Condor, 82:186–192

FOOD SAMPLING BIASES ASSOCIATED WITH THE "LIGATURE METHOD"

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ABSTRACT.—Biases associated with the ligature technique of sampling food from nestlings were quantified for the Gray Catbird and Brown Thrasher. Food samples collected after a one-hour interval did not accurately represent the diet because nestlings with ligatures gaped less intensely, gasped when trying to gape, and disgorged food from their throats.

Food samples collected from ligatured nestlings after one-hour intervals were compared with samples obtained after each feeding trip and with observations of nestlings without ligatures. Biases in the former method were demonstrated by three different analyses: average volume of food delivered/nestling/h, size of individual food items, and number of nestlings fed/h. Ligatures did not seem to affect appreciably diet composition.

Several methods have been employed to collect food samples from nestling passerines. Analysis of stomach contents (e.g., Evans 1964, Crase and DeHaven 1977) has the disadvantages that birds must be sacrificed, food items obtained are greatly fragmented, and samples are biased in favor of items less readily digested (Hartley 1948, Dillery 1965). Also, sizes of individual food items and rate of feeding are difficult to determine. Analysis of feces (Evans 1964) does not require sacrificing birds, but identifying and quantifying diet composition are even more difficult and incomplete than stomach content analysis (Hartley 1948). Nest boxes with windows have been used for cavity-nesting species (Betts 1955, Royama 1966); food items delivered are directly seen or photographed with a perch-triggered camera. When many items are brought to the nest at once, however, only a few can be seen and identified (Betts 1956). Use of artificial nestlings to collect food was moderately successful for Betts (1956), but if many food items were brought to the nest in one trip, not all could be collected. In other studies, this method failed (Evans 1964, Orians 1966).

In many recent studies of nestling food-habits, constrictive ligatures have been placed around the nestlings' necks to prevent them from swallowing food delivered by the parents. Food that accumulates in the nestlings' throats is collected and later analyzed. This method has the advantage that food items are usually intact, which facilitates their identification and size determination. Also, individual nestlings can be used repeatedly. Various materials have been used for ligatures, including plastic-coated wire (Owen 1956), metal bands (Kluyver 1961), pipe-cleaners (Orians 1966), light string (Westerterp 1973), heavy thread (Pinkowski 1978), and enameled copper wire (Best 1974, this study).

Several problems with the ligature technique have been reported. Orians (1966) and Willson (1966), using pipe-cleaners, stated that ligatures could not be used on nestling Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*) less than three days old because either the nestlings were strangled or food items slipped by the ligatures. Food slipped past ligatures in older nestlings of other species (Owen 1956, Walsh 1978).

Ligatures have been reported to affect feeding behavior. Young Black-capped Chickadees (*Parus atricapillus*) would not gape for food when wearing metal collars (Kluyver 1961). Orians and Horn (1969) collected more food from nestling Red-winged (*Agelaius phoeniceus*) and Yellow-headed blackbirds than from Brewer's Blackbirds (*Euphagus cyanocephalus*), possibly because the latter would not beg as vigorously when wearing ligatures. Another problem is that adult birds may remove food from nestlings' mouths and eat it (Van Balen 1973, Robertson 1973). Nestling Yellow-headed Blackbirds disgorged food if the sampling period was too long or food delivery too rapid (Orians 1966).

