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Validation of the Labeled-Water Method for Estimating Food Consumption in Nestling Herons

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ABSTRACT.—Accurate measurements of food consumption by young birds are of great value in a variety of ecological studies, but traditional measurement methods are problematic and prone to high error. In animals that obtain their water primarily through food, it is possible to estimate food consumption from measurements of water influx rate, using radioactively labeled water (^3H_2O), and diet water content. We evaluated that method by comparing actual food consumption with simultaneous estimates of food consumption based on the labeled-water method in captive-reared nestling Tricolored Herons (*Egretta tricolor*). There was good agreement. The average error in the isotope method was −2% (SD = 8%), and that difference was not statistically significant. An errors analysis indicated that the accuracy of this method is sensitive to determinations of dietary water content and animal body water content, and to estimates of rates of metabolic water production and unidirectional water vapor input.

RESUMEN.—Mediciones precisas del consumo de alimento en aves jóvenes presentan un gran valor para una serie de estudios ecológicos, pero los métodos tradicionales de medición son problemáticos y altamente propensos a errores. En animales que obtienen el agua principalmente a través del alimento, es posible estimar el consumo de alimento mediante mediciones del flujo de agua, utilzando agua marcada radiactivamente (^3H_2O) y el contenido de agua en la dieta. Evaluamos este método mediante la comparación del consumo real con estimaciones simultáneas del consumo de alimento basadas en el método de agua marcada en polluelos de *Egretta tricolor* criados en cautiverio. Hubo una buena concordancia. El error promedio mediante el método de isótopos fue del −2% (DE = 8%), una diferencia que no fue estadísticamente significativa. Un análisis de errores indicó que este método es sensible a la determinación del contenido de agua en la dieta y en el cuerpo, y a la estimación de las tasas de producción metabólica de agua y de entrada unidireccional de vapor de agua.

Accurate measurements of food consumption by nestling birds are of potential use for answering a range of ecological questions, including parent–offspring conflict (Mock and Parker 1997), assessment of toxicant accumulation in wild populations of birds (Pulliam 1994, Brewer and Hummell 1994), and development of energetically based simulation models for testing hypotheses related to ecosystem function and dynamics (Wolff 1994). In animals that do not drink and obtain water essentially only through food consumption, use of isotopically labeled water to measure total water influx—and so to estimate food consumption—has provided a tool for quantifying food consumption in many wild animals without severely impairing their normal activities (Nagy 1989a, Gauthier and Thomas 1990). Although the labeled-water method has been used on adult animals in a variety of situations (Nagy 1989b, Nagy and Obst 1992), rapidly growing juveniles have not been studied. Total water influx in birds, as measured by isotopically labeled water, consists of water consumed as food and drink, water produced internally via energy metabolism, and input of ambient water vapor and its mixing with body water in lungs and through skin (Lifson and McClintock 1966, Nagy and Costa 1980). In birds that do not drink for periods of time, or if drinking can be measured separately and accounted for, total water influx is due primarily to water taken in as part of the food, and that forms the basis of estimating the feeding rate from the water influx rate. The metabolic water component of total influx is usually small (<15%) and can be determined from measurements (preferably, such as via doubly labeled water; Nagy 1989a) or from estimates (using predictive equations; Nagy et al. 1999) of metabolic rate, or from the metabolizable energy component of the diet (Shoemaker et al. 1976). Similarly, the vapor input is also usually small (<10%), and the lung component can be estimated from metabolic rate and associated breathing rate. Birds and mammals have skin that is relatively impermeable to water, and many live in relatively dry circumstances

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(Nagy and Costa 1980), so the cutaneous input of water vapor is generally negligible in endotherms. Thus, total water influx, corrected for drinking (if any), and for metabolic water production and vapor input, represents water intake due to dietary water (succulence of food). That rate, in units of milliliters of water consumed per day, can be divided by the water content of the diet, in units of milliliters of water per gram of food, to calculate feeding rate, in units of grams of food consumed per day.

