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Published in:
Physiological and Biochemical Zoology

DOI:
10.1086/316648

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1999

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Dietz, M. W., Dekinga, A., Piersma, T., & Verhulst, S. (1999). Estimating organ size in small migrating shorebirds with ultrasonography: An intercalibration exercise. *Physiological and Biochemical Zoology*, *72*(1), 28-37. https://doi.org/10.1086/316648

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Estimating Organ Size in Small Migrating Shorebirds with Ultrasonography: An Intercalibration Exercise

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Accepted 6/25/98

ABSTRACT

Organs, even of fully grown adult birds, mammals, and reptiles, may show substantial size changes in relation to specific performances. These changes are difficult to study, because measurements usually can only be obtained following the death of the animal. We explored the use of ultrasonographic imaging, a relatively simple noninvasive technique, to measure size of pectoral muscles and stomach in two small shorebird species (red knots *Calidris canutus* and golden plovers *Pluvialis apricaria*). Accuracy of ultrasound measurements in estimating organ mass in red knots was reasonably high. Depending on the equipment used, the error of individual measurements was 20%–25% for the pectoral muscles and 26%–44% for the stomach. In plovers the technique was less accurate, probably because of the low variability of the organs involved. Ultrasound scanning is particularly suited to measure rapidly changing organ sizes over short time intervals. We demonstrate this with an example in which changes in individuals in size of pectoral muscle and stomach were monitored in captive red knots following a change in diet. Ultrasound measures will enable studies on the links between body composition and future behavior and physiology.

Introduction

Although much of physiology is based on the concept of homeostasis, in fact, large physiological and morphological changes may occur throughout an animal's lifetime (Mrosovsky 1990). Organs of adult birds, mammals, or reptiles may show substantial size changes in relation to specific performances (Piersma and Lindström 1997). This often involves reversible adaptations of the digestive system to changes in diet (Walsberg and Thompson 1990; Brugger 1991; Martínez del Rio et al. 1995; Novoa et al. 1996), to changes in energy demands (Dykstra and Karasov 1992; Gammonley 1995; Koteja 1996; Campbell and MacArthur 1996), or even simply to eating a meal (Secor and Diamond 1997). Other organs such as heart, liver, and muscles also show changes in size, for example, during preparations for long-distance flights (Jehl 1997; Piersma and Gill 1998), during molt (Thompson and Drobney 1996; Jehl 1997), during reproduction (Silverin 1981; Gammonley 1995; Speakman and McQeenie 1996), or in response to changes in food intake (Daan et al. 1989). However, considering the generality of this phenomenon and the drastic impact on the animal's body, such changes have received relatively little attention.

Body composition data usually are obtained from carcass analyses (Lindström and Piersma 1993; Van der Meer and Piersma 1994; Brown 1996). This has serious disadvantages. First, it is not possible to use carcass analysis to investigate organ size changes within individuals. For statistical reasons, larger sample sizes will be required in carcass studies, since variation between individuals will increase total variance in the sample, and because the number of animals required in an experiment has to be multiplied with the number of time points at which measurements are carried out. Second, unless animals are used that were not specifically killed for a study, bioethical problems have to be considered in carcass studies. Third, carcass analysis can help us understand the effect of past environmental circumstances on body composition, but future consequences of having a particular body composition cannot be studied. Thus, a simple nondestructive method to monitor organ size would greatly facilitate the study of the physiology of change, or rheostasis (Mrosovsky 1990; Piersma and Lindström 1997).

Some noninvasive methods currently are available to monitor organ size in live animals: x-ray techniques (Fuller et al. 1994; Duke et al. 1997), nuclear magnetic resonance (Fuller et al. 1994; Wasser et al. 1996), and ultrasonographic scanning (Newton 1993; Fuller et al. 1994; Herring et al. 1994). Each method has certain advantages and disadvantages (Fuller et al. 1994), and most techniques are not suitable for estimating the size or mass of particular organs. A particular disadvantage for ecologically oriented studies is that the use of x-ray and nuclear

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Physiological and Biochemical Zoology 72(1):28–37. 1999. 1522-2152/99/7201-9837$03.00

magnetic resonance measurements is generally confined to laboratory settings. Modern ultrasonographic equipment, however, is portable, may be used in the field, and has the added advantage of being relatively safe and inexpensive (Fuller et al. 1994).

Initially, the ultrasound technique involved the "pulse-echo" method, in which the time passed between the input signal and the reflected output signal gives an indication of the thickness of the medium scanned (e.g., Sears 1988; Newton 1993). Today, ultrasound technique also involves ultrasonographic imaging, which has the major advantage that the "landscape" of the internal organs and skeleton is visible. Ultrasonographic measurements distress animals relatively little and are commonly used in humans and animals for diagnostic purposes (e.g., Grooters et al. 1994; Lambertz et al. 1995) and in the context of animal production (e.g., Perkins et al. 1992; Herring et al. 1994; Chiba 1995). Ultrasonography has been used in a few ecophysiological studies (e.g., Sears 1988; Newton 1993; Reimers et al. 1993; Woodroffe 1995; Haefner et al. 1996).