During a study of Gray Catbird (*Dume-*

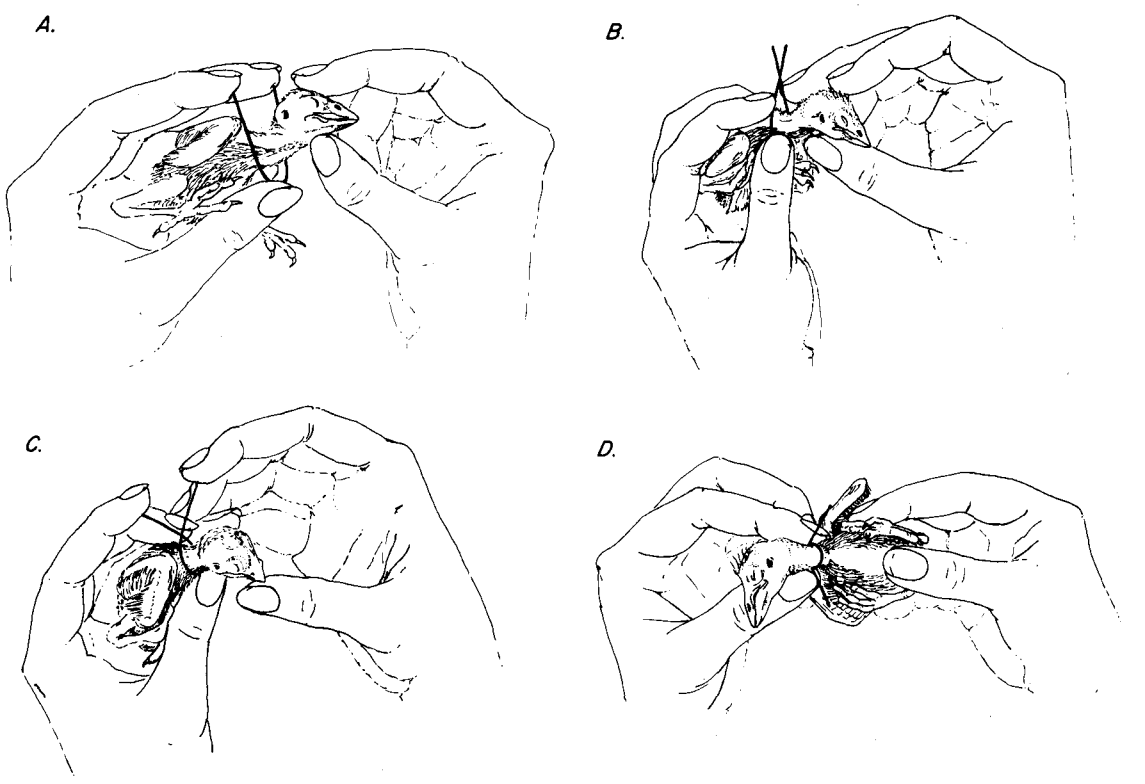

FIGURE 1. Application of ligature. The ligature is positioned on the proximal region of the jugulum (A). The ends of the wire are overlapped (B) and then carefully wrapped around the neck (C) until they extend ventrally (D). Wire ends projecting beyond the neck are clipped.

tella carolinensis) feeding ecology (Johnson and Best, unpubl.), we also observed ligature-induced behavioral changes. These changes can influence the food data collected, but to our knowledge, the extent of the problem has never been reported. In this paper, we have attempted to quantify various biases associated with the ligature technique of collecting food.

METHODS

This study was conducted in a shrubby and partly wooded pasture near Ames, Iowa. Ligatures were used to sample food from nestling Gray Catbirds and Brown Thrashers (*Toxostoma rufum*) during the breeding seasons of 1976–1978. The ligature wire was fashioned into a 'U' shape of approximately the same diameter as the nestling's neck and applied as illustrated in Figure 1. One of four gauges of enameled copper wire was used, depending on nestling size; gauges of 34, 30, 26, and 24 were used on nestlings 0–10, 10–20, 20–40, and 40–60 g, respectively. Ligature tightness was varied by adjusting ventral and lateral pressure while wrapping the wire around the neck. Completed ligatures were circular, without sharp-angled bends, thus avoiding excessive pressure on the neck. The ligature fit could be adjusted so that even the smallest items were collected in the throat, and nestlings (even as young as two hours old) were sampled with less than 5% mortality.

During 1976 and 1977, ligatures were left on nestlings for approximately one hour after the birds were returned to the nest. We then collected the food that had accumulated in their throats and removed the ligatures. Young were sampled from 0–8 days old, after which handling would have caused them to leave the nest prematurely (normal departure occurs at about 11 days for both species). A total of 544 nestling-hours (each nestling of a brood sampled for one hour constituted one nestling-hour) was sampled from 52 nestlings (17 broods) for the catbird and 410 nestling-hours from 57 nestlings (16 broods) for the thrasher.