In this study, we tested the accuracy of the labeled-water method by comparing estimates of food consumption derived from the labeled-water method with actual food consumption measured in captive-raised nestling Tricolored Herons (*Egretta tricolor*). We evaluated assumptions and associated errors in the labeled-water method in an effort to make that method more accurate. We hope this report may stimulate implementation of the labeled-water technique in a variety of field situations with other species.

Assumptions in using the labeled-water method.—Our goal was to develop this method to aid studies of wild nestling wading birds in the Everglades. The use of the labeled-water method in the field or in the laboratory relies on several assumptions, some of which can be justified, and others that needed testing specifically for our study of nestling Tricolored Herons. First, the labeled-water method allows accurate estimation of food consumption in the field only if water consumed is derived almost entirely from the foods eaten. Because wading birds are confined to the nest during the nestling period, they have no access to standing water at ground level. In addition, the wading-bird breeding season in south Florida coincides with the dry season. Finally, we observed nestlings during occasional rainstorms, and they did not attempt to drink rainwater.

Second, the water content of the food items eaten by the organism must be well known. The water content of a variety of fish species from our study site in the Everglades has been measured (Kushlan et al. 1986). In the case of Everglades Tricolored Herons, collections of regurgitant samples confirmed that the diet consists of >95% fish (Frederick 1997). Although similar arguments and information may be applicable to a variety of animals with strict diets (particularly herbivores and carnivores), we emphasize that this assumption may not be applicable to a large number of omnivores.

Methods.—Three broods of Tricolored Heron nestlings (seven nestlings total, ranging in age from 8 to 14 days) were collected on 27 May 1998 from a heron breeding colony located on Lake Griffin, near Fruitland Park, Florida. The birds were individually color banded and transported the same day to a laboratory at the University of Florida. Sibling nestlings were housed indoors in brood-specific cages for the 21 days of the study. Initially, siblings were placed together in 65 × 40 cm plastic boxes containing nest structures made of chicken wire and lined heavily with sticks collected from the field. The nest structures were frozen for 24 h prior to contact with the birds, to kill any ectoparasites that may have been present. It was necessary to wire all sticks firmly to the nest structure to give the nestlings the support needed for normal bone growth. After one week, the nestlings became very mobile, and the plastic boxes were placed in cages (141 × 70 × 43 cm) constructed of plastic sheeting, PVC pipes, and flexible window screening, and equipped with a sturdy perching structure made of PVC pipe and wooden dowels. Room temperature was kept between 24 and 32°C, and humidity was maintained at ~85% with two humidifiers.

All birds were fed thawed fish (Atlantic silversides; *Menidia menidia*), augmented with a vitamin supplement (Vianate®). Each fish was first weighed (to ±0.01 g) with an electronic balance and then hand-fed to the individually color-tagged birds. All birds were fed until satiated (begging ceased), three times daily. To determine the average water content of the food, 30 frozen silversides were weighed individually (to ±0.01 g), wrapped loosely in aluminum foil, and dried to constant weight in an oven at 60°C (about 7 days). Fractional water content of a subsample of silversides was calculated as ([fresh mass − dry mass]/fresh mass). The dietary free water consumed by nestling herons was computed by multiplying the mass of fresh fish eaten by the fractional water content.

After allowing nestlings to acclimate to captivity for 5 days, they were weighed (±1 g), injected intramuscularly (thigh) with 1.0 mCi of tritiated water per kilogram body mass (Williams 1997) in the morning, held for 1 h without food or water, and a blood sample (0.2 mL) was taken from a jugular vein. On the morning of the 5th day after that, each bird was weighed and a blood sample was taken to end the first measurement interval. Immediately thereafter, a second dose of tritiated water was injected as above, and after 1 h, another blood sample was taken to begin the second 5-day measurement interval. A third 5-day measurement interval was completed before ending the experiment.

To prevent contamination and dilution of the blood samples by ambient water vapor, they were transferred immediately from the syringes into 3 mL heparinized evacuated glass tubes. Within 2 h of collection, each blood sample was transferred to capillary tubes, which were then flame-sealed (Nagy 1983).

