*The Auk* 119(4):1167–1173, 2002

# Corticosterone Metabolites can be Measured Noninvasively in Excreta of European Stonechats (*Saxicola torquata rubicola*)

Wolfgang Goymann,[1,3] Erich Möstl,[2] and Eberhard Gwinner[1]

[1]*Max-Planck Research Centre for Ornithology, Von-der-Tann-Str. 7, D-82346 Andechs, Germany; and*
[2]*Institute of Biochemistry, Veterinarian University, Veterinärplatz 1, A-1210 Vienna, Austria*

Abstract.—Measurements of corticosterone levels from blood samples of birds provide accurate snapshots of systemic hormone concentrations. However, those birds must be caught and handled, which may be unfeasible especially when multiple sampling is required. Furthermore, handling causes stress and may therefore interfere with hormone measurements. Therefore a non-invasive technique was developed to measure metabolites of corticosterone in excreta of European Stonechats (*Saxicola torquata rubicola*) using a corticosterone enzyme-immunoassay. High-performance liquid chromatography of excreta of a female and a male stonechat injected with tritiated corticosterone showed that corticosterone is excreted in the form of numerous metabolites and that the corticosterone enzyme-immunoassay cross-reacted with most of those metabolites. Injection of adrenocorticotrophic hormone in one female and seven male stonechats led to a significant increase in the levels of excreted corticosteroid metabolites within 1 h 20 min after administration of adrenocorticotrophic hormone. These results suggest that the corticosterone enzyme-immunoassay used in this study provides a quantitative measure of excreted corticosteroid metabolite levels in European Stonechats and has the potential to replace plasma measurements of these hormones.

Resumen.—Las mediciones de los niveles de corticosterona en muestras de sangre de aves proveen información puntual exacta sobre las concentraciones de hormonas sistémicas. Sin embargo, las aves deben ser capturadas y manipuladas, lo que puede ser difícil especialmente cuando se requieren múltiples muestras. Mas aún, la manipulación causa estrés y podría interferir con las medidas hormonales. Por lo tanto, se desarrolló una técnica no invasora para medir metabolitos de corticosterona en las excretas de *Saxicola torquata rubicola* por medio de un inmunoensayo enzimático. Practicando cromatografía líquida de alto rendimiento en las excretas de una hembra y un macho que fueron inyectados con corticosterona tritriada, se encontró que la corticosterona es excretada en forma de numerosos metabolitos y que el inmunoensayo reaccionó con la mayoría de éstos. La inyección de la hormona adrenocorticotrófica en una hembra y siete machos condujo en 1 h 20 min a un incremento significativo de los niveles de metabolitos corticosteroides excretados. Estos resultados sugieren que el inmunoensayo enzimático de corticosterona empleado en este estudio provee una medida cuantitativa de los niveles de metabolitos corticosteroides excretados en *Saxicola torquata rubicola* y tiene potencial para reemplazar las mediciones plasmáticas de esta hormona.

Birds and other vertebrates respond to unexpected physical or social changes of their environment with a rapid elevation of plasma corticosterone levels. Corticosterone mediates facultative behavioral and physiological responses of the organism to such unpredictable events and helps the individual cope with unexpected changes in an adaptive way (Wingfield et al. 1998). An increasing number of studies shows that birds and other vertebrates are able to modulate production of corticosteroids, depending on season, environment, or their life-history stage (e.g. Wingfield et al. 1992, 1995, 1998; Silverin and Wingfield 1998; Goymann et al. 2001; Moore et al. 2001).

Typically, corticosteroids are measured in blood plasma, but that method has obvious drawbacks because animals must be caught and handled. Particularly in small birds, the practicability of hormone studies is often limited by frequency and volume of blood samples that can be obtained. Also, handling may cause stress (e.g. Wingfield et al. 1992) and therefore may interfere with the hormone measurement. Noninvasive methods, such as measuring corticosteroid metabolites in excreta of birds (=cloacal mixture of feces and urine) have the advantage that sampling does not interfere with behavior and multiple samples can be obtained. Surprisingly, to our knowledge, noninvasive measurement of corticosteroids has not yet been applied in any passerine bird and there are few studies that have investigated corticosteroids noninvasively in nonpasserine bird species (Wasser et al. 1997; Kotrschal et al. 1998, 2000; Hiebert et al. 2000a, b).

[3] Present address: Department of Zoology, University of Washington, Box 351800, Seattle, Washington 98195, USA. E-mail: wgoymann@u.washington.edu

The validity of noninvasive hormone measurement relies on the assumption that steroid hormone concentrations in excreta proportionally reflect production of those hormones. The objective of this study was to validate an enzyme immunoassay (EIA) to quantify corticosteroid metabolites in excreta of European Stonechats (*Saxicola torquata rubicola*).

*Methods.*—Experimental birds were bred and raised in captivity and belonged to the central European Stonechat subspecies *S. t. rubicola*. They were kept under natural light conditions and housed in individual cages, where they could hear but not see each other. Experiments were carried out end of April (adrenocorticotrophic hormone [ACTH] challenge) and June (radiolabeled corticosterone infusion) 2000, when gonads of the stonechats were fully developed. All birds were supplied *ad libitum* with our standard diet (Gwinner et al. 1995) and water.

Tritiated corticosterone ([1,2,6,7]$^3$H-Cc New England Nuclear–Dupont: NET-399) was used to determine how one male and one female stonechat metabolize and excrete that steroid. On 25 June (lights on 0421 hours, lights off 2139 hours) birds were injected i.p. at 0600 hours European standard time with Hamilton syringes. Each bird was injected with 50 μL $^3$H-corticosterone (~550,000 dpm) in sterile isotonic saline including 1 μg cold corticosterone as carrier. Fifty-microliter triplicates of the $^3$H-corticosterone solution were pipetted with the same Hamilton syringe into scintillation vials and counted with a Beckman LS6000 counter with 4 mL scintillation fluid to determine total radioactivity. After isotope administration, syringes were rinsed with ethanol and the residual radioactivity counted and subtracted from the preinjection total. Excreta were collected from nonabsorbant plastic sheets that covered the cage floor and walls and that were discarded after use. The plastic sheets were inserted to the cages in the evening (1700 hours) before the injection and were collected immediately before the injection of $^3$H-corticosterone. Further samples were collected 3 h 40 min, 6 h 25 min, 9 h 5 min, 24 h 10 min, 31 h 25 min, and 47 h 10 min after $^3$H-corticosterone administration. After extraction (see below) radioactivity of each sample was determined by counting a fraction of 100 μL in duplicates with 4 mL scintillation liquid to an accuracy of 2–3% in a Beckman LS 6000 β-counter. The 100 μL fractions represented 5–10% of the total sample volume. The sample with the highest amount of radioactivity was further analyzed with HPLC (see below).

To test whether an increase of corticosteroid metabolites can be traced in Stonechat excreta, seven adult male Stonechats (mean ± sem weight 14.6 ± 0.3 g) and one female (14.8 g) were injected i.p. with 1 μg synthetic ACTH (1–24; Sigma) in 100 μL saline at 0840 hours European standard time on 26 April (lights on 0522 hours, lights off 2037 hours). Adrenocorticotrophic hormone has been demonstrated to reliably increase corticosterone production in birds (e.g. Astheimer et al. 1994, Romero et al. 1998a, b; Sims and Holberton 2000, Wilson and Holberton 2001) and thus should result in an increase of excreted corticosteroid metabolite levels. Handling of birds has been demonstrated to induce a stress response with a subsequent release of corticosterone (Wingfield 1994). Thus, in addition to the effect of ACTH, the handling during the injection was likely to cause an elevation of corticosterone production in itself. Excreta were collected from stainless steel plates underneath the perches of each cage during three hours preceding the ACTH administration (0540–0840 hours), and 1 h 20 min (1000 hours), 2 h 40 min (1120 hours), 4 h (1240 hours), 6 h 20 min (1500 hours) and 8 h 20 min (1700 hours) after the ACTH administration. Two sets of stainless steel plates were used for each bird and exchanged at the respective sampling times. Excreta were removed from the plates using a spatula, collected in small plastic vials, and frozen at −70°C until further processing. Before reuse, each steel plate was washed with hot water and detergent, rinsed with cold water, and wiped with 70% methanol.

Immediately after collection, samples were frozen at −70°C. Then, they were freeze-dried with a Christ Alpha I-5 lyophilizator, pulverized, weighed to the nearest milligram with a Sartorius Research R 160 P balance, and extracted with 75% methanol and 25% double-distilled water (ddH$_2$O). Depending on the sample weight, we used 0.75–2.0 mL of methanol/ddH$_2$O for the extraction. After shaking the samples on a rotating table for 30 min, the samples were centrifuged (2,500 g, 10 min) and the supernatant transferred to a new tube. A fraction of 200 μL was transferred to a further vial and dried under a stream of nitrogen. Then 200 μL sodium acetate buffer (0.2 M, pH 4.8) were added, containing β-glucuronidase/arylsulfatase (1:100), to cleave conjugated steroids. Then the vials were sealed and incubated for 16 h at 39°C. After incubation the samples were stored at −40°C until further analysis.

We used an EIA with an antibody against corticosterone (custom made by E. Möstl) to determine the concentration of immunoreactive metabolites of corticosteroids in stonechat excreta. The corticosterone assay has been described previously (Palme and Möstl 1997, Goymann et al. 1999) and has been successfully used in two species of geese (Kotrschal et al. 1998, 2000). We tested one further antibody (against 11-oxoetiocholanolone) that has been successfully used in geese (Kotrschal et al. 2000) but it did not give satisfying results in stonechats.

Ten microliters of each extracted sample plus 40 μL assay buffer were pipetted into microtiterplate wells (coated with sheep-anti-rabbit IgG) and 100 μL of the antibody and enzyme solution were added. All further steps were conducted in a cold room at 4°C. After overnight incubation plates were washed four

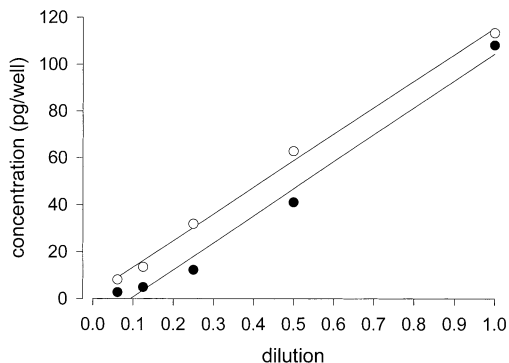


FIG. 1. Parallelism between a dilution of standard corticosterone (open circles) and pooled samples of stonechat excreta (closed circles). The slopes of the regression of the standard ($y = 113.3x + 2.1$) and the pool ($y = 115.2x - 10.8$) are parallel and do not significantly differ from each other ($t = 0.203$, $P = 0.846$).

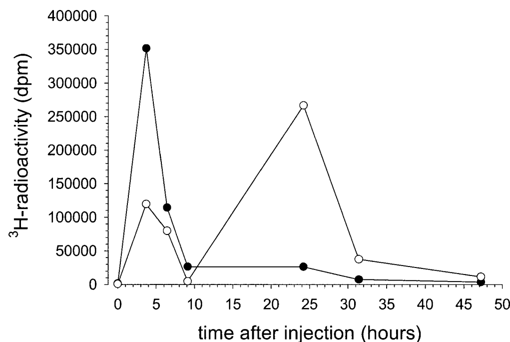


FIG. 2. Time course of excretion of i.p. injected $^{3}$H-corticosterone in a female (open circles) and a male (closed circles) European Stonechat. The female did not feed for the rest of the day following the injection, and thus, excretion of corticosterone may have been delayed. Time points represent the end of the collection period.

times with ddH$_2$O/TWEEN 20 (1:500,000), 250 μL enzyme solution (Streptavidin-POD conjugated, 500 U, 1:30,000; Roche, Mannheim, Germany) was pipetted into each well, and incubated for 45° min at 4°C. Then, plates were washed again and 250 μL tetramethylbenzidine/H$_2$O$_2$-substrate solution was added. After incubation (45 min, 4°C) the reaction was stopped with 50 μL H$_2$SO$_4$ (3M) and absorbance measured at 450 nm/630 nm, using a DIAS reader (Dynatech, Guernsey, Great Britain). Standard curves and sample concentrations were calculated with Immunofit 3.0 (Beckman Inc., Fullerton, California).

Serial dilutions of extracts yielded displacement curves parallel to standard corticosterone (Fig. 1). Assay accuracy was determined by adding a defined amount of corticosterone standard to pooled samples of stonechat excreta. The levels of those samples were compared with pooled samples without corticosterone standard. Accuracy was 104 ± 6%. (mean ± SE, $n = 8$). The detection limit was 2 pg corticosterone per well, mean (±SE) intraassay coefficient of variation was 6.6 ± 3.6% ($n = 6$), and the interassay coefficient of variation for all six assays was 14.9%. Samples were assayed in duplicate and concentrations were expressed as nanograms per gram dried excreta.