The red knot *Calidris canutus* is a good example of a small, long-distance migrating shorebird that shows remarkable seasonal shifts in organ size (Evans et al. 1992; Piersma et al. 1996; Piersma and Lindström 1997). These organs can be divided into two groups: the digestive organs, such as stomach and intestines, and the exercise organs, such as pectoral muscles and heart (Piersma 1998; Piersma and Gill 1998). Usually, a change in size of one organ correlates well with the size change of other organs of its group (Piersma et al. 1996). Therefore, size changes of the stomach and pectoral muscles can be used to predict the magnitude and direction of the size changes of other organs of the digestive and exercise organ group, respectively. In this study, we validate the ultrasonographic imaging technique in one specific application: estimating stomach and pectoral muscles size in two small shorebird species, the red knot *C. canutus* and the Eurasian golden plover *Pluvialis apricaria.* The analysis emphasizes the repeatability of the measurements and the predictive value of calibration curves and compares three brands of equipment.

Material and Methods

Ultrasound Technique and Organ Measurement

For ultrasonographic scanning, sedation of the animal generally is not required. Bones and air are impenetrable for the ultrasound signal, and the use of ultrasonography is thus limited by the skeleton, the air in the fur or feathers, and, in birds, also by the air sacs. A scanning gel has to be used as a medium between the probe and the skin. The ultrasound signal is produced and received in the probe. The penetration depth and amount of details of the image depend on the frequency of the ultrasound signal used and the type of probe. Depth of vision decreases with increasing frequency of the probe, while the amount of visible details increases. As with x-ray and nuclear magnetic resonance, breathing, heartbeat, intestinal movements, and any skeletal muscular movement cause movement artifacts on the images (Fuller et al. 1994).

In this study, pectoral muscle thickness was measured at two locations. At the transversal location, the probe was placed transversally on the left pectoral muscle at an angle of about 90° from the rostral top of the sternum to the shoulder, that is, the joint of the coracoid with the clavicula (Fig. 1*A*). This resulted in white V-shaped images of the keel of the sternum and the coracoid (Fig. 2*A*), in which the pectoral muscle is

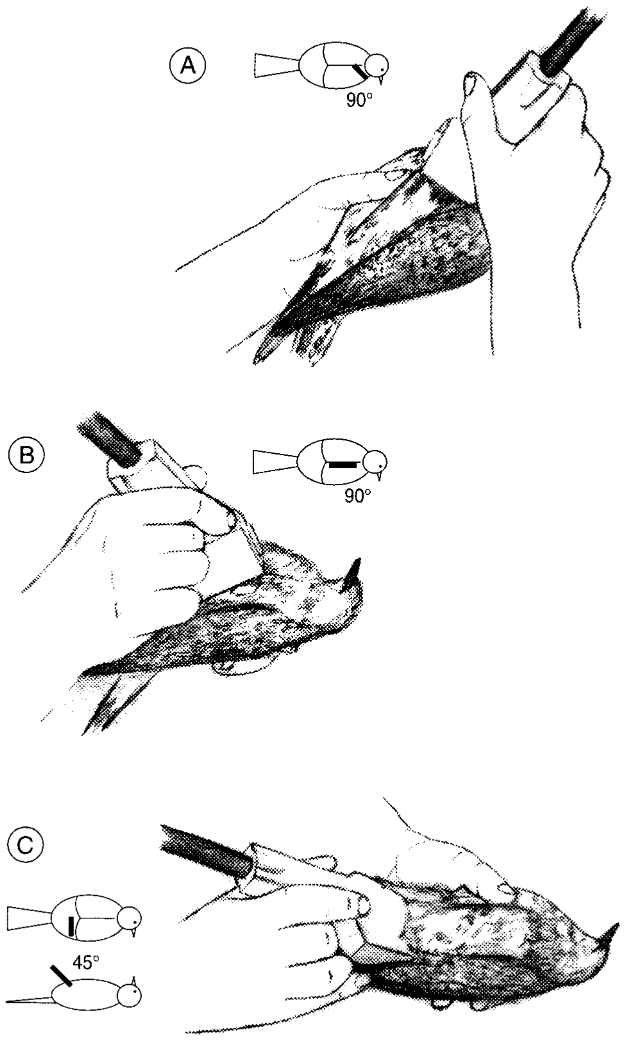

Figure 1. Using the ultrasound on small shorebirds. *A,* Schematic view of the transversal placement of the probe on the pectoral muscle. *B,* Schematic view of the longitudinal placement of the probe on the pectoral muscle. *C,* Schematic view of the placement of the probe while scanning the stomach.