We distilled water from the blood samples using a vacuum line. Each capillary tube was placed in a ball-and-socket joint attached to a Y-shaped tube using Cajon™ joints, and the entire apparatus was attached to a closed-system vacuum manifold with pressure drawn down to 2.5×10^{-2} torr. Then, stopcocks were closed to seal each blood sample under vacuum. Capillary tubes were broken open by turn-

ing the ball-and-socket joints. A gas torch was used to heat the cracked capillary tubes, causing the water in each blood sample to vaporize. The water vapor migrated into ampules attached to the other side of the Y-tube, which were immersed in cold traps of liquid nitrogen. The ampules containing the distilled water were then flame-sealed while under vacuum and removed from the vacuum line. If the vacuum line leaked during the distillation process, causing ambient water to visibly condense in an ampule, the sample was discarded.

After the ampules cooled, they were cracked open, and 10 μL of water were pipetted into 7 mL borosilicate glass scintillation vials. Depending on the amount of sample available, 3 to 7 vials per sample were prepared. Five milliliters of Scintiverse® biodegradeable scintillation cocktail was added to each vial. Vials were sealed and agitated for 30 s with a Vortex-Genie® to thoroughly mix the water and scintillation cocktail. Above-background specific activity was then counted using a Beckman® LSC6500 scintillation counter.

Feeding rates were calculated from isotope data in three steps: calculation of total water loss and gain, correction for metabolic and vapor water gain, and then conversion of dietary water intake to food intake. In the following equations, ''initial'' (*i*) and ''final'' (*f*) are used to indicate measurements taken at the beginning and end of the 5 day sampling intervals, respectively. To calculate the daily rate of total water loss (mL H_2O efflux per animal per day), we used a simplified version of equation 4 of Nagy and Costa (1980) for situations where body water volume changes linearly through time:

$$\text{water efflux} = \frac{(BWf - BWi)\cdot \ln[(Hi\cdot BWi)/(Hf\cdot BWf)]}{\ln(BWf/BWi)\cdot t}$$

where

BWi = initial body water volume of the nestling (g)
BWf = final body water volume of nestling (g)
Hi = initial background-corrected activity of tritium in 10 μL of water from a nestling (counts per minute, or CPM)
Hf = final CPM of tritium in nestling
t = length of interval (=5 days)
ln = natural log

In tritiated water studies, water influx is calculated as the rate of water efflux plus the rate of body water change (Nagy and Costa 1980):

$$\text{body water change} = \frac{(BWf - BWi)}{t}$$

$$\text{water influx} = \text{water efflux} + \text{body water change}$$

Nestling body water contents were not measured by the isotope dilution method (Nagy and Costa 1980) in this study. Instead, we used data from the more accurate method of oven-drying to constant mass for nestlings of a closely related species, the Eastern Great White Egret (*Ardea alba modesta*; Min et al. 1984). Body water in the Tricolored Herons was calculated from their body mass by multiplying by the age-specific percentage body water that Min et al. (1984) determined for Eastern Great White Egrets of the same age.

Because metabolic water (and CO_2) are end products of energy metabolism, we used predicted metabolic rates to estimate rates of metabolic water formation. Field metabolic rates (FMR) of adult birds can be predicted from body mass using the equation kJ $day^{-1} = 10.9\ (g)^{0.64}$, where g is body mass in grams (Nagy 1987). However, nestlings are less active than adults (especially captive nestlings), and although they have basal metabolic rates (BMRs) that are typical for their body mass (Ellis 1980), they probably have relatively lower FMRs than expected. We assumed that the FMRs of nestlings averaged 2.5 × BMR (vs. the 3 × BMR expected for adults), and adjusted the FMR predicted from the above equation by multiplying it by 0.83 (=2.5/3.0). The result, in units of kilojoules per day, was then converted to units of equivalent metabolic water production using the factors 25.8 kJ L^{-1} CO_2 and 0.617 mL metabolic H_2O formed per liter of CO_2 for a fish diet (Nagy 1983). That value was then subtracted from the total water influx rate to correct for water produced through metabolism.