The number and relative proportions of immunoreactive corticosteroid metabolites in extracts of excreta were determined with high performance liquid chromatography (HPLC). Methanol/ddH$_2$O extracted samples (1 mL) of the $^{3}$H-corticosterone injected male and female stonechat were diluted with ddH$_2$O (10 mL) and passed through C-18 matrix columns (Waters, Milford, Massachusetts). Columns were then washed with ddH$_2$O (2 × 5 mL) and eluted with methanol (4 mL). The methanol was evaporated to dryness under a stream of nitrogen, the sample resuspended in 50% methanol, and separated on a Novapak C-18 column using a linear gradient of 50–75% methanol (flow rate 1 mL min$^{-1}$; run time 40 min). For each bird ninety-five 330 μL fractions were collected, dried under a stream of nitrogen and reconstituted in sodium acetate buffer with β-glucuronidase/arylsulfatase. After cleavage of conjugated steroids (see processing of samples), immunoreactive corticosterone metabolites of each fraction were quantified with the corticosterone EIA. Elution of $^{3}$H-corticosterone was measured by liquid scintillation counting. The HPLC elution profile of corticosterone was determined by adding pure standard corticosterone to the HPLC column and eluting it in the same way as described for the samples. Standard corticosterone eluted in fraction 68.

Because excreted corticosteroid data did not follow a normal distribution, we used distribution-free statistics. A Friedman test (the nonparametric equivalent of a repeated measures ANOVA) was used to establish whether there was an overall difference in excreted corticosteroid levels at different points of time before and after the ACTH injection (six repeated measures, see above). *Post-hoc* multiple comparisons between repeated measures were calculated following Conover (1980) with a custom-made computer program (J. Lamprecht and B. Knauer, MPI Seewiesen, Germany). Results were considered significant at $\alpha = 0.05$ and $P$-values are two-tailed.

*Results.*—Recoveries in excreta of $^{3}$H-corticosterone were 94.5% in the female and 96.2% in the male over a 47 h 10 min period of collecting samples. In the male, metabolites of i.p. injected $^{3}$H-corticosterone were excreted mainly within 3–6 h after administration (Fig. 2). Because the female did not feed for a whole day after administration of $^{3}$H-corticoste-

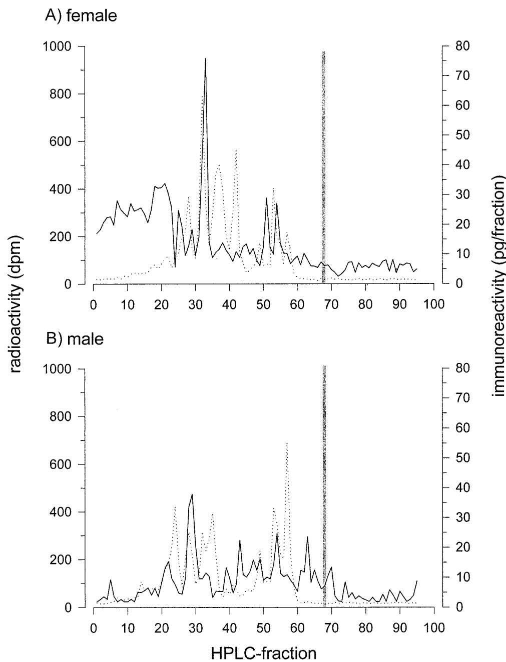


FIG. 3. HPLC profile of metabolites of i.p. injected $^3$H-corticosterone (dotted line) and immunoreactive substances measured with the corticosterone EIA (solid line) in a female (a) and a male (b) Stonechat. $^3$H-corticosterone was metabolized into a number of substances eluting between fractions 20 and 60 (dotted line). The corticosterone EIA cross-reacted with most of those metabolites (solid line). Note that the magnitude of radioactive peaks do not necessarily indicate the quantity of the respective $^3$H-corticosterone metabolites. A $^3$H-metabolite which retained all four tritium atoms of the original $^3$H-corticosterone may elicit a larger peak than a $^3$H-metabolite of the same concentration which was partially hydrolized. The gray bar indicates in which fraction standard corticosterone eluted.

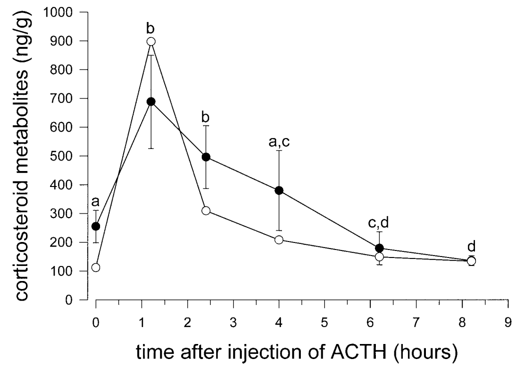


FIG. 4. Profile of excreted corticosterone metabolite concentrations in one female (open circles) and seven male (closed circles) European Stonechats injected with ACTH (mean ± SE). The data of the female were included in the statistical analysis, but graphed separately to demonstrate that the female followed the same pattern as the males. Data points that do not share the same lower case letter are significantly different from each other (*post-hoc* comparisons). Time points represent the end of the collection period.

rone, she had a low defecation rate and thus showed a delayed excretion pattern of $^3$H-corticosterone. In the female, the metabolites of corticosterone were excreted mainly between 9 and 24 h after the treatment (Fig. 2). Levels of radioactivity in excreta of both birds had returned to background levels between 31 h 25 min and 47 h 10 min after the treatment (Fig. 2).

HPLC separation of the excreta containing the highest amount of radioactivity (3 h 40 min sample of the male, 24 h 10 min sample of the female) showed that $^3$H-corticosterone was completely metabolized in excreta of both birds. Corticosterone, which should elute in fraction 68, did not contribute a radioactive peak (Fig. 3). Instead, there were numerous metabolites of $^3$H-corticosterone, eluting mainly between fractions 20 and 60 (Fig. 3). Compared to corticosterone those metabolites showed elution patterns of more polar substances. Although the polarity of the $^3$H-corticosterone metabolites was similar in the female and the male, the magnitude of the peaks differed.

Subsequent analysis of the HPLC fractions with the corticosterone EIA indicated that the antibody showed high cross-reactivity with two of six major radioactive peaks in the female (Fig. 3, a) and with three of the seven major radioactive peaks in the male (Fig. 3, b). The antibody also cross-reacted with several of the other major radioactive peaks in those animals, but to a lower degree (Fig. 3). These results indicate that the EIA picked up substances that qualitatively represent metabolized corticosterone. Faecal corticosterone metabolites significantly increased after challenging the birds with ACTH (Friedman test, $\chi^2 = 25.86$, $P < 0.0001$; Fig. 4). *Post-hoc* comparisons revealed that samples taken 1 h 20 min (1000 hours) and 2 h 40 min (1120 hours) after the ACTH challenge had significantly higher corticosteroid metabolite levels than samples taken before the ACTH challenge (0540–0840 hours) or samples taken later than 4 h after the challenge (Fig. 4). Furthermore, there might have been a negative feedback effect, because corticosteroid metabolite levels were significantly lower than preinjection levels after 6 h 20 min (1500 hours) and after 8 h 20 min (1700 hours).

*Discussion.*—In this study, we investigated whether excreted corticosteroid metabolites of European Stonechats reflect the adrenal production of those hormones. HPLC analysis indicated, that $^3$H-corti-

costerone injected to a female and a male stonechat was metabolized and excreted in the form of numerous metabolites. Peaks in the female and the male were of similar polarity but differed in magnitude. Possibly that indicated that there were differences in corticosterone metabolism between sexes or between individuals. Further analysis of the HPLC fractions with the corticosterone EIA confirmed, that the EIA cross-reacted with some of those metabolites, confirming a qualitative measure of corticosterone metabolites in stonechat excreta. Administration of ACTH and handling of birds typically stimulate the adrenals to increase production of corticosterone which peaks after ~30 min in plasma and reaches levels that are typically 3–10× higher than baseline levels (e.g. Astheimer et al. 1994, Wingfield et al. 1997, Romero et al. 1998a, b). In stonechats, the combined effects of ACTH administration and handling during that treatment led to a significant increase of excreted corticosteroid metabolites within 1 h 20 min. Postinjection corticosterone metabolite levels were ~3× higher than control levels before the challenge. Hence, the corticosterone EIA detected ACTH-induced changes of corticosterone production in stonechat excreta and the magnitude of the increase in excreted metabolites is within the range of expected changes in blood plasma. The corticosterone EIA can thus be used as a tool to detect quantitative changes in corticosterone metabolite levels.

The time-lag between a plasma hormone peak and its appearance in bird excreta depends on kidney and gut passage times (Goldin et al. 1981, Wasser et al. 1988). In the male, $^{3}$H-corticosteroid metabolite levels peaked within 3 h 40 min, when excreta were collected for the first time after the administration of $^{3}$H-corticosterone. Also the female showed a peak within 3 h 40 min, but she showed a second and higher peak of $^{3}$H-corticosterone metabolites that were excreted between 9 h and 24 h after administration of the radiolabeled hormone. Most likely, that bimodal excretion pattern was caused by the fact that the female did not feed for the rest of the day after the injection and only started feeding again the next day. Because the female did not feed, her defecation rate decreased and the excreta collected 6 h 25 min and 9 h 5 min after the injection were completely white and did not contain a visible fecal fraction. Thus, gut motility may have shut down for a couple of hours and the respective excreta consisted of the renal fraction only. The retention of corticosteroid metabolites due to disrupted feeding behavior may indicate that corticosteroids are excreted mainly through the gut rather than through the kidneys in Stonechats. Excreted corticosteroid metabolite levels peaked between 1 h 20 min and 2 h 40 min after the combined effects of administration of ACTH and handling of birds during the injection. Taken together, these results indicate that excreted corticosteroid metabolites usually integrate plasma hormone levels of 1–3 h in stonechats and thus parallel the time course of androgen metabolite excretion in this species (Goymann et al. 2002). However, when feeding behavior was disrupted, as in the case of the nonfeeding female, that pattern may change. Then, gut motility may be reduced or shut down and corticosteroid metabolites may be retained in the gut for a longer period of time. To our knowledge, the Rufous Hummingbird (*Selasphorus rufus*) is the only other small, although nonpasserine bird species for which comparative excretion data are available. Corticosteroid metabolite concentrations in cloacal fluid of Rufous Hummingbirds represent pooled plasma levels of those hormones of 30 min (Hiebert et al. 2000a).

It is currently unclear whether measuring corticosteroid metabolites in excreta decreases or increases the variability of corticosteroid measurements. On the one hand, variability of circulating corticosteroid levels may be muted in excreta due to the integrative nature of the measurement, that is, the pooling effect of corticosteroid secretions (1–3 h in the case of stonechats). On the other hand, the variability may increase in excreta, because differences in metabolic rate and gut passage time changes in relative proportion to which the renal and intestinal pathway contribute to an actual sample, or individual differences in corticosterone metabolism may introduce additional variance. However, the noninvasive nature of this method makes it relatively easy to collect multiple samples from one individual and thus, a larger sample size may compensate for a potentially higher variability.

Enzymatic breakdown may cause changes in corticosteroid metabolite levels in excreta after defecation. There are few studies in mammals that show that the first 6–24 h may be (Wasser et al. 1988) or may not be (Goymann 2000) critical regarding changes in fecal steroid concentrations. To avoid that potential for variability in field studies, samples should be collected immediately after the bird has left the perch, kept cool (e.g. in a thermos with ice), and then stored in a freezer within a couple of hours.

In summary, the corticosterone EIA presented here is able to detect corticosteroid metabolites in excreta of European Stonechats and quantitatively measures the levels of those hormones. The same corticosterone EIA also reflected corticosteroid metabolite levels in two nonpasserine bird species, the Greylag Goose (*Anser anser*; Kotrschal et al. 1998) and the Domestic Goose (*Anser domesticus*; Kotrschal et al. 2000). It is thus likely that the corticosterone EIA presented in this study has the potential to detect corticosteroid metabolite levels in a number of other passerine and nonpasserine bird species. Further work is needed to investigate, for example, the effect of different food types, feeding rates, and metabolic rate on excreted corticosteroid levels.

*Acknowledgments.*—We thank S. Kuchar-Schulz for assistance with the HPLC analysis and C. Vleck and

an anonymous referee for helping to improve earlier versions of the manuscript. The experiments described in this study were approved by the government of Upper Bavaria, Germany (Aktenzeichen 211-2531.2-20/2000).

## Literature Cited

ASTHEIMER, L. B., W. A. BUTTEMER, AND J. C. WINGFIELD. 1994. Gender and seasonal differences in the adrenocortical response to ACTH challenge in an Arctic passerine, *Zonotrichia leucophrys gambelii*. General and Comparative Endocrinology 94:33–43.

CONOVER, W. J. 1980. Practical Non-Parametric Statistics. Wiley and Sons, New York.

GOLDIN, B. R., H. ADLERCREUTZ, S. L. GORBACH, J. H. WARRAM, J. T. DWYER, L. SWENSON, AND M. N. WOODS. 1981. Effect of diet on excretion of estrogen in pre- and post-menopausal women. Cancer Research 41:3771–3773.

