Vasta, James D. , Fried, Bernard and Sherma, Joseph(2008) 'Characterization and Quantification of the Neutral Lipids in the Lizard *Uta stansburiana stansburiana* by HPTLC-Densitometry', Journal of Liquid Chromatography & Related Technologies, 31: 13, 1881 — 1891
To link to this Article: DOI: 10.1080/10826070802189310
URL: http://dx.doi.org/10.1080/10826070802189310

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Liquid Chromatography & Related Technologies®, 31: 1881–1891, 2008
Copyright © Taylor & Francis Group, LLC
ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802189310

Downloaded At: 16:56 23 January 2011

Characterization and Quantification of the Neutral Lipids in the Lizard *Uta stansburiana stansburiana* by HPTLC-Densitometry

Peter A. Zani,[1] Jessica L. Counihan,[1] James D. Vasta,[1] Bernard Fried,[1] and Joseph Sherma[2]

[1]Department of Biology, Lafayette College, Easton, Pennsylvania, USA
[2]Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA

Abstract: We performed high-performance thin-layer chromatography (HPTLC) to characterize and quantify the various neutral lipid classes in organs (livers, abdominal fat bodies, and tails) of 6 male and 6 female northern side-blotched lizards (*Uta stansburiana stansburiana*) with a range of body sizes (from young-of-the-year to adult). We determined the percentage of each organ that was comprised of free sterols, free fatty acids, triacylglycerols, methyl esters, and steryl esters. Regardless of lizard sex or body size, the greatest concentrations of neutral lipids in all organs examined were triacylglycerols. Statistical analyses revealed that the organs of females contained roughly twice the percentage of triacylglycerols compared to males. No other neutral lipid amounts were significantly different between the sexes. We detected only one significant relationship based on size: larger lizards contained more steryl esters in their livers than smaller lizards. Our results are consistent with previous studies showing that females contain more energy reserves (particularly triacylglycerols) than males. This is the first report of the use of TLC or HPTLC with densitometry to analyze any type of analytes in lizard samples.

Keywords: Bioenergetics, High-performance thin-layer chromatography (HPTLC), Lipids, Northern side-blotched lizards, Organs, Reptiles, Squamates

Correspondence: Peter Zani, Department of Biology, Lafayette College, Easton, PA, USA. E-mail: zanip@lafayette.edu

Downloaded At: 16:56 23 January 2011

INTRODUCTION

The study of lipid (fat) storage and utilization in squamate reptiles (especially lizards) has a long history that stretches well over five decades.[1–3] Numerous previous studies have examined the role that fat stores play in reptilian hibernation[4–9] and reproduction.[10–14] Studies of reproduction have focused on several broad topics such as the fluctuations of lipids related to reproductive cycles[15–29] and the lipid components related to offspring (egg/embryo) provisioning.[30–32]

Previous research has also compared the lipid content of various organs in diverse lizard lineages such as polychrotids (*Anolis*),[21] teiids (*Cnemidophorus* [=*Aspidoscelis*]),[33] and lacertids (*Lacerta*),[34] as well as in Sphenodontia (=Rhynchocephalia),[35] the sister lineage to squamates. These previous studies consistently found that large amounts of energy are stored in the liver, paired abdominal fat bodies, and proximal portions of the tail. To quantify lipid content, many of the studies cited above used gravimetric techniques in which a homogenized sample was dissolved in a solvent (e.g., petroleum ether or chloroform-methanol) to extract lipids. However, a few other studies have used gas- or thin-layer-chromatographic techniques to quantify lipid classes in reptiles.[10,30–34] In the past few decades, advancements in analytical chemistry have allowed for characterization and increasingly precise and accurate quantification of the various neutral lipid classes in animal organs. However, some of the most recent techniques have yet to be applied to comparative lizard physiology. In this study, high-performance thin-layer chromatography (HPTLC) was used to quantify the neutral lipids in the organs (liver, fat body, and tail) of the northern side-blotched lizard (*Uta stansburiana stansburiana*).