Vapor input rate was also estimated from FMR, by assuming that vapor input across the skin (which is relatively impermeable in endotherms) was negligible, and that vapor input via the lungs could be estimated from ambient humidity data and rates of air movement during breathing. We assumed that mixed, exhaled air contained 2.9% CO_2 (derived from data in Dejours 1975), and calculated from this that the birds must inhale 34.5× as much air (inverse of 0.029) as they produce CO_2. At the ambient conditions during our experiments, air contained an average of 0.0176 g of H_2O vapor per liter of air. All of the inhaled vapor probably condenses into and mixes with body water (Pinson and Langham 1957). Thus, we estimated vapor influx as 0.83 × predicted FMR in kilojoules per day divided by 25.8 kJ L^{-1} CO_2 × 34.5 L air L^{-1} CO_2 × 0.0176 mL H_2O L^{-1} air. That value was also subtracted from the total water influx rate to correct for vapor input.

After correcting for metabolic water production and water vapor input, food consumption could be estimated. Nestling diet consisted of 74% water (SD = ±1%, n = 30). Thus,

$$\text{food intake rate (grams wet-weight per day)} = \text{whole-animal influx corrected for metabolic water production and water vapor} / 0.74.$$

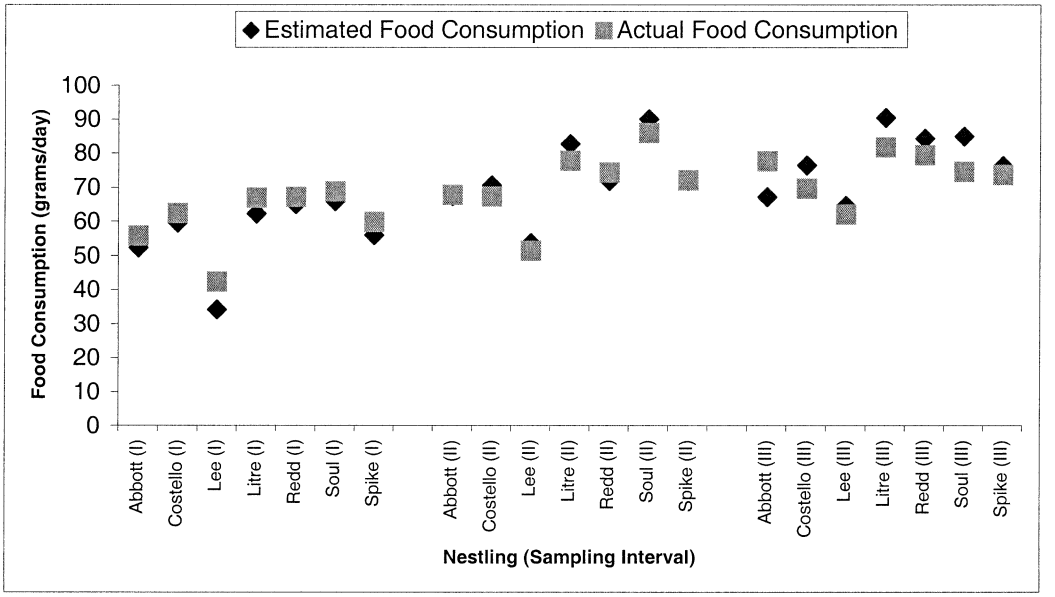

FIG. 1. Estimated and actual rates of food consumption by nestling Tricolored Herons. Values for each bird are shown for each of the three sampling intervals.

The accuracy of the labeled-water method was measured for each nestling and for each sampling interval, allowing us to conduct a repeated-measures statistical analysis. We were interested in determining whether differences in accuracy were due to either sampling interval or to differences in individual nestling attributes (age, sex, or other individual attributes). We first tested the null hypotheses of no effect of sampling interval on accuracy, and no effect of individual chick attributes on accuracy. The repeated-measures analysis was the ideal test for those two hypotheses, because three measurements of accuracy were obtained for each of seven experimental subjects (Zar 1996). A paired, two-tailed *t*-test was also conducted to determine if the error was significantly different from zero error (Zar 1996).