GOYMANN, W. 2000. Hormone physiology and life history of spotted hyaenas, *Crocuta crocuta*. Ph.D. dissertation, University of Munich, Munich, Germany.

GOYMANN, W., E. MÖSTL, T. J. VAN'T HOF, M. L. EAST, AND H. HOFER. 1999. Noninvasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta*. General and Comparative Endocrinology 114:340–348.

GOYMANN, W., M. L. EAST, B. WACHTER, O. P. HÖNER, E. MÖSTL, T. J. VAN'T HOF, AND H. HOFER. 2001. Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas. Proceedings of the Royal Society London, Series B 268: 2453–2459.

GOYMANN, W., E. MÖSTL, AND E. GWINNER. 2002. Non-invasive methods to measure androgen metabolites in excrements of European Stonechats, *Saxicola torquata rubicola* General and Comparative Endocrinology 129:80–87.

GWINNER, E., S. KOENIG, AND C. HALEY. 1995. Genetic and environmental factors influencing clutch size in equatorial and temperature zone stonechats (*Saxicola torquata axillaris* and *S.t. rubicola*): An experimental study. Auk 112:748–755.

HIEBERT, S. M., M. RAMENOFSKY, K. SALVANTE, J. C. WINGFIELD, AND C. L. GASS. 2000a. Noninvasive methods for measuring and manipulating corticosterone in hummingbirds. General and Comparative Endocrinology 120:235–247.

HIEBERT, S. M., K. G. SALVANTE, M. RAMENOFSKY, AND J. C. WINGFIELD. 2000b. Corticosterone and nocturnal torpor in the Rufous Hummingbird (*Selasphorus rufus*). General and Comparative Endocrinology 120:220–234.

KOTRSCHAL, K., J. DITTAMI, K. HIRSCHENHAUSER, E. MÖSTL, AND P. PECZELY. 2000. Effects of physiological and social challenges in different seasons on fecal testosterone and corticosterone in male Domestic Geese (*Anser domesticus*). Acta Ethologica 2:115–122.

KOTRSCHAL, K., K. HIRSCHENHAUSER, AND E. MÖSTL. 1998. The relationship between social stress and dominance is seasonal in Greylag Geese. Animal Behaviour 55:171–176.

MOORE, I. T., M. J. GREENE, AND R. T. MASON. 2001. Environmental and seasonal adaptations of the adrenocortical and gonadal responses to capture stress in two populations of the male garter snake, *Thamnophis sirtalis*. Journal of Experimental Zoology 289:99–108.

PALME, R., AND E. MÖSTL. 1997. Measurement of cortisol metabolites in feces of sheep as a parameter of cortisol concentration in blood. Zeitschrift für Säugetierkunde 62(Supplement 2):192–197.

ROMERO, L. M., K. K. SOMA, AND J. C. WINGFIELD. 1998a. The hypothalamus and adrenals regulate modulation of corticosterone release in redpolls (*Carduelis flammea*), an Arctic breeding songbird. General and Comparative Endocrinology 109: 347–355.

ROMERO, L. M., K. K. SOMA, AND J. C. WINGFIELD. 1998b. Hypothalamic-pituitary-adrenal axis changes allow seasonal modulation of corticosterone in a bird. American Journal of Physiology 274:R1338–R1344.

SILVERIN, B., AND J. C. WINGFIELD. 1998. Adrenocortical responses to stress in breeding flycatchers, *Ficedula hypoleuca*: Relation to latitude, sex, and mating status. Journal of Avian Biology 29:228–234.

SIMS, C. G., AND R. L. HOLBERTON. 2000. Development of the corticosterone stress response in young Northern Mockingbirds (*Mimus polyglottos*). General and Comparative Endocrinology 119:193–201.

WASSER, S., L. RISLER, AND R. A. STEINER. 1988. Excreted steroids in primate feces over the menstrual cycle and pregnancy. Biology of Reproduction 39:862–872.

WASSER, S. K., R. THOMAS, P. P. NAIR, C. GUIDRY, J. SOUTHERS, J. LUCAS, D. E. WILDT, AND S. L. MONFORT. 1993. Effects of dietary fibre on faecal steroid measurements in baboons (*Papio cynocephalus cynocephalus*). Journal of Reproduction and Fertility 97:569–574.

WASSER, S. K., K. BEVIS, G. KING, AND E. HANSON. 1997. Noninvasive physiological measures of disturbance in the Northern Spotted Owl. Conservation Biology 11:1019–1022.

WILSON, C. G., AND R. L. HOLBERTON. 2001. An alternative method for delivering adrenocorticotropin hormone in birds. General and Comparative Endocrinology 122:349–353.

Wingfield, J. C. 1994. Modulation of the adrenocortical response to stress in birds. Pages 520–528 *in* Perspectives in Comparative Endocrinology (K. G. Davey, R. E. Peter, and S. S. Tobe, Eds.). National Research Council Canada, Ottawa, Ontario.

Wingfield, J. C., K. Hunt, C. W. Breuner, K. Dunlap, G. S. Fowler, L. Freed, and J. Lepson. 1997. Environmental stress, field endocrinology, and conservation biology. Pages 95–131 *in* Behavioral Approaches to Conservation in the Wild (J. R. Clemmons and R. Buchholz, Eds.). Cambridge University Press, Cambridge, United Kingdom.

Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, and R. D. Richardson. 1998. Ecological bases of hormone–behavior interactions: The emergency life-history stage. American Zoologist 38:191–206.

Wingfield, J. C., K. M. O'reilly, and L. B. Astheimer. 1995. Modulation of the adrenocortical responses to acute stress in Arctic birds: A possible ecological basis. American Zoologist 35:285–294.

Wingfield, J. C., C. M. Vleck, and M. C. Moore. 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran Desert. Journal of Experimental Zoology 264:419–428.

*Received 7 December 2001, accepted 31 July 2002.*
*Associate Editor: W. H. Karasov*

---

*The Auk* 119(4):1173–1179, 2002

## Daily Energy Expenditure of Ovenbirds (*Seiurus aurocapillus*) Feeding Nestlings

W. Matthew Vander Haegen[1] and Richard M. DeGraaf
*U.S. Department of Agriculture Forest Service, Northeastern Research Station, University of Massachusetts, Amherst, Massachusetts 01003, USA*

Abstract.—The doubly labeled water method was used to measure daily energy expenditure of adult Ovenbirds (*Seiurus aurocapillus*) feeding nestlings in large and small forests in northern New England. Carbon dioxide production for all birds averaged 7.67 ± 1.29 mL $g^{-1}$ $hr^{-1}$ ($n$ = 27), equivalent to 90.1 ± 15.0 kJ $day^{-1}$. Carbon dioxide production did not differ between males and females within the same pair measured over the same period. Ratio of daily energy expenditure to basal metabolic rate (BMR) for Ovenbirds (3.4) was closer to the mean value derived for aerial foragers (3.8) than that for ground foragers (2.3) in a sample of passerines feeding nestlings. High daily energy expenditure for Ovenbirds may be related to their relatively brief breeding season in northern climates and their use of cool, closed-canopy forests. Although daily energy expenditure for Ovenbirds was high when compared to other ground-foraging passerines, most individuals were not working near the hypothetical maximum of 4× BMR (Drent and Daan 1980). Daily energy expenditure for Ovenbirds feeding young was greater in large forests than small forests, although the difference was not statistically significant. We speculate that time used for foraging (and hence energy expenditure) may be lower in small forests as a result of increased prey density, or alternatively, greater risk of nest predation in forest fragments leads to greater vigilance at the nest site and less time available for foraging. Further studies of avian energetics in large and small forests, including detailed time–activity budgets, may reveal hidden costs of forest fragmentation.

Resumen.—Utilizamos el método de agua isotópica, para medir el gasto de energía diario de individuos adultos de la especie *Seiurus aurocapillus* que se encuentran alimentando a sus polluelos en bosques grandes y pequeños en el norte de Nueva Inglaterra. La producción de $CO_2$ para todas las aves promedió 7.67 ± 1.29 mL $g^{-1}$ $hr^{-1}$ ($n$ = 27), equivalente a 90.1 ± 15.0 kJ $día^{-1}$. La producción de $CO_2$ medida durante un mismo período no difirió entre machos y hembras pertenecientes a una misma pareja. La razón entre el gasto energético diario y la tasa metabólica basal para *Seiurus aurocapillus* (3.4) fue más similar al valor obtenido para aves que forrajean durante el vuelo (3.8) que para las que forrajean en el suelo (2.3) de una muestra de paserinos que alimentan a sus polluelos. El alto gasto energético diario para *Seiurus aurocapillus* puede relacionarse con el período de cría relativamente breve que

[1] Present address: Washington Department of Fish and Wildlife, Wildlife Program, Science Division, 600 Capitol Way North, Olympia, Washington 98501, USA. E-mail: vandemwv@dfw.wa.gov

ellos presentan en los climas norteños y con el uso de bosques frescos de dosel cerrado. Aunque el gasto energético diario para *Seiurus aurocapillus* fue alto al ser comparado con otros paserinos que forrajean en el suelo, la mayoría de los individuos no alcanzaron el valor máximo hipotético de 4 veces la tasa metabólica basal (Drent y Daan 1980). El gasto energético diario para individuos de *Seiurus aurocapillus* que se encuentran alimentando a juveniles fue mayor en los bosques de mayor tamaño que en los bosques pequeños, sin embargo, la diferencia no fue estadísticamente significantiva. Especulamos que el tiempo usado para forrajear (y el gasto de energía) puede ser menor en los bosques pequeños como resultado del aumento de la densidad de presas, o alternativamente, que el mayor riesgo de depredación de nidos en fragmentos de bosque lleva a una mayor vigilancia en el sitio del nido lo que puede resultar en un menor tiempo disponible para forrajear. Estudios adicionales sobre la energética de aves en fragmentos de bosque grandes y pequeños que incluyan el análisis detallado del presupuesto de tiempo de actividades, pueden revelar costos no conocidos de los efectos de la fragmentación del bosque.

Accumulation and use of energy is a fundamental cycle in nature. For some species of birds, energy use may be greatest during the breeding season when gametes are produced and nestlings and fledglings must be provided with food (Masman et al. 1988, 1989; Weathers and Sullivan 1993). It has been hypothesized that adults feeding young may be working maximally, approaching a threshold where additional energy costs could reduce productivity (Drent and Daan 1980). Although field studies suggest that some passerines do not attain such high levels of energy expenditure (Weathers and Sullivan 1993, Weathers et al. 1999), the number of species with quantitative measurements is low. Moreover, there is evidence that both taxonomy (Walsberg 1983) and life-history traits such as foraging method (Weathers and Sullivan 1989) influence energy expenditure, making broad generalizations unwise.

Daily energy expenditure (DEE) has been measured for numerous species using the doubly labeled water (DLW) method. That technique uses turnover rates of isotopes introduced into body water to calculate production of $CO_2$ and, in turn, energy expenditure (Nagy 1983). Much of that research has focused on seabirds and aerial-foraging passerines, with little work on forest passerines (Weathers and Sullivan 1989). To our knowledge, DEE has not been measured using DLW for any species of Parulidae, for example.

In this article, DEE measurements are presented for the Ovenbird (*Seiurus aurocapillus*), a ground-nesting forest warbler that gleans invertebrate foods from the forest floor. We tested for sexual differences were tested for in DEE between members of the same pair feeding advanced nestlings, and we examined relationships between environmental variables and DEE. Last, DEE was compared for this forest interior specialist in forest stands of different sizes. Fragmentation of previously extensive forests can lead to reduced reproductive success of forest interior passerines (Robinson et al. 1995, Donovan et al. 1997), although little is known about energetics of birds using large versus small forests.

*Methods.*—Daily energy expenditure of Ovenbirds was measured on two study plots in White Mountain National Forest in northern New Hampshire (1991) and on eight plots in industrial timberlands of north-central Maine (1992–1993). Two New Hampshire plots were 12 km apart; eight Maine plots were scattered over a 38 $km^2$ study area. All sites were mature, hardwood or mixed hardwood and softwood forests with upper canopies dominated by species such as maple (*Acer saccharum*, *A. rubrum*), American beech (*Fagus grandifolia*), paper birch (*Betula papyrifera*), white pine (*Pinus strobus*), balsam fir (*Abies balsamea*), and spruce (*Picea rubens*, *P. mariana*). Four of the Maine plots were within large blocks (>1,000 ha) of continuous forest; the remaining four were within smaller, mature forest fragments in a matrix of recently harvested and regenerating stands. Several stands adjacent to those fragments had been harvested by clear-cut logging one to two years prior to this study. Tree-species composition and structure were similar between large and small plots in Maine (Hagan et al. 1996). During May, June, and July of each year we searched for nests on study plots and collected data on energy expenditure of Ovenbirds.