EXPERIMENTAL

Lizard Collection and Maintenance

Lizards were collected from a large population (Wright's Point) 20 km south of Burns, OR (1318 m elevation, 43.44°N Lat., 118.93°W Long.) as part of a long-term study of the life-history evolution of this species by one of us (PAZ). All animals were collected during the fall of 2007 (6–10 October), approximately one month prior to the onset of the winter inactive period (daily high temperatures <8°C). Lizards were shipped via overnight mail to Lafayette College and maintained in 1 m diameter circular cages made of aluminum flashing. The bottom of the cage was filled with 10 cm of masonry sand. A cluster of rocks in the center of the cage provided ample retreats. Animals were provided with 40 W

fluorescent shop lights suspended 0.5 m above the surface of the sand (14:10 light:dark) and 120 W mercury vapor spot lights suspended 0.4 m above one end of the cage (10:14 light:dark). This provided both light and heat. The sand temperature under the heat lamp regularly reached 40°C, while the room temperature only reached 25°C. Over the course of the fall we attempted to mimic the onset of winter by reducing both photoperiod (~15 min) and temperature (~1–2°C) each week. Lizards were fed crickets and mealworms two or three times per week. However, since they were fall acclimatized most animals did not eat.

Downloaded At: 16:56 23 January 2011

Euthanasia and Dissection

After approximately two months of captive care, 12 lizards were killed by decapitation. For each lizard the following measurements were recorded: snout-vent length (SVL) to nearest mm using a linear ruler; mass to 0.1 g using an Acculab model PP2060D electronic balance; and sex as determined by presence of enlarged post-anal scales in males. We then dissected out the liver, abdominal fat body, and a portion of the tail for lipid extraction. Each organ was rinsed using a general reptile ringer solution, blotted dry, and weighed using a Mettler College 150 electronic balance. Organs were immediately frozen at −80°C to await lipid extraction, which occurred within two weeks.

Lipid Extraction

Tissue samples of less than 100 mg in blotted wet mass were homogenized in 2 mL of chloroform-methanol (2:1) in a 7 mL capacity Wheaton (Millville, NJ) glass homogenizer. This solvent-sample ratio assured complete extraction of the lipids.[36] The Folch wash (0.88% KCl, w/v, in deionized water) was used in a ratio of 4 parts to 1 part salt solution to remove non-lipophilic material. Samples were vortex mixed for 30 s and the top (aqueous) layer removed and discarded. The samples were dried in a warm water bath (40–60°C) using a stream of nitrogen gas and stored at −20°C until use. To obtain appropriate densitometry scan areas within the calibration curves, samples were reconstituted prior to HPTLC tissue analysis in 1.5–10 mL of chloroform-methanol (2:1), as necessary.

HPTLC Analysis

The neutral lipid standard, non-polar lipid mixture B (Matreya, Inc., Pleasant Gap, PA), was dissolved in chloroform-methanol (2:1).

The standard contained 20.0% each of cholesterol (CH), oleic acid (OA), triolein (TR), methyl oleate (MO), and cholesteryl oleate (CO) and a total lipid concentration of 25.0 mg/mL. This standard was used to represent the sample neutral classes of free sterols (FS), free fatty acids (FFA), triacylglycerols (TG), methyl esters (ME), and steryl esters (SE), respectively, and was prepared at a concentration of 0.200 μg/μL.

HPTLC analysis was performed on 10 × 20 cm HPTLC-HLF silica gel plates (Analtech, Inc., Newark, DE), which contained 19 scored lanes and a concentration zone spotting area. Before use, plates were prewashed by development to the top with dichloromethane-methanol (1:1) and dried with a stream of air.

Downloaded At: 16:56 23 January 2011

Standard and reconstituted sample solutions (2.00, 4.00, 8.00, and 16.0 μL) were applied to the concentration zone of separate lanes on the HPTLC plates using a 10-μL (Drummond, Broomall, PA) digital microdispenser. We developed plates to a distance of 8 cm beyond the concentration-zone silica gel interface with 25 mL of petroleum ether-diethyl ether-glacial acetic acid (80:20:1) mobile phase (Mangold, 1969). This development was carried out in a twin trough TLC chamber (Camag, Wilmington, NC) containing a saturation pad (Analtech, Inc.). Prior to development, the chamber was equilibrated with the mobile phase for 20 min. Development, which required approximately 8–9 min, was carried out at 21 ± 1°C and a relative humidity of ~25%.