As part of another project (Johnson and Best, unpubl.), feeding frequencies of nestlings without ligatures were studied from blinds. Because young were seen being fed much more food/h than food samples indicated, feeding behavior of nestlings with ligatures was observed from blinds in 1977. Ligatures greatly affected behavior of the young and, consequently, behavior of the parent birds (see Results and Discussion). Because of potential biases caused by these behavioral changes, food was sampled by using another procedure in 1978. Nestlings were fitted with ligatures as before but were watched from a blind, and food was collected after each parental delivery. Thus, volume of food delivered/feeding trip could be determined. Using this method, 212 samples were collected from 34 Gray Catbird nestlings (8 broods) and 78 samples from 23 Brown Thrashers (8 broods).

The effects of ligatures on feeding behavior of adult and nestling catbirds and thrashers were quantified in 1979. During the first hour of a two-hour observation period, nestlings were not ligatured; during the second, ligatures were fitted loosely on half the brood members and tightly on the other half. Loose ligatures may have allowed very small items to be swallowed; tight ones prevented this. Nestlings in several age

TABLE 1. Effects of ligatures on the feeding behavior of adult and nestling Gray Catbirds. Tight ligatures prevented the passage of all food items down the throat; loose ligatures may have allowed very small items to be swallowed.

	Nestling age								
	0–2 days old (3/14)[a]			3–5 days old (3/12)			6–7 days old (3/10)		
	No ligature	Loose ligature	Tight ligature	No ligature	Loose ligature	Tight ligature	No ligature	Loose ligature	Tight ligature
Time spent gasping (min/h)	$0^{L,T,C}$[b]	3	3	$0^{L,T,C}$	3	6	$0^{T,C}$	3	12
Gaping intensity[c]	1.2	0.9	1.1	1.6	1.3	1.2	$2.0^{T,C}$	1.9	0.9
Feedings/nestling/h	2.0	2.2	2.5	1.8	3.2	3.1	2.4^{L}	7.3^{T}	2.0
Attempted feedings/nestling/h	0.3	0.7	1.0	0.0	0.4	0.4	0.1	0.5	0.6
Food removals/nestling/h	0.0^{C}	1.0	1.9	0.0	1.0	1.3	0.0^{L}	2.0^{T}	0.2
Food disgorgements/nestling/h	0.0	0.0	0.0	0.0	0.4	0.4	0.0^{L}	1.3	0.5

[a] Number of broods sampled/nestling observations (number of nestlings × number of 2-h observation periods).
[b] Statistically different ($P \leq 0.05$) from loose ligature (L), tight ligature (T), or both ligature types combined (C); using the Kruskal-Wallis multiple comparisons test (Gibbons 1976:182).
[c] Intensity estimates ranged from 0 through 3; see text for explanation.

classes were observed. Behaviors recorded were: time spent gasping (gasping was distinguished from panting to dissipate heat), gaping intensity, feedings and attempted feedings, food removals, and food disgorgements. Estimates of gaping intensity (including both the height reached for food and the duration of begging) ranged from 0 (no gape) to 3, with 2 being average gaping intensity. During an attempted feeding, the adult would momentarily place food into the nestling's mouth and then remove it. During a food removal, food that previously had been fed to the nestling was removed.

Volume of food delivered/h could not be measured directly from food samples but was determined indirectly for the catbird. Both food sampling procedures underestimated the normal quantity of food delivered/h; samples collected after one-hour intervals were low because of biases induced by behavioral changes, and samples collected after each feeding trip were low because natural feeding rhythms were disrupted. The average number of feeding trips/nestling/h (obtained from feeding frequency observations) and the average volume delivered/feeding trip were regressed on nestling age (mean values obtained from brood sizes 3, 4, and 5 were not significantly different [Johnson and Best, unpubl.] and were combined in the regression analysis). Information obtained from the two regression lines then was used to plot the total volume of food delivered/h versus age.

A comparison of diet composition using the two methods of sampling with ligatures could not be made because each method was conducted during a different year. A drought occurred in 1977 so that availability of food was different than in 1978. To determine the influence of ligatures on the types of food collected, food items delivered to Gray Catbird nestlings without ligatures, as observed from blinds, were compared with food samples collected after one-hour sampling periods. The frequencies of caterpillars, moths, and grasshoppers (three food types common in the diet and readily identified from a blind) in the nestling diet were determined for each method and the results were compared by chi-square contingency analyses for each possible age group and time of season.

