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Obtaining accurate measurements of the size and volume of insects fed to nestlings from video-recordings

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ABSTRACT. Video-recordings are commonly used to study the types, amount, and size of food items provided to nestling birds. However, the accuracy and repeatability of estimates of the size of food items from video-recordings has not been examined. We assessed three aspects of the reliability of measuring prey size from video-recordings of Great Tits (*Parus major*) provisioning nestlings. To test the accuracy of measurements of prey size (length and width) used to determine prey volume, we molded artificial plasticine caterpillars and compared their size and volume as determined using measurements of length and width on screenshots of video-recordings (using the vertical diameter of nest-box entrance holes as a size reference) to their actual size and volume. We also examined within- and among-observer repeatability of measurements of the size and volume of actual prey items delivered to nestlings by adult Great Tits. We found that observers were able to accurately measure prey size and determine volume, with high agreement between the actual size and volume of plasticine caterpillars and the size and volume as determined from measurements made on screenshots from video-recordings (r_{ICC} = 0.99), and, in addition, within- and among-observer repeatability were also high (r_{ICC} = 0.98 and 0.93, respectively). Overall, our results suggest that the size of prey items delivered to nestlings by adults in video-recordings can be accurately measured and those measurements, in turn, can be used to accurately determine the volume of those insect prey.

Key words: nestling food, insectivorous bird, method, *Parus major*, caterpillar

The number and condition of nestlings is an important component of reproductive success for birds, and critically depend on the quality and amount of food provided by the parents (Naef-Daenzer and Keller 1999). Food quality is usually studied by identifying components of nestling diet, whereas the quantity of food is most commonly described by feeding rates. However, feeding rate is not always a reliable proxy for the amount of food provided to nestlings because the size of prey items may vary. For example, studies of House Sparrows (*Passer domesticus*) have revealed that the rate at which particularly large food items were delivered was the best predictor of nestling mass (Schwagmeyer and Mock 2008) and survival (Seress et al. 2012) prior to fledging.

Several methods have been used to investigate composition and quantity of nestling food, including direct behavioral observations (Schwagmeyer and Mock 2008, Seress et al. 2012), neck-collars (Barba and Gil-Delgado 1990, Pagani-Núñez et al. 2011), artificial nestling gape (Gibb and Betts 1963), and fecal analysis (Deagle et al. 2010, Orłowski et al. 2015). Video-cameras placed near or inside nest-boxes have also been used by many investigators, allowing researchers to collect data without disturbing birds (Seress et al. 2017). Another advantage of video-recordings is that they allow researchers to estimate the size of food items. In general, however, size estimation requires something to which the size of food items can be compared. Some investigators have used the length of adult bills as a reference (e.g., Navalpotro et al. 2016), whereas others have placed a scale bar above the entrance holes of nest boxes (García-Navas and Sanz 2010). Investigators have also determined prey size in different ways, e.g., some have only used broad size categories (Seress et al. 2012), others estimated prey length (Banbura et al. 2001, García-Navas and Sanz 2010), and still others have estimated the length and width of food items and calculated prey volume (Slagsvold and Wiebe 2007, Wiebe and Slagsvold 2014).

[RG1] megjegyzést írt: I'm not sure what this is. Clarify.

Although analysis of video-recordings can provide useful information about both the composition and size of food items provided to nestlings, the repeatability and reliability of this method has not been tested. Our objectives, therefore, were to 1) determine how often observers can identify prey items and determine their size from video-recordings of Great Tits (*Parus major*) provisioning their young, 2) test the accuracy of prey size measurements by comparing the actual sizes of known-sized artificial food items to their sizes as measured from screenshots of video-recordings, and 3) test the repeatability of the size measurements both within and among observers.