After development, plates were dried with a stream of cool air from a hair dryer, sprayed with 5% ethanolic phosphomolybdic acid solution, and heated on a Camag plate heater at 115°C for 10 min to detect neutral lipids as blue zones on a yellow background. For a single liver sample, a plate was developed in hexanes-petroleum ether-diethyl ether-glacial acetic acid (50:20:5:1) mobile phase[37] to confirm or reject the presence of the fast moving ME and SE zones. This solvent system is excellent for resolving neutral lipids that migrate at or near the mobile phase front in the Mangold solvent system.[38]

Quantitative densitometric analysis was carried out using a TLC Scanner II (Camag) with the tungsten light source set at a wavelength of 610 nm, slit width 4, slit length 4, and scanning rate of 4 mm/s. The CATS-3 software automatically generated polynomial calibration curves (standard zone masses versus peak areas) and interpolated sample masses based on their peak areas. We calculated the percentage by mass of lipid in each tissue sample using the equation:

$$\text{percent neutral lipid} = \frac{w * R * \text{ dilution factor } * 100}{\text{initial tissue sample mass } (\mu g)}$$

where w = lipid mass (μg) of sample interpolated from calibration curve and R = reconstituted volume (μL)/spotted volume (μL). For samples that were diluted or concentrated to obtain bracketed scan areas within

the calibration curve, an appropriate dilution factor was included in the calculation of percent neutral lipid.

Statistical Comparisons

Differences in the amount of neutral lipid classes present in the different organs were compared by conducting a separate one-factor analysis of variance on each organ in which sex (male/female) was included as the factor. In addition, relationships between lipid classes in each organ and body size were evaluated by conducting regression analysis using lizard body mass. All statistical analyses were conducted using JMP v 5.1[39] for Macintosh computer.

Downloaded At: 16:56 23 January 2011

RESULTS

Neutral lipids were identified based upon comigration of standard zones with corresponding zones in the liver, abdominal fat body, and tail samples of lizards (Fig. 1). Neutral lipid classes (FS, FFA, TG, and SE) were identified in liver samples and gave retention factor (R_F) values of 0.19, 0.35, 0.61, and 0.87, respectively. The major neutral lipids in the liver were TG, SE, FS; trace amounts of FFA were also found. All of these neutral lipids migrated identically with the neutral lipid standard. TG and trace amounts of FS (not seen in Fig. 1) were found in the fat-body sample. The R_F value of TG in the fat body was 0.61. In the tail TG ($R_F = 0.61$) and occasionally low amounts of FS ($R_F = 0.19$) were identified. For the liver sample analyzed in the Smith et al. mobile phase[37], the presence of SE was confirmed based on the comigration of its zone with the corresponding standard; no ME was found.

Calibration curves relating densitometric scan areas to the mass of neutral lipid standard zones consistently produced very high polynomial regression correlation coefficients ($r = 0.99$). We determined the percentage of neutral lipids found in the liver, fat body, and tail of *Uta stansburiana* (Table 1). Sample zones with scan areas below the area of the lowest standard were considered to be not quantifiable.

When lipid classes were compared between the sexes, significant differences in percent triacylglycerols were found between males and females in the liver ($F_{1,10} = 6.66$, $P = 0.027$) and fat body ($F_{1,10} = 5.21$, $P = 0.045$), but not the tail ($F_{1,10} = 3.52$, $P = 0.090$). These relationships are qualitatively identical when body mass is considered as a covariate in this analysis (liver: $F_{1,10} = 5.54$, $P = 0.043$; fat body: $F_{1,10} = 5.21$, $P = 0.046$; tail: $F_{1,10} = 4.36$, $P = 0.067$). Thus, the major differences in triacylglycerols appear to be due to lizard sex rather than body size.