RESULTS AND DISCUSSION

EFFECTS OF LIGATURES ON BEHAVIOR

Normal behavior of nestling catbirds was compared with the behavior of birds wearing ligatures (Table 1). When nestlings without ligatures were 0–1 day old, adults called several times before the young reacted; usually the young would gape within one-half minute. Ligatures did not seem to affect gaping intensity of young nestlings. In older nestlings, those without ligatures gaped almost immediately after the parent landed on the nest rim or called. Those with ligatures gaped less, especially nestlings in the oldest age class fitted with tight ligatures. Gaping often caused nestlings to start gasping, with the result that the parents could not put food into their mouths. On hot days, ligatured nestlings usually gasped and (or) tossed about in the nest during much of the sampling period. Gasping also was common when food was present in the throat, causing the young to disgorge the food. A parent would attempt to feed a disgorged item to a nestling again and, if unsuccessful, would either eat the food or carry it away. Food items not below the sphincter muscle in the throat and visible when nestlings opened their mouths were removed by the adults. Thus, much of the food brought to the nest was not collected in the food samples. Because food items removed from nestlings' mouths sometimes were fed to nestlings again, the frequency of feedings was greater for ligatured nestlings than for those without ligatures. Attempted feedings also occurred more often for nestlings with ligatures, probably because they could not swallow normally. Based upon a limited sample, ligature-induced behavioral changes in Brown Thrashers were similar to those of catbirds.

ANALYSES OF BIASES IN THE ORIGINAL SAMPLING METHOD

Biases in the original sampling method, in which food was collected after a one-hour

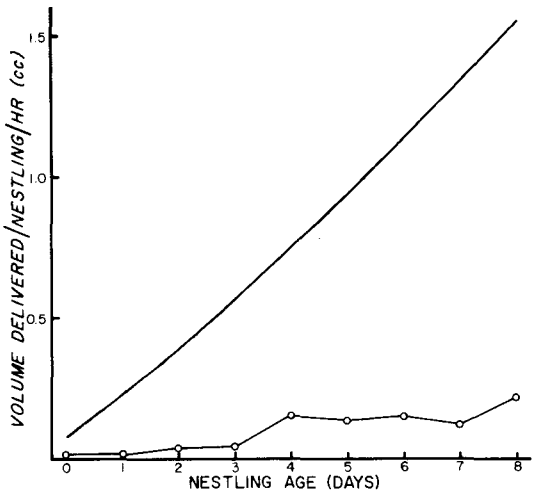

FIGURE 2. Volume of food delivered/nestling/h for the Gray Catbird as collected from ligatured nestlings after 1-h intervals (open circles) and as estimated from regression lines of average number of feeding trips/h and average volume delivered/feeding trip (solid line).

interval, were analyzed in four ways: average volume of food delivered/nestling/hour, size of individual food items, number of nestlings fed/h, and diet composition.

The average volume of food collected/nestling after a one-hour interval was much less than the volume delivered/h to unligatured nestlings, as estimated from information on feeding frequency and volume delivered/trip (Fig. 2). This was because food was disgorged and gaping was weaker. Larger food items probably were disgorged more readily than smaller ones because samples collected after a one-hour interval seemed biased in favor of smaller individual food items when compared with samples collected after each feeding trip (Fig. 3). In nearly all age classes for both species, food items 0.0–0.1 cc in size were over-represented in food samples collected after one-hour intervals. The reverse was generally true for the next three size classes. We found no consistent pattern of difference between the two methods in the largest food-size categories. In only two instances, however, were the size distributions obtained by the two methods significantly different.