METHODS

Study sites, populations, and data collection. Our study was conducted at two urban and two forest sites in Hungary. The urban study sites were located in Veszprém (47°05’17”N, 17°54’29”E) and Balatonfüred (46°57’30”N, 17°53’34”E), where nest boxes were placed in public parks, a cemetery, and university campuses where vegetation consists of both native and introduced species. The forest study sites were in a downy oak (*Quercus pubescens*) and South European flowering ash (*Fraxinus ornus*) forest at Vilma-puszta (47°05'06.7"N, 17°51'51.4"E) and in a European beech (*Fagus sylvatica*) and European hornbeam (*Carpinus betulus*) forest near Szentgál (47°06’39”N, 17°41’17”E). Nest boxes at all study sites were checked at least twice a week throughout each breeding season (March-July) to determine laying dates, clutch sizes, hatching dates, and brood sizes of breeding Great Tits.

[RG2] megjegyzést írt: What years?

We collected one 60-min-long video-recording per breeding pair when nestlings were 8-12 days old (mean ± SE = 9.6 ± 0.1 days) because one-hour observation periods are reported to be sufficient for quantifying the provisioning behavior of Great Tits (Pagani-Núñez and Senar 2013). We video-recorded first broods, with eggs hatching between 11 April and 3 May. At the start of each recording session, we placed a small video-camera (GoPro HD HERO 2) in a black plastic box outside the nest-box (~15 cm from the entrance hole; Fig. 1a) to minimize the possible effect of the camera's presence. Camera boxes were already attached to nest-boxes so the birds were familiar with them. During video-recordings, observers stayed away from nest-boxes to avoid disturbing the parents. We never captured or banded adults or measured and banded nestlings prior to video-recording to avoid the possible disturbances caused by these processes (Seress et al. 2017).

Determining the type and volume of prey items from video-recordings. We visually scanned 53 video recordings using VLC media player 2.1.5. (Free Software Foundation) and took a screenshot of each feeding event when a parent bird held a prey item in front of the entrance hole (mean = 21.9 ± 1.7 feeding events per video-recording). Adult Great Tits are usually single prey loaders (Kluijver 1950), and we did not record any feeding event when a parent carried multiple prey items. From screenshots, we determined prey type and also measured the size of food items that were clearly visible. We divided food items into three categories: (1) caterpillar, (2) other arthropods, and (3) non-arthropods, e.g., seeds and eggshells. We then used the software Fiji (Schindelin et al. 2012) to measure the length and width of each prey item to the nearest 0.001 mm (excluding wings and legs), and used the vertical diameter of the entrance hole (32 mm and clearly visible in each screenshot) as a size reference. We measured the length of food items, and their average width was calculated as the mean of three measurements at each third of the item's length because width can vary along the body of some types of prey (Fig. 1b). We then calculated prey volume, assuming prey had the shape of a cylinder (Slagsvold and Wiebe 2007), using the following equation:

$$V = \pi l (0.5w)^2$$

where V is prey volume, and l and w are the length and average width of a prey item, respectively.

[RG3] megjegyzést írt: A brief explanation of how the software is used to measure items would be useful to readers.

[RG4] megjegyzést írt: For open-cup nesting species, couldn't investigators use the length of some unobtrusive object placed somewhere near nests when video-recording?

Measuring the volume of artificial caterpillars. To test the accuracy of measuring prey size from images, we molded 40 artificial caterpillars from colored plasticine to resemble living *Lepidoptera* larvae, which are the main component of the diet of nestling Great Tits in our study population and other populations (Perrins 1991, Sinkovics 2014). Because our earlier field observations revealed that adult Great Tits delivered caterpillars between 2.67–36.17 mm in length and between 1.19–8.35 mm in width to nestlings, the size of plasticine caterpillars varied within these ranges. Plasticine caterpillars were made in the characteristic curved position similarly to that when birds hold caterpillars in their beaks (Fig. 1b). We held the artificial caterpillars with tweezers (to mimic a bird's beak) at the front of a nest box entrance and recorded these presentations with a video-camera placed in the camera-box used to record provisioning behavior (see above). Artificial caterpillars were presented in random order. The measuring process was the same as described above (i.e., we took screenshots and measured length and average width to calculate volume), and the person doing the measuring did not know the actual size of these artificial food items. Finally, we measured the length and width of the plasticine caterpillars with calipers and calculated their true volume.