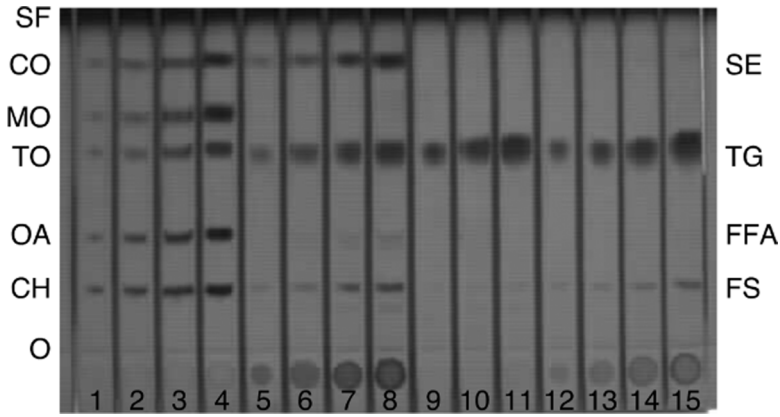


Figure 1. Chromatograms on an Analtech HPTLC-HLF plate photographed in white light with a Camag VideoStore Documentation System showing the neutral lipid profiles of liver, fat body, and tail samples from a single lizard. Lanes 1–4 contain the neutral lipid standard spotted at 2, 4, 8, and 16 μL aliquots, respectively. Lanes 5–8 contain liver sample spotted in 2, 4, 8, and 16 μL aliquots, respectively. Lanes 9–11 contain fat body sample spotted in 4, 8, and 16 μL aliquots, respectively. Lanes 12–15 contain tail sample spotted in 2, 4, 8, and 16 μL aliquots, respectively. SF = solvent front, CO = cholesteryl oleate, MO = methyl oleate, TO = triolein, OA = oleic acid, CH = cholesterol, SE = steryl esters, TG = triacylglycerols, FFA = free fatty acids, FS = free sterols, O = origin. The labels to the right of the chromatogram refer to the neutral lipid classes in the samples as seen in lanes 5–15, while the labels to the left refer to the standard in lanes 1–4.

The only relationship among the neutral lipids and lizard body size was for steryl esters: the livers of larger lizards contained more absolute amounts of steryl esters ($F_{1,10} = 6.97$, $P = 0.025$), but not necessarily when considered as a percentage of the liver ($F_{1,10} = 4.51$, $P = 0.060$).

DISCUSSION

Although the literature on lipid storage and utilization in squamate reptiles covers many species and numerous aspects of organismal physiology and biochemistry, recent advances in the characterization and quantification of neutral lipid classes using high-performance thin layer chromatography allow for an even greater understanding of vertebrate physiology and life history. This is the first paper to utilize HPTLC to study neutral lipids in lizards. Our findings indicate that triacylglycerols are the primary neutral lipid class used for storage in the organs of *Uta* and that the paired abdominal fat bodies are the primary storage

Downloaded At: 16:56 23 January 2011

Downloaded At: 16:56 23 January 2011

Table 1. Percent by mass (mean ± standard error of mean) of neutral lipids in organs of 6 male and 6 female *Uta stansburiana* lizards

	Liver (% of organ mass)		Fat body (% of organ mass)		Tail (% of organ mass)		Sum of lipids in organs (% of total lizard mass)	
Neutral lipid	Male	Female	Male	Female	Male	Female	Male	Female
Steryl esters	1 ± 1	1.2 ± 0.7	N.D.[a]	N.D.	N.Q.[b]	N.Q.	0.02 ± 0.01	0.02 ± 0.01
Triacylglycerols	3 ± 2	7 ± 3	40 ± 30	90 ± 40	4 ± 3	7 ± 3	1.7 ± 0.5	1.4 ± 0.6
Free fatty acids	0.2 ± 0.1	0.4 ± 0.3	N.D.	N.D.	N.D.	N.D.	0.004 ± 0.003	0.007 ± 0.004
Free sterols	0.16 ± 0.06	0.16 ± 0.07	N.D.	N.D.	N.Q.	0.193[c]	0.003 ± 0.002	0.003 ± 0.001

[a]Not detected (no visible chromatographic zone).
[b]Below limits of quantification.
[c]Only one sample was quantifiable.

organs (Table 1). In all tissues, the remaining classes of neutral lipids (free sterols, free fatty acids, methyl esters, and steryl esters) were present in relatively smaller amounts, if at all. These findings are consistent with previous attempts to classify the neutral lipids in the different organs of lizards.[10,30,33,34] These previous studies found that fat bodies are typically the tissue with the highest lipid content and are 66–97% lipid by mass. Thus, *Uta* (40–90% lipid) appears to be a typical lizard with regards to the types and amounts of lipids stored.

An additional important aspect of this study is the difference in lipids between the sexes. Consistent with previous research,[1,25,33] we found that females had higher lipid contents than males in all tissues. Previous research on side-blotched lizards indicates that females have higher amounts of stored energy during all seasons and that these lipid stores peak late in the growing season.[25] Thus, the difference in lipid content at the end of the growing season we detected between the sexes likely represents a maximum difference that decreases overwinter and during the subsequent spring reproductive cycle as stored lipids are utilized for ovarian follicle development.[25]

In summary, it was demonstrated that HPTLC is an essential and modern technique for quantifying neutral lipid classes in squamate reptiles and that side-blotched lizards exhibit patterns of lipid storage that are consistent with numerous previous studies on lizard species. The study of lipid storage and utilization remains an important aspect of our understanding of physiological and life-historical variation.