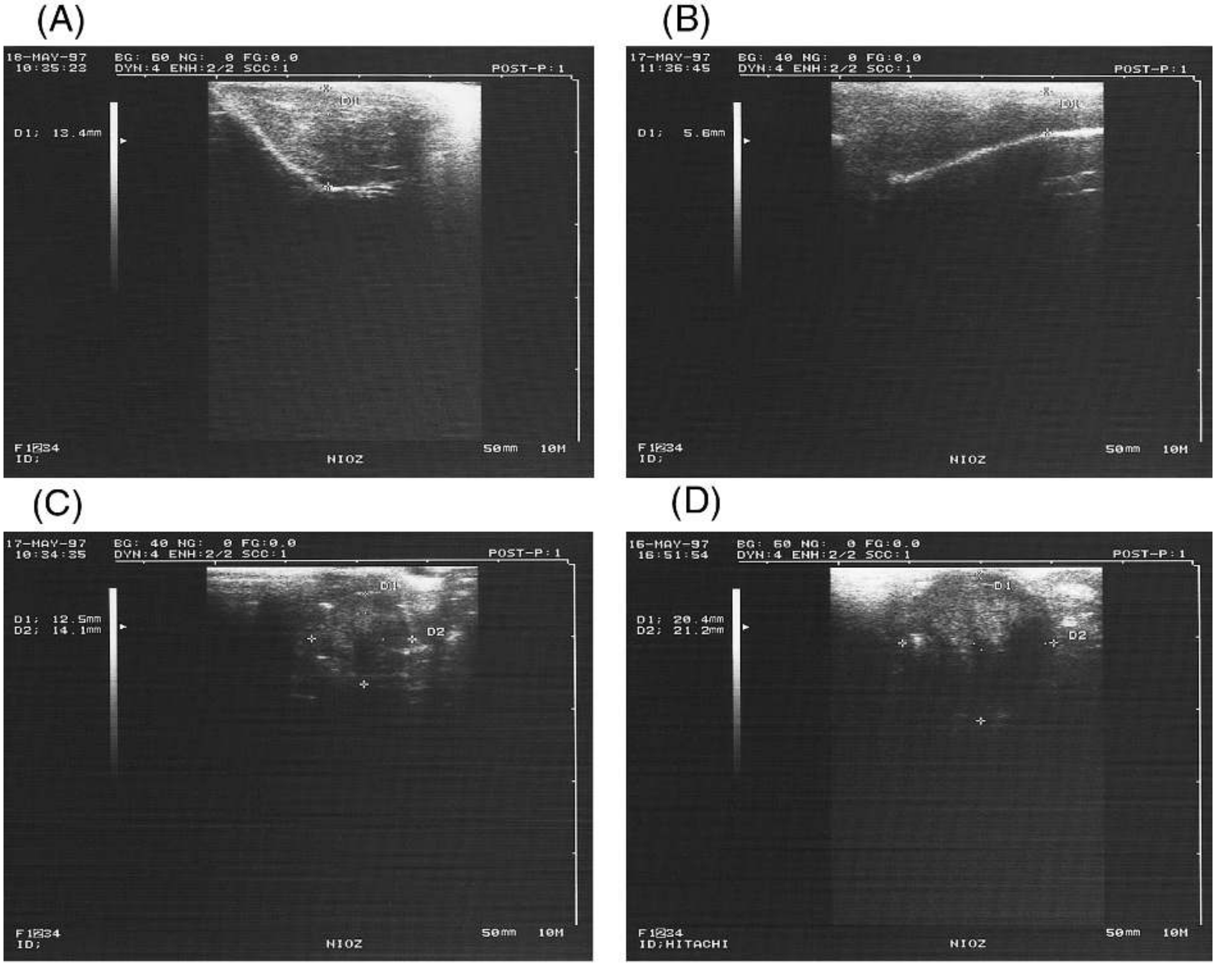

Figure 2. Examples of ultrasound images. *A*, Example of the ultrasonographic image of the left pectoral muscle from the transversal location in knots (the right side of the image is the direction of the shoulder). *B*, Example of the ultrasonographic image of the pectoral muscles from the longitudinal location in knots. *C*, Example of the ultrasonographic image of a small stomach in knots. *D*, Example of the ultrasonographic image of a large stomach in knots. Note that printing always yields a lower-quality image than the screen.

located. The thickness was measured from the bottom of the V to the top of the muscle (±0.1 mm). At the longitudinal location, the probe was placed longitudinally on the muscle parallel to the sternum (Fig. 1*B*). This way the keel became clearly visible, and the pectoral muscle thickness was measured from the horizontal part of the keel toward the top of the muscle (Fig. 2*B*).

Stomach size was determined by measuring its diameter. The probe was placed transversally on the belly of the bird at an angle of about 45° just below the sternum (Fig. 1*C*). The stomach was visible as a round, slightly ellipse-shaped image (Fig. 2*C*, *D*). Both the horizontal (stomach width) and vertical (stomach height) diameter were measured (±0.1 mm).

Experimental Setup and Animals

The first experiment focused on the repeatability and predictive value of the ultrasonic measurements. We used dead knots, a shorebird species with a particularly large variation in stomach size (Piersma et al. 1993*b*). Pilot tests in knots had shown that there were no differences in the ultrasound images of both pectoral muscles and stomach between live and dead birds, in accordance with Sears (1988). One observer (M.D.) measured with ultrasound scanning the thickness of the left pectoral muscles and the stomach diameter in three very different groups of dead knots (normal-condition lighthouse victims, starved winter victims, and laboratory birds fed a soft diet; all groups $n = 7$, so 21 birds total). The large variation in pectoral muscles and stomach masses between the groups is confirmed by the results of dissections (Table 1).