The average number of ligatured nestlings from which food was obtained after a one-hour interval was consistently much less than the average number fed during one-hour periods as observed from blinds (Fig. 4). Observations from blinds showed that the average number of nestlings fed/h increased with age. In the catbird, this average approached the actual brood size at age 2–3 days and remained quite constant thereafter. The average for the thrasher increased more gradually, and the maximum was not as high as in the catbird. The average number of ligatured nestlings from which food was obtained after a one-hour interval decreased in all instances from the first age class to the second, after which there was a gradual increase. The decrease is probably because musculature of the youngest nestlings is not developed enough to disgorge food from their throats. No food disgorgements were noted in nestlings 0–2 days old (Table 1). The main source of bias at this age probably results from a relatively small throat capacity. Musculature evidently develops by 2–3 days of age such that food is disgorged frequently. The increase after this age in the average number of nestlings with food probably is attributable to greater throat capacity and a better ability to hold food down. As a consequence, the disparity between the two curves decreased with age (Fig. 4). The number of nestlings from which food was collected by using the ligature technique also was more variable than the observed number fed during one-hour periods. This was probably because the disgorging and refeeding of food items resulted in flux in the number of ligatured nestlings with food in their throats.

The composition of prey items in the diet as determined from one-hour sampling intervals did not seem biased when compared with food items fed to non-ligatured nestlings, with the exception of early June samples collected from nestlings 2–5 days old (Table 2). (No reason is apparent for the under-representation of caterpillars in these samples.) The three kinds of food used for comparison (moths, caterpillars, and grasshoppers) usually were relatively large and more likely would be disgorged or removed from the throat than smaller items. The fact that samples of these larger taxa were representative of the diet would suggest even less likelihood of bias with smaller food types. The apparent absence of sampling bias may be because catbirds and thrashers fed their nestlings mostly soft-bodied prey.

The diet of nestlings 0–1 day old included few of the food types compared because these items generally were too large to be eaten. Items fed to these nestlings were too small to identify from a blind, thus biases in diet composition as determined from one-hour sampling intervals could not be evaluated for this age group.