Downloaded At: 16:56 23 January 2011

ACKNOWLEDGMENTS

The authors acknowledge Kathy Flannery for lodging during field work and Mary Rollyson for help in maintaining animals in captivity. Travel by PAZ was supported by the Lafayette College Biology Department Stableford endowment. JLC and JDV were supported by the Lafayette College EXCEL scholar program. JDV was partially supported by a Camille and Henry Dreyfus Foundation Senior Scientist Mentor Program award to JS. This study was approved by the Lafayette College Animal Care and Use Committee.

REFERENCES

1. Derickson, W.K. Lipid storage and utilization in reptiles. Am. Zool. **1976**, *16*, 711–723.
2. Sheridan, M.A. Regulation of lipid metabolism in poikilothermic vertebrates. Comp. Biochem. Physiol. B **1994**, *107*, 495–508.

Downloaded At: 16:56 23 January 2011

3. Thompson, M.B.; Speake, B.K. Energy and nutrient utilisation by embryonic reptiles. Comp. Biochem. Physiol. A **2003**, *133*, 529–538.
4. Afroz, H., Ishaq, M., Ali, S.S. Seasonal changes in the lipids of adipose tissue in a hibernating lizard (*Uromastix hardwickii*). Proc. Soc. Exp. Biol. Med. **1971**, *136*, 894–898.
5. Avery, R.A. Utilization of caudal fat by hibernating common lizards, *Lacerta vivipara*. Comp. Biochem. Physiol. **1970**, *37*, 119–121.
6. Barwick, R.E.; Bryant, C. Physiological and biochemical aspects of hibernation in the scincid lizard *Egernia cunninghami* (Gray, 1832). Physiol. Zool. **1966**, *39*, 1–20.
7. Brown, W.S.; Parker, W.S.; Endler, J.A. Thermal and spatial relationships of two species of colubrid snakes during hibernation. Herpetologica **1974**, *30*, 32–38.
8. Dessauer, H.C. Hibernation of the lizard *Anolis carolinensis*. Proc. Soc. Exp. Biol. Med. **1953**, *82*, 351–353.
9. Zain, B.K.; Zain-Ul-Abedin, M. Characterizations of the abdominal fat pads of a lizard. Comp. Biochem. Physiol. **1967**, *23*, 173–177.
10. Ballinger, R.E.; Holy, L., Rowe, J.W.; Karst, F., Ogg, C.L.; Stanley-Samuelson, D.W. Seasonal changes in lipid composition during the reproductive cycle of the red-chinned lizard, *Sceloporus undulatus erythrocheilus*. Comp. Biochem. Physiol. B **1992**, *103*, 527–531.
11. Derickson, W.K. Lipid deposition and utilization in the sagebrush lizard, *Sceloporus graciosus*: its significance for reproduction and maintenance. Comp. Biochem. Physiol. A **1974**, *49*, 267–272.
12. Lacy, E.L.; Sheridan, M.A.; Moore, M.C. Sex differences in lipid metabolism during reproduction in free-living tree lizards (*Urosaurus ornatus*). Gen. Comp. Endocrinol. **2002**, *128*, 180–192.
13. Smith, R.E. Experimental evidence for a gonadal-fat body relationship in two teiid lizards (*Ameiva festiva, Ameiva quadrilineata*). Biol. Bull. **1968**, *134*, 325–331.
14. Vitt, L.J.; Cooper, W.E., Jr. The relationship between reproduction and lipid cycling in the skink *Eumeces laticeps* with comments on brooding ecology. Herpetologica **1985**, *41*, 419–432.
15. Ataev, C. Characteristics of hibernation of the caucasian agama in the Kopet-Dag range. Sov. J. Ecol. **1974**, *5*, 76–78.
16. Avery, R.A. Storage lipids in the lizard *Lacerta vivipara*: a quantitative study. J. Zool. (Lond.) **1974**, *173*, 419–425.
17. Ballinger, R.E.; Congdon, J.D. Population ecology and life history strategy of a montane lizard (*Sceloporus scalaris*) in southeastern Arizona. J. Nat. Hist. **1981**, *15*, 213–222.
18. Benabib, M. Reproduction and lipid utilization of tropical populations of *Sceloporus variabilis*. Herpetol. Monogr. **1994**, *8*, 160–180.
19. Castilla, A.M.; Bauwens, D. Reproductive and fat body cycles of the lizard, *Lacerta lepida*, in central Spain. J. Herpetol. **1990**, *24*, 261–266.