Results.—Overall, the labeled-water method estimated food consumption with an average error of −2.1% for all birds and ages (SD ± 8%). That difference is not significantly different from zero error ($P = 0.38$ via a paired, two-tailed *t*-test). Average errors for the three 5-day intervals ranged from a significant underestimate of −9.4% (Interval I; $P = 0.0003$) to a nonsignificant +2.7% (Interval III, $P = 0.47$). Errors for individual measurements ($n = 21$) ranged from −21.7% to +11.6% (Fig. 1).

There were no significant differences in accuracy due to individual chick effects (repeated measures ANOVA, $P = 0.25$). However, significant differences in accuracy did exist due to sampling intervals (repeated measures ANOVA, $P = 0.0055$). Error differed between sampling intervals in the following manner: Interval I (−9.4%), Interval II (+0.3%), Interval III (+2.7%).

Discussion.—Overall, our error in using the labeled-water method to estimate food consumption was low and statistically insignificant. However, we did find a significant underestimate of feeding rate during the first measurement period, and the individual variation in accuracy (−22 to +12%) was substantial. We suspect that some portion of those errors was due to our use of estimated, rather than measured, body-water volumes. Estimates based on average water contents do not account for probable variation between individuals, and that may introduce errors of around 4–5%. Also, even though we used age-specific water content data from a conspecific heron, the pattern of change in body-water percentage with age may have differed in the two species, yielding the consistent error that we found in the first five-day period. Even though tritium dilution spaces typically overestimate body-water volumes by about 3–4% (Nagy and Costa 1980, Speakman 1997), and thereby probably introduce error into water flux calculations, that can be accounted for by doing additional measurements (drying carcasses), if possible with the species being studied, to quantify the error and correct for it. That would probably improve accuracy.

Correcting for water-vapor input and metabolic water production were important in reducing overall error. If not accounted for, those two potential sources of error would cause an overestimate in food consumption by an average of 32%: 17% error if water

vapor input were not accounted for, and 15% error due to metabolic water input. Additional error could result from metabolic water input if FMR of the nestlings was different from the 2.5 × BMR, as we assumed. For example, if FMR was only 1.7 × BMR, food consumption would have been overestimated by 6%. Clearly, the assumptions made in estimations of metabolic and vapor water input can have a substantial effect on the accuracy of this method. We recommend that these issues be evaluated carefully in future labeled-water studies.

The estimates of food consumption are also strongly influenced by the value used for water content of the food. Increasing or decreasing the water content of the food by 10% resulted in errors of the same magnitude. Therefore, it is imperative that the diet composition of study animals be assessed thoroughly to determine what the diet is, and if it is consistent in the field, because variability in the water content of food can substantially influence the accuracy of estimates.

We have demonstrated that isotopically labeled water turnover can yield accurate estimates of food consumption in nestling Tricolored Herons. If the assumptions and conditions in this study apply to other animals, this method may work well on a variety of organisms studied in the field. It is important that the animals do not drink water, but rather derive nearly all of their water from food. The study organisms should also be sedentary or easily recaptured. Particularly in humid environments, it is important to account accurately for the water vapor input from ambient air.

The labeled-water method has many advantages over other methods for estimating food consumption. Collaring has been used in some cases to estimate food intake, but this method restricts the chick from actually swallowing and digesting food and should probably not be used for more than one or two feeding intervals. That method could also influence mass measurements. In contrast, the labeled-water technique allows collection of information for nestling birds over periods of several days, with researcher disturbance limited to <10 min for each bird. The nestlings need only to be weighed, injected with a small amount of tritiated water, blood sampled 1 h later, and then visited again several days later to obtain a final blood sample. In addition, the nestling could be reinjected at that time and the process repeated for another sampling interval, if so desired. The method has already been used to successfully determine amount of food consumed by wild, free-ranging Great Egret (*Ardea alba*; Williams 1997), Snowy Egret (*Egretta thula*), Little Blue Heron (*E. caerula*), and Tricolored Heron nestlings in the Everglades (Salatas 2000).

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