Three sets of equipment were used: an Aloka SSD 500 with a 7.5-MHz linear probe (Biomedic BV, Almere-Stad, The Netherlands), a Hitachi EUB405 with a 10-MHz linear probe (Ecoscan Ultrasound BV, Reeuwijk, The Netherlands), and a Pie 200 with a 7.5-MHz linear probe (Pie Medical Benelux BV, Maastricht, The Netherlands). Each bird was scanned twice with each set. Scanning order (bird, equipment) was randomized by assigning a unique number to each treatment combination (bird, equipment, measurement 1 or 2) and drawing these numbers at random. The observer did not know which bird was scanned or what the results of previous measurements were. The knots were kept in a refrigerator at about 4°C between scans. Scanning duration varied between 5 min and 15 min per bird, and the whole experiment lasted 4 d.

In the second experiment, we looked at the differences between two observers (M.D. and A.D.). A.D. had less experience with the ultrasound technique than M.D. Unfortunately, due to circumstances beyond our control, the measurements were done with two sets of equipment (M.D. used Pie and A.D. used the Hitachi equipment). A good comparison between observers thus was not possible. Both observers measured the thickness of the left pectoral muscles and the stomach diameter in dead golden plovers (M.D. scanned six and A.D. scanned 10 birds). Each observer scanned each bird twice. For each observer, scanning order of the birds was randomized with the same procedure as in knots. The plovers were kept in a refrigerator at about 4°C between scannings. The duration of the ultrasonographic examination varied between 5 min and 15 min per bird, and the whole experiment lasted 3 d.

In addition, we measured pectoral muscle thickness (transversal location) and stomach diameter (height and width) in wild knots captured in July–August 1997 in the Dutch Wadden Sea (Pie equipment, observer, A.D.). Nine birds, captured August 5, 1997, were taken to the laboratory (Netherlands Institute for Sea Research [NIOZ], Texel) and fed with a pellet food (Trouvit). Wild birds feed on small bivalves and are expected to have large stomachs, since they ingest the bivalves whole and crush them within their stomachs (Piersma et al. 1993*a*, 1993*b*). In the laboratory, however, the birds are fed a soft food, which induces a stomach mass decrease of about 50% (Piersma et al. 1993*b*). After 3 mo in captivity, the knots were scanned again. Pectoral muscles and stomach mass (using stomach height) were calculated using the prediction equations for knots determined in the calibration experiment. The ultrasonographic examination took about 10–15 min per bird. The scanning gel was easily and completely removed from the feathers with lukewarm water. The birds remained in perfect shape after the procedure.

Dissection

After the experimental ultrasound measurements, the dead knots and plovers were dissected, and the birds were processed according to the methodological details in Piersma et al. (1996). Wet masses of both pectoral muscles and the stomach were determined (summarized in Table 1). In the analyses and graphs below, pectoral muscle mass thus represents the mass of the left and right pectoral muscles taken together.

Statistics

Repeatabilities were calculated following Lessells and Boag (1987), with standard error following Becker (1984). The relationship between measurements obtained with the ultrasound scanning and the true organ mass was determined using linear regression (Model I following Sokal and Rohlf [1995]; more complex equations, using, e.g., linear measurements cubed, did not explain a larger proportion of the variance). In this and subsequent analysis, we used the mean of the two replicates of each organ location. For both organs, ultrasound measurements were made at two locations, and multiple regression was used to investigate how pectoral muscle mass or stomach mass could

Table 1: Average body mass, pectoral muscles (both sides), and stomach mass (g) for three groups of red knots and one group of Eurasian golden plovers

	Body Mass	Pectoral Muscles	Stomach
Red knots:			
Lighthouse victims	145.6 (4.1)^a	27.5 (1.0)^a	12.3 (.7)^a
Winter victims	116.1 (11.5)^b	18.3 (1.0)^b	10.6 (1.2)^a
Laboratory birds	99.4 (10.5)^c	16.6 (7.8)^b	3.2 (.2)^b
All birds	120.4 (6.6)	20.8 (1.8)	8.7 (1.0)
Plovers	211.1 (5.7)	48.0 (1.2)	5.5 (.2)

Note. Values are presented as mean and SEM. $n = 7$ for each group of knots ($n = 21$ for all birds), and $n = 10$ for the plovers. Averages of groups of knots with different indices (within a column) differ significantly from each other (Student's t-test, $P < 0.05$).