Within- and between-person reliability of prey size measurements. To test whether the measurements of actual prey items from video-recordings (described above) were reproducible, we selected 40 prey items from 12 video-recordings that were clearly visible. These prey items were selected to represent the whole prey-size spectrum. For testing within-observer reliability, one person measured these items twice, whereas, for testing among-observer reliability, three people measured each item.

Statistical analyses. We used intraclass correlation (ICC) to test the repeatability between measurements (Lessells and Boag 1987, Koo and Li 2016). Various ICC coefficient (r_{ICC}) values were proposed as thresholds for reliable measurements: for example, Lee et al. (1989) suggest that ICC indicates a reliable method if the lower limit of the 95% confidence interval of r_{ICC} is at least 0.75; Chinn (1991) recommends that a useful measurement should have an r_{ICC} of at least 0.6; Koo and Li (2016) suggested that an r_{ICC} between 0.5 - 0.75 indicates moderate, 0.75 - 0.9 indicates good, and > 0.90 indicates excellent reliability. Here we used the most often-used criterion, i.e., whether r_{ICC} was significantly higher than 0.75.

In addition to calculating ICCs, we also tested whether there was any consistent bias between repeated measurements by comparing the mean values of these measurement series (Lee et al. 1989). In the artificial caterpillar experiment and within-observer reliability test where two sets of measurements were compared, we used paired *t*-tests to examine possible differences between the means. In the among-observer reliability test with three observers, we used linear mixed-effects models (LMM, using package 'nlme'), where the dependent variable was prey volume, the explanatory variable was observer ID (i.e., the IDs of the three people who measured the same prey items), and the random factor was the prey item ID. To summarize, in each case, we used ICC to examine the correlation between two series of measurements as well as using a paired *t*-test or LMM to compare the means of the measurements. Statistical analyses were conducted using R statistical software (version 3.2.2). ICC estimates and their 95% confident intervals were calculated using the 'irr' R package.

RESULTS

We identified 68.3% of the prey items from the videos of Great Tits provisioning nestlings, and were able to measure the volume of 32.4% of the prey items (N = 1170 feeding events). When prey type, prey size, or both could not be determined, adults either entered nest boxes too fast, resulting in a blurry image, or held prey items so they were not clearly visible (e.g., partially blocked from view by a bird's bill).

Comparison of the true volume of plasticine caterpillars with their volume as determined using measurements on screenshots of video-recordings revealed that our method allowed accurate estimates of prey volume. The r_{ICC} values were high (Table 1, Fig. 2a) and we found no significant difference between the means of the two sets of measurements (Table 2). We also found that estimates of prey volume were highly repeatable. The r_{ICC} values were high for both within- and among-obsever repeatability (Table 1, Fig. 2b-e), and we found no significant differences between mean volumes estimated by repeated measurements made by either the same or different observers (Table 2).

DISCUSSION

Our plasticine-caterpillar experiment confirmed that the volume of prey items provided to nestlings can be accurately determined suing measurements made on screenshots from video-recordings, and our reliability analyses revealed that measurements were highly repeatable and unbiased both within and among observers. We also found that the diameter of nest-box entrances can provide a good size standard for measuring prey size.

A disadvantage of using a camera outside of nest boxes in our study was the difficulty in determining the type and size of prey items because adults either entered nest boxes too fast or prey were not clearly visible. We suggest three possible ways to overcome these issues. First, video-recordings in some studies have been made using cameras placed inside nest boxes (e.g., Pagani-Núñez and Senar 2014, Navalpotro et al. 2016). This may make identification and measurement of prey items easier because video-recorders can be placed in a more favorable position (e.g., in front of adults rather than on the side) and because adults may move more slowly once they enter a nest-box.

Second, Currie et al. (1996) also used video-cameras placed outside of nest boxes, but attached a small wire cage to the front of the entrance. Because parent birds could only access their nest through the wire cage, they moved more slowly and improved the likelihood that prey items could be identified and their size measured. Using this method, Currie et al. (1996) were able to identify 94.4% of the prey items delivered to nestling Great Tits from video-recordings.