20. de Souza, S.C.R.; de Carvalho, J.E.; Abe, A.S.; Bicudo, J.E.P.W.; Bianconcini, M.S.C. Seasonal metabolic depression, substrate utilization and changes in scaling patterns during the first year cycle of tegu lizards (*Tupinambis merianae*). J. Exp. Biol. **2004**, *207*, 307–318.
21. Dessauer, H.C. Seasonal changes in the gross organ composition of the lizard, *Anolis carolinensis*. J. Exp. Zool. **1955**, *128*, 1–12.
22. Fleming, T.H.; Hooker, R.S. *Anolis cupreus*: the reponse of a lizard to tropical seasonality. Ecology **1975**, *56*, 1243–1261.
23. Gaffney, F.G.; Fitzpatrick, L.C. Energetics and lipid cycles in the lizard, *Cnemidophorus tigris*. Copeia **1973**, *1973*, 446–452.
24. Goldberg, S.R. Reproduction in mountain and lowland populations of the lizard *Sceloporus occidentalis*. Copeia **1974**, *1974*, 176–182.
25. Hahn, W.E.; Tinkle, D.W. Fat body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard *Uta stansburiana*. J. Exp. Zool. **1965**, *158*, 79–86.
26. Jameson, E.W.; Jr. Fat and breeding cycles in a montane population of *Sceloporus graciosus*. J. Herpetol. **1974**, *8*, 311–322.
27. Jameson, E.W.; Jr., Allison, A. Fat and breeding cycles in two montane populations of *Sceloporus occidentalis* (Reptilia, Lacertilia, Iguanidae). J. Herpetol. **1976**, *10*, 211–220.
28. Magnusson, W.E. Reproductive cycles of teiid lizards in Amazonian savanna. J. Herpetol. **1987**, *21*, 307–316.
29. McKinney, R.B.; Marion, K.R. Reproductive and fat body cycles in the male lizard, *Sceloporus undulatus*, from Alabama, with comparisons of geographic variation. J. Herpetol. **1985**, *19*, 208–217.
30. Hadley, N.F.; Christie, W.W. The lipid composition and triglyceride structure of eggs and fat bodies of the lizard *Sceloporus jarrovi*. Comp. Biochem. Physiol B **1974**, *48*, 275–284.
31. Jones, S.M.; Bennett, E.J.; Swadling, K.M. Lipids in yolks and neonates of the viviparous lizard *Niveoscincus metallicus*. Comp. Biochem. Physiol. B **1998**, *121*, 465–470.
32. Speake, B.K.; Thompson, M.B. Lipids of the eggs and neonates of oviparous and viviparous lizards. Comp. Biochem. Physiol. A **2000**, *127*, 453–467.
33. Brian, B.L.; Gaffney, F.G.; Fitzpatrick, L.C.; Scholes, V.E. Fatty acid distribution of lipids from carcass, liver and fat bodies of the lizard, *Cnemidophorus tigris*, prior to hibernation. Comp. Biochem. Physiol. B **1972**, *41*, 661–664.
34. Avery, R.A.; Shewry, P.R.; Stobart, A.K. A comparison of lipids from the fat body and tail of the common lizard, *Lacerta vivipara*. Brit. J. Herpetol. **1974**, *5*, 410–412.
35. Body, D.R.; Newman, D.G. The lipid composition of liver, lung and adipose tissues from tuatara (*Sphenodon punctatus*) (Reptilia: Sphenodontia). Comp. Biochem. Physiol. B **1989**, *93*, 223–227.
36. Folch, J., Lees, M., Sloane Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. **1957**, *226*, 497–509.

Downloaded At: 16:56 23 January 2011

37. Smith, M.C.; Webster, C.L.; Sherma, J., Fried, B. Determination of neutral lipids in regular and low-fat eggs by high-performance TLC with densitometry. J. Liq. Chromatogr. **1995**, *18*, 527–535.
38. Mangold, H.K. *Thin-Layer Chromatography*; 2nd ed; Springer: New York, 1969.
39. SAS. JMP V 5.1. SAS Institute Inc: Pacific Grove, California, 2003.

Received March 31, 2008
Accepted April 11, 2008
Manuscript 6311N

Downloaded At: 16:56 23 January 2011