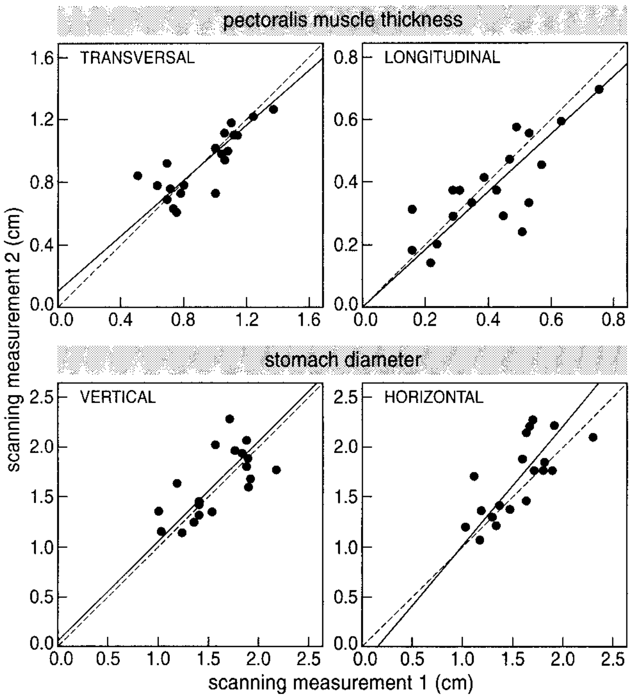

Figure 3. Relationship between the first scanning and second scanning of the thickness of left pectoral muscle at the transversal and longitudinal location and the stomach height and width in knots. The dotted line indicates the line of equality ($y = x$); the solid line represents the reduced major axis equation fitted through the data. Equipment used: Pie 200.

be best predicted. For stomachs, the adding of the multiplication of stomach width × height was also tested, since on theoretical grounds one might expect this variable to be the best predictor.

To assess the value of the obtained regression lines to predict organ mass for a new sample, we omitted some randomly chosen cases from the calculation of the regression line and used the regression line obtained with the remaining data to predict organ mass for the omitted cases. This was repeated for 1,000 rounds, because preliminary analysis showed this to be sufficient to yield a stable estimate. In each round, the predicted values were compared with the true values, and the mean absolute ($|y_{\mathrm{obs}} - y_{\mathrm{pred}}|$) and relative ($|y_{\mathrm{pred}}/y_{\mathrm{obs}} - 1|$) discrepancy between predicted and observed values were calculated for the omitted cases. The discrepancy values obtained in this way were averaged over all rounds to obtain an overall estimate of the predictive power of the regression line. In each round, five out of 21 cases were omitted for the knot sample and three out of 10 cases were omitted for the plovers. However, preliminary analysis showed that the value obtained is independent of this number over a wide range of values.

In the procedure described above, averages of the two replicates were used. To evaluate the effect of replicated measurements, a "one-measurement" data set was formed by randomly choosing one of the two available cases per bird. For this new data set, discrepancy values were calculated. This procedure was repeated 10,000 rounds to obtain an overall estimate of the predictive power of a regression based on one measurement only.

Results

Repeatability of Ultrasound Measures

Knots. Repeatabilities of the two scans of transversal pectoral muscle thickness and stomach width and height were generally fairly high in all three sets of equipment (Table 2; see Fig. 3 for an example). An exception was the longitudinal scanning location for pectoral muscle thickness, where repeatabilities obtained with the Aloka and Hitachi equipment were substantially lower than all other values (0.51 and 0.52 vs. 0.68–0.83).

Plovers. Repeatabilities with the Pie equipment were significant in most cases, although they tended to be lower than in the knot. Repeatabilities obtained with the Hitachi equipment were somewhat lower, but this is probably due to the observer's lack of experience, as no systematic variation between equipment was observed in the knots (Table 2).

Regression and Predictability

Knots. Linear regression was used to estimate organ size on the basis of ultrasound measurements, and these regressions were highly significant in all cases (Table 3; see Fig. 4 for an example). Two different measurements were taken of both organs (pectoral: transversal and longitudinal; stomach: height and width), but using both measurements in a multiple regression analysis did not yield a significant increase in explained variance in any organ/equipment combination. Also, adding the multiplication term width × height in the multiple regression of the stomach did not yield a significant increase in the explained variation.

The absolute and relative discrepancy values are direct and unbiased estimates of the errors made when using these regression lines to predict organ mass in other individuals of the same species. The discrepancy when estimating pectoral muscle mass was 20%–25%, or 3–4 g (Table 3). This is approximately 45% of the standard deviation. The discrepancy when estimating stomach mass was slightly higher: 26%–44%, or 1.7–2.5 g (approximately 47% of the standard deviation).