The DLW method was used to measure DEE of adult Ovenbirds feeding nestlings. We used single-sample variation of that method (Webster and Weathers 1989) to minimize handling time. Nests were located by systematically walking each study area searching for nests and by flushing incubating or brooding females. Nests were visited periodically until eggs hatched, and every two to three days thereafter. When nestlings were six to eight days old, mist nets were set 5 to 8 m from the nest, and adults were captured as they flew to or from the nest while feeding nestlings. Captured adults were weighed to the nearest 0.1 g and banded with numbered metal federal bands and unique combinations of color bands, then injected with 64 μL of distilled water containing 3.0 $\mu L^{-1}g$ 97 atom-percent $^{18}O$ and 0.09 $\mu L^{-1}g$ deuterium into the pectoral muscle. Birds were then immediately released to resume care of young. Adults were recaptured 24–48 h later, reweighed, and 50 μL of blood was drawn from a brachial vein into each of two 70 μL heparinized glass capillary tubes. Tubes were flame-sealed and kept refrigerated until analysis. All birds were released at their nest site. Five nests were monitered from a distance of >20 m, and adults that had been injected were observed returning within 60 min. Of all birds

trapped at the nest and injected with isotope ($n$ = 36), four never were resighted, whereas five were seen visiting the nest but not recaptured.

Initial isotope levels were derived from a control group of male Ovenbirds captured away from the main study areas, injected with isotope mixture, and bled 1 h later ($n$ = 10 in 1991, 7 in 1992, and 8 in 1993). Measures were derived for total body water in 1991 from a sample of 10 Ovenbirds collected and dried to constant weight. Total body water in 1992 and 1993 was derived from $^{18}O$ dilution in the sample of birds used to obtain initial isotope levels. Background isotope levels were obtained from individuals captured off the main study areas ($n$ = 2 per year). Blood samples were analyzed for isotope levels at the University of California, Los Angeles, by K. Nagy.

Daily energy expenditure was derived from measurements of $CO_2$ production and body weight using a conversion (26.2 kJ $L^{-1}$ $CO_2$) developed for insectivorous birds (Weathers and Sullivan 1989). Aschoff and Pohl's (1970) allometric equation for resting-phase basal metabolic rate (BMR) was used to derive ratios of DEE to BMR.

Microclimate variables were measured within the forest understory of both study plots in 1992 and in three of eight study plots in 1992 and 1993. Air temperature ($T_a$) was measured using shielded thermocouples placed 1 m above the ground. Wind speed was measured (1992 and 1993 only) 1 m above the ground using low-threshold cup anemometers (0.27 m $s^{-1}$ stall speed). Data were collected every 60 s and averaged each hour by CR-10 dataloggers (Campbell Scientific, Logan, Utah). Measurement of energy expenditure for each bird was matched to microclimate data (mean $T_a$, minimum $T_a$, mean wind speed) from the nearest plot. In 1992, four of five nests were in plots with microclimate measuring stations; the fifth nest was in a plot 7 km from the nearest microclimate station. In 1993, 5 of 12 nests were in plots with microclimate stations; the remaining 7 nests were in two plots that were 8.5 and 12 km from the nearest microclimate station.

*T*-tests were used to examine differences in mass between male and female Ovenbirds and to examine differences in DEE of Ovenbirds using large and small forests in Maine. Analysis of variance (ANOVA) was used to test for differences in DEE among adults feeding broods of different sizes. In the above comparisons, only data from one adult was used from each nest to avoid inflating sample sizes through pseudoreplication (females were used for all but one nest). A paired *t*-test was used to compare energy expenditure of males versus females measured simultaneously within the same pair. Pearson correlation was used to examine relationships between microclimate variables and energy expenditure. Means are reported ±SD.

*Results.*—We measured daily energy expenditure for 27 Ovenbirds feeding advanced nestlings ranging from six to nine days old (10 in 1991, 5 in 1992, and 12 in 1993). Dates of measurement ranged from 15 May to 20 July. Mass of males (18.7 ± 0.96 g, $n$ = 12) and females (18.7 ± 1.03 g, $n$ = 15) did not differ ($t$ = 0.049, df = 25, $P$ = 0.96). Most birds maintained weight between injection of the isotopes and recapture to draw blood; percentage change in mass over the measurement period averaged 0.5 ± 2.35 (range 5.23 to −4.59, $n$ = 27). Recapture intervals ranged from 1.0 to 2.14 days. Mean deviation from 24 ($n$ = 24) or 48 ($n$ = 3) h recapture interval was 0.9 ± 1.1 h (range 0 to 3.36, $n$ = 27).

TABLE 1. Carbon dioxide production (milliliters per gram per hour) for adult Ovenbirds feeding broods containing different numbers of advanced nestlings, northern New England, 1991–1993.

| Number of chicks | Number of adults | Mean | SD |
|---|---|---|---|
| 3 | 6 | 7.26 | 0.85 |
| 4 | 8 | 7.34 | 1.40 |
| 5 | 4 | 7.51 | 0.21 |

Carbon dioxide production for all birds averaged 7.67 ± 1.29 mL $g^{-1}$ $h^{-1}$ ($n$ = 27), equivalent to 90.1 ± 15.0 kJ $day^{-1}$. Carbon dioxide production did not differ between males (7.65 ± 1.58) and females (7.24 ± 1.07) within the same pair measured over the same period ($t$ = 1.45, df = 8, $P$ = 0.164). Power of this test was sufficient to detect a difference of 1.26 mL $CO_2$ $g^{-1}$ $h^{-1}$ or 16% of the combined mean ($\alpha$ = 0.05, power = 0.80). Carbon dioxide production of adults was not significantly different among those with three, four or five nestlings ($F$ = 0.06, df = 2 and 15, $P$ = 0.94; Table 1). Power of this test was sufficient to detect a difference of 1.8 mL $CO_2$ $g^{-1}$ $h^{-1}$ or 24% of the combined mean ($\alpha$ = 0.05, power = 0.80). Ratio of DEE to BMR ranged from 2.15 to 5.21 and averaged 3.37 ± 0.56 ($n$ = 27). Ratio of DEE to BMR averaged 3.59 ± 0.59 ($n$ = 12) for males and 3.20 ± 0.48 ($n$ = 15) for females.

Mean daily air temperature at three sites where microclimate was measured in 1992 and 1993 generally varied by <0.5°C among sites each year (Fig. 1). In 1992, mean air temperature averaged 15.5 ± 0.1°C during daylight hours (0500–2000 hours) and 12.8 ± 0.4°C at night; in 1993, mean air temperature averaged 18.4 ± 1.1°C during daylight hours (0500–2000 hours) and 15.1 ± 0.7°C at night. Average wind speed among sites was more variable than air temperature, but followed a similar pattern among sites (Fig. 1). Differences in mean air temperature among sites on a given day ranged from 0 to 1.7°C; differences in mean wind speed ranged from 0 to 0.35 m $s^{-1}$. Carbon dioxide production of Ovenbirds was not correlated with mean air temperature ($r$ = −0.01, $n$ = 27, $P$ = 0.96), minimum air temperature ($r$ = 0.12, $n$ = 27, $P$ = 0.58), mean wind speed ($r$ =

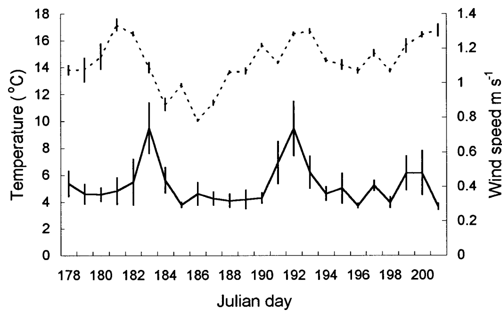


FIG. 1. Daily mean air temperature (dashed line; mean ± 1 SD) and wind speed (solid line) at three study plots where DEE was measured for Ovenbirds feeding nestlings in northern Maine, 1992.

$-0.23$, $n = 17$, $P = 0.38$), or Julian day ($r = -0.29$, $n = 27$, $P = 0.14$).

Within the Maine study plots, data on DEE was obtained from adults at one nest in each of four small forests, and from adults at two nests in each of three large forests and from a single nest in a fourth, large forest. Brood size was similar between nests in large (3.7 ± 0.76 chicks, $n = 7$) and small (4.0 ± 0.82, $n = 4$) forests ($t = 0.59$, df = 9, $P = 0.57$). Carbon dioxide production for Ovenbirds feeding young was greater in large forests (7.48 ± 1.12 mL $g^{-1}$ $h^{-1}$, $n = 7$) than small forests (6.36 ± 0.61 mL $g^{-1}$ $h^{-1}$, $n = 4$) ($t = 1.6$, df = 9, $P = 0.14$), although the difference was not statistically significant.

*Discussion.*—Ovenbirds feeding advanced nestlings had high DEE relative to other ground-foraging passerines that have been studied. The ratio of DEE to BMR for Ovenbirds (3.4) was closer to the mean value derived for aerial foragers (3.8) than that for ground-foragers (2.3) in a sample of passerines feeding nestlings (Weathers and Sullivan 1989). Similar studies of ground-foraging passerines reported ratios of 2.7 for Savannah Sparrows (*Passerculus sandwichensis*) feeding nestlings in a California salt marsh (Williams and Nagy 1985) and 2.1 for Yellow-eyed Juncos (*Junco phaeonotus*) feeding nestlings in an Arizona pine forest (Weathers and Sullivan 1989). The ratio for Ovenbirds was closest to that reported for the Northern Wheatear (*Oenanthe oenanthe*; 3.0), a ground-foraging passerine of open moorland and scree habitats (Tatner 1990). Daily energy expenditure for Ovenbirds was essentially identical to the value predicted from a published (Masman et al. 1989) allometric equation for passerines feeding young (90.5 kJ $day^{-1}$); an equation based largely on aerial-foraging species.

Ratios of DEE to BMR are sensitive to measurements of BMR (Weathers and Sullivan 1989) and this study's value for Ovenbirds was derived from an allometric equation based on body weight; however, increasing derived value for BMR in this study by 10% still resulted in a ratio $>3.0$. Moreover, use of a more recently derived allometric equation for BMR that corrects for phylogeny (Reynolds and Lee 1996) resulted in a mean change of only 1.4% in this study's ratios of BMR to DEE. Although DEE for Ovenbirds was high when compared to other ground-foraging passerines, most individuals measured in northern New England were not working near the hypothetical maximum of 4× BMR (Drent and Daan 1980). Kirkwood (1983) developed an allometric equation for maximum daily metabolizable energy intake, a likely upper threshold for DEE. When calculated using this study's mean mass for Ovenbirds, the value from this allometric equation results in a maximum DEE to BMR ratio of 3.7, similar to the hypothetical maximum and greater than the measured value for most Ovenbirds.

No comparative data on DEE are available for other ground-foraging warblers. However, DEE of Black-throated Blue Warblers (*Dendroica caerulescens*; two male, two female) measured while feeding advanced nestlings was 71.5 ± 8.6 kJ $day^{-1}$ (W. M. Vander Haegen and R. M. DeGraaf unpubl. data) or 4.5 ± 0.50× the measured value for BMR (Holmes et al. 1979). Black-throated Blue Warblers are half the mass of Ovenbirds and forage primarily by gleaning insects and fly catching within the shrub and tree canopies (Black 1975). These small warblers likely use flight in their foraging strategy more than Ovenbirds, contributing to greater DEE. Ratio of DEE to BMR for Black-throated Blue Warblers was greater than that measured for some true aerial foragers such as Tree Swallows (*Tachycineta bicolor*, 4.0; Williams 1988) and Pied Flycatchers (*Ficedula hypoleuca*, 4.2; Moreno et al. 1995). High energy needs may be related to the short breeding season that these Neotropical migrants face upon arrival on northern breeding grounds and the fact that both species inhabit mature, closed-canopy forests that present cool environments. Mean daytime and night air temperature on our study sites were below both the estimated lower critical temperature for Ovenbirds (23.7°C; allometric equation of Kendeigh et al. 1977) and the measured lower critical temprature (24°C) for the Black-throated Blue Warbler (Black 1975); moreover, thermal benefits of direct solar radiation likely are limited in these closed-canopy forests. Previous studies of ground-foraging passerines have focused on populations of short-distance migrants and those using more open habitats (e.g. Savannah Sparrows and Yellow-eyed Juncos); in contrast, the Wheatear, with a DEE and BMR ratio close to that of the Ovenbird, is an Arctic nester and long-distance migrant. Comparative studies that use resident species and short-distance migrants that breed in closed-canopy forests, and Neotropical migrants that inhabit more open habitats, may help explain patterns of energy use.

No difference was found in DEE between male and female Ovenbirds, a result similar to that found for other species that share parental duties (Williams 1988, Weathers and Sullivan 1989, Tatner 1990). Although activity budgets were not quantified for adult Ovenbirds, our limited observations concur with previously reported findings that both members of the pair spend equal time feeding advanced nestlings (Van Horn and Donovan 1994). Similarly, although sample sizes were low no difference in DEE was found for females feeding different numbers of chicks. Several other studies reported no difference in DEE among females feeding natural broods of different sizes (Ricklefs and Williams 1984, Williams and Nagy 1985, Tatner 1990). However, Sanz et al. (1998) found that DEE of female Great Tits (*Parus major*) was positively associated with both brood size and proportion of the day spent active; apparently, females with larger broods worked longer days. Studies where workloads were increased by artificially increasing brood size have documented increased DEE of adults (Moreno et al. 1995, Sanz and Tinbergen 1999).