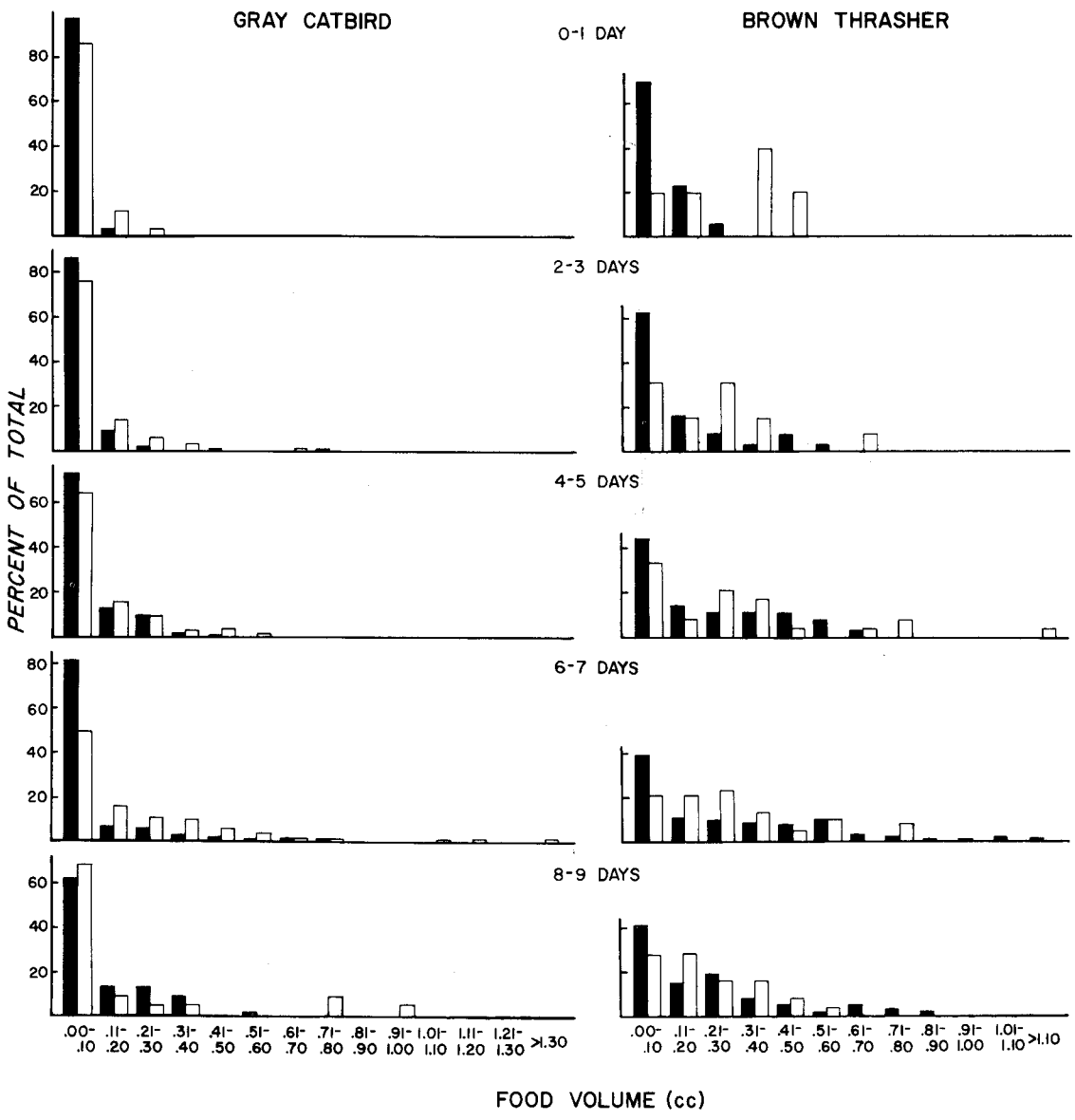

FIGURE 3. Size distribution of individual food items collected from ligatured Gray Catbird and Brown Thrasher nestlings after 1-h intervals (solid bars) and after each feeding trip (open bars). Significantly different ($P \leq 0.05$) size distributions, as determined by chi-square contingency analysis, occurred in thrashers 0–1 day old and catbirds 6–7 days old.

CONCLUSIONS

Our observations show that, at least for Gray Catbirds and Brown Thrashers, food is often disgorged from ligatured nestlings soon after feeding. As a consequence, sampling over any interval, even as brief as 15 min, does not accurately represent the diet. A true portrayal of volumes of individual food items and total food volume delivered/trip can be obtained only by collecting food immediately after each feeding visit. Each feeding must be watched carefully, however, because some items that have been brought to the nest may not be fed to the nestlings due to ligature-induced behavioral changes. Volume of food delivered per unit time cannot be determined by this method unless feeding frequency of non-ligatured nestlings also is known. This is because feeding ceases each time young are removed from the nest to collect food until the parents recover from the disturbance. The extent of disruption in feeding depends on the individual birds and varies both intra- and interspecifically. Some individuals would not feed for more than one hour after such a disturbance, whereas from others, as many as 11 food samples could be collected