Plovers. Only for the results of the Hitachi equipment were predictive regressions of organ mass calculated, as the data set of the Pie equipment was too limited ($n = 6$). The regressions were not significant, except for the regression of stomach mass on stomach width (Table 3). The discrepancy when estimating pectoral muscle mass was 9%, or 4.2 g (Table 3), which is

Table 2: Repeatability values for the ultrasonographic measurements of the pectoral muscle thickness and stomach diameter for each set of equipment in red knots and Eurasian golden plovers

	Aloka	Hitachi	Pie
Red knot:[a]			
Pectoral muscle, transversal	$.72^{***}$ (.11, 21)	$.68^{***}$ (.13, 19)	$.83^{***}$ (.07, 21)
Pectoral muscle, longitudinal	$.51^{**}$ (.16, 21)	$.52^{*}$ (.17, 19)	$.76^{***}$ (.10, 19)
Stomach height	$.72^{***}$ (.11, 21)	$.86^{***}$ (.06, 18)	$.68^{***}$ (.13, 19)
Stomach width	$.73^{***}$ (.10, 21)	$.83^{***}$ (.07, 18)	$.69^{***}$ (.12, 19)
Eurasian golden plover:[b]			
Pectoral muscle, transversal	…	.29 (.30, 10)	$.78^{**}$ (.17, 6)
Pectoral muscle, longitudinal	…	$.68^{**}$ (.17, 10)	$.49^{+}$ (.32, 6)
Stomach height	…	.32 (.29, 10)	$.65^{*}$ (.25, 6)
Stomach width	…	$.48^{+}$ (.25, 10)	$.48^{*}$ (.33, 6)

Note. SEM and number of birds are given in parentheses.

[a] All measured by M.D.

[b] Hitachi measurements by A.D.; Pie measurements by M.D.

$^{+}$ $P < 0.1$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

approximately 111% of the standard deviation. The discrepancy when estimating stomach mass was slightly lower: 5%–8%, or 0.3–0.4 g (approximately 70% of the standard deviation). Note that although the absolute and relative discrepancies are lower than the data obtained in knots, relative to the standard deviation, they are substantially higher. This indicates that the ultrasound technique is less suitable for plovers than for knots. The difference between the species is probably related to the variability of the traits studied, as the coefficient of variation for muscle and stomach mass was substantially higher in knots (breast 38%, stomach 52%) than in plovers (breast 8%, stomach 9%).

Effect of Replicated Measurements (*Knots Only*)

Since the taking of four ultrasound measures can take up to 15 min, it is important to establish whether replicated measures of the same birds yield a substantial increase in the predictive power of the measures. To investigate the effect of replication we recalculated the absolute and relative discrepancies for the knots, but using only one of the two replicate measurements (in previous analyses the mean of the two replicates was used throughout). For each bird, one of the two replicate measurements was randomly selected.

In general, using only one ultrasound measurement to estimate organ mass resulted in a modest increase in relative and absolute discrepancy (Table 3). For the pectoral muscles, the discrepancy increased from 20%–25% (3–4 g) to 23%–31% (4–5 g). This is approximately 55% of the standard deviation (this was 45% when using both replicates). The discrepancy when estimating stomach mass increased from 26%–44% (1.7–2.5 g) to 30%–49% (1.9–2.7 g). This is approximately 50% of the standard deviation (this was 47% when using both replicates).

Example of an Application in Live Knots

To investigate the use of the ultrasound technique to monitor intraindividual changes in body composition, we captured nine red knots at the end of July in the Dutch Wadden Sea, near Richel. At that time, knots fed mainly on bivalves (Piersma et al. 1993*a*), which we know is associated with large stomach sizes (Piersma et al. 1993*b*). Ultrasound measurements were taken at capture and after 3 mo in captivity, where the birds were fed only food pellets. Body mass increased during captivity, on average from 140 to 156 g (Fig. 5*A*; paired *t*-test, $t_8 = 2.11$, $P = 0.068$). Pectoral muscle size increased over the same period, on average from 34 to 44 g (Fig. 5*B*; paired *t*-test, $t_8 = 3.659$, $P < 0.01$). Since no subcutaneous fat was visible at the scanning location, the increase in pectoral muscle thickness probably reflects an increase in muscle mass. Stomach mass of the knots decreased to less than half of the mass at capture, on average from 20 to 7.5 g (Fig. 5*C*; paired *t*-test, $t_5 = 9.559$, $P < 0.001$). Thus, intraindividual changes in body composition, probably caused by the change in diet (at least for the stomach), were detectable using the ultrasound technique.

Discussion

Reversible variation in organ size is attracting increasing attention (e.g., Evans et al. 1992; Gammonley 1995; Speakman and McQueenie 1996; Piersma and Lindström 1997), but the study

Table 3: Linear regression correlation coefficients between pectoral muscles or stomach mass and the average ultrasound measure in red knots and Eurasian golden plovers and the discrepancy of the prediction obtained from these regressions from the true value