Daily energy expenditure of Ovenbirds feeding nestlings was predicted to be greater in forest fragments than in large forests on the basis of the following plausible relationships: (1) increased thermoregulatory costs due to wind infiltration from the forest edge (Webster and Weathers 1988); (2) increased foraging time resulting from lower availability of invertebrate prey on the forest floor (Gibbs and Faaborg 1990, Burke and Nol 1998); (3) increased competition and need for territorial defense caused by greater density of conspecifics (Hagan et al. 1996); and (4) increased biotic interaction with forest-edge species (Freemark and Collins 1992). Although low sample size prevented us from adequately testing our prediction, there was a trend towards greater DEE for Ovenbirds using continuous forest stands that ran counter to expectation.

Why would Ovenbirds raising broods in small forests experience lower DEE than those raising broods in large forests? We offer two hypotheses that might warrant investigation. First, invertebrate foods may be more abundant in small forests, reducing the time needed for foraging. Although forest fragments in agricultural landscapes have fewer litter-inhabiting invertebrates compared to extensive forest (Burke and Nol 1998), recently fragmented forest in commercial timberlands act as refugia for some mature forest species (Hagan et al. 1996) and may harbor increased densities of invertebrate prey. If this phenomenon occurs, it may be limited to forest fragments adjacent to young clearcuts and likely would be short-lived, as desiccation of the leaf litter or other changes related to fragmentation alter the forest floor environment (Lovejoy et al. 1986). Alternatively, lower DEE in forest fragments may be related to relative numbers of nest predators and risk of nest predation. Overall, Ovenbird pairs on this study site appeared to have lower reproductive success in forest fragments than in continuous forests (Hagan et al. 1996), a pattern also reported for Ovenbirds in Missouri (Porneluzi and Faaborg 1999) and possibly linked to a greater density of red squirrels (*Tamiasciurus hudsonicus*) in forest fragments (Hagan et al. 1996). Indeed, sample size for DEE measurements in this study was limited by early predation of nests, particularly in small forests. Greater risk of nest predation in forest fragments might stimulate greater vigilance at the nest site (Martin 1992, Schmidt 1999), reducing time spent in other perhaps more energy-expensive activities such as foraging. Thus, fitness consequences of a given level of activity may differ for Ovenbirds nesting in forests of different sizes (e.g. Ovenbirds in large forests could forage longer before incurring the same risk of nest predation; Weathers et al. 1999). Birds that can forage longer and still maintain body mass may have an advantage during the brief breeding season characteristic of Neotropical migrants. These hypotheses are not mutually exclusive, because greater food availability likely would increase foraging efficiency, allowing more time for nest defense. Further studies of avian energetics in large and small forests, including detailed time–activity budgets, may reveal hidden costs of forest fragmentation.

*Acknowledgments.*—We thank Bowater Inc. and S. D. Warren Co. for access to their lands in Maine and managers of the Bartlett Experimental Forest for providing housing in New Hampshire. We also thank W. Weathers for advice on the use of DLW, A. Hoffman for statistical advice, and K. Nagy for analyzing blood samples. We acknowledge the many field biologists who helped in our studies of northern forest passerines, including R. Brown, K. Cameron, F. Currie, J. Dodge, S. Dooling, S. Gende, S. Plentovich, E. Schauber, S. Shulze, S. Shuman, and S. Strzalkowska. We appreciate the collaboration of J. Hagan and P. McKinley from Manomet Center for Conservation Sciences. C. Dykstra, R. Holmes, and D. King provided helpful reviews of a previous draft of the manuscript. Use of trade names does not constituted endorsement by the U.S. Government.

## Literature Cited

Aschoff, J., and H. Pohl. 1970. Der Ruheumsatz von Vogelen als Funktion der Tageszeit und der Korpergross. Journal für Ornithologie 111:38–47.

Black, C. P. 1975. The ecology and bioenergetics of the northern Black-throated Blue Warbler (*Dendroica caerulescens caerulescens*). Ph.D. dissertation, Dartmouth College, Hanover, New Hampshire.

Burke, D. M., and E. Nol. 1998. Influence of food abundance, nest-site habitat, and forest fragmentation on breeding Ovenbirds. Auk 115:96–104.

Donovan, T. M., P. W. Jones, E. M. Annand, and F. R. Thompson III. 1997. Variation in local-scale edge effects: Mechanisms and landscape context. Ecology 78:2064–2075.

Drent, R. H., and S. Daan. 1980. The prudent parent: Energetic adjustments in avian breeding. Ardea 68:225–252.

Freemark, K., and B. Collins. 1992. Landscape ecology of birds breeding in temperate forest fragments. Pages 443–454 *in* Ecology and Conservation of Neotropical Migrant Landbirds. (J. M. Hagan III and D. W. Johnson, Eds.). Smithsonian Institution Press, Washington, D.C.

Gibbs, J. P., and J. Faaborg. 1990. Estimating the viability of Ovenbird and Kentucky Warbler populations in forest fragments. Conservation Biology 4:193–196.

Hagan, J. M., W. M. Vander Haegen, and P. S. McKinley. 1996. The early development of forest fragmentation effects on birds. Conservation Biology 10:188–202.

Holmes, R. T., C. P. Black, and T. W. Sherry. 1979. Comparative population bioenergetics of three insectivorous passerines in a deciduous forest. Condor 81:9–20.

Kendeigh, S. C., V. R. Dol'nik, and V. M. Gavrilov. 1977. Avian energetics. Pages 127–204 *in* Granivorous Birds in Ecosystems (J. Pinowski and S. C. Kendeigh, Eds.). Cambridge University Press, Cambridge, United Kingdom.

Kirkwood, J. K. 1983. A limit to metabolizable energy intake in mammals and birds. Comparative Biochemistry and Physiology A 75:1–3.

Lovejoy, T. E., R. O. Bierregaard, Jr., A. B. Rylands, J. R. Malcolm, C. E. Quintela, L. H. Harper, K. S. Brown, Jr., A. H. Powell, G. V. N. Powell, H. O. Schubart, and M. B. Hays. 1986. Edge and other effects of isolation on Amazon forest fragments. Pages 257–285 *in* Conservation Biology: The Science of Scarcity and Diversity. (M. E. Soule, Ed.). Sinauer Associates, Sunderland, Massachusetts.

Martin, T. E. 1992. Interaction of nest predation and food limitation in reproductive strategies. Current Ornithology 9:163–197.

Masman, D., S. Daan, and H. J. A. Beldhuis. 1988. Ecological energetics of the Kestrel: Daily energy expenditure throughout the year based on time–energy budget, food intake and doubly labeled water methods. Ardea 76:64–81.

Masman, D., C. Dijkstra, S. Daan, and A. Bult. 1989. Energetic limitation of avian parental effort: Field experiments in the Kestrel (*Falco tinnunculus*). Journal of Evolutionary Biology 2:435–455.

Moreno, J., R. J. Cowie, J. J. Sanz, and R. S. Williams. 1995. Differential response by males and females to brood manipulations in the Pied Flycatcher: Energy expenditure and nestling diet. Journal of Animal Ecology 64:721–732.

Nagy, K. A. 1983. The doubly labeled water ($^2H^{18}O$) method: A guide to its use. University of California at Los Angeles Publication, no. 12–1417.

Porneluzi, P. A., and J. Faaborg. 1999. Season-long fecundity, survival, and viability of Ovenbirds in fragmented and unfragmented landscapes. Conservation Biology 13:1151–1161.

Reynolds, P. S., and R. M. Lee III. 1996. Phylogenetic analysis of avian energetics: Passerines and nonpasserines do not differ. American Naturalist 147:735–759.

Ricklefs, R., and J. Williams. 1984. Daily energy expenditure and water turnover rate of adult European Starlings (*Sturnus vulgaris*) during the nesting cycle. Auk 101:707–716.

Robinson, S. K., F. R. Thompson III, T. M. Donovan, D. R. Whitehead, and J. Faaborg. 1995. Regional forest fragmentation and the nesting success of migratory birds. Science 267:1987–1990.

Sanz, J. J., and J. M. Tinbergen. 1999. Energy expenditure, nestling age, and brood size: An experimental study of parental behavior in the Great Tit *Parus major*. Behavioral Ecology 10:598–606.

Sanz, J. J., J. M. Tinbergen, M. Orell, and S. Rytkonen. 1998. Daily energy expenditure during brood rearing of Great Tits *Parus major* in northern Finland. Ardea 86:101–107.

Schmidt, K. A. 1999. Foraging theory as a conceptual framework for studying nest predation. Oikos 85:151–160.

Tatner, P. 1990. Energetic demands during brood rearing in the Wheatear *Oenanthe oenanthe*. Ibis 132:423–435.

Van Horn, M. A., and T. M. Donovan. 1994. Ovenbird (*Seiurus aurocapillus*). *In* The Birds of North America, no. 88 (A. Poole and F. Gill, Eds.). Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, D.C.

Walsberg, G. E. 1983. Avian ecological energetics. Pages 161–220 *in* Avian Biology, vol. 7 (D. S. Farner, J. R. King, and K. C. Parks, Eds.). Academic Press, New York.

Weathers, W. W., C. R. Olson, R. B. Siegel, C. L. Davidson, and T. R. Famula. 1999. Winter and breeding-season energetics of nonmigratory White-crowned Sparrows. Auk 116:842–847.

Weathers, W. W., and K. A. Sullivan. 1989. Juvenile foraging proficiency, parental effort, and avian reproductive success. Ecological Monographs 59:223–246.

Weathers, W. W., and K. A. Sullivan. 1993. Seasonal patterns of time and energy allocation by birds. Physiological Zoology 66:511–536.

Webster, M. D., and W. W. Weathers. 1988. Effect of wind and air temperature on metabolic rate in Verdins, *Auriparus flaviceps*. Physiological Zoology 61:543–554.

Webster, M. D., and W. W. Weathers. 1989. Validation of the single-sample doubly labeled wa-

ter method for measuring energy metabolism. American Journal of Physiology 256:R572–R576.

Williams, J. B. 1988. Field metabolism of Tree Swallows during the breeding season. Auk 105:706–714.

Williams, J. B., and K. A. Nagy. 1985. Daily energy expenditure by female Savannah Sparrows feeding nestlings. Auk 102:187–190.

*Received 29 July 2001, accepted 16 August 2002.*
*Associate Editor: W. H. Karasov*

---

*The Auk* 119(4):1179–1186, 2002

# A Geometric Method for Determining Shape of Bird Eggs

Douglas E. Baker[1]
*9 Old Orchard Road, Wilbraham, Massachusetts 01095, USA*

Abstract.—Precise quantification of the oval of a bird egg can provide a powerful tool for the analysis of egg shape for various biological problems. A new approach to the geometry of a bird egg oval is presented here using a simple algebraic equation to fit all normal bird egg shapes. Only two parameters are needed in the equation for complete shape description of an egg oval to quantify the equation's capacity for curve fitting all species and shapes of bird egg ovals. The equation was fitted to egg silhouettes from a sample of 250 different bird egg species containing one egg per species. Standard regression analysis was used to fit the equation to each egg profile. The 99% CI for the curve fit acceptance rate was calculated to determine the equation's statistical significance for all species of bird eggs. Compared to the power series multiequation models (Preston 1968, Todd and Smart 1984), the equation used here is the simplest analytic description of a bird egg oval.

Resumen.—La cuantificación exacta del óvalo de un huevo de ave puede proporcionar una herramienta poderosa para el análisis de la forma del huevo para varios problemas biológicos. Se presenta aquí un nuevo acercamiento a la geometría del óvalo del huevo de un ave, el cual usa una ecuación algebraica simple que se ajusta a todas las formas normales de huevos de aves. En la ecuación sólo dos parámetros son necesarios para la descripción de la forma completa del óvalo del huevo, y para cuantificar la capacidad de la ecuación de ajustar las curvas a todas las especies y formas de óvalo de huevos de ave. La ecuación fue ajustada a siluetas de una muestra de 250 huevos de diferentes especies, con un huevo por especie. Se utilizó un análisis de regresión estándar para ajustar la ecuación a cada perfil de huevo. Se calculó el intervalo de confianza del 99% para la aceptación del ajuste de la curva, para determinar la significancia estadística de la ecuación para todas las especies de huevos de ave. Comparada con los modelos de múltiples ecuaciones de series de potencia (Preston 1968, Todd y Escoce 1984), la ecuación usada aquí es la descripción analítica más simple de un óvalo de huevo de ave. Esta ecuación podría ser útil para la aplicación del análisis de la forma del huevo en varios problemas biológicos.

Past approaches to bird-egg geometry involved curve fitting power series to actual egg outlines (Preston 1968, Todd and Smart 1984). Those methods lacked a single closed form equation because they required truncations at different points for different species. Ideally, a single closed form equation should fit all eight normal bird egg shapes (Walters 1994). The single algebraic equation described here achieves that ideal with sufficient accuracy and statistical significance for fitting oval profiles of all bird egg species.