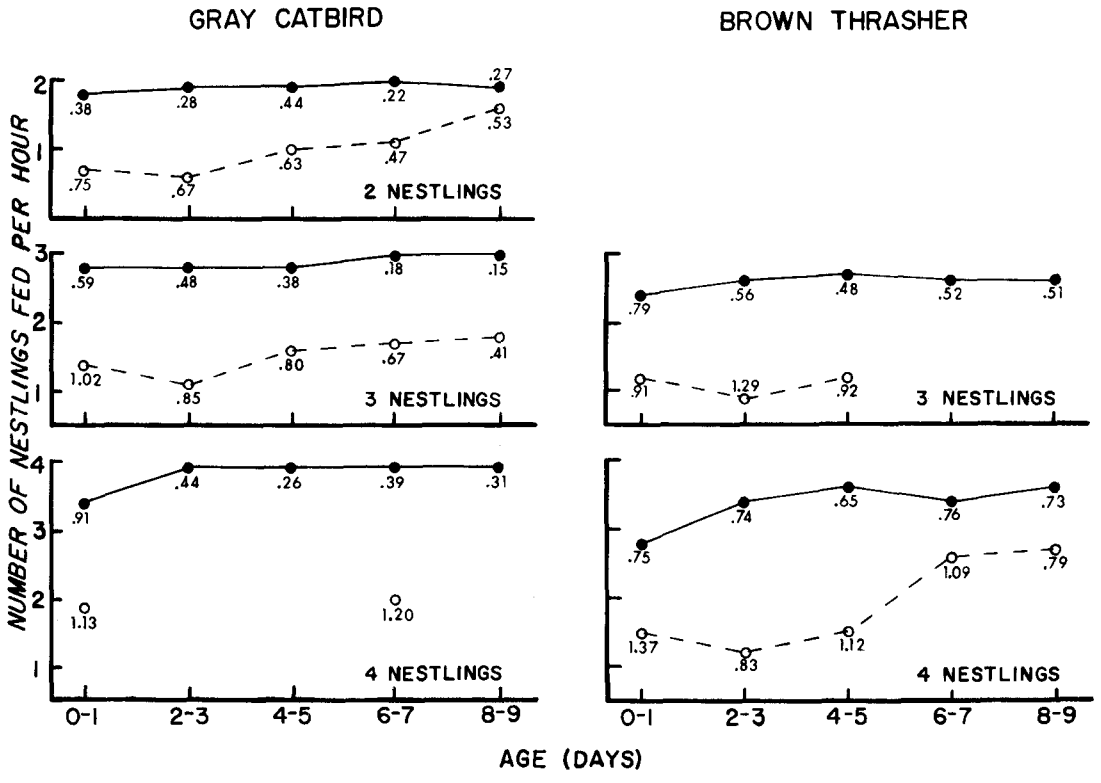

FIGURE 4. Mean number of nestling Gray Catbirds and Brown Thrashers fed/h for different brood sizes, comparing observations made from a blind (closed circles) with food sampled using throat ligatures (open circles). Values below circles represent standard deviations. Data are presented only for age categories where greater than five hours were sampled.

in a one-hour period. Disturbances disrupted the feeding of thrashers more than they disrupted catbirds.

The gaping response is related to the tightness of the ligature. If the wire is adjusted tightly enough to prevent tiny items from slipping by, the gaping response is reduced, especially in older nestlings. We

TABLE 2. Frequencies of feeding moths, caterpillars, and grasshoppers to nestling Gray Catbirds without ligatures, as observed from blinds, and with ligatures, as collected after 1-h periods of sampling. Chi-square contingency analyses were conducted for each possible age class and time of season to test for differences in diet composition as determined by the two methods.

	Number of food items					
	Age 0–1 day		Age 2–5 days		Age 6–9 days	
	Observed from blinds	Collected in samples	Observed from blinds	Collected in samples	Observed from blinds	Collected in samples
1–14 June	(27)[a]	(42)[b,c]	(30) **	(125)	(31)	(115)
Moth	4	0	84	15	172	18
Caterpillar	77	1	149	12	59	10
Grasshopper	1	1	1	3	0	0
15–28 June	(3)	(19)	(22)	(63)	(24)	(78)
Moth	2	1	83	13	30	19
Caterpillar	4	3	29	3	15	5
Grasshopper	0	0	2	1	7	1
29 June–12 July	(20)	(5)[c]	(30)	(43)	(42)	(32)
Moth	2	0	12	2	9	0
Caterpillar	10	0	25	4	13	2
Grasshopper	7	0	37	5	71	4

[a] Number of hours of observation.
[b] Number of food items collected; these included taxa other than those listed.
[c] The number of items collected in these samples was too small to test by chi-square contingency analysis.
** Statistically significant at $P \leq 0.01$.

found that ligatures could be applied more loosely only if food was retrieved immediately after the nestlings were fed and before small items had slipped past the ligature.

Although ligatures did not seem to affect appreciably the diet composition, this needs more study.

The biases we have documented may be similar for other species, thus, caution should be taken before attempting to use ligatures in investigating nestling food-habits. Nestlings fitted with ligatures should be watched from a blind to determine how their behavior is affected, and then sampling methods should be adjusted to minimize biases.

ACKNOWLEDGMENTS

We thank Gaylan Crim, Cindy Hartmann, Diane Boellstorff, and Valerie Miller for assisting with the field work. The constructive suggestions of two anonymous referees for improving the manuscript are gratefully acknowledged. Financial support for this study was received from the Iowa Agriculture and Home Economics Experiment Station, Sigma Xi, the Frank M. Chapman Memorial Fund, and the Josselyn Van Tyne Memorial Fund. Journal Paper No. J-9527 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2168.

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