				Absolute Discrepancy (g)		Relative Discrepancy (%)		Discrepancy Relative to SD	
	n	*r*	*P*	I	II	I	II	I	II
Red knots:									
Pectoral muscle, transversal:									
Aloka	21	.884	.0000	4.06	3.51	26.8	23.7	50.6	43.7
Hitachi	19	.892	.0000	4.18	3.26	27.4	20.4	52.0	40.6
Pie	21	.914	.0000	3.43	2.95	22.6	18.9	42.7	42.7
Pectoral muscle, longitudinal:									
Aloka	21	.837	.0000	4.94	3.95	31.2	24.9	61.5	49.2
Hitachi	19	.834	.0000	4.78	3.76	30.5	22.4	59.5	46.8
Pie	19	.837	.0000	4.16	3.80	27.0	24.4	51.8	47.3
Stomach height:									
Aloka	21	.831	.0000	2.56	2.35	46.7	41.3	56.3	51.7
Hitachi	18	.928	.0000	1.94	1.70	30.4	26.6	42.7	37.4
Pie	19	.820	.0000	2.43	2.00	34.4	27.0	53.5	44.0
Stomach width:									
Aloka	21	.792	.0000	2.66	2.51	48.5	44.1	58.6	55.2
Hitachi	18	.924	.0000	2.13	1.91	33.1	30.3	46.9	42.0
Pie	19	.840	.0000	2.55	2.30	39.0	33.4	56.1	50.1
Eurasian golden plovers:									
Pectoral muscle, transversal:									
Hitachi[a]	10	.118	.746	...	4.36	...	9.3	...	114.5
Pectoral muscle, longitudinal:									
Hitachi[a]	10	.483	.157	...	4.11	...	8.6	...	108.0
Stomach height:									
Hitachi[a]	10	.554	.096	...	.42	...	8.1	...	80.9
Stomach width:									
Hitachi[a]	10	.82	.004	...	.28	...	5.2	...	54.0

Note. See "Material and Methods" section for the calculation of the absolute and relative discrepancies between the predicted organ mass values and the true values. Columns I and II under the discrepancy headings refer to the calculation of discrepancies using only one (I) or an average value of two (II) ultrasound measures for the prediction equations.

[a] Observed by A.D.

of this phenomenon is hampered by the lack of nondestructive techniques for estimating organ size. In fact, the field of organ adaptation would benefit greatly from a nondestructive technique for measuring organ size. In this article, we explore whether ultrasonographic scanning can fulfill this role.

In earlier validation studies, assessment of the accuracy of the ultrasound method for predicting organ mass usually was restricted to the calculation of correlation coefficients between the ultrasound measure and organ mass. Correlation coefficients of the relationship between ultrasound measure and organ mass in knots (0.792–0.928) are comparable with those found for swans (*Cygnus olor*, 0.929; Sears 1988), dippers (*Cinclus cinclus*, 0.859; Newton 1993), and canaries (*Serinus canaria*, 0.927; Newton 1993) and also with the range of the correlation coefficients between the ultrasound measure and fat thickness or muscle area in beef cattle (Perkins et al. 1992; Herring et al. 1994). Values obtained in plovers were substantially lower (0.118–0.820), however. Variation between the different equipment brands used was small (Table 3).

Correlation coefficients are not sufficient to evaluate the value of a predictive equation; this requires an independent sample in which the prediction error is quantified. We calculated the discrepancy between real and predicted values by resampling our data (Table 3) and compared the observed discrepancies with the overall standard deviation of the sample. As our results show, the mass of the pectoral muscles and the

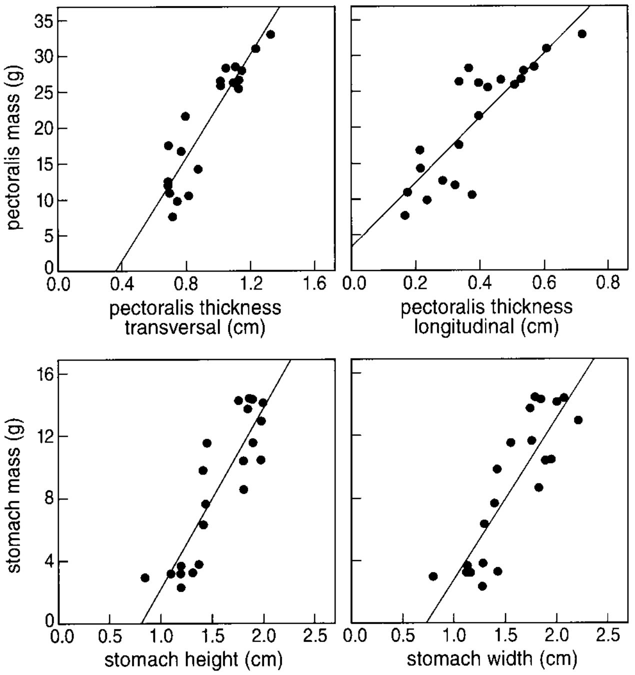

Figure 4. Relationship between true organ mass and average ultrasonographic scanning value of the pectoral muscle thickness at the transversal and longitudinal location and the stomach height and width in knots. The solid line represents the linear regression equation fitted through the data. Equipment used: Pie 200.

stomach of knots could be estimated reasonably well from ultrasonographic scanning measurements, while reliability was relatively low for the plovers. We suggest that the difference between the species is due to the coefficient of variation in the morphological trait studied, and that accuracy will increase with increasing variation.

Our validation was based on comparison between individuals because you cannot kill a bird twice. However, it seems likely that at least part of the error in predicting organ mass is due to interindividual variation in aspects such as organ shape. Such error will be relatively less important when comparisons are made within individuals. In the presented application of the ultrasound technique, changes in organ mass were extremely clear in spite of limited sample sizes (Fig. 5). Thus, it seems that a change in organ mass within an individual bird is measured with greater accuracy than our between-individual validation would suggest, reducing the required sample size.