*Methods.*—The algebraic equation that generates egg contours is based on a fundamental transformation from projective geometry. Those contours are called ''path curves'' and were developed by Edwards (1993).

Geometric sequences of points are plotted on lines ''a'' and ''b'' perpendicular to the oval axis PQ (Fig. 1A). Those sequences of points are joined by segments to the poles P and Q to form a field of quadrilaterals. Intersections of those two sets of line segments are points on path curve ovals. Only one of those ovals is shown in Figure 1A.

Embedding the oval in $x$,$y$ coordinates (Fig. 1B) enables the analytic path curve equation to be derived. (All egg profiles are scaled to the unit circle for uniform shape comparisons.) First, however, a system of equations in $x$ and $y$ variables must be developed in terms of a third variable, $t$, called a parameter. This parameter steps an intersection point on a path curve oval (Fig. 1A) to the next diagonal intersection

[1] E-mail: douglasebaker@earthlink.net

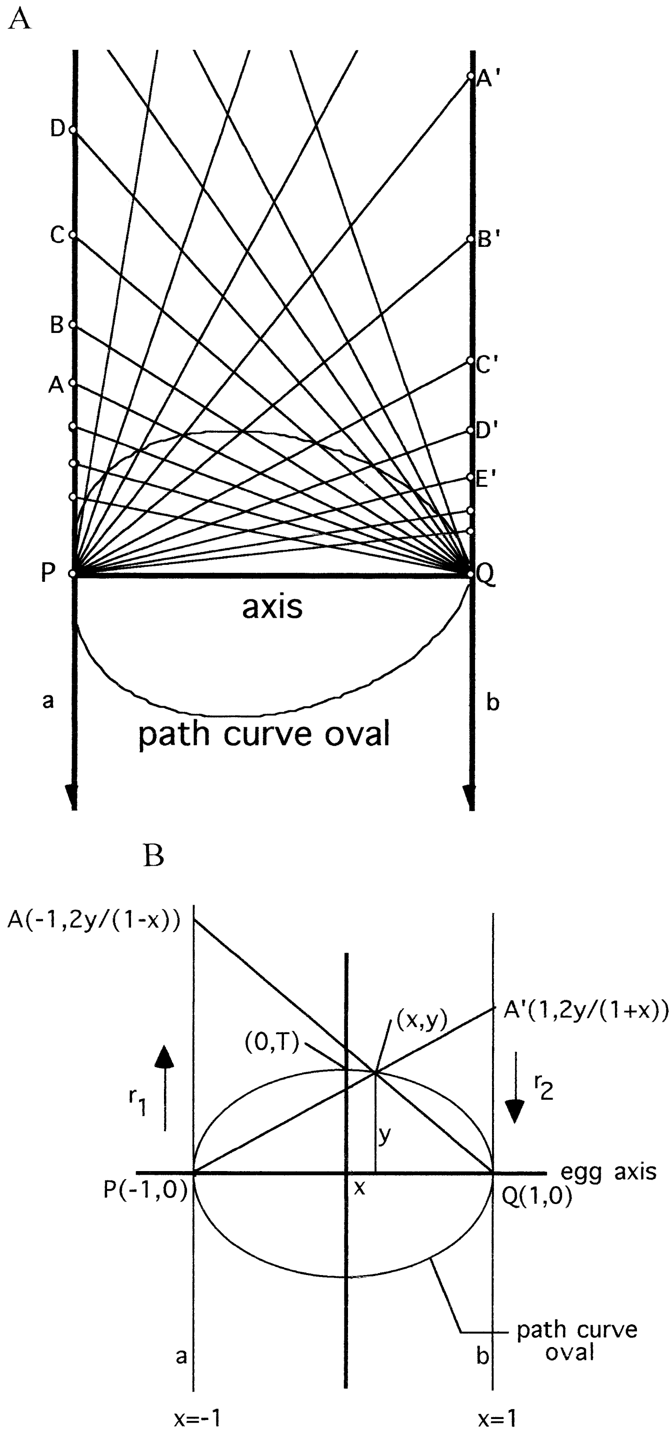


FIG. 1. (A) Construction of a path curve oval. PQ is the symmetry axis of the egg. Geometric sequences A,B,C,. . . and A′,B′,C′,. . . are constructed along lines "a" and "b", respectively. The common ratios of the geometric sequences are: $r_1$ = PB/PA = PC/PB = . . . ; $r_2$ = QB′/QA′ = QC′/QB′ = . . . . (B) Path curve oval scaled to a unit circle. AQ and A′P intersect at a point with coordinates $(x,y)$ on the path curve oval.

point in repeated succession to complete a half oval. Each equation represents the vertical coordinate of a point on one of the two geometric sequences on lines "a" and "b".

$$2y/(1 - x) = c r_1^{t} \quad \text{and}$$

$$2y/(1 + x) = d r_2^{-t} \qquad (1)$$

The first equation is for the sequence of points on line "a" and the second equation for the sequence on line "b" (Fig. 1B). The right side of each equation is the geometric sequence formula for the vertical coordinate of A and A′ respectively with $c$ and $d$ the initial points on each sequence when $t = 0$. The left sides of Equation (1) give the same vertical coordinates of A and A′ as before, but now expressed in $x,y$ coordinates of a point on the path curve oval. This is done using two pairs of similar triangles, one pair pointing to pole P and the other to pole Q.

Eliminating the parameter $t$ in Equation (1) produces the equation of a path curve for the top half of an egg oval sized to a unit circle:

$$y = T(1 + x)^{1/(1+\lambda)}(1 - x)^{\lambda/(1+\lambda)} \qquad (2)$$

where $\lambda$ is $\ln(r_2)/\ln(r_1)$ and $T$ is the scaled equatorial radius of the egg.

The complete shape of a path curve oval is fixed by the $\lambda$ and $T$ shape parameters, $\lambda$ being a measure of the asymmetry of an egg and $T$ the reciprocal of the equatorial elongation of an egg. The axis length is used only for sizing the eggs to the unit circle. For $\lambda = 1.0$, the path curve oval is an ellipse. As $\lambda$ increases to $>1.0$, the path curve oval becomes blunter at one end and reciprocally sharper at the other.

Least-square nonlinear regression can be applied to the egg equation with respect to $T$ and $\lambda$ to determine the path curve that best fits the actual egg contour. The regression equations for calculating values of the two egg-shape parameters are:

$$\lambda' = 10.51/[\ln(7)\ln(7F/A) + \ln(3)\ln(3E/B) + \ln(5/3)\ln(5D/3C)] - 1 \qquad (3)$$

$$T' = \ln(3.25 \cdot ABCTDEF)/7 \qquad (4)$$

where $A$, $B$, $C$, $T$, $D$, $E$, and $F$ are the seven measured radii of a bird egg, each one perpendicular to the egg axis (Fig. 2) and scaled to the unit circle. $\lambda'$ and $T'$ indicate regression estimates for the shape parameters in contrast to the exact or theoretical $\lambda$ and $T$ values used in Equation (2).

The path curve regression error, $\varepsilon$, is the measure used to accept or reject the least-squares path curve fit to any egg oval and is based on the standard regression error formula for the sum of square deviations between the path curve radius and the actual egg radius at each of the seven diameter levels (Fig. 2). This sum is normalized by the axis half-length squared. The regression error $\varepsilon$ measures the goodness-of-fit of the path curve to an actual egg oval.

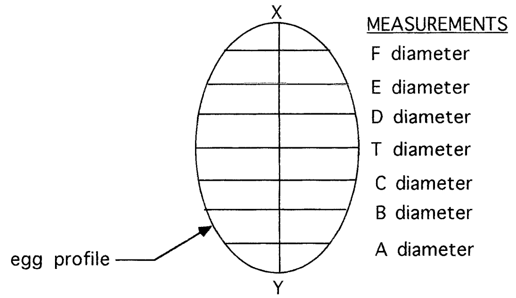


FIG. 2. Seven equispaced radii measurements of an egg scaled to the unit circle. XY is the scaled longitudinal axis of the egg with length two units.

A regression error limit (0.001) is set. A path curve of an egg species is rejected if $\varepsilon$ exceeds the limit, and accepted otherwise. The limit value is chosen to be in the middle of an uncertain interval ($0.0003 < \varepsilon < 0.0016$) where a noticeable visual discrepancy between a path curve and the actual egg profile normally occurs. Below 0.0003 there is little if any visual difference between a path curve oval and the actual egg profile and there's no doubt that an excellent path curve fit for an egg species exists. The right end of this $\varepsilon$ interval (0.0016) was the maximum $\varepsilon$ for an egg path curve fit that occurred in the sample. The maximum $\varepsilon$ egg at the right in Figure 3 illustrates the visual difference between the seven egg-diameter measurements (solid horizontal lines) and the path curve values at those levels. Consequently, this path curve fit must be rejected because too much discrepancy exists in the middle and blunt end sections of the egg. Having determined that 0.0003 to 0.0016 is the interval of uncertainty in which a path curve fit is acceptable or not, the midpoint of that $\varepsilon$ interval, 0.001, was chosen as the upper error limit for acceptable path curve fits.

The left egg in Figure 3 illustrates the visual discrepancies when an egg barely exceeds 0.001. This egg's curve fit is also rejected, because $\varepsilon$ is $>0.001$. All eggs with acceptable path curves (i.e. with $\varepsilon \leq 0.001$) should have visual discrepancies less than those shown in the left egg in Figure 3. An equivalent 0.001 error limit was used by Edwards (1993) in his work with path curve fitting to plant bud forms.

Egg-shape data for 250 species representing 21 orders came from photographs of egg collections from Cramp (1980), Harrison (1975), and Peterson (1967). The sample included eggs from all eight classes of normal egg shapes found in Walters (1994). Abnormal egg shapes were excluded.

Egg selection did not involve finding a typical egg shape for each species. Where a group of eggs from a single species was available from the collections, the egg selected for the sample was one with a profile

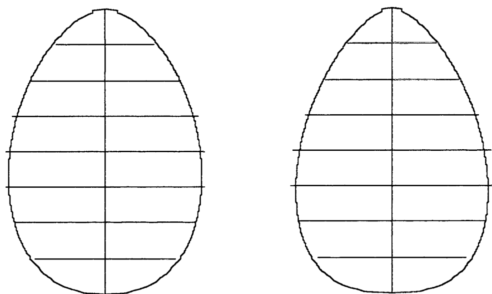

FIG. 3. Visual discrepancies between path curves and egg measurements. Solid horizontal lines indicate the actual diameter measurements of an egg. Visual differences between the diameter end points and corresponding path curve values indicate the error at each diameter level. The left egg has a path curve $\varepsilon$ barely exceeding 0.001, the regression error limit. On the right, the egg has a path curve fit with maximum regression error ($\varepsilon$ = 0 .00158) encountered in the sample.

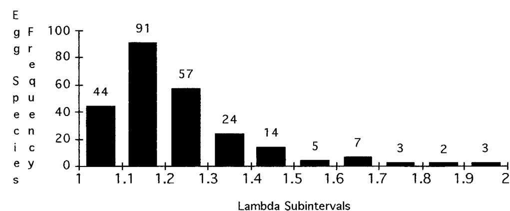


FIG. 4. Path curve fit frequency versus $\lambda$ histogram. The sample $\lambda$ range is divided into 10 subintervals between $\lambda$ = 1 and $\lambda$ = 2. The number above each bar indicates the number of egg species from the sample whose path curves have $\lambda$ values within a given subinterval.

difficult for the egg equation to fit. That was done by visually selecting the most asymmetric egg (i.e. the one with the sharpest end) from eggs of a single species. (The larger an egg's asymmetry the more likely it is to have a higher curve fit error.) If a group of eggs had bilateral symmetry, then either the most extreme cylindrical or biconic egg was chosen to represent the species. In most species groups, no discernible shape discrepancies were detectable and egg selection was arbitrary.

Path curves for passerine eggs are relatively easy to calculate, but highly asymmetric shorebird and alcid eggs provide the most challenging tests. For that reason, the sample contains more nonpasserine (165) than passerine (85) species.

*Results.*—The results of the curve fitting of 250 bird egg ovals are listed in the Appendix. Species order is from Sibley and Monroe (1990). The average curve fit error ($\varepsilon$) for the 250 egg sample was 0.00024 (SD = 0.00029) and ~90% (226 species) of the regression errors were <0.0003, meaning little (if any) perceptible discrepancies from the actual egg silhouettes could be observed. Only 11 egg fits (4.4%) were rejected for exceeding the $\varepsilon$ limit. The species giving the maximum $\varepsilon$ (0.00158) was the Black Turnstone (*Arenaria melanocephala*; the egg on the right in Fig. 3).

The path curve $\lambda$ range for the sample was between 1.0 and 2.0, average $\lambda$ was 1.23 (SD = 0.18, $n$ = 250), which means the typical bird egg in the sample was oval shaped and slightly sharper at one end than the other. The most difficult eggs to fit were highly asymmetrical ($\lambda > 1.4$) murre and shorebird species. The most asymmetrical was the egg of the Common Murre (*Uria alge*) egg with $\lambda$ =1.97. Only 6% (15) of the eggs possessed spherical or ellipsoidal shapes with $\lambda$'s between 1.00 and 1.03.