Finally, the number of prey items that can be measured can be increased by extrapolating volume from estimates of size. For example, we noted several feeding events where a prey item was clearly visible, but the size could not be measured because the adult did not hold it in front of the entrance hole that we used to scale prey size. In many such cases (37%), however, we were able to estimate approximate prey size relative to the length and width of bird bills, and then use these values to estimate prey volume (C. Sinkovics et al., unpubl. data). To do this, we created four length categories, including small (shorter than the bill), medium (same length as the bill), large (longer than the bill), and very large (at least twice as long as the bill), and three width categories, including thin (not as wide as the bill), medium (same width as the bill), and thick (wider than the bill), and then placed the prey items into one 12 size categories (small x thin, small x medium, and so on). Then, we placed prey items into the same categories using their exact length (small = < 7 mm, medium = 7 – 14 mm, big = 14-21 mm, and extra = > 21 mm) and width (thin = < 3 mm, medium = 3 – 6 mm, and thick = > 6 mm) and calculated the average of the measured volume for each category. Finally, we assigned these average volume values to all prey items categorized by comparison to beak length. Using this method, we were able to estimate the volume of a greater percentage of prey items (57.4% rather than 32.4%).

[RG5] megjegyzést írt: What were the length and width of the bills?

[RG6] megjegyzést írt: Where do these numbers come from and why, if they're 'exact' do these categories have a range of lengths and widths? Clarify.

In conclusion, we found that accurate measures of the volume of prey items can be made from video-recordings, allowing investigators to characterize the provisioning efforts of adults by prey volume rather than just provisioning rates – at least for single prey-loading species that deliver one food item per feeding visit. We also found that accurate measurements can be made by multiple observers, given our high among-observer repeatability, when observers are provided with a detailed description of the protocol for making measurement. Finally, our results show that the location of video-cameras is important and can potentially limit the ability of observers to identify and accurately measure the size of prey items delivered to nestlings by adults.

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Tables

Table 1. Intraclass correlation (ICC) tests of the repeatability between actual volumes and those determined using measurements from screenshots from video-recordings, and of within- and among-observer measures of prey volume from video-recordings. For plasticine caterpillars (*N* = 40), one person first measured length and width from a screenshot and then measured their real size with a caliper. For within-observer reliability, one person measured each prey item (*N* = 40) on screenshots twice and, for among-observer reliability, three people measured the same prey item once. We tested the null hypothesis that $r_{ICC} > 0.75$, so the reported *P* values refer to the significance of this test.

Comparison	Intraclass correlation			
	r_{ICC}	95% confidence interval of r_{ICC}	*F*	*P*
Plasticine caterpillars	0.99	0.985-0.995	31.6	< 0.001
Within-observer reliability	0.98	0.97-0.99	16.2	< 0.001
Among-observer reliability	0.93	0.90-0.96	4.3	< 0.001

Table 2. Comparison of actual volumes of plasticine caterpillars and those determined using measurements from screenshots of video-recordings, and the within- and among-observer reliability of determining prey volumes determined using measurements from screenshots. We used paired *t*-tests for the comparisons of plasticine caterpillars and within-rater repeatability, and used a linear mixed-effect model for between-rater repeatability.

Comparison		Mean difference or intercept (mm^3)	SE	*t*	*P*
Plasticine caterpillars	-	0.92	7.42	0.1	0.90
Within-observer reliability	-	-1.86	6.26	-0.3	0.77
Among-observer reliability	Intercept (Observer 1)	225.08	34.68	6.5	< 0.001
	Observer 2	1.85	12.78	0.1	0.89
	Observer 3	-4.33	12.78	-0.3	0.74

Provide Figure legends. Combine Figs. 1a and 1b into a single figure and label as (a) and (b). Also, on Fig. 2, a) and b) at the top are only partly visible. Also, for (a), the y-axis label should be 'Volume determined from video (mm^3)', for (b), the axes should be 'Volume, determined first time' and 'Volume, determined second time', and, for (c), (d), and (e), the axis labels should be 'Volume determined by Observer 1', etc. Finally, move and center (e) below (c) and (d)