Sources of Error

When using ultrasound to estimate organ mass in live birds, there are two steps that each introduce error in the obtained estimates: first, inaccuracy in the measurement of the ultrasound images, and second, the error made when predicting organ mass on the basis of these measurements. Here we discuss factors that determine the magnitude of these errors and how they can be minimized.

The error introduced when measuring ultrasound images is reflected in the repeatabilities (Table 2). This error can be reduced by taking repeated measurements (Table 3), and the reduction in discrepancy obtained in this way decreases with increasing repeatability. Variation between replicate measurements is probably due, to a large extent, to probe placement. Variation in location—but also variation in the angle of probe placement—changes the plane of view through the animal, thereby affecting the ultrasound image.

The three brands of equipment used in this study were equipped with different probes and screens, but repeatabilities generally were comparable (Table 2) and in the same range as found in previous studies (Perkins et al. 1992; Herring et al. 1994). However, while variation between equipment in the discrepancy between observed and predicted organ mass was small

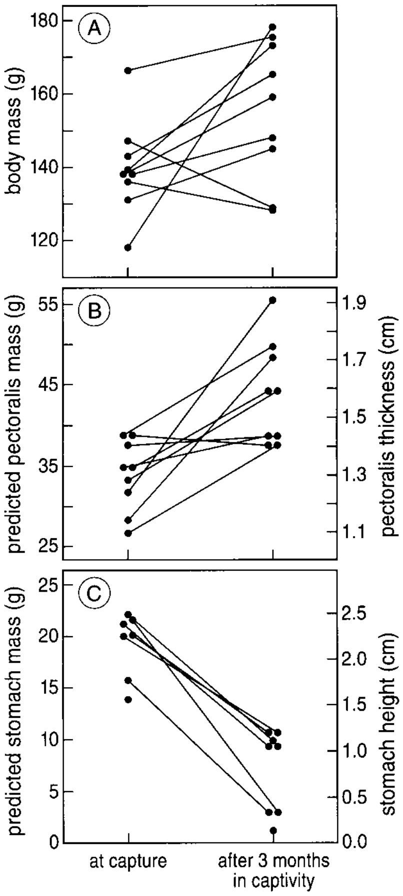

Figure 5. Body mass (*A*), predicted pectoral muscle mass (*B*), and predicted stomach mass (*C*) in knots over a 3-mo period of captivity, while being fed food pellets.

for pectoral muscles, when estimating stomach mass, the discrepancies appeared slightly higher for the Aloka equipment compared with Hitachi and Pie (Table 3). Explanation of this variation would require further study.

Variation between observers was not investigated here, but previous studies have suggested that observer effects decrease as observers gain more experience (Perkins et al. 1992; Herring et al. 1994). Furthermore, we expect that the experience required is specific for particular organ/species combinations. Thus, a period of training is required in order to fully benefit from the advantages of the ultrasound technique. Another observer effect may occur when the observer has a certain expectation of the outcome, for example, about differences between groups. Rigorous methodology would require that the observer is blind with respect to the expected outcome, but this may not always be possible.

Even if ultrasound measurements could be taken without error, there would still be error in the estimate of organ mass due to variation in organ shape; hence, some organs will be more suitable than others to estimate using ultrasound. The location where an organ is measured can affect the accuracy of the mass prediction, although different measurement locations did not differ systematically in their predictive value in our study (Table 3). Nevertheless, this may be worth exploring when developing a calibration curve. In swans, for example, when estimating pectoral muscle mass, the accuracy was higher at anterior than at posterior locations (Sears 1988).

Conclusion

Ultrasound imaging is a simple and promising noninvasive technique for determining organ sizes in individual animals in the laboratory and in the field, though the method will never be as straightforward as using a scale. Ultrasound scanning is particularly suitable for measuring rapid changes in organ size over short time intervals, without sacrificing large numbers of animals. Furthermore, ultrasound measures will enable studies of the interactions between body composition and behavioral and physiological characteristics and fitness measures. Hitherto, this has been impossible.

Acknowledgments

We thank Jan Schouten and Biomedic BV, Rico van Dalen and Ecoscan Ultrasound BV, and Yvonne Soons and Pie Medical Benelux BV for kindly lending us their ultrasonographic scanning equipment. We are particularly grateful to Yvonne Soons, who enthusiastically helped us to understand the ultrasound technique and who lent us her own Pie 200 when other equipment was unavailable. We thank Bernard Spaans for his help with the scanning of the birds. Ciska Raaijmakers helped with the compositional analysis of the carcasses made available by Joop Jukema, Peter L. Meininger, and Hans-Ulrich Rösner. We thank Dick Visser for drawing the figures. The experiments complied with the Dutch laws regarding animal experiments. M.D., A.D., and T.P. are supported by a PIONIER grant to T.P. from the Netherlands Organization for Scientific Research (NWO). S.V. is supported by NWO-SLW grant 803-30.165. This is NIOZ publication 3224.

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