The frequency distribution of curve fit $\lambda$ values (Fig. 4) was skewed strongly towards the lower part of this range (between 1.0 and 1.4). About 87% of the eggs had $\lambda$ values <1.4 and most of those were passerine eggs, which were slightly blunter at one end than the other. Only the Eurasian Jackdaw (*Corvus monedula*) and Yellow Wagtail (*Motacilla citreola*) had $\lambda$ values >1.4. No path curves among the 85 passerine species were rejected and all possessed path curves with little or no perceivable discrepancies from their actual egg profiles.

The only rejected species with a $\lambda < 1.4$ was the Broad-billed Hummingbird (*Cyanthus latirostris*, $\lambda$ = 1.16) which had an excessively cylindrical contour. The remaining 10 reject eggs ($\lambda > 1.40$) were nonpasserine species (nine shorebirds and one gull) with high asymmetries and almost flat tapers.

Although the mean $\lambda$ value (asymmetry) for nonpasserines matches the passerines, the $\lambda$ SD of nonpasserines was twice the passerines value, indicating a much greater shape variability among nonpasserine species. All except two of the 85 passerine eggs were slightly blunter at one end than the other.

Curve fitting is considered a set of binomial trials, one trial per egg species, and the overall acceptance rate in this sample was 95.6% (or 4.4% rejection). A 250 egg sample is more than sufficient to calculate a 99% CI, and for this sample it is 92 to 99%. Ninety-nine out of 100 CIs found in this way will contain the actual population accept for all bird egg species. Consequently, the sample's curve fit results are statistically highly significant for all bird egg species.

*Discussion.*—Over 90% of the egg species measured have path curve ovals with little visual discrepancy from actual egg contours using this simple algebraic equation. Because this model accurately fits a wide diversity of bird eggs tested with a high level of statistical significance, it represents a universal equation for calculating egg profiles for all bird species. (In an unpublished study of 75 highly asymmetric eggs [33 murre and 42 shorebird eggs], I calculated a 12% rejection rate for each group with a 95% CI ranging from 81 to 95% for the combined

sample. Almost 9 out of 10 of those eggs have acceptable path curve ovals and because these extreme asymmetric egg species are the most difficult to fit, the egg equation can justifiably be applied to all egg species.) Because the egg species are selected to provide a much more severe test of the egg equation, the sample's path curve acceptance rate (95.4%) for the sample is conservative.

Compared to the power series multiequation models (Preston 1968, Todd and Smart 1984), the path curve equation is the simplest analytic description of a bird egg's profile geometry. Only two independent parameters are needed to specify the physical shape of a complete bird egg oval, $\lambda$ and $T$. Unlike the purely abstract constants used in power series curve fitting of egg profiles, path curve parameters directly relate to two of the natural geometric shape characteristics of an egg oval, asymmetry and elongation. Hence, the path curve model is geometrically much more natural and useful for quantitative comparison of different egg shapes than analytic series approaches.

Because $\lambda$ derives from Equation (3) and the geometric transformation in Figure 1A, $\lambda$ provides a more insightful and accurate measure of egg asymmetry than the empirically measured difference of end curvatures used to calculate asymmetry in the past.

The egg equation could be very helpful in making egg shape analyses of various kinds beyond those done in this investigation. Those include (1) examination of eggs for an individual bird and single species samples; (2) dynamic shape analysis of an egg moving down an oviduct; (3) correlation of species egg shape with different egg-laying environments; (4) contrasting egg shapes between vertebrate classes such as bird and dinosaur; and (5) accurate calculations of quantities such as volume and surface area of an egg.

With the egg equation, additional shape-related quantities such as taper, taper bending, longitudinal cross section, surface area, and volume of an egg ovoid can be developed as simple approximation formulas. As an example, the exact volume of an egg would be calculated from the integral:

$$V = \frac{\pi L^3}{8} \int_{-1}^{1} y^2 \, dx \tag{5}$$

where $y$ is from the egg Equation (3) and $L$ is the actual axis length. This volume formula is not integrable in closed form; consequently, a numerical approximation formula must be used. Simpson's rule is a simple, yet effective technique. If the step size is 1/4, the function $f(x) = y^2$ is evaluated at the seven measurement levels. The egg volume approximation formula would be:

$$V \approx \frac{\pi L^3}{96}[4f(-0.75) + 2f(-0.5) + 4f(-0.25) + 2f(0) + 4f(0.25) + 2f(0.5) + 4f(0.75)] \tag{6}$$

Alternately, the integral in Equation (5) could be numerically evaluated using mathematical software (e.g. MATHEMATICA) instead of using Equation (6). The other shape quantities can be derived similarly.

*Acknowledgments.*—I thank L. Edwards from Strontian, Scotland, for his inspired teaching of path curves and their application to natural organic forms. Professor D. W. Winkler, Cornell University, provided invaluable guidance and review for this work. The manuscript benefited from comments of M. Wesson.

## Literature Cited

Cramp, S., Ed. 1980. Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic, vols. I and II. Oxford University Press, London.

Edwards, L. 1993. The Vortex of Life. Floris Books, Edinburgh, Scotland.

Harrison, C. 1975. A Field Guide to the Nests, Eggs and Nestlings of British and European birds. William Collins and Sons Ltd., London.

Peterson, R. T. 1967. The Birds. Time-Life Books, New York.

Preston, F. W. 1968. The shapes of birds' eggs: Mathematical aspects. Auk 85:454–463.

Sibley, C., and B. Monroe, Jr. 1990. Distribution and Taxonomy of Birds of the World. Yale University Press, New Haven, Connecticut.

Todd, P. H., and I. H. Smart. 1984. The shape of birds' eggs. Journal of Theoretical Biology 106: 239–243.

Walters, M. 1994. Birds' Eggs. Dorling-Kindersley, New York.

*Received 14 October 1999, accepted 8 June 2002.*
*Associate Editor: J. Brawn*

APPENDIX. Path curve shape parameters and regression error for each egg species in the sample. The first and second columns following each species name list the regression values of the shape parameters for λ′ and $T'$ of the unit circle path curve which fits the species egg. The third column indicates the path curve regression error (ε). Order follows Sibley and Monroe (1990).

| Species | λ′ | $T'$ | ε × $10^{-4}$ |
|---|---|---|---|
| *Struthio camelus* | 1.01 | 0.822 | 0.60 |
| *Rhea americana* | 1.07 | 0.692 | 2.92 |
| *Dromaius novaehollandiae* | 1.07 | 0.721 | 1.27 |
| *Apteryx australis* | 1.19 | 0.595 | 0.97 |
| *Eudromia elegans* | 1.14 | 0.711 | 0.60 |
| *Megapodius freycinet* | 1.16 | 0.638 | 0.52 |
| *Tetraogallus caucasicus* | 1.41 | 0.646 | 5.08 |
| *Tetraogallus caspius* | 1.25 | 0.693 | 2.56 |
| *Lagopus lagopus* | 1.47 | 0.641 | 2.61 |
| *Lagopus mutus* | 1.24 | 0.683 | 2.25 |
| *Tetrao tetrix* | 1.17 | 0.758 | 0.99 |
| *Tetrao mlokosiewiczi* | 1.34 | 0.670 | 0.64 |
| *Tetrao urogallus* | 1.28 | 0.707 | 1.32 |
| *Bonasa bonasia* | 1.43 | 0.652 | 2.80 |
| *Oxyura leucocephala* | 1.13 | 0.746 | 1.50 |
| *Cygnus olor* | 1.12 | 0.575 | 1.87 |
| *Cygnus cygnus* | 1.12 | 0.623 | 0.11 |
| *Cygnus columbianus* | 1.01 | 0.597 | 2.30 |
| *Anser brachyrhynchus* | 1.11 | 0.648 | 2.62 |
| *Anser fabalis* | 1.11 | 0.640 | 0.45 |
| *Anser albifrons* | 1.10 | 0.708 | 0.68 |
| *Anser erythropus* | 1.15 | 0.606 | 1.10 |
| *Anser anser* | 1.06 | 0.668 | 0.34 |
| *Branta canadensis* | 1.18 | 0.653 | 3.81 |
| *Branta leucopsis* | 1.18 | 0.668 | 0.22 |
| *Branta bernicla* | 1.17 | 0.642 | 0.98 |
| *Tadorna ferruginea* | 1.11 | 0.686 | 0.86 |
| *Aix galericulata* | 1.11 | 0.718 | 0.65 |
| *Anas strepera* | 1.03 | 0.732 | 0.03 |
| *Anas crecca* | 1.14 | 0.702 | 0.41 |
| *Anas platyrhynchos* | 1.12 | 0.714 | 0.19 |
| *Anas acuta* | 1.06 | 0.657 | 0.23 |
| *Anas clypeata* | 1.06 | 0.721 | 0.28 |
| *Aythya ferina* | 1.29 | 0.700 | 1.40 |
| *Aythya nyroca* | 1.06 | 0.724 | 2.68 |
| *Aythya fuligula* | 1.07 | 0.699 | 2.60 |
| *Aythya marila* | 1.14 | 0.670 | 0.10 |
| *Bucephala clangula* | 1.20 | 0.691 | 0.80 |
| *Bucephalo islandica* | 1.18 | 0.728 | 1.00 |
| *Somateria mollissima* | 1.41 | 0.652 | 2.01 |
| *Somateria spectabilis* | 1.17 | 0.682 | 3.38 |
| *Polysticta stelleri* | 1.11 | 0.684 | 0.60 |
| *Histrionicus histrionicus* | 1.23 | 0.687 | 0.50 |
| *Melanitta nigra* | 1.10 | 0.665 | 0.98 |
| *Melanitta fusca* | 1.28 | 0.656 | 0.99 |
| *Mergellus albellus* | 1.10 | 0.729 | 0.21 |
| *Cuculus canorus* | 1.27 | 0.734 | 1.69 |
| *Coccyzus americanus* | 1.16 | 0.714 | 0.73 |
| *Cyanthus latirotris* | 1.16 | 0.648 | 14.8 |
| *Bubo virgianus* | 1.02 | 0.859 | 0.63 |
| *Strix uralensis* | 1.03 | 0.856 | 0.15 |
| *Strix nebulosa* | 1.00 | 0.789 | 0.88 |
| *Caprimulgus europaeus* | 1.18 | 0.758 | 2.94 |
| *Caprimulgus aegyptius* | 1.16 | 0.696 | 0.26 |

APPENDIX. Continued.

| Species | λ′ | $T'$ | ε × $10^{-4}$ |
|---|---|---|---|
| *Caprimulgus nubicus* | 1.07 | 0.785 | 0.46 |
| *Columba palumbus* | 1.01 | 0.730 | 0.58 |
| *Columba trocaz* | 1.04 | 0.664 | 0.56 |
| *Tetrax tetrax* | 1.25 | 0.772 | 1.59 |
| *Otis tarda* | 1.18 | 0.752 | 5.37 |
| *Chlamydotis undulata* | 1.16 | 0.718 | 4.35 |
| *Grus vigo* | 1.24 | 0.622 | 2.45 |
| *Porphyrio martinicus* | 1.27 | 0.644 | 4.65 |
| *Fulica cristata* | 1.33 | 0.729 | 0.73 |
| *Fulica atra* | 1.15 | 0.721 | 0.59 |
| *Syrrhaptes paradoxus* | 1.02 | 0.714 | 1.66 |
| *Pterocles alchata* | 1.10 | 0.653 | 0.60 |
| *Pterocles exustus* | 1.09 | 0.715 | 0.26 |
| *Pterocles senegallus* | 1.06 | 0.691 | 0.51 |
| *Pterocles orientalis* | 1.01 | 0.677 | 0.34 |
| *Pterocles coronatus* | 1.01 | 0.96 | 4.83 |
| *Pterocles lichtensteinii* | 1.05 | 0.691 | 1.05 |
| *Scolopax rusticola* | 1.28 | 0.690 | 7.13 |
| *Gallinago media* | 1.83 | 0.628 | 11.3 |
| *Limosa limosa* | 1.71 | 0.651 | 0.93 |
| *Limosa lapponica* | 1.65 | 0.626 | 5.62 |
| *Numenius phaeopus* | 1.57 | 0.619 | 6.26 |
| *Nemenius arquata* | 1.64 | 0.590 | 13.2 |
| *Tringa erythropus* | 1.66 | 0.653 | 6.44 |
| *Tringa nebularia* | 1.95 | 0.623 | 9.54 |
| *Tringa glareola* | 1.77 | 0.634 | 14.6 |
| *Tringa cinerea* | 1.41 | 0.640 | 11.6 |
| *Tringa hypoleucos* | 1.30 | 0.674 | 8.54 |
| *Arenaria melanocephala* | 1.80 | 0.633 | 15.8 |
| *Calidris alba* | 1.56 | 0.672 | 6.38 |
| *Philomachus pugnax* | 1.67 | 0.626 | 12.3 |
| *Phalaropus fulicaria* | 1.90 | 0.593 | 11.3 |
| *Recurvirostra avosetta* | 1.61 | 0.666 | 10.6 |
| *Pluvalis apricaria* | 1.56 | 0.611 | 6.18 |
| *Himantopus himantopus* | 1.45 | 0.699 | 8.07 |
| *Pluvalis squatarola* | 1.93 | 0.641 | 12.8 |
| *Vanellus vanellus* | 1.69 | 0.681 | 9.37 |
| *Catharacta skua* | 1.38 | 0.652 | 3.39 |
| *Larus hyperboreus* | 1.19 | 0.643 | 0.43 |
| *Larus argentatus* | 1.35 | 0.679 | 2.86 |
| *Larus fuscus* | 1.37 | 0.678 | 4.73 |
| *Larus ichthyaetus* | 1.41 | 0.670 | 1.31 |
| *Larus genei* | 1.29 | 0.682 | 1.71 |
| *Larus melanocephalus* | 1.41 | 0.677 | 2.85 |
| *Rissa tridactyla* | 1.28 | 0.669 | 0.89 |
| *Sterna caspia* | 1.37 | 0.671 | 0.68 |
| *Sterna bengalensis* | 1.35 | 0.683 | 0.12 |
| *Sterna bergii* | 1.38 | 0.675 | 2.37 |
| *Sterna sandvicensis* | 1.41 | 0.621 | 1.24 |
| *Sterna dougallii* | 1.67 | 0.646 | 0.21 |
| *Sterna hirundo* | 1.36 | 0.640 | 1.46 |
| *Sterna paradisaea* | 1.43 | 0.653 | 10.02 |
| *Sterna repressa* | 1.31 | 0.704 | 0.98 |
| *Sterna anaethetus* | 1.24 | 0.705 | 0.56 |
| *Uria aalge* | 1.96 | 0.583 | 5.58 |
| *Uria lomvia* | 1.41 | 0.577 | 7.47 |
| *Alca torda* | 1.41 | 0.587 | 0.47 |
| *Cepphus grylle* | 1.38 | 0.666 | 1.59 |
| *Fratercula arctica* | 1.42 | 0.676 | 0.45 |
| *Pandion haliaetus* | 1.21 | 0.685 | 0.21 |
| *Pernis apivorus* | 1.24 | 0.769 | 0.49 |

APPENDIX. Continued.

| Species | $\lambda'$ | $T'$ | $\varepsilon \times 10^{-4}$ |
|---|---|---|---|
| *Elanus caeruleus* | 1.10 | 0.750 | 0.14 |
| *Milvus milvus* | 1.22 | 0.774 | 0.94 |
| *Gypaetus barbatus* | 1.18 | 0.773 | 5.14 |
| *Neophron percnopterus* | 1.13 | 0.979 | 1.22 |
| *Gyps fulvus* | 1.14 | 0.731 | 2.62 |
| *Circaetus gallicus* | 1.03 | 0.822 | 0.18 |
| *Torgos tracheliotus* | 1.09 | 0.744 | 1.16 |
| *Circus cyaneus* | 1.24 | 0.710 | 3.55 |
| *Circus pygargus* | 1.07 | 0.826 | 0.12 |
| *Accipter badius* | 1.18 | 0.783 | 0.18 |
| *Accipter brevipes* | 1.12 | 0.808 | 1.28 |
| *Accipter nisus* | 1.02 | 0.786 | 2.91 |
| *Accipter gentilus* | 1.15 | 0.809 | 0.90 |
| *Buteo buteo* | 1.10 | 0.811 | 0.37 |
| *Buteo rufinus* | 1.14 | 0.773 | 1.81 |
| *Buteo lagopus* | 1.16 | 0.837 | 0.67 |
| *Aquila pomarina* | 1.09 | 0.843 | 0.45 |
| *Aquila clanga* | 1.17 | 0.807 | 1.26 |
| *Aquila rapax* | 1.11 | 0.777 | 0.54 |
| *Aquila heliaca* | 1.14 | 0.810 | 1.05 |
| *Aquila chrysaetos* | 1.14 | 0.737 | 4.53 |
| *Aquila verreauxii* | 1.23 | 0.760 | 0.28 |
| *Hieraaetus fasciatus* | 1.09 | 0.789 | 0.51 |
| *Hieraaetus pennatus* | 1.07 | 0.826 | 0.28 |
| *Falco eleonaorae* | 1.18 | 0.781 | 0.29 |
| *Falco biarmicus* | 1.10 | 0.779 | 1.83 |
| *Falco cherrug* | 1.18 | 0.719 | 0.32 |
| *Falco rusticolus* | 1.20 | 0.765 | 1.54 |
| *Falco peregrinus* | 1.16 | 0.805 | 3.30 |
| *Falco pelegrinoides* | 1.14 | 0.787 | 0.76 |
| *Tachybaptus rufficollis* | 1.25 | 0.651 | 6.36 |
| *Podiceps grisegena* | 1.15 | 0.696 | 2.63 |
| *Podiceps cristatus* | 1.18 | 0.604 | 0.55 |
| *Podiceps auritus* | 1.20 | 0.649 | 5.43 |
| *Podiceps nigricollis* | 1.21 | 0.657 | 1.57 |
| *Anhinga melanogaster* | 1.17 | 0.598 | 3.28 |
| *Phalacrocorax pygmaeus* | 1.23 | 0.632 | 0.11 |
| *Phalacrocorax carbo* | 1.20 | 0.637 | 0.44 |
| *Phalacrocorax aristotelis* | 1.22 | 0.647 | 0.59 |
| *Ardea cinerea* | 1.11 | 0.675 | 0.78 |
| *Phoenicopterus ruber* | 1.22 | 0.551 | 2.38 |
| *Platalea leucorodia* | 1.04 | 0.652 | 0.70 |
| *Pelecanus crispus* | 1.12 | 0.658 | 0.51 |
| *Pelecanus erythrorhynchos* | 1.17 | 0.642 | 1.84 |
| *Coragyps atratus* | 1.23 | 0.734 | 0.16 |
| *Ciconia nigra* | 1.00 | 0.812 | 5.35 |
| *Ciconia ciconia* | 1.07 | 0.721 | 0.42 |
| *Gavia stellata* | 1.30 | 0.614 | 1.12 |
| *Gavia arctica* | 1.28 | 0.636 | 0.78 |
| *Gavia immer* | 1.18 | 5.99 | 0.41 |
| *Fulmarus glacialis* | 1.15 | 0.703 | 0.19 |
| *Calonectris diomedea* | 1.22 | 0.700 | 0.26 |
| *Puffinus puffinus* | 1.20 | 0.751 | 0.65 |
| *Pyrocephalus rubinus* | 1.14 | 0.719 | 1.26 |
| *Perisoreus infaustus* | 1.18 | 0.673 | 2.21 |
| *Nucifraga caryocatactus* | 1.25 | 0.704 | 4.73 |
| *Pyrrhocorax pyrrhocorax* | 1.26 | 0.659 | 1.22 |
| *Pyrrhocorax graculus* | 1.40 | 0.619 | 0.88 |
| *Corvus monedula* | 1.53 | 0.597 | 0.89 |
| *Corvus frugilegus* | 1.34 | 0.632 | 3.48 |
| *Corvus rufficollis* | 1.35 | 0.633 | 1.31 |
| *Corvus corax* | 1.39 | 0.656 | 1.68 |
| *Corvus rhipurdis* | 1.39 | 0.638 | 1.22 |
| *Oriolus oriolus* | 1.34 | 0.674 | 0.83 |
| *Pica pica* | 1.36 | 0.688 | 0.11 |
| *Monticola saxatilis* | 1.20 | 0.668 | 1.53 |
| *Turdus torquatus* | 1.35 | 0.673 | 1.60 |
| *Turdus ruficollis* | 1.24 | 0.710 | 3.71 |
| *Turdus pilaris* | 1.24 | 0.751 | 0.80 |
| *Turdus migratorius* | 1.05 | 0.752 | 1.38 |
| *Muscicapa striata* | 1.26 | 0.685 | 2.76 |
| *Ficedula hypoleuca* | 1.11 | 0.716 | 3.96 |
| *Ficedula albicollis* | 1.20 | 0.688 | 0.76 |
| *Luscinia luscinia* | 1.23 | 0.734 | 2.32 |
| *Luscinia megarhynchos* | 1.17 | 0.739 | 1.03 |
| *Luscinia calliope* | 1.12 | 0.709 | 2.90 |
| *Luscinia svecica* | 1.22 | 0.735 | 1.08 |
| *Phoenicurus phoenicurus* | 1.21 | 0.709 | 0.39 |
| *Phoenicurus moussieri* | 1.19 | 0.714 | 3.18 |
| *Saxicola rubetra* | 1.13 | 0.766 | 1.14 |
| *Saxicola dacotiae* | 1.18 | 0.803 | 1.00 |
| *Saxicola torquata* | 1.13 | 0.765 | 2.91 |
| *Saxicola jerdoni* | 1.20 | 0.706 | 0.68 |
| *Oenanthe leucopyga* | 1.23 | 0.72 | 1.20 |
| *Oenanthe monacha* | 1.04 | 0.739 | 1.83 |
| *Oenanthe leucura* | 1.11 | 0.759 | 2.06 |
| *Oenanthe lugens* | 1.12 | 0.768 | 0.78 |
| *Oenanthe finschii* | 1.24 | 0.764 | 0.79 |
| *Oenanthe moesta* | 1.21 | 0.734 | 3.40 |
| *Oenanthe pleschanka* | 1.13 | 0.739 | 1.45 |
| *Oenanthe hispanica* | 1.26 | 0.726 | 0.94 |
| *Oenanthe deserti* | 1.19 | 0.745 | 0.71 |
| *Cercomela melanura* | 1.16 | 0.698 | 1.26 |
| *Sturnus vulgaris* | 1.28 | 0.757 | 1.57 |
| *Sitta krueperi* | 1.16 | 0.712 | 0.58 |
| *Sitta tephronata* | 1.28 | 0.665 | 1.04 |
| *Parus ater* | 1.17 | 0.725 | 2.92 |
| *Parus caeruleus* | 1.31 | 0.749 | 2.04 |
| *Acrocephalus scirpaceus* | 1.24 | 0.774 | 0.37 |
| *Acrocephalus dumetorum* | 1.07 | 0.725 | 2.58 |
| *Acrocephalus palustris* | 1.09 | 0.690 | 1.55 |
| *Acrocephalus arundinaceus* | 1.21 | 0.638 | 0.52 |
| *Acrocephalus stentoreus* | 1.13 | 0.747 | 0.40 |
| *Hippolais pallida* | 1.12 | 0.699 | 0.240 |
| *Hippolais languida* | 1.10 | 0.679 | 2.26 |
| *Hippolais olivetorum* | 1.20 | 0.686 | 1.83 |
| *Hippolais icterina* | 1.17 | 0.750 | 0.75 |
| *Sylvia borin* | 1.06 | 0.768 | 2.04 |
| *Sylvia melanocephala* | 1.19 | 0.740 | 3.03 |
| *Sylvia mystecea* | 1.30 | 0.708 | 0.44 |
| *Passer domesticus* | 1.21 | 0.655 | 1.64 |
| *Passer hispaniolensis* | 1.10 | 0.673 | 0.40 |
| *Passer moabiticus* | 1.22 | 0.694 | 0.66 |
| *Motacilla aguimp* | 1.23 | 0.757 | 1.10 |
| *Motacilla citreola* | 1.37 | 0.725 | 1.56 |
| *Motacilla flava* | 1.51 | 0.696 | 3.92 |
| *Anthus campestris* | 1.14 | 0.713 | 0.18 |
| *Anthus berthelotii* | 1.01 | 0.706 | 2.44 |
| *Anthus similis* | 1.22 | 0.696 | 0.62 |
| *Anthus trivialis* | 1.18 | 0.90 | 7.32 |
| *Anthus gustavi* | 1.22 | 0.643 | 1.81 |
| *Anthus pratensis* | 1.32 | 0.687 | 1.11 |

APPENDIX. Continued.

| Species | $\lambda'$ | $T'$ | $\varepsilon \times 10^{-4}$ |
|---|---|---|---|
| *Anthus cervinus* | 1.22 | 0.669 | 2.89 |
| *Anthus spinoletta* | 1.17 | 0.675 | 0.92 |
| *Prunella modularis* | 1.37 | 0.696 | 7.13 |
| *Fringilla coelebs* | 1.37 | 0.751 | 0.80 |
| *Coccothraustes coccothraustes* | 1.30 | 0.695 | 3.40 |
| *Emberiza cintrinella* | 1.05 | 0.756 | 2.04 |
| *Emberiza leucocephalos* | 1.01 | 0.790 | 1.90 |
| *Emberiza cirlus* | 1.12 | 0.744 | 2.06 |
| *Emberiza cia* | 1.09 | 0.717 | 4.54 |
| *Emberiza caesia* | 1.13 | 0.778 | 5.39 |
| *Emberiza elegans* | 1.12 | 0.712 | 1.57 |
| *Miliaria calandra* | 1.24 | 0.681 | 5.40 |
| *Spizella arborea* | 1.17 | 0.761 | 2.52 |